Biotherapy Journal Review

Volume 2, Number 2; Spring 2016












BioTherapeutics, Education & Research Foundation

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The BTER Foundation's

Biotherapy Journal Review

Vol 2, No 2; Spring 2016

Table of Contents

Publication Credits p. ii

Letter from the Editor p. 3

Section I: Journal Reviews p. 5

Wilson MR, Nigam Y, Jung W, Knight J, and Pritchard DI. **The impacts of larval density and protease inhibition on feeding in medicinal larvae of the greenbottle fly Lucilia sericata.** Med.Vet.Entomol. 2016; 30(1):1-7.

d'Ettorre G, Ceccarelli G, Giustini N, Serafino S, Calantone N, De Girolamo G, Bianchi L, Bellelli V, Ascoli-Bartoli T, Marcellini S, Turriziani O, Brenchley JM, Vullo V. **Probiotics Reduce Inflammation in Antiretroviral Treated, HIV-Infected Individuals: Results of the "Probio-HIV" Clinical Trial.** PLoS One. 2015 Sep 16;10(9):e0137200.

Leung S, Parumasivam T, Gao F, Carrigy NB, Vehring R, Finlay WH, Morales S, Britton WJ, Kutter E, Chan H. **Production of Inhalation Phage Powders Using Spray Freeze Drying and Spray Drying Techniques for Treatment of Respiratory Infections.** Pharm Res. 2016; Published on-line, February, 2016

Stein M, Greenberg Z, Boaz M, Handzel ZT, Meshesha MK, and Bentwich Z. **The Role of Helminth Infection and Environment in the Development of Allergy: A Prospective Study of Newly-Arrived Ethiopian Immigrants in Israel.** PLoS.Negl.Trop.Dis. 2016; 10(1):e0004208

Section II: Biotherapy Citations for Spring 2016 p. 21

A. Apitherapy/Bee Venom Therapy p. 21

B. Helminthic Therapy p. 22

C. Ichthyotherapy p. 23

D. Leech Therapy p. 24

E. Mammals- Service & Therapy Animals p. 27

F. Maggot Debridement Therapy p. 30

G. Olfactory Detection and Diagnosis p. 32

H. Phage Therapy p. 33

I. Probiotics and Bacteriotherapy p. 111

From the Editor's Desk:

Each new season brings with it some changes, and some consistency. Fortunately each new season also brings another issue of the BTER Foundation's Biotherapy Journal Review. In this Spring issue, you will find some comforting consistency, but also some novelty. As in all issues, we have posted an extensive list of new biotherapy publications, organized by specialty. We have also provided in-depth reviews of four important new research articles. The articles chosen for review represent four different fields: maggot therapy, helminthic therapy, phage therapy and probiotics (bacteriotherapy). They also represent several different strategies within the Scientific Method: observational studies (particularly helpful with questions of epidemiology), laboratory-based or "basic science" hypothesis testing, and translational studies, which bridge the gap between laboratory and clinic.

The Spring issue of the Biotherapy Journal Review also introduces the expansion of our artistic contributions. Between each review and wherever space permits, you will find a scientific or/or artistic image related to biotherapy. Currently these images are provided by the BTER Foundation's media archive, the personal collections of the Editorial Board, or from the public domain. We welcome contributions from our readers as well, and hope to have enough contributions from the community to be able to feature your work for a long time to come. For more information or to submit your images, contact the editor@BTERFoundation.org.

The BTER Foundation seeks to keep its members - and the general community - up to date on events, activities, and new developments in the field of biotherapy. Therefore, beginning with the Spring Issue, another feature you will notice in the Biotherapy Journal Review is the addition of public service announcements, selected conference notices, and promotional information. If you have an announcement that would be of interest to the Biotherapy Community, let us know and we will consider it for the Biotherapy Journal Review and/or for our newsletter, the B*e*TER L*e*TTER.



Ronald A. Sherman, MD, MSc, DTM&H
Director, BTER Foundation

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Section I: Journal Reviews

The BTER Foundation's

Biotherapy Journal Review

Volume 2, Number 2; Spring 2016

Title: The impacts of larval density and protease inhibition on feeding in medicinal larvae of the greenbottle fly Lucilia sericata.
Authors: Wilson MR, Nigam Y, Jung W, Knight J, and Pritchard DI.
Reviewer: Frank Stadler
To view the article review, visit page 6

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Authors: d'Ettorre G, Ceccarelli G, Giustini N, Serafino S, Calantone N, De Girolamo G, Bianchi L, Bellelli V, Ascoli-Bartoli T, Marcellini S, Turriziani O, Brenchley JM, Vullo V.
Reviewer: Dr. Ronald Sherman
To view the article review, visit page 9

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Authors: Leung S, Parumasivam T, Gao F, Carrigy NB, Vehring R, Finlay WH, Morales S, Britton WJ, Kutter E, Chan H.
Reviewer: Dr. Ronald Sherman
To view the article review, visit page 13

Title: The Role of Helminth Infection and Environment in the Development of Allergy: A Prospective Study of Newly-Arrived Ethiopian Immigrants in Israel.
Authors: Stein, M., Greenberg, Z., Boaz, M., Handzel, Z. T., Meshesha, M. K., and Bentwich, Z.
Reviewer: Dr. Ronald Sherman
To view the article review, visit page 16

Maggot Debridement Therapy Reviews

Journal Citation:

Wilson MR, Nigam Y, Jung W, Knight J, and Pritchard DI. **The impacts of larval density and protease inhibition on feeding in medicinal larvae of the greenbottle fly Lucilia sericata.** Med.Vet.Entomol. 2016; 30(1):1-7.

Published Abstract

Larval therapy, the therapeutic use of blowfly larvae to treat chronic wounds, is primarily used in debridement. There are, however, gaps in current knowledge of the optimal clinical application of the therapy and mechanisms of action in the debridement process. Using an artificial assay, two studies were undertaken to investigate these aspects of larval debridement by Lucilia sericata Meigen (Diptera: Calliphoridae); the first studied the effects of the density of larvae on tissue digestion and larval mass, and the second considered the effects on the same parameters of incorporating protease inhibitors into the feeding substrate. The total mass of tissue digested increased with larval density until saturation was observed at 5.0-7.5 larvae/cm(2) . This range was considered optimal as lower doses resulted in the removal of less tissue and higher doses offered no additional tissue removal and appeared to exacerbate competition for feeding. In the second study, increased protease inhibitor concentration led to significant decreases in tissue digestion and larval mass, suggesting that serine proteases, particularly trypsin, may play major roles in larval digestion. Such information is important in elucidating the main constituents that make up larval digestive products and may be significant in the development of new therapies.

Introduction & Background to Study

The authors revisit the background to larval therapy which is increasingly used to remove necrotic and devitalized tissue from wounds, to prevent and fight infection and to stimulate wound healing. They briefly review recent biochemical and molecular approaches to the characterization of proteases and their corresponding gene sequences, and discuss the potential for these advances to translate into the development of new wound care therapies. However, Wilson et al. appear to be primarily motivated to shed light on the relationship between larval density and debridement activity, and on the role of proteases in larval debridement. They argue that determining optimal larval density and a better understanding of larval activity will lead to greater efficacy and cost-effectiveness in larval therapy

Materials & Methods

Larval activity assays were conducted using bagged medical maggots that were placed on pork substrate. Both the change in larval and substrate mass was recorded for the 48 hour feeding period at 32 °C, because larval mass gain

is influenced by competition and the ability to digest/ingest substrate (i.e. debride the wound), and substrate mass loss is a direct measure of debridement activity.

In the density experiment, treatments included densities of between 0 and 15 larvae per cm^2 applied via BioBag to 20g of pork. In the protease activity experiment, the authors added Kunitz soybean trypsin inhibitor and a high-activity soybean trypsin inhibitor to the pork substrate at concentrations of 1 to 20 mg per gram of pork. The authors used an optimal larval density of 5 larvae per cm^2 for this experiment.

Study Results

As for the larval density and debridement efficiency experiment, the authors clearly demonstrated that tissue loss increased for densities from 0 and 7.5 larvae per cm^2 but that there was no further increase in tissue loss beyond 7.5 larvae per cm^2. Larval mass was unaffected by increasing larval density until 5 larvae per cm^2 beyond which larval mass decreased. Increasing larval densities did not lead to increased mortality of larvae.

In the protease inhibitor study, the addition of inhibitor had an inversely proportional effect on tissue loss and larval mass gain, meaning that increasing concentrations of inhibitor led to less tissue being consumed and less larval mass gained over the experimental period. There was no difference between the two types of soybean trypsin inhibitor nor did their concentration affect mortality rates. Interestingly, inhibitors also had an affect when there were no larvae present.

Author's Conclusions

The authors conclude that the ideal application density for bagged *L. sericata* is between 5 and 7.5 larvae per cm^2 of covered wound area, based on the maximal debridement activity at 7.5 larvae per cm^2 and optimal weight gain for larvae up to a density of 5 larvae per cm^2. The authors explain this with a steady increase in resource competition within the confined environment of the BioBag which eventually affects food availability. Over-application of larvae therefore does not convey additional debridement gain.

In the eyes of the authors, the inhibitor study confirmed the importance of trypsin for medical maggots to digest tissue and grow, which confirms the findings of other studies that investigated the relationship between trypsin and protein digestion. Moreover, results suggest that chymotrypsin has a lesser effect on larval digestion. The authors conclude "*The present assays provide insight into effective dosing and how larval density affects debridement. In addition, the study confirms the significance of specific serine proteases to the mechanism of larval debridement.*"

Reviewer's Conclusions

This is an important study because it provides an experimentally verified optimal larval density for application of bagged larvae, for which there was hitherto little experimental data available in the public arena. The authors freely acknowledge the limitations of such an assay in that it can only approximate the wound environment. Provided these limitations are kept in mind, the wound assay is an adequate approach and the results appear to be sound and conclusive.

What deserves discussion, however, is the influence of maggot enclosure on density-dependent debridement efficiency. Wilson and colleagues correctly pointed out that the BioBag restricts larval access to the wound and renders what is in nature a three-dimensional system into a flat two-dimensional system. In the wound, medical maggots can bury into the slough and necrotic tissue and therefore spread out in three dimensions and gain better access to their food source. Therefore, in BioBags one should expect density-dependent negative effects to occur at lower densities than under 'free-range' conditions, which has implications for larval dosage advice for free-range versus bagged application of medical maggots.

Furthermore, the authors suggest that in addition to being clinically inefficient, overapplication is also wasteful. Whether a few maggots more or less in a BioBag is a cost concern is a matter for producers to decide. However, reducing larval densities to levels recommended in this study without considering potential larval mortality in the wound may indeed decrease efficiency rather than improve it. Larval survival has been shown to be affected by wound aetiology (Čičková, et al. 2013, Evidence-based Comp Alt Med 2013), and other factors such as healthcare provider skill in applying and maintaining dressings, and patient behavior, also have an impact. A moderate surplus of maggots applied to wounds whether free-range or bagged should compensate for such mortality and still deliver adequate therapeutic benefit. Besides, the study suggests that over-application within BioBags has no detrimental effect on wound debridement efficiency. It merely reduces the growth rate of larvae which is of little consequence because they are disposed of after treatment.

To the reviewer's mind, the paper would have been a more coherent body of work if the inhibitor study had been omitted and discussed in a separate publication with additional experimentation elucidating the relative roles of trypsin and chymotrypsin. More importantly, the density study has direct and practical implications for larval therapy while the inhibitor study aims to understand the role of larval proteases with a long-term view toward the harnessing of biochemical attributes of larval excretions and secretions for wound care pharmaceuticals.

In summary, the authors are to be congratulated for generating the experimental evidence for effective and efficient larval densities in the application of bagged maggots in maggot therapy.

Probiotics and Bacteriotherapy Reviews

Journal Citation:

d'Ettorre G, Ceccarelli G, Giustini N, Serafino S, Calantone N, De Girolamo G, Bianchi L, Bellelli V, Ascoli-Bartoli T, Marcellini S, Turriziani O, Brenchley JM, Vullo V. **Probiotics Reduce Inflammation in Antiretroviral Treated, HIV-Infected Individuals: Results of the "Probio-HIV" Clinical Trial.** PLoS One. 2015 Sep 16;10(9):e0137200.

Published Abstract

BACKGROUND: HIV infection results in damage to the gastrointestinal (GI) tract, microbial translocation and immune activation. These are not completely normalized with combined antiretroviral therapy (cART). Moreover, increate morbidity and mortality of cART-treated HIV-infected individuals is associated with inflammation. ***METHODS***: In order to enhance GI tract immunity, we recruited and treated 20 HIV-infected humans with cART supplemented with probiotics and followed inflammation and immunological parameters (clinical trial number NCT02164344). 11 HIV seronegative subjects were included as control group. The enumeration of CD4+, CD8+, CD38+ and HLA-DR+ lymphocytes were evaluated on peripheral blood; HIV-RNA levels, sCD14, d-dimer, C-reactive protein (CRP) high sensitivity C-reactive protein (hsCRP), IL-6 and Lipopolysaccharide Binding Protein (LBP) were assayed on plasma. ***RESULTS***: We observe that cART does not normalize the levels of immune activation in HIV positive patients anyway inflammation and markers of microbial translocation were significantly reduced with probiotic supplementation. Patients show a clear and statistically significant reduction in the levels of immune activation on CD4 T-lymphocytes, for both markers CD38 and HLA-DR and their simultaneous expression, LBP and hsCRP plasma levels after probiotic diet supplementation settling to values comparable to controls. ***CONCLUSIONS***: Supplementing cART with probiotics in HIV-infected individuals may improve GI tract immunity and there by mitigate inflammatory sequelae, ultimately improving prognosis.

Introduction & Background to Study

Infection with the retrovirus known as Human Immunodeficiency Virus (HIV) often begins insidiously as a "cold" or "flu." Gastrointestinal symptoms like diarrhea and abdominal distress often follow, as the virus multiplies in the white blood cells, which are incredibly numerous in the lining of the intestinal tract. It is no wonder, then, that the early stages of HIV infection are associated with a perturbation ("dysbiosis") of the GI tract microbiome, with reduced numbers of *bifidobacteria* and *lactobacillus* species, along with increased numbers of potentially pathogenic *proteobacteria* species. As the retroviral disease advances, so does the destruction of the cellular immune system, until we can no longer defend ourselves from the microbial world outside or within us, nor can we effectively find and destroy those mutated cells that we call cancers.

The development of antiretroviral therapy (ART) – and in particular, the use of multiple agents simultaneously to create a highly active or combined antiretroviral therapy "cocktail" (HAART or cART) – have effectively converted HIV infection from a rapidly fatal infection into a chronic disease. Still, patients treated with cART have a shorter life span than non-infected persons, and they have a variety of complications that are not directly attributable to the drugs themselves. These complications include gastrointestinal, hepatic, renal, cardiac, and neurological systems. One mechanism that may play a role in at least some of these disorders may be inflammation, for increased inflammation and immune activation have been common in HIV positive individuals, even when the virus appears completely suppressed by appropriate therapy.

HIV-positive persons – even those with completely "controlled infection" – have higher levels of activated T cells, inflammatory monocytes and pro-inflammatory cytokines than seronegative individuals. Possible causes include ongoing HIV replication at low levels (less than what can be detected by our current technology), the presence of coinfections, and microbial translocation.

The authors of this paper Hypothesized that, if microbial translocation is a cause, and if some of the microbes responsible were those in the dysbiotic gut, then probiotics might be able to control or reverse the inflammatory state. To that end, they provided HIV-positive subjects with both cART and a probiotic supplement for 48 weeks, and monitored immune function and activation status.

Materials & Methods

Study subjects: Subjects were eligible for the study if they were HIV-positive but did not have a diagnosis of AIDS nor nor any current acute infection. They had to be on their first cART regimen with good viral control (viral load less than 37 copies/ml). "Control" subjects were selected from among the HIV-negative community, though beyond that, the precise selection criteria were not described.

Intervention: The HIV-positive subjects were given twice daily dietary supplements with 1 g packets containing *Streptococcus salivarius* ssp. *Termophilus*, *Bifidobacteria* (represented by *B. breve*, *B. infantis* and *B. longum*), *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus delbrueckii ssp. Bulgaricus*, and *Streptococcus faecium* for 48 weeks. The HIV-negative subjects were not given any such supplements.

Data collection: At the beginning of the study and again at the end (48 weeks later), blood tests were drawn to evaluate inflammatory and immunological parameters. Commercially available enzyme-linked immunosorbent assays (ELISAs) were used to quantify plasma levels of soluble CD14 (sCD14), D-dimer, C-reactive protein (CRP), high sensitivity CRP (hsCRP), interleukin 6 (IL-6) and lipopolysaccharide binding protein (LBP). White blood cells (CD4+, CD8+, CD38+ and HLA-DR+ lymphocytes) were measured in the peripheral blood. HIV rna was

measured at the beginning and end of the study; the limit of detection was 1 copy/ml. Additionally, a self-administered questionnaire solicited demographic information, history of prior probiotic use, adherence to the study interventions, and bowel symptoms.

Study Results

A total of 20 HIV-positive subjects were enrolled: 17 men and 3 women, median age was 54 years (range 27–72). All subjects were on cART from at least 3 years (median 12,5 years; range 3–20) and had a median of CD4+ T cell count of 542 cells/µl. (range 285–1402) and HIV RNA of <40 copies/mL at the time of enrolment. Seven women and 4 men comprised the HIV-negative control arm, with an average age of 43 (ranging from 28 to 73 years). Only 29% of patients certified that they had taken lactic acid bacteria in the past, and only a third of the sample regularly consumed yogurt.

CD4+ T cells among HIV+ subjects were lower than for seronegative subjects, consistent with reports in the literature. Also predictably, immune activation was higher in HIV+ subjects than in healthy controls.

Some markers of immune activation decreased significantly over the 48 weeks of probiotic treatment, approaching the lower level of the control group by the end of the study. A small increase in CD4 numbers and percentages was seen over the 48 weeks of probiotics, but this was not considered statistically significant ($p = 0.065$).

Regarding subjective symptoms of gastrointestinal health, all subjects treated with probiotics reported substantial improvement in digestion, reduced intestinal bloating, reduced episodes of acute diarrhea or constipation.

Probiotics were associated with no adverse events and no drop-outs from the study. The cost of probiotics (borne by the study subjects themselves) was described by all subjects as "too expensive."

Author's Conclusions

The authors concluded that "Supplementing cART with probiotics in HIV-infected individuals may improve GI tract immunity and there by mitigate inflammatory sequelae, ultimately improving prognosis . . . . However, the suggestions provided by this pilot study and other researches on the topic are still preliminary and fragmentary to draw any firm conclusions. Instead they represent an important incentive to justify larger studies to assess whether probiotic administration truly could decrease inflammation and improve the prognosis of cART-treated, HIV-infected, individuals . . . . In conclusion current and emerging studies support the concept that probiotic bacteria can provide specific benefit in HIV infected patients during antiretroviral treatment by improvement of the microbiota and reducing mucosal and systemic inflammation."

Reviewer's Conclusions

Probiotics were clearly associated with a better sense of gastrointestinal health. There was also a suggestion that probiotics might decrease the immune activation seen in HIV+ patients. But because the study design called for no control arm of HIV+ subjects who did NOT receive probiotics, we can not deduce with any certainty that the probiotics were responsible for these outcomes. In addition to an HIV+ control arm, I would suggest that any follow-up study examine the immunological parameters more frequently than once every two years (for example, check at 3 months, 6 months, and every 6 months thereafter) just in case there is an effect that occurs early but then wanes.

This reviewer agrees with the authors that probiotics just may decrease the inflammatory state seen in chronic HIV infection. This pilot study deserves to be followed by a larger and better controlled study, and hopefully they are already on it.



Phage Therapy Reviews

Journal Citation:

Leung S, Parumasivam T, Gao F, Carrigy NB, Vehring R, Finlay WH, Morales S, Britton WJ, Kutter E, Chan H. **Production of Inhalation Phage Powders Using Spray Freeze Drying and Spray Drying Techniques for Treatment of Respiratory Infections.** Pharm Res. 2016; Published on-line, February, 2016

Published Abstract

Purpose The potential of aerosol phage therapy for treating lung infections has been demonstrated in animal models and clinical studies. This work compared the performance of two dry powder formation techniques, spray freeze drying (SFD) and spray drying (SD), in producing inhalable phage powders. ***Method*** A Pseudomonas podoviridae phage, PEV2, was incorporated into multi-component formulation systems consisting of trehalose, mannitol and L-leucine (F1=60:20:20 and F2=40:40:20). The phage titer loss after the SFD and SD processes and in vitro aerosol performance of the produced powders were assessed. ***Results*** A significant titer loss (~2 log) was noted for droplet generation using an ultrasonic nozzle employed in the SFD method, but the conventional two-fluid nozzle used in the SD method was less destructive for the phage (~0.75 log loss). The phage were more vulnerable during the evaporative drying process (~0.75 log further loss) compared with the freeze drying step, which caused negligible phage loss. In vitro aerosol performance showed that the SFD powders (~80% phage recovery) provided better phage protection than the SD powders (~20% phage recovery) during the aerosolization process. Despite this, higher total lung doses were obtained for the SD formulations (SD-F1=13.1±1.7×104 pfu and SD-F2=11.0±1.4×104 pfu) than from their counterpart SFD formulations (SFD-F1=8.3±1.8×104 pfu and SFDF2= 2.1±0.3×104 pfu). ***Conclusion*** Overall, the SD method caused less phage reduction during the powder formation process and the resulted powders achieved better aerosol performance for PEV2.

Introduction & Background to Study

Pulmonary (lung) infections are deadly and communicable. These two facts alone make them an important target for antimicrobial therapy. Unfortunately, the frequent and repeated use of antibiotics for pulmonary infections such as tuberculosis, hospital-acquired pneumonias, or the pneumonias in patients with cystic fibrosis or lung cancers has created multi-resistant strains of those infections. Clearly, new treatments are needed for many of these resistant infections.

Bacteriophages (phages) are viruses that are bacterial parasites; they infect and replicate inside bacterial hosts, killing the bacteria in the process. There already exists a number of phages known to kill multi-resistant pulmonary pathogens; but getting those viruses to the site of infection remains a challenge.

Delivery of phages through inhalation could be an effective means of treating pneumonias. Most preparations for pulmonary delivery have been liquid aerosols delivered through intranasal instillation or nebulization. Formulating the phage as a dry powder has the advantage that it could be easily transported and stored until it could be inhaled.

In the past, the efficacy of dry powder phage formulations have been dependent on phage type, the type and concentration of formulation excipients, and the production process. The aim of the current research was to compare and evaluate two methods of preparation – spray drying (SD) and spray freeze drying (SFD) – using different ratios of trehalose, mannitol and leucine as the bulking and stabilizing agents. The stability of phage after the powder production process and the in vitro aerosol performance of the formulation were both assessed.

Materials & Methods

A Pseudomonas podoviridae phage, PEV2, was incorporated into multi-component formulation systems consisting of trehalose, mannitol and L-leucine. The phage suspension was spray dried (SD) or sprayed, frozen in liquid nitrogen and then lyophilized (SFD).

The phage titer was calculated after each process, using the Miles-Misra surface droplet technique of spreading the phage preparation over a lawn of host bacteria (*Pseudomonas aeruginosa*) and counting plaques of bacterial killing after incubation overnight).

In vitro aerosol performance was assessed by dispersing 20 mg of powder into a Multi-stage Liquid Impinger (MSLI) using an Osmohaler™ at 100 l/ min for 2.4 s. The fine particle fraction (FPF) was defined as the mass fraction of particles ≤5.0 µm with respect to the recovered dose.

Study Results

Each method had its advantages and disadvantages. The SFD method of production led to much greater loss of phage during droplet generation with the ultrasonic nozzle, but the phage were less vulnerable during the evaporative drying process. The SFD product also were associated with better phage recovery (80%) in the aerosol performance assay.

The two-fluid nozzle used in the SD method, on the other hand, was less destructive for the phage, but the phage were more vulnerable during the method's evaporative drying process (0.75 log loss). The SD product was

associated with lower phage recovery (20%) in the aerosol performance assay. Still, because of the lower powder loss during the powder production process, higher total lung doses were obtained for the SD formulations, compared with the SFD method overall.

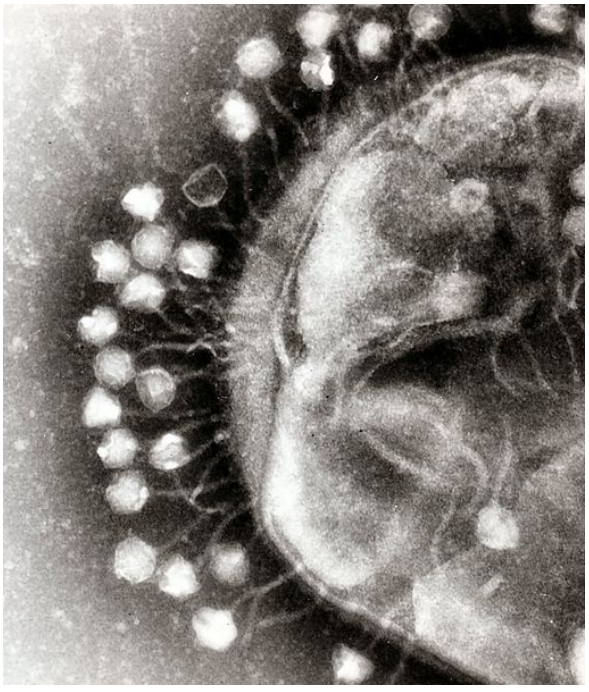
Author's Conclusions

"Overall, the SD method caused less phage reduction during the powder formation process and the resulted powders achieved better aerosol performance for PEV2."

In summary, the SD method produced more stable phage powder formulations and achieved better aerosol performance for the bacteriophage PEV2, and has the potential for providing viable phage for treating lung Pseudomonas infections.

Reviewer's Conclusions

The data speaks for itself. For the PEV2 phage, SD is a better method for efficiently producing stable, aerosolizable powdered product for lung inhalation. The take home message should also be that preparation of phage must be individualized and carefully monitored throughout the process. Had the results of preparation been assessed only after spraying, or only after drying, a different conclusion may have been reached. Only by measuring the effect of each step in the preparation process was it possible to determine the efficacy of that process, and ultimately the optimal method for producing viable, inhalable, dry powdered phage for the treatment of pulmonary infections.



Helminthic Therapy Reviews

Journal Citation

Stein M, Greenberg Z, Boaz M, Handzel ZT, Meshesha MK, and Bentwich Z. **The Role of Helminth Infection and Environment in the Development of Allergy: A Prospective Study of Newly-Arrived Ethiopian Immigrants in Israel.** PLoS.Negl.Trop.Dis. 2016; 10(1):e0004208

Published Abstract

Helminth infection may be protective against allergy and account for the low prevalence of allergy in developing countries. We studied prospectively the prevalence of allergy in Ethiopian immigrants with heavy helminth infection on arrival in Israel, and again after a year of adjustment to an urban industrialized setting, to explore the roles of helminth infection, changed environment and background immunity on the manifestations of allergy. 126 newly arrived Ethiopian immigrants were studied at baseline and 115 after a year of follow up in Israel. Allergic symptoms, Skin prick tests (SPT), Tuberculin (PPD) skin tests, stool and blood samples were obtained for determining parasites, blood IgE and eosinophil levels, respectively. Anti-helminthic therapy was offered to the entire infected individuals, but only 50/108 (46.3%) took the medication. At baseline, there was a significant negative association between helminth infection and allergy, 4/18 (22.2%) of uninfected participants were allergic compared to 7/108 (6.5%) of helminth-infected participants ($p = 0.028$), as well as between helminth infection and SPT reactivity, 12/18 (66.6%) of uninfected participants compared to 43/108 (39.8%) of helminth-infected participants ($p = 0.033$). After one year, a significant general increase in allergy and SPT was observed. While only 11/126 (8.7%) were allergic at baseline, 30/115 (26.1%) became allergic at follow-up ($p<0.0001$), and while 55/126 (43.7%) were SPT+ at baseline, 79/115 (68.7%) became SPT+ at follow-up ($p<0.001$). A twofold increase in allergen sensitization was also observed after one year in Israel, particularly for dust mites, grasses and olive tree ($p<0.001$). These results show that: a) Helminth infection is significantly associated with low allergy and low SPT reactivity; b) One year after immigration to Israel, allergy and SPT reactivity increased significantly in all immigrants; c) Higher increases in positive SPT and allergy were observed after a year in the group that remained infected with helminths, even though they had a lowered helminth load; d) The reasons for the increased allergy one year after immigration needs further investigation but probably reflects the combined influence of the decreased helminth load and novel environmental factors.

Introduction & Background to Study

It is well documented that allergic symptoms are more common in people living in developed countries than in people living in developing countries. Studies have shown an association between allergy, affluence, environment. The often

quoted "hygiene hypothesis" suggests that improved sanitary conditions of the developed world – or more specific, fewer parasitic infections – is a major contributing factor behind this discrepancy in the prevalence of allergies. But data to this effect has been conflicting.

Over 60,000 Ethiopian Jews immigrated from Ethiopia to Israel in recent years, primarily in a large airlift in the mid-eighties and again in 1991. These immigrants came mostly from rural areas in Northern Ethiopia, and after arrival in Israel, they underwent medical evaluations and treatment. On arrival of both waves of immigration, they were found to have a very high prevalence of helminth infections with a very low prevalence of allergy, while after a year in Israel, many of them developed allergy. The aim of this study was to investigate the prevalence of allergy in relation to helminth infection and a changing environment in a population of Ethiopian immigrants to Israel.

Materials & Methods

Study Design: Prospective randomized longitudinal epidemiologic study, conducted between 1997–2001.

Study population: Ethiopian immigrants were recruited during their first three months of arrival, in Israel. They were selected randomly from three immigrant absorption centers.

Exclusion Criteria: Subjects found to have tuberculosis, syphilis, or HIV infections were excluded from the study.

Clinical and laboratory testing: The immigrants were interviewed by the physician with the help of an experienced Ethiopian social worker. A questionnaire about respiratory symptoms relating to asthma and allergic rhinitis (based on the International Union Against Tuberculosis and Lung Disease questionnaire and the European Community Respiratory Health Survey) was administered to each subject. Laboratory testing was performed at the time of enrollment and 1 year later. Testing included a skin prick test (SPT) for ten common allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, feather mix, cockroach, cat pelt, dog epithelium, grass pollens mix, weed mix, olive, and cypress trees). Mantoux skin test for delayed type hypersensitivity with intracutaneous injection of tuberculin (PPD) were administered, and induration of 10 mm or more was interpreted as a positive response. Helminth infections were identified by stool studies (quantitative parasite egg counts). Complete blood count, total eosinophil count and total serum IgE levels were also evaluated.

Antihelminthic treatment: Helminth infected subjects were offered appropriate infection-specific treatment. Those who consented to treatment were treated directly by the physician and/or by the health worker.

Definitions: Allergic individuals were defined as those having both, positive SPT and clinical history of asthma and/or allergic rhinitis. Individuals with a negative clinical history of asthma or rhinitis, but with a positive SPT were defined as non-allergic SPT-positives (sensitized asymptomatic atopic individuals). Individuals with a negative clinical history and a negative SPT were defined as non-allergic normal individuals.

Study Results

A total of 126 participants were enrolled in the study (66 male, 60 female, mean age 31.2 ± 14.5). At baseline, helminthic infections were detected in 108 out of 126 (85.7%). Hookworm was the most prevalent species, being found in 79 individuals (73.1%). *Schistosoma mansoni* was found in 51 (47.2%), *Ascaris* species in 50 (46.3%), and *Trichuris trichiura* in 16 subjects (14.8%). Multiple worm infections were found in 70 (64.8%) of the infected subjects.

Eosinophil counts were elevated across the entire cohort, whether or not they were infected with helminths. However, helminth-infected individuals had significantly higher levels of eosinophilia than uninfected individuals ($p = 0.01$). Similarly, IgE levels were elevated across the entire cohort, but tended to be higher in the helminth-infected group ($p = 0.06$).

Allergy was present in 11/126 (8.7%) subjects at baseline. There were 3/126 (2.4%) subjects with clinical rhinitis but with negative SPT, who were not categorized as allergic individuals. Allergic individuals were predominantly male (9/11, $p = 0.041$) and older (mean age 41.2 ± 14.4 versus 29.7 ± 14.1, $p = 0.01$). There was a significant negative association between helminth infection and allergy. Seven of 108 (6.5%) of helminth infected subjects were allergic, while only 4 of 18 (22.2%) of uninfected subjects were allergic ($p = 0.028$). Helminth infected subjects were also less likely to have SPT reactivity at baseline ($p = 0.033$) and less likely to have PPD skin test reactivity at baseline ($p = 0.019$). Among helminth infected subjects, those with multiple infections and/or high egg burdens were less likely to be allergic.

Out of the 126 original study participants, 115 individuals were available for follow-up testing one year later. The 11 individuals who withdrew from the study were all helminth infected and all non-allergic. Only 50 (46.3%) of the original 108 helminth infected individuals agreed to receive treatment for their helminth infection; 28 (56%) of these achieved successful eradication.

In the group of subjects with helminths whose infections were not successfully eradicated, peripheral blood eosinophilia did not change significantly, and total IgE increased slightly. The mean egg burden in this group decreased from a mean of 1327 to a mean of 699 eggs/cm3 of stool, ($p = 0.02$).

Allergy increased significantly in the overall cohort from 11/126 (8.7%) allergic individuals at baseline to 30/115 (26.1%) at follow-up ($p<0.001$). Clinical history of

allergy but with negative SPT was found in two individuals with rhinitis that were considered as non-allergic rhinitis patients.

Several new asthma cases arose by the end of the year, but this rise was statistically significant only in the group of subjects who were initially infected with helminths and remained so ($p = 0.002$). SPT reactivity also increased significantly in the overall cohort, but again that increase was significant only in the groups that was initially infected with helminths and either remained infected or was successfully treated.

The mean allergen sensitization increased by 2 fold, with an increase in the number of allergens sensitizations per individual after a year in all the groups. Individuals who were infected with helminths, whether or not that infection was cured, showed an increase in the numbers of allergens to which they were sensitive; but those were not infected showed no significant increase.

Author's Conclusions

"The results of the present study suggest that helminth infection confers protection from allergy but also point to the possible role of the new environment and the change in the helminth load, in increasing allergy . . . . Additional studies . . . would be important in gaining a better understanding of these phenomena and will be of great importance to public health in the coming years."

Reviewer's Conclusions

For many years is has been clear that populations infected with helminths had a significantly lower prevalence of allergies (and other diseases). But it has been difficult to demonstrate that it was the helminth infection that was somehow protective. Some researchers suggested that the reason it was difficult to prove this relationship was because it did not exist. In this study we have evidence to indicate a more common alternative explanation: the relationship holds, but it is not the only factor in the relationship. Clearly, environmental factors (and/or behavioral factors triggered by the environment) were at play in this cohort, in which the prevalence of allergies increased whether or not the subjects were infected with helminths. Indeed, for some of the tests, those who were initially infected with helminths and remained infected demonstrated an increase in allergic signs more so than did their uninfected cohorts. This reviewer agrees completely that further study is necessary to understand the factors at play in the development of allergy, as well as the directly and/or indirectly protective roles that helminths appear to play.

The ability to study and compare individuals who both cleared their helminthic infection and those who did not was a particularly strong point in this study. Hopefully future studies will be large enough to look even deeper into these cohorts and separate meaningful differences in behavior (i.e., cultural adaptations), environmental exposures (i.e., changes in diet, animal and

material exposures), and even sort out differences between those immigrants to remained infected because their antibiotic treatment failed and those who remained infected because they refused treatment. Refusing treatment on the basis of cultural beliefs may itself correlate with a difference in the level of integration with their new society and environment.



Section II: Biotherapy Citations for Spring 2016

A. Apitherapy and Bee Venom Therapy

Abdulsalam, M. A., Ebrahim, B. E., and Abdulsalam, A. J. Immune thrombocytopenia after bee venom therapy: a case report. BMC.Complement Altern.Med. 2016; 16(1):107

ABSTRACT: BACKGROUND: Immune thrombocytopenia (ITP) is a hematological disorder with an isolated decrease in number of circulating platelets. Bee venom therapy (BVT) is a form of alternative medicine. It is still being practiced in the Middle East and other parts of Asia. In BVT, acupuncture points are used to inject diluted bee venom into the body. The pharmacological basis behind BVT is not fully understood. However, it has been used to treat various medical conditions such as arthritis and low back pain. On the other hand there have been a number of reported complications of BVT use such as ITP. We present a case report on ITP after BVT. CASE PRESENTATION: A 61 year old lady presented with gum bleeding and ecchymosis and found to have isolated thrombocytopenia (platelet count of 9 x 10(9)/L) after receiving four direct bee sting sessions. There was no evidence of any other risk factors of ITP. CONCLUSION: Bee venom components and toxicity may be associated with thrombocytopenia as a complication. Further research is needed to postulate guidelines and protocol for BVT. In the meantime, monitoring of the practice of BVT should be made, with an emphasis on patient education regarding the safety profile and associated risks compared to the gained benefits.

Lee, G. and Bae, H. Bee Venom Phospholipase A(2): Yesterday's Enemy Becomes Today's Friend. Toxins.(Basel). 2016; 8(2)

ABSTRACT: Bee venom therapy has been used to treat immune-related diseases such as arthritis for a long time. Recently, it has revealed that group III secretory phospholipase A(2) from bee venom (bee venom group III sPLA(2)) has in vitro and in vivo immunomodulatory effects. A growing number of reports have demonstrated the therapeutic effects of bee venom group III sPLA(2). Notably, new experimental data have shown protective immune responses of bee venom group III sPLA(2) against a wide range of diseases including asthma, Parkinson's disease, and drug-induced organ inflammation. It is critical to evaluate the beneficial and adverse effects of bee venom group III sPLA(2) because this enzyme is known to be the major allergen of bee venom that can cause anaphylactic shock. For many decades, efforts have been made to avoid its adverse effects. At high concentrations, exposure to bee venom group III sPLA(2) can result in damage to cellular membranes and necrotic cell death. In this review, we summarized the current knowledge about the therapeutic effects of bee venom group III sPLA(2) on several immunological diseases and described the detailed mechanisms of bee venom group III sPLA(2) in regulating various immune responses and physiopathological changes.

B. Helminthic Therapy

Nair, M. G. and Herbert, D. R. Immune polarization by hookworms: taking cues from T 2, ILC2 and M2. Immunology. 2-29-2016;

ABSTRACT: Cellular and molecular investigation of parasitic helminth infections has greatly accelerated the understanding of Type 2 immune responses. However, there remains considerable debate regarding the specific leukocytes that kill parasites and whether these mechanisms are distinct from those responsible for tissue repair. Herein, we chronicle discoveries over the past decade highlighting current paradigms in Type 2 immunity with a particular emphasis upon how CD4+ T helper 2 cells (TH 2), type 2 innate lymphoid cells (ILC2), and alternatively activated macrophages (M2) coordinately control helminth-induced parasitism. Primarily, this review will draw from studies of the murine nematode parasite Nippostrongylus brasiliensis, which bears important similarities to the human hookworms Ancylostoma duodenale and Necator americanus. Given that one or more hookworm species currently infect millions of individuals across the globe, we propose that vaccine and/or pharmaceutical-based cure strategies targeting these affected human populations should incorporate the conceptual advances outlined herein. This article is protected by copyright. All rights reserved.

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C. Ichthyotherapy

No New Publications for Spring 2016.



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D. Leech Therapy

Baylan, J. M., Chambers, J. A., McMullin, N., Fletcher, J. L., Sinha, I., Lundy, J., King, B. T., and Chan, R. K. Reverse posterior interosseous flap for defects of the dorsal ulnar wrist using previously burned and recently grafted skin. Burns. 2016; 42(2):e24-e30.

ABSTRACT: BACKGROUND: In the severely burned patient, coverage of exposed bone in the dorsal ulnar wrist can be a difficult problem. This is especially challenging in patients with a high percentage total body surface area (TBSA) where donor flaps can be scarce. The use of previously burned and/or recently grafted skin as flaps is an option. It has been postulated that use of previously burned skin can result in higher rates of local or distant flap failures. The reverse posterior interosseous flap (PIF) is an axial flap, based on the retrograde posterior interosseous artery, to provide coverage of the hand. Here we describe utilization of the PIF, using previously burned and/or recently grafted skin for coverage of dorsal ulnar wrist defects. METHODS: This is a case series of three patients, with extensive burns (range 35-83%TBSA), where defects of the dorsal ulnar wrist necessitated coverage. Each patient underwent PIF(s) utilizing previously burned and/or grafted skin, all within three months after their initial burn event. RESULTS: Case 1: 28 year old male who suffered 35% TBSA via blast mechanism developed a chronic open wound over the dorsal ulnar wrist with exposed tendon. The patient successfully underwent a left PIF using previously grafted skin. Case 2: 23 year old male with 83% TBSA. Bilateral ulnar styloids were exposed. PIFs were performed bilaterally, using previously burned and recently grafted skin. Coverage was successful but received leech therapy post-operatively for venous congestion. Case 3: 37 year old male with 52% TBSA, with the most severe burns isolated to his bilateral upper extremities; the ulnar head was exposed. The posterior interroseous artery was explored and PIF was attempted, but there was no retrograde flow in the distal artery due to a deeper injury than previously recognized. The patient ultimately underwent a pedicled abdominal flap for coverage. CONCLUSIONS: Defects of the distal ulnar wrist after deep and extensive burns can be problematic. Use of the reverse PIF using previously burned skin, even those that has just been recently grafted is a viable option for this difficult patient population. However, it may not be possible in all patients. Vigilance and early intervention for post-operative venous congestion are important.

Oregaard, J. S., Lang, C. L., and Venzo, A. Partial salvage of avulsed tissue after dog bite. Ann.R.Coll.Surg.Engl. 2016; 98(2):e22-e25.

ABSTRACT: Injuries to the nose can be severe from both a functional and cosmetic perspective. After suffering a dog bite to the central part of the face, an 18-year old woman underwent replantation of the avulsed tissue with the help of microsurgical arterial anastomosis. A venous anastomosis was impossible and venous congestion was treated with leech therapy. Subsequent skin necrosis occurred after a few days and the replantation was revised, revealing healthy tissue immediately below. The defect was covered with a full-thickness skin graft. At follow-up review eight months later, the functional and cosmetic result was satisfactory. To our knowledge, this is one of few cases where an injury of this severity healed with a cosmetically acceptable result.

Rajic, A. J. and Deleyiannis, F. W. Determining the Duration of Leech Therapy in the Treatment of Acute Venous Congestion in Prefabricated Free Flaps. Plast.Reconstr.Surg. 2016; 137(2):495e-496e.

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Abstract Not Available.

Sadeghi, A., Navabakhsh, B., and Izadi, Vahedi N. Leech Induced Pyoderma Gangrenosum in an Ulcerative Colitis Patient: A Case Report. Middle East J.Dig.Dis. 2016; 8(1):63-66.

ABSTRACT: Pyoderma gangrenosum (PG) is a painful skin lesion that results from excessive inflammatory response to a host of traumatic, inflammatory, or neoplastic processes in susceptible individuals. A clear pathogenetic mechanism as well as an exhaustive list of potential triggers for PG is yet to be fully characterized. This case documents the occurrence of pyoderma gangrenosum following leech-therapy in a patient who is a known case of ulcerative colitis and it deserves attention because leeches have been part of medical armamentarium since ancient times and have re-emerged in the last century relying on their ancient charm and modern research revealing potential benefits of several bioactive substances in their saliva.

Verriere, B., Sabatier, B., Carbonnelle, E., Mainardi, J. L., Prognon, P., Whitaker, I., Lantieri, L., and Hivelin, M. Medicinal leech therapy and Aeromonas spp. infection. Eur.J.Clin.Microbiol.Infect.Dis. 4-2-2016;

ABSTRACT: While the use of medicinal leech therapy (MLT) in reconstructive and orthopaedic surgery is widely described, post-operative complications related to leeches remain a major concern. Aeromonas spp. strains are involved in the majority of reported cases. As surgical success rate is directly impacted, an adapted antibiotic prophylaxis should be instituted in order to minimize these complications. We assessed pharmaceutical process, microbiological control and related infections in order to provide data and choose the appropriate antibiotherapy for patients requiring MLT. We report a clinical and microbiological study over a 24-month period. Clinical data were collected from patients' database, and microbiological analysis both on leeches' tank water and crushed leeches were performed to characterize isolated strains and their susceptibility to antibiotics. A total of 595 leeches were used to treat 28 patients (12 in plastic surgery and 16 in orthopaedic surgery), and three documented cases of post-operative infections were reported. Aeromonas spp. isolates yielded from 62 % of analyzed batches (75 % of Aeromonas veronii). Eighteen Aeromonas spp. isolates yielded from 23 water samples and three crushed leeches. Isolates were similar in tank and crushed leeches. Strains were susceptible to fluoroquinolones, sulfamethoxazole/trimethoprim, aminosides, and third-generation cephalosporins but resistant to amoxicillin/clavulanic acid and second-generation cephalosporins. According to collected data, routine tank water microbiological analyses are mandatory in order to identify leeches' batches containing resistant strains and to discard them. In this context, the surgeon is able to select an appropriated antibiotic prophylaxis in order to avoid MLT associated serious post-operative complications.

Wagenheim, G. N., Au, J., and Gargollo, P. C. Medicinal Leech Therapy for Glans Penis Congestion After Primary Bladder Exstrophy-Epispadias Repair in an Infant: A Case Report. Urology. 2016; 87:193-195.

ABSTRACT: Many postoperative complications have been reported after repair of classic bladder exstrophy. We present a case of medicinal leech therapy for glans penis congestion following exstrophy repair in an infant. A 2-week-old male with classic bladder exstrophy underwent complete primary repair. On postoperative day 1, he developed rapidly worsening glans penis venous congestion. Medicinal leech therapy was instituted with antibiotics and blood transfusions to maintain a hematocrit >30%. After 24 hours, venous congestion improved and therapy was discontinued. The patient's remaining hospital course was uncomplicated. Medicinal leeches are an effective therapy to relieve glans penis venous congestion.

Zaidi, S. A. Unani treatment and leech therapy saved the diabetic foot of a patient from amputation. Int.Wound.J. 2016; 13(2):263-264.

ABSTRACT: Every 30 seconds, a lower limb amputation is carried out due to diabetes throughout the world. The mortality rate due to diabetic foot gangrene is just next to that of cancer. As tissue death cannot be reversed, surgical removal of the affected tissue (debridement) or amputation of the limb is the only treatment option left when gangrene has advanced. The present case study illustrates an option to treat poorly healing diabetic wounds with Unani medicine (blood purifier and deobstruent) besides hirudotherapy. The study was performed on a 60-year-old woman suffering from diabetic foot (on the left) grade 5 and facing the prospect of imminent amputation. The patient was having severe pain (80 mm on a 100 mm visual analogue scale) in the gangrenous foot and foul-smelling with necrosed areas. Wound dressing was done with unripe papaya as it has a very good role in clearing necrotising area and hirudotherapy was also used in poorly healing wounds. The pain score decreased to 0-10 mm on a 100 mm visual analogue scale within 20 days and no further pain relieving medication was required. Over a time interval of nearly 3.5 months, necrotic areas disappeared and the wound was completely healed.



E. Mammals- Service & Therapeutic Animals

Burres, S., Edwards, N. E., Beck, A. M., and Richards, E. Incorporating Pets into Acute Inpatient Rehabilitation: A Case Study. Rehabil.Nurs. 3-9-2016;

ABSTRACT: PURPOSE: The use of animals in various healthcare settings dates as far back as the 19th century, and is still a widely practiced intervention even today. The use of animals in the acute rehabilitation setting is a common practice that benefits both the patient's therapy progression and allows the opportunity for financial reimbursement for the facility. As acute rehabilitation facilities continue to cope with ever changing rules and guidelines, the use of alternate modalities can help the facility overcome difficult challenges while focusing on the needs of the patients. The use of animal assisted therapy is illustrated with a stroke patient at an acute rehabilitation facility who benefited from implementing a pet therapy regimen when regular therapy modalities were not helping. CLINICAL RELEVANCE: Incorporating animal assisted therapy in acute rehabilitation settings is described to obtain greater satisfaction for patients and staff and to facilitate reimbursement for rehabilitation settings.

Cabiddu, R., Borghi-Silva, A., Trimer, R., Trimer, V., Ricci, P. A., Italiano, Monteiro C., Camargo Magalhaes, Maniglia M., Silva Pereira, A. M., Rodrigues das, Chagas G., and Carvalho, E. M. Hippotherapy acute impact on heart rate variability non-linear dynamics in neurological disorders. Physiol Behav. 3-15-2016; 159:88-94.

ABSTRACT: Neurological disorders are associated with autonomic dysfunction. Hippotherapy (HT) is a therapy treatment strategy that utilizes a horse in an interdisciplinary approach for the physical and mental rehabilitation of people with physical, mental and/or psychological disabilities. However, no studies have been carried out which evaluated the effects of HT on the autonomic control in these patients. Therefore, the objective of the present study was to investigate the effects of a single HT session on cardiovascular autonomic control by time domain and non-linear analysis of heart rate variability (HRV). The HRV signal was recorded continuously in twelve children affected by neurological disorders during a HT session, consisting in a 10-minute sitting position rest (P1), a 15-minute preparatory phase sitting on the horse (P2), a 15-minute HT session (P3) and a final 10-minute sitting position recovery (P4). Time domain and non-linear HRV indices, including Sample Entropy (SampEn), Lempel-Ziv Complexity (LZC) and Detrended Fluctuation Analysis (DFA), were calculated for each treatment phase. We observed that SampEn increased during P3 (SampEn=0.56+/-0.10) with respect to P1 (SampEn=0.40+/-0.14, $p<0.05$), while DFA decreased during P3 (DFA=1.10+/-0.10) with respect to P1 (DFA=1.26+/-0.14, $p<0.05$). A significant SDRR increase ($p<0.05$) was observed during the recovery period P4 (SDRR=50+/-30ms) with respect to the HT session period P3 (SDRR=30+/-10ms). Our results suggest that HT might benefit children with disabilities attributable to neurological disorders by eliciting an acute autonomic response during the therapy and during the recovery period.

Champagne, D., Corriveau, H., and Dugas, C. Effect of Hippotherapy on Motor Proficiency and Function in Children with Cerebral Palsy Who Walk. Phys.Occup.Ther.Pediatr. 3-1-2016; 1-13.

ABSTRACT: AIMS: To evaluate the effects of hippotherapy on physical capacities of children with cerebral palsy. METHODS: Thirteen children (4-12 years old) with cerebral palsy classified in Gross Motor Function Classification System Level I or II were included in this prospective quasi-experimental ABA design study. Participants received 10 weeks of hippotherapy (30 min per week). Gross motor function and proficiency were measured with the Bruininks-Oseretski Motor Proficiency short form [BOT2-SF]) and the Gross Motor Function Measure-88 [GMFM-88] (Dimension D and E) twice before the program (T1 and T1'), immediately after (T2), and 10 weeks following the end of the program (T3). RESULTS: Mean scores for dimensions D and E of the GMFM-88 Dimension scores (p = .005) and three out of the eight items of the BOT2-SF (fine motor precision (p = .013), balance (p = .025), and strength (p = .012) improved between baseline and immediately after intervention; mean scores immediately following and 10 weeks following intervention did not differ. CONCLUSIONS: Hippotherapy provided by a trained therapist who applies an intense and graded session for 10 weeks can improve body functions and performance of gross motor and fine motor activities in children with cerebral palsy.

Krause-Parello, C. A. and Kolassa, J. Pet Therapy: Enhancing Social and Cardiovascular Wellness in Community Dwelling Older Adults. J.Community Health Nurs. 2016; 33(1):1-10.

ABSTRACT: Pet therapy can be therapeutic for older adults living in the community. A crossover design was used to examine changes in blood pressure and heart rate before and after a pet therapy visit versus a volunteer-only visit in 28 community dwelling older adults. Relationships among stress, pet attitude, social support, and health status were also examined. Study findings supported that pet therapy significantly decreased blood pressure and heart rate. Ultimately, the findings supported the notion that community health nurses should consider developing and implementing pet therapy programs in the communities they serve. Further implications for community health nurses are discussed.

Rigby, B. R. and Grandjean, P. W. The Efficacy of Equine-Assisted Activities and Therapies on Improving Physical Function. J.Altern.Complement Med. 2016; 22(1):9-24.

ABSTRACT: OBJECTIVE: To summarize the physical benefits of therapeutic horseback riding and hippotherapy and suggest directions for future research. METHODS: Review of databases for peer-reviewed articles related to equine-assisted activities and therapies. Databases included MEDLINE via EBSCO, Web of Science, PubMed, Google Scholar, and Academic Search Complete. Articles were limited to those with full-text access published in English since 1987. RESULTS: Acute and residual improvements in physical benefits, such as gross motor function (e.g., walking, running, jumping), spasticity, muscle symmetry, posture, balance, and gait occur in adults and children with varying disabilities. The benefits appear to be greatest following multiweek interventions with one or more sessions per week. Modest acute cardiovascular responses are observed during equine-assisted activities and therapies with little or no evidence for training improvements in heart rate or blood pressure at rest or during riding. CONCLUSION: The present body of literature provides evidence that equine-assisted activities and therapies are an effective means of improving many measures of physical health. However, more controlled trials are urgently needed to strengthen the current knowledge base, establish dose-response characteristics of equine-assisted activities and therapies, and explore the physiologic basis for the promising results suggested from the literature.

Strom, B. S., Ytrehus, S., and Grov, E. K. Sensory stimulation for persons with dementia: a review of the literature. J.Clin.Nurs. 3-31-2016;

ABSTRACT: AIMS AND OBJECTIVES: To provide an overview of available sensory stimulation interventions, and their effect on persons with dementia and to present theoretical and methodological characteristics of the studies included. BACKGROUND: Different sensory stimulation interventions are used for persons with dementia to increase alertness, reduce agitation and improve quality of life. However, the effect of these interventions is not clear, neither are their characteristics. DESIGN: A systematic search and review of the literature with description of the content and an evaluation of theoretical and methodological approaches. METHODS: Systematic searches in CINAHL, PubMed (Medline), The Cochrane library and PsycINFO. Studies included have been subject to quality assessment by means of Critical Appraisal Skills Programme. RESULTS: Fifty-five studies were included and thirty of these documented significant effect. The effect of the sensory stimulation interventions mainly reported on negative behaviours, except from five studies assessing quality of life and well-being. The majority of the studies had methodological limitations. The different sensory stimulation interventions were organised into eight categories: music, light therapy, acupressure/reflexology, massage/aromatherapy and doll therapy/pet therapy/toy therapy, the Sonas programme and Snoezelen. CONCLUSIONS: More studies are needed to clarify appropriate substantial background for the specific interventions. However, most of the studies based their interventions on a theoretical foundation. Furthermore, more research is needed to measure the effect of sensory stimulation on communication as well as quality of life. In addition, studies are to focus on whether the effect depends on the stage of dementia. RELEVANCE TO CLINICAL PRACTICE: Nurses are to be aware of sensory stimulation as a possible intervention to improve persons' quality of life.

Thomas, R. and Matusitz, J. Pet Therapy in Correctional Institutions: A Perspective From Relational-Cultural Theory. J.Evid.Inf.Soc.Work. 2016; 13(2):228-235.

ABSTRACT: In this article the authors apply Relational-Cultural Theory to pet therapy in correctional institutions. An important premise is that when pet therapy is used in prisons a symbiotic relationship develops between pets and prison inmates which, at the same time, improve their relationships with people themselves. Relational-Cultural Theory posits that relationships with individuals are not just a means to an end. Rather, good relationships promote growth and healthy development; they also cultivate reciprocal empathy. Hence, a major reason of suffering for most people is their experience of isolation; healing can occur in growth-fostering relationships.

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F. Maggot Debridement Therapy

Linger RJ, Belikoff EJ, Yan Y, Li F, Wantuch HA, Fitzsimons HL, Scott MJ. Towards next generation maggot debridement therapy: transgenic Lucilia sericata larvae that produce and secrete a human growth factor. BMC Biotechnol. 2016 Mar 22;16:30.

ABSTRACT: BACKGROUND: Diabetes and its concurrent complications impact a significant proportion of the population of the US and create a large financial burden on the American health care system. FDA-approved maggot debridement therapy (MDT), the application of sterile laboratory-reared Lucilia sericata (green bottle fly) larvae to wounds, is a cost-effective and successful treatment for diabetic foot ulcers and other medical conditions. Human platelet derived growth factor-BB (PDGF-BB) is a secreted dimeric peptide growth factor that binds the PDGF receptor. PDGF-BB stimulates cell proliferation and survival, promotes wound healing, and has been investigated as a possible topical treatment for non-healing wounds. Genetic engineering has allowed for expression and secretion of human growth factors and other proteins in transgenic insects. Here, we present a novel concept in MDT technology that combines the established benefits of MDT with the power of genetic engineering to promote healing. The focus of this study is to create and characterize strains of transgenic L. sericata that express and secrete PDGF-BB at detectable levels in adult hemolymph, whole larval lysate, and maggot excretions/ secretions (ES), with potential for clinical utility in wound healing. RESULTS: We have engineered and confirmed transgene insertion in several strains of L. sericata that express human PDGF-BB. Using a heat-inducible promoter to control the pdgf-b gene, pdgf-b mRNA was detected via semi-quantitative PCR upon heat shock. PDGF-BB protein was also detectable in larval lysates and adult hemolymph but not larval ES. An alternative, tetracycline-repressible pdgf-b system mediated expression of pdgf-b mRNA when maggots were raised on diet that lacked tetracycline. Further, PDGF-BB protein was readily detected in whole larval lysate as well as larval ES. CONCLUSIONS: Here we show robust, inducible expression and production of human PDGF-BB protein from two conditional expression systems in transgenic L. sericata larvae. The tetracycline-repressible system appears to be the most promising as PDGF-BB protein was detectable in larval ES following induction. Our system could potentially be used to deliver a variety of growth factors and anti-microbial peptides to the wound environment with the aim of enhancing wound healing, thereby improving patient outcome in a cost-effective manner.

Masiero, FS and Thyssen PJ. Evaluation of conventional therapeutic methods versus maggot therapy in the evolution of healing of tegumental injuries in Wistar rats with and without diabetes mellitus. Parasitol.Res. 3-15-2016.

ABSTRACT: Larval therapy consists on the application of sterilized carrion flies larvae, reared in laboratory, on acute, chronic, and/or infected wounds in order to promote healing. Conventional methods for treating injuries include mechanical debridement or silver-based dressings; however, they are not always effective for wound healing. Larval therapy is a feasible and safe treatment for therapeutic application and, in many cases, the only and the most recommended alternative for difficult healing injuries. Thus, this study aimed to evaluate the competence of Cochliomyia macellaria F. (Diptera: Calliphoridae) as a suitable species for therapeutic application and evaluate time and effectiveness of the types of treatments most commonly used to treat integumental

injuries. C. macellaria eggs were obtained from colonies established in laboratory and sterilized prior to application. Twenty-five larvae were applied for each centimeter squared of lesion. Lesions were induced in 24 Wistar rats; type 1 diabetes mellitus was induced in 12 of them. Animals were divided in four groups with three individuals each, being denominated: larval therapy, larval therapy associated with foam dressing with silver release, mechanical debridement with foam dressing silver and control group, without treatment. All treatments were applied once and held for 24 h. Medical application of larvae was found to be safe, as only dead tissue was removed, and efficient to accelerate healing process when compared to other treatments.

Wilson MR, Nigam Y, Jung W, Knight J, and Pritchard DI. The impacts of larval density and protease inhibition on feeding in medicinal larvae of the greenbottle fly Lucilia sericata. Med.Vet.Entomol. 2016; 30(1):1-7.

ABSTRACT: Larval therapy, the therapeutic use of blowfly larvae to treat chronic wounds, is primarily used in debridement. There are, however, gaps in current knowledge of the optimal clinical application of the therapy and mechanisms of action in the debridement process. Using an artificial assay, two studies were undertaken to investigate these aspects of larval debridement by Lucilia sericata Meigen (Diptera: Calliphoridae); the first studied the effects of the density of larvae on tissue digestion and larval mass, and the second considered the effects on the same parameters of incorporating protease inhibitors into the feeding substrate. The total mass of tissue digested increased with larval density until saturation was observed at 5.0-7.5 larvae/cm(2) . This range was considered optimal as lower doses resulted in the removal of less tissue and higher doses offered no additional tissue removal and appeared to exacerbate competition for feeding. In the second study, increased protease inhibitor concentration led to significant decreases in tissue digestion and larval mass, suggesting that serine proteases, particularly trypsin, may play major roles in larval digestion. Such information is important in elucidating the main constituents that make up larval digestive products and may be significant in the development of new therapies.



Ange Gllroy, Beaker of Maggots, July 9 2010, Creative Commons License

G. Olfactory Detection and Diagnosis

Roddick, K. M., Roberts, A. D., Schellinck, H. M., and Brown, R. E. Sex and Genotype Differences in Odor Detection in the 3xTg-AD and 5XFAD Mouse Models of Alzheimer's Disease at 6 Months of Age. Chem.Senses. 3-11-2016;

ABSTRACT: Deficits in odor identification and detection are early symptoms of Alzheimer's disease (AD). Two transgenic mouse models of AD, the 5XFAD and the 3xTg-AD mice and their wildtype controls, were assessed for olfactory detection with decreasing concentrations of ethyl acetate in a go no-go operant olfactometer task at 6 months of age. For both the 5XFAD and their B6SJLF1 wildtype littermates, females made fewer errors in detecting the ethyl acetate than males on all but the lowest odor concentrations. Female 5XFAD mice performed slightly better than their female wildtype littermates on the higher odor concentrations, though not at the lowest concentration. The 3xTg-AD females showed decreased olfactory detection compared with their wildtype B6129S1 controls, whereas there was no difference in the males. Therefore, although the 5XFAD mice showed no olfactory detection deficits, female 3xTg-AD mice had impaired olfactory detection at low odor concentrations but males did not. This difference in odor detection should be considered in studies of olfactory learning and memory, as differences in performance may be due to sensory rather than cognitive factors, though detection seems unimpaired at high odor concentrations.

Sufang, Z., Xiangbo, K., Hongbin, W., Gang, Z., Jinxiu, Y., Fu, L., and Zhen, Z. Sensory and immune genes identification and analysis in a widely used parasitoid wasp Trichogramma dendrolimi Matsumura (Hymenoptera: Trichogrammatidae). Insect Sci. 3-4-2016;

ABSTRACT: Trichogramma dendrolimi Matsumura (Hymenoptera: Trichogrammatidae) is one of the preponderant egg parasitoids of Dendrolimus spp., which are important defoliators of coniferous forests. This parasitoid wasp has been widely released to control pine caterpillar and other lepidopteran pests, but its control efficiency needs to be improved. Sensory systems are crucial for T. dendrolimi to locate hosts, and immunity is probably involved after egg deposition in the host cavity; however, few reports have focused on the molecular mechanism of olfactory detection and survival of T. dendrolimi. It is necessary to identify these genes before further functional research is conducted. In this study, we assembled and analyzed the transcriptome of T. dendrolimi using next-generation sequencing technology. The sequencing and assembly resulted in 38,565 contigs with N50 of 3,422 bp. Sequence comparison indicate that T. dendrolimi sequences are very similar to those of another parasitoid Nasonia vitripennis. Then the Olfactory, vision, and immune-related gene families were identified, and phylogenetic analyses were performed with these genes from T. dendrolimi and other model insect species. Furthermore, phylogenetic tree with odorant binding proteins of T. dendrolimi and their host Dendrolimus was constructed to determine whether convergent evolution exists. These genes can be valid targets for further gene function research. The present study may help us to understand host location and survival mechanisms of T. dendrolimi and to use them more efficiently for pest control in the future.

H. Phage Therapy

Abouhmad, A., Mamo, G., Dishisha, T., Amin, M. A., and Hatti-Kaul, R. T4 lysozyme fused with cellulose binding module for antimicrobial cellulosic wound dressing materials. J.Appl.Microbiol. 3-29-2016;

ABSTRACT: AIMS: Bacterial infection is a major challenge in wound care. Antimicrobial wound dressings are of great value for treating wound infections. Endolysins are evolving as a new class of antimicrobials with multiple applications. This study describes the production and evaluation of T4 lysozyme (T4Lyz), product of gene e of the T4 bacteriophage, fused with Cellulose Binding Module (CBM) for facile immobilization to cellulosic wound dressing. METHODS AND RESULTS: Genes encoding T4Lyz-CBM and T4Lyz were cloned and expressed in Escherichia coli and the enzymes were purified by cation exchange chromatography. While the CBM tag did not alter the optimum pH and stability features of T4Lyz, the lytic activity of the fusion protein was lowered. The bactericidal activity of T4Lyz-CBM, determined by viable count plating assay after 1 hour incubation with Micrococcus lysodeikticus was 97.5% with 10 mug ml-1 , and 99.96% and 95% for E. coli and Pseudomonas mendocina, respectively, with 200 mug ml-1 enzyme. T4Lyz-CBM was immobilized to wound dressing gauze with a capacity of 5.5 mug mg-1 matrix whereas the unmodified T4Lyz did not exhibit any binding. The immobilized protein retained its bactericidal activity against Gram-positive and -negative bacteria. Both free and immobilized T4Lyz-CBM, after heat denaturation, retained their bactericidal activities against Gram-negative bacteria only. The immobilized enzyme exhibited higher stability than the free enzyme when stored in dry form or in the presence of polyol stabilizers. CONCLUSION: Tagging T4Lyz with CBM provides a facile, irreversible binding to cellulosic wound dressing while retaining its activity. This approach may be suitable even for other antimicrobial enzymes and -peptides. SIGNIFICANCE AND IMPACT OF THE STUDY: The spread of antibiotic resistance requires innovative strategies for discovery and development of effective antimicrobial alternatives. This report presents a novel strategy for producing antimicrobial wound dressing materials. This article is protected by copyright. All rights reserved.

Adamu, Ahmad K., Sabo, Mohammed A., and Abas, F. Chitosan Nanoparticles as Carriers for the Delivery of PhiKAZ14 Bacteriophage for Oral Biological Control of Colibacillosis in Chickens. Molecules. 2016; 21(3)

ABSTRACT: The use of chitosan as a delivery carrier has attracted much attention in recent years. In this study, chitosan nanoparticles (CS-NP) and chitosan-PhiKAZ14 bacteriophage-loaded nanoparticles (C-PhiKAZ14 NP) were prepared by a simple coercavation method and characterized. The objective was to achieve an effective protection of bacteriophage from gastric acids and enzymes in the chicken gastrointestinal tract. The average particle sizes for CS-NP and C-PhiKAZ14 NP were 188 +/- 7.4 and 176 +/- 3.2 nm, respectively. The zeta potentials for CS-NP and C-PhiKAZ14 NP were 50 and 60 mV, respectively. Differential scanning calorimetry (DSC) of C-PhiKAZ14 NP gave an onset temperature of -17.17 degrees C with a peak at 17.32 degrees C and final end set of 17.41 degrees C, while blank chitosan NP had an onset of -20.00 degrees C with a peak at -19.78 degrees C and final end set at -20.47. FT-IR spectroscopy data of both CS-NP and C-PhiKAZ14 NP were the same. Chitosan nanoparticles showed

considerable protection of PhiKAZ14 bacteriophage against degradation by enzymes as evidenced in gel electrophoresis, whereby PhiKAZ14 bacteriophage encapsulated in chitosan nanoparticles were protected whereas the naked PhiKAZ14 bacteriophage were degraded. C-PhiKAZ14 NP was non-toxic as shown by a chorioallantoic membrane (CAM) toxicity assay. It was concluded that chitosan nanoparticles could be a potent carrier of PhiKAZ14 bacteriophage for oral therapy against colibacillosis in poultry.

Alcaine, S. D., Law, K., Ho, S., Kinchla, A. J., Sela, D. A., and Nugen, S. R. Bioengineering bacteriophages to enhance the sensitivity of phage amplification-based paper fluidic detection of bacteria. Biosens.Bioelectron. 3-22-2016; 82:14-19.

ABSTRACT: Bacteriophage (phage) amplification is an attractive method for the detection of bacteria due to a narrow phage-host specificity, short amplification times, and the phages' ability to differentiate between viable and non-viable bacterial cells. The next step in phage-based bacteria detection is leveraging bioengineered phages to create low-cost, rapid, and easy-to-use detection platforms such as lateral flow assays. Our work establishes the proof-of-concept for the use of bioengineered T7 phage strains to increase the sensitivity of phage amplification-based lateral flow assays. We have demonstrated a greater than 10-fold increase in sensitivity using a phage-based protein reporter, maltose-binding protein, over the detection of replicated T7 phage viron itself, and a greater then 100-fold increase in sensitivity using a phage-based enzymatic reporter, alkaline phosphatase. This increase in sensitivity enabled us to detect 103CFU/mL of Escherichia coli in broth after 7h, and by adding a filter concentration step, the ability to detect a regulatory relevant E. coli concentration of 100CFU/100mL in inoculated river water after 9h, where the current standard requires days for results. The combination of the paper fluidic format with phage-based detection provides a platform for the development of novel diagnostics that are sensitive, rapid, and easy to use.

Alves, D. R., Perez-Esteban, P., Kot, W., Bean, J. E., Arnot, T., Hansen, L. H., Enright, M. C., and Jenkins, A. T. A novel bacteriophage cocktail reduces and disperses Pseudomonas aeruginosa biofilms under static and flow conditions. Microb.Biotechnol. 2016; 9(1):61-74.

ABSTRACT: Pseudomonas aeruginosa is an opportunistic human pathogen that forms highly stable communities - biofilms, which contribute to the establishment and maintenance of infections. The biofilm state and intrinsic/acquired bacterial resistance mechanisms contribute to resistance/tolerance to antibiotics that is frequently observed in P. aeruginosa isolates. Here we describe the isolation and characterization of six novel lytic bacteriophages: viruses that infect bacteria, which together efficiently infect and kill a wide range of P. aeruginosa clinical isolates. The phages were used to formulate a cocktail with the potential to eliminate P. aeruginosa PAO1 planktonic cultures. Two biofilm models were studied, one static and one dynamic, and the phage cocktail was assessed for its ability to reduce and disperse the biofilm biomass. For the static model, after 4 h of contact with the phage suspension (MOI 10) more than 95% of biofilm biomass was eliminated. In the flow biofilm model, a slower rate of activity by the phage was observed, but 48 h after addition of the phage cocktail the biofilm was dispersed, with most cells eliminated (> 4 logs) comparing with the control. This cocktail has the potential for development as a therapeutic to control P. aeruginosa infections, which are predominantly biofilm centred.

Arnold, J. W., Spacht, D., and Koudelka, G. B. Determinants that govern the recognition and uptake of E. coli O157:H7 by Acanthamoeba castellanii. Cell Microbiol. 3-15-2016;

ABSTRACT: Predation by phagocytic predators is a major source of bacterial mortality. The first steps in protozoan predation are recognition and consumption of their bacterial prey. However, the precise mechanisms governing prey recognition and phagocytosis by protists and the identities of the molecular and cellular factors involved in these processes are, as yet, ill-characterized. Here we show that that the ability of the phagocytic bacterivorous amoebae, Acanthamoeba castellanii to recognize and internalize E. coli, a bacterial prey, varies with LPS structure and composition. The presence of an O-antigen carbohydrate is not required for uptake of E. coli by A castellanii. However O1, but not, O157 O-antigen types inhibit recognition and uptake of bacteria by amoeba. This finding implies O-antigen may function as an anti-predator defense molecule. Recognition and uptake of E. coli by A castellanii is mediated by the interaction of mannose binding protein located on amoebae's surface (aMBP) with LPS carbohydrate. Phagocytic mammalian cells also use mannose-binding lectins to recognize and/or mediate phagocytosis of E. coli. Nonetheless, aMBP apparently displays no sequence similarity with any known metazoan mannose binding protein. Hence the similarity in bacterial recognition mechanisms of amoebae and mammalian phagocytes may be a result of convergent evolution.

Baker, C. W., Miller, C. R., Thaweethai, T., Yuan, J., Hollibaugh, Baker M., Joyce, P., and Weinreich, D. M. Genetically Determined Variation in Lysis Time Variance in the Bacteriophage varphiX174. G3.(Bethesda.). 2-26-2016;

ABSTRACT: Researchers in evolutionary genetics recently have recognized an exciting opportunity in decomposing beneficial mutations into their proximal, mechanistic determinants. The application of methods and concepts from molecular biology and life history theory to studies of lytic bacteriophages (phages) has allowed them to understand how natural selection sees mutations influencing life history. This work motivated the research presented here, in which we explored whether, under consistent experimental conditions, small differences in the genome of bacteriophage phiX174 could lead to altered life history phenotypes among a panel of eight genetically distinct clones. We assessed the clones' phenotypes by applying a novel statistical framework to the results of a serially sampled parallel infection assay, in which we simultaneously inoculated each of a large number of replicate host volumes with ~1 phage particle. We sequentially plated the volumes over the course of infection and counted the plaques that formed after incubation. These counts served as a proxy for the number of phage particles in a single volume as a function of time. From repeated assays, we inferred significant, genetically determined heterogeneity in lysis time and burst size, including lysis time variance. These findings are interesting in light of the genetic and phenotypic constraints on phiX174's single-protein lysis mechanism. We speculate briefly on the mechanisms underlying our results and discuss the potential importance of lysis time variance in viral evolution.

Ball, C. S., Light, Y. K., Koh, C. Y., Wheeler, S. S., Coffey, L. L., and Meagher, R. J. Quenching of Unincorporated Amplification Signal Reporters in Reverse-Transcription Loop-Mediated Isothermal Amplification Enabling Bright, Single-Step, Closed-Tube, and Multiplexed Detection of RNA Viruses. Anal.Chem. 4-5-2016; 88(7):3562-3568.

ABSTRACT: Reverse-transcription-loop-mediated isothermal amplification (RT-LAMP) has frequently been proposed as an enabling technology for simplified diagnostic tests for RNA viruses. However, common detection techniques used for LAMP and RT-LAMP have drawbacks, including poor discrimination capability, inability to multiplex targets, high rates of false positives, and (in some cases) the requirement of opening reaction tubes postamplification. Here, we present a simple technique that allows closed-tube, target-specific detection, based on inclusion of a dye-labeled primer that is incorporated into a target-specific amplicon if the target is present. A short, complementary quencher hybridizes to unincorporated primer upon cooling down at the end of the reaction, thereby quenching fluorescence of any unincorporated primer. Our technique, which we term QUASR (for quenching of unincorporated amplification signal reporters, read "quasar"), does not significantly reduce the amplification efficiency or sensitivity of RT-LAMP. Equipped with a simple LED excitation source and a colored plastic gel filter, the naked eye or a camera can easily discriminate between positive and negative QUASR reactions, which produce a difference in signal of approximately 10:1 without background subtraction. We demonstrate that QUASR detection is compatible with complex sample matrices such as human blood, using a novel LAMP primer set for bacteriophage MS2 (a model RNA virus particle). Furthermore, we demonstrate single-tube duplex detection of West Nile virus (WNV) and chikungunya virus (CHIKV) RNA.

Batonick, M., Kiss, M. M., Fuller, E. P., Magadan, C. M., Holland, E. G., Zhao, Q., Wang, D., Kay, B. K., and Weiner, M. P. pMINERVA: A donor-acceptor system for the in vivo recombineering of scFv into IgG molecules. J.Immunol.Methods. 2016; 431:22-30.

ABSTRACT: Phage display is the most widely used method for selecting binding molecules from recombinant antibody libraries. However, validation of the phage antibodies often requires early production of the cognate full-length immunoglobulin G (IgG). The conversion of phage library outputs to a full immunoglobulin via standard subcloning is time-consuming and limits the number of clones that can be evaluated. We have developed a novel system to convert scFvs from a phage display vector directly into IgGs without any in vitro subcloning steps. This new vector system, named pMINERVA, makes clever use of site-specific bacteriophage integrases that are expressed in Escherichia coli and intron splicing that occurs within mammalian cells. Using this system, a phage display vector contains both bacterial and mammalian regulatory regions that support antibody expression in E. coli and mammalian cells. A single-chain variable fragment (scFv) antibody is expressed on the surface of bacteriophage M13 as a genetic fusion to the gpIII coat protein. The scFv is converted to an IgG that can be expressed in mammalian cells by transducing a second E. coli strain. In that strain, the phiC31 recombinase fuses the heavy chain constant domain from an acceptor plasmid to the heavy chain variable domain and introduces controlling elements upstream of the light chain variable domain. Splicing in mammalian cells removes a synthetic intron containing the M13 gpIII gene to produce the fusion of the light chain variable domain to the constant domain. We show that phage displaying a scFv and recombinant IgGs generated using this system are expressed at wild-type levels and retain normal function. Use of the pMINERVA completely eliminates the labor-intensive subcloning and DNA sequence confirmation steps currently needed to convert a scFv into a functional IgG Ab.

Bernheim, A. G., Libis, V. K., Lindner, A. B., and Wintermute, E. H. Phage-mediated Delivery of Targeted sRNA Constructs to Knock Down Gene Expression in E. coli. J.Vis.Exp. 2016; (109)

ABSTRACT: RNA-mediated knockdowns are widely used to control gene expression. This versatile family of techniques makes use of short RNA (sRNA) that can be synthesized with any sequence and designed to complement any gene targeted for silencing. Because sRNA constructs can be introduced to many cell types directly or using a variety of vectors, gene expression can be repressed in living cells without laborious genetic modification. The most common RNA knockdown technology, RNA interference (RNAi), makes use of the endogenous RNA-induced silencing complex (RISC) to mediate sequence recognition and cleavage of the target mRNA. Applications of this technique are therefore limited to RISC-expressing organisms, primarily eukaryotes. Recently, a new generation of RNA biotechnologists have developed alternative mechanisms for controlling gene expression through RNA, and so made possible RNA-mediated gene knockdowns in bacteria. Here we describe a method for silencing gene expression in E. coli that functionally resembles RNAi. In this system a synthetic phagemid is designed to express sRNA, which may designed to target any sequence. The expression construct is delivered to a population of E. coli cells with non-lytic M13 phage, after which it is able to stably replicate as a plasmid. Antisense recognition and silencing of the target mRNA is mediated by the Hfq protein, endogenous to E. coli. This protocol includes methods for designing the antisense sRNA, constructing the phagemid vector, packaging the phagemid into M13 bacteriophage, preparing a live cell population for infection, and performing the infection itself. The fluorescent protein mKate2 and the antibiotic resistance gene chloramphenicol acetyltransferase (CAT) are targeted to generate representative data and to quantify knockdown effectiveness.

Bertozzi, Silva J., Storms, Z., and Sauvageau, D. Host receptors for bacteriophage adsorption. FEMS Microbiol.Lett. 2016; 363(4)

ABSTRACT: The adsorption of bacteriophages (phages) onto host cells is, in all but a few rare cases, a sine qua non condition for the onset of the infection process. Understanding the mechanisms involved and the factors affecting it is, thus, crucial for the investigation of host-phage interactions. This review provides a survey of the phage host receptors involved in recognition and adsorption and their interactions during attachment. Comprehension of the whole infection process, starting with the adsorption step, can enable and accelerate our understanding of phage ecology and the development of phage-based technologies. To assist in this effort, we have established an open-access resource-the Phage Receptor Database (PhReD)-to serve as a repository for information on known and newly identified phage receptors.

Betts, A., Gifford, D. R., MacLean, R. C., and King, K. C. Parasite diversity drives rapid host dynamics and evolution of resistance in a bacteria-phage system. Evolution. 3-23-2016;

ABSTRACT: Host-parasite evolutionary interactions are typically considered in a pairwise species framework. However, natural infections frequently involve multiple parasites. Altering parasite diversity alters ecological and evolutionary dynamics as parasites compete and hosts resist multiple infection. We investigated the effects of parasite diversity on host-parasite population dynamics and evolution using the pathogen P. aeruginosa and five lytic bacteriophage parasites. To manipulate parasite diversity, bacterial populations were exposed for 24 hours to either phage monocultures or diverse

communities containing up to five phages. Phage communities suppressed host populations more rapidly but also showed reduced phage density, likely due to inter-phage competition. The evolution of resistance allowed rapid bacterial recovery that was greater in magnitude with increases in phage diversity. We observed no difference in the extent of resistance with increased parasite diversity, but there was a profound impact on the specificity of resistance; specialized resistance evolved to monocultures through mutations in a diverse set of genes. In summary, we demonstrate that parasite diversity has rapid effects on host-parasite population dynamics and evolution by selecting for different resistance mutations and affecting the magnitude of bacterial suppression and recovery. Finally, we discuss the implications of phage diversity for their use as biological control agents. This article is protected by copyright. All rights reserved.

Bischof, L. F., Friedrich, C., Harms, A., Sogaard-Andersen, L., and van der, Does C. The Type IV Pilus Assembly ATPase PilB of Myxococcus xanthus Interacts with the Inner Membrane Platform Protein PilC and the Nucleotide-binding Protein PilM. J.Biol.Chem. 3-25-2016; 291(13):6946-6957.

ABSTRACT: Type IV pili (T4P) are ubiquitous bacterial cell surface structures, involved in processes such as twitching motility, biofilm formation, bacteriophage infection, surface attachment, virulence, and natural transformation. T4P are assembled by machinery that can be divided into the outer membrane pore complex, the alignment complex that connects components in the inner and outer membrane, and the motor complex in the inner membrane and cytoplasm. Here, we characterize the inner membrane platform protein PilC, the cytosolic assembly ATPase PilB of the motor complex, and the cytosolic nucleotide-binding protein PilM of the alignment complex of the T4P machinery ofMyxococcus xanthus PilC was purified as a dimer and reconstituted into liposomes. PilB was isolated as a monomer and bound ATP in a non-cooperative manner, but PilB fused to Hcp1 ofPseudomonas aeruginosaformed a hexamer and bound ATP in a cooperative manner. Hexameric but not monomeric PilB bound to PilC reconstituted in liposomes, and this binding stimulated PilB ATPase activity. PilM could only be purified when it was stabilized by a fusion with a peptide corresponding to the first 16 amino acids of PilN, supporting an interaction between PilM and PilN(1-16). PilM-N(1-16) was isolated as a monomer that bound but did not hydrolyze ATP. PilM interacted directly with PilB, but only with PilC in the presence of PilB, suggesting an indirect interaction. We propose that PilB interacts with PilC and with PilM, thus establishing the connection between the alignment and the motor complex.

Britton, L. E., Cassidy, J. P., O'Donovan, J., Gordon, S. V., and Markey, B. Potential application of emerging diagnostic techniques to the diagnosis of bovine Johne's disease (paratuberculosis). Vet.J. 2016; 209:32-39.

ABSTRACT: Mycobacterium avium subspecies paratuberculosis (MAP) causes Johne's disease (paratuberculosis), a chronic wasting disease in cattle with important welfare, economic and potential public health implications. Current tests are unable to recognise all stages of the disease, which makes it difficult to diagnose and control. This review explores emerging diagnostic techniques that could complement and enhance the diagnosis of MAP infection, including bacteriophage analysis, new MAP-specific antigens, host protein expression in response to infection, transcriptomic studies, analysis of microRNAs and investigation of the gastrointestinal microbiome. It emphasises the inherent challenges of diagnosing bovine Johne's disease and investigates novel areas

which may have the potential both to advance our understanding of the immunopathology of MAP infection and to augment current diagnostic tests.

Brown, T. L., Petrovski, S., Dyson, Z. A., Seviour, R., and Tucci, J. The Formulation of Bacteriophage in a Semi Solid Preparation for Control of Propionibacterium acnes Growth. PLoS.One. 2016; 11(3):e0151184

ABSTRACT: AIMS: To isolate and characterise phage which could lyse P. acnes and to formulate the phage into a delivery form for potential application in topical treatment of acne infection. METHODS AND RESULTS: Using standard phage isolation techniques, ten phage capable of lysing P. acnes were isolated from human skin microflora. Their genomes showed high homology to previously reported P. acnes phage. These phage were formulated into cetomacrogol cream aqueous at a concentration of 2.5x108 PFU per gram, and shown to lyse underlying P. acnes cells grown as lawn cultures. These phage formulations remained active for at least 90 days when stored at four degrees Celsius in a light protected container. CONCLUSIONS: P. acnes phage formulated into cetomacrogol cream aqueous will lyse surrounding and underlying P. acnes bacteria, and are effective for at least 90 days if stored appropriately. SIGNIFICANCE AND IMPACT OF THE STUDY: There are few reports of phage formulation into semi solid preparations for application as phage therapy. The formulation method described here could potentially be applied topically to treat human acne infections. The potential exists for this model to be extended to other phage applied to treat other bacterial skin infections.

Bugg, T. D., Rodolis, M. T., Mihalyi, A., and Jamshidi, S. Inhibition of phospho-MurNAc-pentapeptide translocase (MraY) by nucleoside natural product antibiotics, bacteriophage varphiX174 lysis protein E, and cationic antibacterial peptides. Bioorg.Med.Chem. 3-9-2016;

ABSTRACT: This review covers recent developments in the inhibition of translocase MraY and related phospho-GlcNAc transferases WecA and TagO, and insight into the inhibition and catalytic mechanism of this class of integral membrane proteins from the structure of Aquifex aeolicus MraY. Recent studies have also identified a protein-protein interaction site in Escherichia coli MraY, that is targeted by bacteriophage varphiX174 lysis protein E, and also by cationic antimicrobial peptides containing Arg-Trp close to their N- or C-termini.

Byvalov, A. A., Dudina, L. G., Konyshev, I. V., Litvinets, S. G., and Martinson, E. A. Immunochemical Nature of Receptors of Pseudotuberculosis Diagnostic Bacteriophage. Bull.Exp.Biol.Med. 3-29-2016;

ABSTRACT: The effect of treatment of Yersinia pseudotuberculosis cells with antibodies of various specificities on adhesiveness of pseudotuberculosis bacteriophage was analyzed by competitive inhibition technique. Bacteriophage adsorption to bacteria was sterically inhibited by monoclonal antibodies to protein epitopes of Y. pseudotuberculosis outer membrane. These results suggest that receptors of pseudotuberculosis diagnostic bacteriophage are localized on the LPS core of microbial cell.

Cafini, F., Nguyen le, T. T., Higashide, M., Roman, F., Prieto, J., and Morikawa, K. Horizontal gene transmission of the cfr gene to MRSA and Enterococcus: role of Staphylococcus epidermidis as a reservoir and alternative pathway for the spread of linezolid resistance. J.Antimicrob.Chemother. 2016; 71(3):587-592.

ABSTRACT: OBJECTIVES: Linezolid resistance mediated by the cfr gene represents a global concern due to its dissemination among multiresistant nosocomial pathogens such as MRSA and Enterococcus. In the present work, we have evaluated the in vitro transmission of cfr pSCFS7-like plasmids from two Staphylococcus epidermidis ST2 strains (SE45 and SE50) isolated in Spanish hospitals, to clinical MRSA and Enterococcus spp. isolates obtained in Japan, a country in which cfr has not been detected yet. We have also investigated alternative mechanisms of horizontal gene transfer involved in the spread of the cfr gene. METHODS: MRSA (n = 16) and Enterococcus spp. (n = 8) clinical isolates were used as recipients in conjugative experiments. Bacteriophage-mediated transmission was tested using MR83a phage and N315, COL and Mu50 strains. A transformation assay was carried out using a natural competent strain derived from N315. RESULTS: The SE45 strain was able to transfer the cfr gene to all strains tested, while transmission from SE50 was observed only to a few strains and with less efficiency. No transmission was observed to Enterococcus spp. isolates. Even though conjugation is thought to be the main mechanism of cfr dissemination, we have demonstrated that transduction can be considered an alternative pathway for transmission of the cfr gene between MRSA strains. However, the results suggest an absence of transmission by natural transformation. CONCLUSIONS: Linezolid resistance mediated by cfr vectors, such as pSCFS7-like plasmids, can be efficiently transferred to clinical MRSA in Japanese isolates. After reaching the staphylococcal pool, the cfr gene could be spread among MRSA strains by either conjugation or transduction.

Calero-Caceres, W. and Muniesa, M. Persistence of naturally occurring antibiotic resistance genes in the bacteria and bacteriophage fractions of wastewater. Water Res. 3-3-2016; 95:11-18.

ABSTRACT: The emergence and prevalence of antibiotic resistance genes (ARGs) in the environment is a serious global health concern. ARGs from bacteria can be mobilized by mobile genetic elements, and recent studies indicate that phages and phage-derived particles, among others, could play a role in the spread of ARGs through the environment. ARGs are abundant in the bacterial and bacteriophage fractions of water bodies and for successful transfer of the ARGs, their persistence in these environments is crucial. In this study, three ARGs (blaTEM, blaCTX-M and sul1) that naturally occur in the bacterial and phage fractions of raw wastewater were used to evaluate the persistence of ARGs at different temperatures (4 degrees C, 22 degrees C and 37 degrees C) and pH values (3, 7 and 9), as well as after various disinfection treatments (thermal treatment, chlorination and UV) and natural inactivation in a mesocosm. Gene copies (GC) were quantified by qPCR; then the logarithmic reduction and significance of the differences between their numbers were evaluated. The ARGs persisted for a long time with minimal reductions after all the treatments. In general, they showed greater persistence in the bacteriophage fraction than in the bacterial fraction. Comparisons showed that the ARGs persisted under conditions that reduced culturable Escherichia coli and infectious coliphages below the limit of detection. The prevalence of ARGs, particularly in the bacteriophage fraction, poses the threat of the spread of ARGs and their incorporation into a new bacterial background that could lead to the emergence of new resistant clones.

Chan, W. T., Espinosa, M., and Yeo, C. C. Keeping the Wolves at Bay: Antitoxins of Prokaryotic Type II Toxin-Antitoxin Systems. Front Mol.Biosci. 2016; 3:9

ABSTRACT: In their initial stages of discovery, prokaryotic toxin-antitoxin (TA) systems were confined to bacterial plasmids where they function to mediate the maintenance and stability of usually low- to medium-copy number plasmids through the post-segregational killing of any plasmid-free daughter cells that developed. Their eventual discovery as nearly ubiquitous and repetitive elements in bacterial chromosomes led to a wealth of knowledge and scientific debate as to their diversity and functionality in the prokaryotic lifestyle. Currently categorized into six different types designated types I-VI, type II TA systems are the best characterized. These generally comprised of two genes encoding a proteic toxin and its corresponding proteic antitoxin, respectively. Under normal growth conditions, the stable toxin is prevented from exerting its lethal effect through tight binding with the less stable antitoxin partner, forming a non-lethal TA protein complex. Besides binding with its cognate toxin, the antitoxin also plays a role in regulating the expression of the type II TA operon by binding to the operator site, thereby repressing transcription from the TA promoter. In most cases, full repression is observed in the presence of the TA complex as binding of the toxin enhances the DNA binding capability of the antitoxin. TA systems have been implicated in a gamut of prokaryotic cellular functions such as being mediators of programmed cell death as well as persistence or dormancy, biofilm formation, as defensive weapons against bacteriophage infections and as virulence factors in pathogenic bacteria. It is thus apparent that these antitoxins, as DNA-binding proteins, play an essential role in modulating the prokaryotic lifestyle whilst at the same time preventing the lethal action of the toxins under normal growth conditions, i.e., keeping the proverbial wolves at bay. In this review, we will cover the diversity and characteristics of various type II TA antitoxins. We shall also look into some interesting deviations from the canonical type II TA systems such as tripartite TA systems where the regulatory role is played by a third party protein and not the antitoxin, and a unique TA system encoding a single protein with both toxin as well as antitoxin domains.

Chang, L., Wang, G., Jia, T., Zhang, L., Li, Y., Han, Y., Zhang, K., Lin, G., Zhang, R., Li, J., and Wang, L. Armored long non-coding RNA MEG3 targeting EGFR based on recombinant MS2 bacteriophage virus-like particles against hepatocellular carcinoma. Oncotarget. 3-16-2016;

ABSTRACT: Hepatocellular carcinoma (HCC) is one of the most frequently diagnosed cancers worldwide. However, the treatment of patients with HCC is particularly challenging. Long non-coding RNA maternally expressed gene 3 (MEG3) has been identified as a potential suppressor of several types of tumors, but the delivery of long RNA remains problematic, limiting its applications. In the present study, we designed a novel delivery system based on MS2 virus-like particles (VLPs) crosslinked with GE11 polypeptide. This vector was found to be fast, effective and safe for the targeted delivery of lncRNA MEG3 RNA to the epidermal growth factor receptor (EGFR)-positive HCC cell lines without the activation of EGFR downstream pathways, and significantly attenuated both in vitro and in vivo tumor cell growth. Our study also revealed that the targeted delivery was mainly dependent on clathrin-mediated endocytosis and MEG3 RNA suppresses tumor growth mainly via increasing the expression of p53 and its downstream gene GDF15, but decreasing the expression of MDM2. Thus, this vector is

promising as a novel delivery system and may facilitate a new approach to lncRNA based cancer therapy.

Chehoud, C., Dryga, A., Hwang, Y., Nagy-Szakal, D., Hollister, E. B., Luna, R. A., Versalovic, J., Kellermayer, R., and Bushman, F. D. Transfer of Viral Communities between Human Individuals during Fecal Microbiota Transplantation. MBio. 2016; 7(2)

ABSTRACT: Fecal microbiota transplantation (FMT) is a highly effective treatment for refractoryClostridium difficileinfections. However, concerns persist about unwanted cotransfer of pathogenic microbes such as viruses. Here we studed FMT from a single healthy human donor to three pediatric ulcerative colitis patients, each of whom received a course of 22 to 30 FMT treatments. Viral particles were purified from donor and recipient stool samples and sequenced; the reads were then assembled into contigs corresponding to viral genomes or partial genomes. Transfer of selected viruses was confirmed by quantitative PCR. Viral contigs present in the donor could be readily detected in recipients, with up to 32 different donor viral contigs appearing in a recipient sample. Reassuringly, none of these were viruses are known to replicate on human cells. Instead, viral contigs either scored as bacteriophage or could not be attributed taxonomically, suggestive of unstudied phage. The two most frequently transferred gene types were associated with temperate-phage replication. In addition, members ofSiphoviridae, the group of typically temperate phages that includes phage lambda, were found to be transferred with significantly greater efficiency than other groups. On the basis of these findings, we propose that the temperate-phage replication style may promote efficient phage transfer between human individuals. In summary, we documented transfer of multiple viral lineages between human individuals through FMT, but in this case series, none were from viral groups known to infect human cells. IMPORTANCE: Transfer of whole communities of viruses between humans has rarely been studied but is of likely medical importance. Here we studied fecal microbiota transplantation (FMT), a highly successful treatment for relapsingClostridium difficileinfection and, potentially, other gastrointestinal (GI) diseases. We investigated the transfer of viral communities during FMT and documented transfer of multiple viral lineages between humans. None of these were viruses that replicated on animal cells or that are known to be pathogenic. We found that temperate bacteriophage, which form stable associations with their hosts, were significantly more likely to be transferred during FMT. This supports a model in which the viral temperate replication style may have evolved in part to support efficient viral transmission between environments.

Chen, J., Jackson, A. A., Rotello, V. M., and Nugen, S. R. Colorimetric Detection of Escherichia coli Based on the Enzyme-Induced Metallization of Gold Nanorods. Small. 3-21-2016;

ABSTRACT: A novel enzyme-induced metallization colorimetric assay is developed to monitor and measure beta-galactosidase (beta-gal) activity, and is further employed for colorimetric bacteriophage (phage)-enabled detection of Escherichia coli (E. coli). This assay relies on enzymatic reaction-induced silver deposition on the surface of gold nanorods (AuNRs). In the presence of beta-gal, the substrate p-aminophenyl beta-d-galactopyranoside is hydrolyzed to produce p-aminophenol (PAP). Reduction of silver ions by PAP generates a silver shell on the surface of AuNRs, resulting in the blue shift of the longitudinal localized surface plasmon resonance peak and multicolor changes of the detection solution from light green to orange-red. Under optimized conditions, the detection limit for beta-gal is 128 pM, which is lower than the conventional colorimetric

assay. Additionally, the assay has a broader dynamic range for beta-gal detection. The specificity of this assay for the detection of beta-gal is demonstrated against several protein competitors. Additionally, this technique is successfully applied to detect E. coli bacteria cells in combination with bacteriophage infection. Due to the simplicity and short incubation time of this enzyme-induced metallization colorimetric method, the assay is well suited for the detection of bacteria in low-resource settings.

Chen, J. L., Yang, Y., Zhang, L. L., Liang, H., Huber, T., Su, X. C., and Otting, G. Analysis of the solution conformations of T4 lysozyme by paramagnetic NMR spectroscopy. Phys.Chem.Chem.Phys. 2-17-2016; 18(8):5850-5859.

ABSTRACT: A large number of crystal structures of bacteriophage T4 lysozyme (T4-L) have shown that it contains two subdomains, which can arrange in a compact conformation (closed state) or, in mutants of T4-L, more extended structures (open state). In solution, wild-type T4-L displays only a single set of nuclear magnetic resonance (NMR) signals, masking any conformational heterogeneity. To probe the conformational space of T4-L, we generated a site-specific lanthanide binding site by attaching 4-mercaptomethyl dipicolinic acid via a disulfide bond to Cys44 in the triple-mutant C54T/C97A/S44C of T4-L and measured pseudocontact shifts (PCS) and magnetically induced residual dipolar couplings (RDC). The data indicate that, in solution and in the absence of substrate, the structure of T4-L is on average more open than suggested by the closed conformation of the crystal structure of wild-type T4-L. A slightly improved fit was obtained by assuming a population-weighted two-state model involving an even more open conformation and the closed state, but paramagnetic relaxation enhancements measured with Gd(3+) argue against such a conformational equilibrium. The fit could not be improved by including a third conformation picked from the hundreds of crystal structures available for T4-L mutants.

Choi, J. S., Dasari, A., Hu, P., Benkovic, S. J., and Berdis, A. J. The use of modified and non-natural nucleotides provide unique insights into pro-mutagenic replication catalyzed by polymerase eta. Nucleic Acids Res. 2-18-2016; 44(3):1022-1035.

ABSTRACT: This report evaluates the pro-mutagenic behavior of 8-oxo-guanine (8-oxo-G) by quantifying the ability of high-fidelity and specialized DNA polymerases to incorporate natural and modified nucleotides opposite this lesion. Although high-fidelity DNA polymerases such as pol delta and the bacteriophage T4 DNA polymerase replicating 8-oxo-G in an error-prone manner, they display remarkably low efficiencies for TLS compared to normal DNA synthesis. In contrast, pol eta shows a combination of high efficiency and low fidelity when replicating 8-oxo-G. These combined properties are consistent with a pro-mutagenic role for pol eta when replicating this DNA lesion. Studies using modified nucleotide analogs show that pol eta relies heavily on hydrogen-bonding interactions during translesion DNA synthesis. However, nucleobase modifications such as alkylation to the N2 position of guanine significantly increase error-prone synthesis catalyzed by pol eta when replicating 8-oxo-G. Molecular modeling studies demonstrate the existence of a hydrophobic pocket in pol eta that participates in the increased utilization of certain hydrophobic nucleotides. A model is proposed for enhanced pro-mutagenic replication catalyzed by pol eta that couples efficient incorporation of damaged nucleotides opposite oxidized DNA lesions created by reactive oxygen species. The biological implications of this model toward increasing mutagenic events in lung cancer are discussed.

Christakos, K. J., Chapman, J. A., Fane, B. A., and Campos, S. K. PhiXing-it, displaying foreign peptides on bacteriophage PhiX174. Virology. 1-15-2016; 488:242-248.

ABSTRACT: Although bacteriophage phiX174 is easy to propagate and genetically tractable, it is use as a peptide display platform has not been explored. One region within the phiX174 major spike protein G tolerated 13 of 16 assayed insertions, ranging from 10 to 75 amino acids. The recombinant proteins were functional and incorporated into infectious virions. In the folded protein, the peptides would be icosahedrally displayed within loops that extend from the proteins beta-barrel core. The well-honed genetics of phiX174 allowed permissive insertions to be quickly identified by the cellular phenotypes associated with cloned gene expression. The cloned genes were easily transferred from plasmids to phage genomes via recombination rescue. Direct ELISA validated several recombinant virions for epitope display. Some insertions conferred a temperature-sensitive (ts) protein folding defect, which was suppressed by global suppressors in protein G, located too far away from the insertion to directly alter peptide display.

Christen, M., Beusch, C., Bosch, Y., Cerletti, D., Flores-Tinoco, C. E., Del, Medico L., Tschan, F., and Christen, B. Quantitative Selection Analysis of Bacteriophage phiCbK Susceptibility in Caulobacter crescentus. J.Mol.Biol. 1-29-2016; 428(2 Pt B):419-430.

ABSTRACT: Classical molecular genetics uses stringent selective conditions to identify mutants with distinct phenotypic responses. Mutations giving rise to less pronounced phenotypes are often missed. However, to gain systems-level insights into complex genetic interaction networks requires genome-wide assignment of quantitative phenotypic traits. In this paper, we present a quantitative selection approach coupled with transposon sequencing (QS-TnSeq) to globally identify the cellular components that orchestrate susceptibility of the cell cycle model bacterium Caulobacter crescentus toward bacteriophage phiCbK infection. We found that 135 genes representing 3.30% of the Caulobacter genome exhibit significant accumulation of transposon insertions upon phiCbK selection. More than 85% thereof consist of new factors not previously associated with phage phiCbK susceptibility. Using hierarchical clustering of dose-dependent TnSeq datasets, we grouped these genes into functional modules that correlate with different stages of the phiCbK infection process. We assign phiCbK susceptibility to eight new genes that represent novel components of the pilus secretion machinery. Further, we demonstrate that, from 86 motility genes, only seven genes encoding structural and regulatory components of the flagellar hook increase phage resistance when disrupted by transposons, suggesting a link between flagellar hook assembly and pili biogenesis. In addition, we observe high recovery of Tn5 insertions within regulatory sequences of the genes encoding the essential NADH:ubiquinone oxidoreductase complex indicating that intact proton motive force is crucial for effective phage propagation. In sum, QS-TnSeq is broadly applicable to perform quantitative and genome-wide systems-genetics analysis of complex phenotypic traits.

Cochrane, K., Manson, McGuire A., Priest, M. E., Abouelleil, A., Cerqueira, G. C., Lo, R., Earl, A. M., and len-Vercoe, E. Complete genome sequences and analysis of the Fusobacterium nucleatum subspecies animalis 7-1 bacteriophage Funu1 and Funu2. Anaerobe. 2016; 38:125-129.

ABSTRACT: Fusobacterium nucleatum is a strictly anaerobic, Gram negative bacterial species that has been associated with dental infections, pre-term labor, appendicitis, inflammatory bowel disease, and, more recently, colorectal cancer. The species is unusual in its phenotypic and genotypic heterogeneity, with some strains demonstrating a more virulent phenotype than others; however, as yet the genetic basis for these differences is not understood. Bacteriophage are known to contribute to the virulence phenotype of several bacterial species. In this work, we set out to characterize the bacteriophage associated with F. nucleatum subsp. animalis strain 7-1, a highly invasive isolate from the human gastrointestinal tract. As well, we used computational approaches to predict and compare bacteriophage signatures across available sequenced F. nucleatum genomes.

Colon, M. P., Chakraborty, D., Pevzner, Y., and Koudelka, G. B. Mechanisms that Determine the Differential Stability of Stx(+) and Stx(-) Lysogens. Toxins.(Basel). 2016; 8(4)

ABSTRACT: Phages 933W, BAA2326, 434, and lambda are evolutionarily-related temperate lambdoid phages that infect Escherichia coli. Although these are highly-similar phages, BAA2326 and 933W naturally encode Shiga toxin 2 (Stx(+)), but phage 434 and lambda do not (Stx(-)). Previous reports suggest that the 933W Stx(+) prophage forms less stable lysogens in E. coli than does the Stx(-) prophages lambda, P22, and 434. The higher spontaneous induction frequency of the Stx(+) prophage may be correlated with both virulence and dispersion of the Stx2-encoding phage. Here, we examined the hypothesis that lysogen instability is a common feature of Stx(+) prophages. We found in both the absence and presence of prophage inducers (DNA damaging agents, salts), the Stx(+) prophages induce at higher frequencies than do Stx(-) prophages. The observed instability of Stx(+) prophages does not appear to be the result of any differences in phage development properties between Stx(+) and Stx(-) phages. Our results indicate that differential stability of Stx(+) and Stx(-) prophages results from both RecA-dependent and RecA-independent effects on the intracellular concentration of the respective cI repressors.

Curtis, A., Blackburn, J. K., Smiley, S. L., Yen, M., Camilli, A., Alam, M. T., Ali, A., and Morris, J. G. Mapping to Support Fine Scale Epidemiological Cholera Investigations: A Case Study of Spatial Video in Haiti. Int.J.Environ.Res.Public Health. 2016; 13(2)

ABSTRACT: The cartographic challenge in many developing world environments suffering a high disease burden is a lack of granular environmental covariates suitable for modeling disease outcomes. As a result, epidemiological questions, such as how disease diffuses at intra urban scales are extremely difficult to answer. This paper presents a novel geospatial methodology, spatial video, which can be used to collect and map environmental covariates, while also supporting field epidemiology. An example of epidemic cholera in a coastal town of Haiti is used to illustrate the potential of this new method. Water risks from a 2012 spatial video collection are used to guide a 2014 survey, which concurrently included the collection of water samples, two of which resulted in positive lab results "of interest" (bacteriophage specific for clinical cholera strains) to the current cholera situation. By overlaying sample sites on 2012 water risk maps, a further fifteen proposed water sample locations are suggested. These resulted in a third spatial video survey and an additional "of interest" positive water sample. A potential spatial connection between the "of interest" water samples is suggested. The paper concludes

with how spatial video can be an integral part of future fine-scale epidemiological investigations for different pathogens.

Das, S., Pettersson, B. M., Behra, P. R., Ramesh, M., Dasgupta, S., Bhattacharya, A., and Kirsebom, L. A. The Mycobacterium phlei genome: expectations and surprises. Genome Biol.Evol. 3-3-2016;

ABSTRACT: Mycobacterium phlei, a non-tuberculosis mycobacterial species was first described in 1898-99. We present the complete genome sequence for the M. phlei CCUG21000T type strain and the draft genomes for four additional strains. The genome size for all five is 5.3 Mb with 69.4% GC content. This is approximately 0.35 Mbps smaller than the previously reported M. phlei RIVM draft genome. The size difference is attributed partly to large bacteriophage sequence fragments in the M. phlei RIVM genome. Comparative analysis revealed: i) a CRISPR system similar to Type-1E (cas3) in M. phlei RIVM; ii) genes involved in polyamine metabolism and transport (potAD, potF) that are absent in other mycobacteria, and iii) strain-specific variations in the number of sigma-factor genes. Moreover, M. phlei has as many as 82 mce (mammalian cell entry) homologs and many of the horizontally acquired genes in M. phlei are present in other environmental bacteria including mycobacteria that share similar habitat. Phylogenetic analysis based on 693 Mycobacterium core genes present in all complete mycobacterial genomes suggested that its closest neighbor is Mycobacterium smegmatis JS623 and Mycobacterium rhodesiae NBB3 while it is more distant to M. smegmatis mc2 155.

Day, C. R., Chen, H., Coulon, A., Meier, J. L., and Larson, D. R. High-throughput single-molecule screen for small-molecule perturbation of splicing and transcription kinetics. Methods. 3-1-2016; 96:59-68.

ABSTRACT: In eukaryotes, mRNA synthesis is catalyzed by RNA polymerase II and involves several distinct steps, including transcript initiation, elongation, cleavage, and transcript release. Splicing of RNA can occur during (co-transcriptional) or after (post-transcriptional) RNA synthesis. Thus, RNA synthesis and processing occurs through the concerted activity of dozens of enzymes, each of which is potentially susceptible to perturbation by small molecules. However, there are few, if any, high-throughput screening strategies for identifying drugs which perturb a specific step in RNA synthesis and processing. Here we have developed a high-throughput fluorescence microscopy approach in single cells to screen for inhibitors of specific enzymatic steps in RNA synthesis and processing. By utilizing the high affinity interaction between bacteriophage capsid proteins (MS2, PP7) and RNA stem loops, we are able to fluorescently label the intron and exon of a beta-globin reporter gene in human cells. This approach allows one to measure the kinetics of transcription, splicing and release in both fixed and living cells using a tractable, genetically encoded assay in a stable cell line. We tested this reagent in a targeted screen of molecules that target chromatin readers and writers and identified three compounds that slow transcription elongation without changing transcription initiation.

Defraine, V., Scheurmans, J., Grymonprez, B., Govers, S. K., Aertsen, A., Fauvart, M., Michiels, J., Lavigne, R., and Briers, Y. Efficacy of Artilysin(R) Art-175 against resistant and persistent Acinetobacter baumannii. Antimicrob.Agents Chemother. 3-28-2016;

ABSTRACT: Bacteriophage-encoded endolysins have shown promise as a novel class of antibacterials with a unique mode of action, i.e. peptidoglycan degradation. However, Gram-negative pathogens are generally not susceptible due to their protective outer membrane. The concept of Artilysin(R)s overcomes this barrier. Artilysin(R)s are optimized, engineered fusions of selected endolysins with specific outer membrane destabilizing peptides. Artilysin(R) Art-175 comprises a modified variant of endolysin KZ144 with an N-terminal fusion to SMAP-29. Previously, we have shown the high susceptibility ofP. aeruginosafor Art-175. Here, we report that Art-175 is highly bactericidal against stationary phase cells of multidrug-resistantAcinetobacter baumannii, even resulting in a complete elimination of large inocula (>/= 108CFU/ml). Besides actively-dividing cells, also persisters are killed. Instantaneous killing ofA. baumanniiupon contact with Art-175 could be visualized after immobilization of the bacteria in a microfluidic flow cell. Effective killing of a cell takes place through osmotic lysis after peptidoglycan degradation. The killing rate is enhanced by the addition of 0.5 mM EDTA. No resistance development against Art-175 under selection pressure or no cross-resistance with existing resistance mechanisms could be observed. In conclusion, Art-175 represents a highly active Artilysin(R) against bothA. baumanniiandP. aeruginosa, two of the most life-threatening pathogens of the order of thePseudomonadales.

Ding, H., Liang, Z. Y., Guo, F. B., Huang, J., Chen, W., and Lin, H. Predicting bacteriophage proteins located in host cell with feature selection technique. Comput.Biol.Med. 4-1-2016; 71:156-161.

ABSTRACT: A bacteriophage is a virus that can infect a bacterium. The fate of an infected bacterium is determined by the bacteriophage proteins located in the host cell. Thus, reliably identifying bacteriophage proteins located in the host cell is extremely important to understand their functions and discover potential anti-bacterial drugs. Thus, in this paper, a computational method was developed to recognize bacteriophage proteins located in host cells based only on their amino acid sequences. The analysis of variance (ANOVA) combined with incremental feature selection (IFS) was proposed to optimize the feature set. Using a jackknife cross-validation, our method can discriminate between bacteriophage proteins located in a host cell and the bacteriophage proteins not located in a host cell with a maximum overall accuracy of 84.2%, and can further classify bacteriophage proteins located in host cell cytoplasm and in host cell membranes with a maximum overall accuracy of 92.4%. To enhance the value of the practical applications of the method, we built a web server called PHPred (<http://lin.uestc.edu.cn/server/PHPred>). We believe that the PHPred will become a powerful tool to study bacteriophage proteins located in host cells and to guide related drug discovery.

Domingo-Calap, P., Georgel, P., and Bahram, S. Back to the future: bacteriophages as promising therapeutic tools. HLA. 2016; 87(3):133-140.

ABSTRACT: Bacteriophages (phages), natural predators of bacteria, are becoming increasingly attractive in medical and pharmaceutical applications. After their discovery almost a century ago, they have been particularly instrumental in the comprehension of basic molecular biology and genetics processes. The more recent emergence of multi-drug-resistant bacteria requires novel therapeutic strategies, and phages are being (re)considered as promising potential antibacterial tools. Furthermore, phages are also

used for other purposes, e.g. vaccine production, gene/drug carriers, bacterial detection and typing. These new alternative approaches using phages are of major interest and have allowed unexpected developments, from the decipherment of fundamental biological processes to potential clinical applications.

Doore, S. M. and Fane, B. A. The microviridae: Diversity, assembly, and experimental evolution. Virology. 2016; 491:45-55.

ABSTRACT: The Microviridae, comprised of ssDNA, icosahedral bacteriophages, are a model system for studying morphogenesis and the evolution of assembly. Historically limited to the phiX174-like viruses, recent results demonstrate that this richly diverse family is broadly divided into two groups. The defining feature appears to be whether one or two scaffolding proteins are required for assembly. The single-scaffolding systems contain an internal scaffolding protein, similar to many dsDNA viruses, and have a more complex coat protein fold. The two-scaffolding protein systems (phiX174-like) encode an internal and external species, as well as an additional structural protein: a spike on the icosahedral vertices. Here, we discuss recent in silico and in vivo evolutionary analyses conducted with chimeric viruses and/or chimeric proteins. The results suggest 1) how double scaffolding systems can evolve into single and triple scaffolding systems; and 2) how assembly is the critical factor governing adaptation and the maintenance of species boundaries.

Drilling, A. J., Cooksley, C., Chan, C., Wormald, P. J., and Vreugde, S. Fighting sinus-derived Staphylococcus aureus biofilms in vitro with a bacteriophage-derived muralytic enzyme. Int.Forum Allergy Rhinol. 2016; 6(4):349-355.

ABSTRACT: BACKGROUND: Staphylococcus aureus biofilms are a nidus for exacerbation of infectious conditions including chronic rhinosinusitis (CRS). Resistance of biofilms to current therapeutics stresses the need for the development of novel anti-biofilm strategies. The chimeric muralytic enzyme P128 was specifically engineered to target Staphylococcal sp. by combining the cell wall binding domain of lysostaphin and the peptidoglycan-degrading murein hydrolase derived from phage K. This study assessed the anti-biofilm activity of P128 against sinus-derived S. aureus. METHODS: Biofilms from S. aureus ATCC 25923 and 3 sinus-derived methicillin-sensitive and methicillin-resistant CRS clinical isolates were grown for 48 hours and treated with various concentrations of P128 (0 to 100 mug/mL) for 2 and 24 hours, using the minimum biofilm eradication concentration (MBEC) assay and Alamar Blue (AB) assay. Biofilm present on the MBEC pegs was stained with LIVE/DEAD BacLight stain, imaged using confocal scanning laser microscopy and biomass determined by COMSTAT2 computation. In the AB assay, biofilm was measured by assessing the cell viability. Results were assessed using a Kruskal-Wallis test, with a Wilcoxon post hoc test and Bonferroni correction. RESULTS: Both the MBEC and AB assay indicated that P128 was effective against in vitro S. aureus biofilms with significant reductions in biofilm of up to 95.5% at concentrations >/=12.5 mug/mL for all tested strains. CONCLUSION: The engineered chimeric endolysin P128 was observed to be an effective anti-biofilm agent against S. aureus. Further study will proceed into the appropriate application of P128 to ensure both an economically and clinically feasible preparation.

Dunne, M., Leicht, S., Krichel, B., Mertens, H. D., Thompson, A., Krijgsveld, J., Svergun, D. I., Gomez-Torres, N., Garde, S., Uetrecht, C., Narbad, A., Mayer, M. J., and Meijers, R. Crystal Structure of the CTP1L Endolysin Reveals How Its Activity Is Regulated by a Secondary Translation Product. J.Biol.Chem. 3-4-2016; 291(10):4882-4893.

ABSTRACT: Bacteriophages produce endolysins, which lyse the bacterial host cell to release newly produced virions. The timing of lysis is regulated and is thought to involve the activation of a molecular switch. We present a crystal structure of the activated endolysin CTP1L that targets Clostridium tyrobutyricum, consisting of a complex between the full-length protein and an N-terminally truncated C-terminal cell wall binding domain (CBD). The truncated CBD is produced through an internal translation start site within the endolysin gene. Mutants affecting the internal translation site change the oligomeric state of the endolysin and reduce lytic activity. The activity can be modulated by reconstitution of the full-length endolysin-CBD complex with free CBD. The same oligomerization mechanism applies to the CD27L endolysin that targets Clostridium difficile and the CS74L endolysin that targets Clostridium sporogenes. When the CTP1L endolysin gene is introduced into the commensal bacterium Lactococcus lactis, the truncated CBD is also produced, showing that the alternative start codon can be used in other bacterial species. The identification of a translational switch affecting oligomerization presented here has implications for the design of effective endolysins for the treatment of bacterial infections.

Edwards, R. A., McNair, K., Faust, K., Raes, J., and Dutilh, B. E. Computational approaches to predict bacteriophage-host relationships. FEMS Microbiol.Rev. 2016; 40(2):258-272.

ABSTRACT: Metagenomics has changed the face of virus discovery by enabling the accurate identification of viral genome sequences without requiring isolation of the viruses. As a result, metagenomic virus discovery leaves the first and most fundamental question about any novel virus unanswered: What host does the virus infect? The diversity of the global virosphere and the volumes of data obtained in metagenomic sequencing projects demand computational tools for virus-host prediction. We focus on bacteriophages (phages, viruses that infect bacteria), the most abundant and diverse group of viruses found in environmental metagenomes. By analyzing 820 phages with annotated hosts, we review and assess the predictive power of in silico phage-host signals. Sequence homology approaches are the most effective at identifying known phage-host pairs. Compositional and abundance-based methods contain significant signal for phage-host classification, providing opportunities for analyzing the unknowns in viral metagenomes. Together, these computational approaches further our knowledge of the interactions between phages and their hosts. Importantly, we find that all reviewed signals significantly link phages to their hosts, illustrating how current knowledge and insights about the interaction mechanisms and ecology of coevolving phages and bacteria can be exploited to predict phage-host relationships, with potential relevance for medical and industrial applications.

Elena, C. M., Leticia, F., Emilia, T., Patricia, E., and Mariella, T. Evaluation of a Labelled Bacteriophage with 99mTc as a Potential Agent for Infection Diagnosis. Curr.Radiopharm. 2-15-2016;

ABSTRACT: The design of target-specific molecular imaging probes to determine infection sites are mainly based on the biochemistry of the inflammatory response that

may lead to an ideal agent for infection imaging. Infectious diseases timely and specifically diagnosed can be clinically challenging but essential for the patient's recovery. Laboratory tests can detect the responsible microorganism but cannot discriminate between sterile inflammatory disease and truly infectious disease. On the other hand, scintigraphic images, can pinpoint the infection in the body. Bacteriophages (phages) are viruses that infect specific bacterial strains. Given the composition of the protein capsid, they could be used as radiopharmaceuticals to diagnose bacterial infection. In this case, PP7 phage was labelled and evaluated as a specific tracer for Pseudomonas aeruginosa infections.99mTc-Phage synthesis used HYNIC as a bifunctional agent. Physicochemical evaluation included studies such as stability in time, ligand exchange, lipophilicity and bacterial binding assay. The radiolabelling process required size exclusion purification of the 99mTc-Phage, which was obtained with a radiochemical purity higher than 90%, during 18 hours post labelling. Three groups of animals namely; healthy, infected with Pseudomonas aeruginosa and induced sterile inflammation were used to conduct biological evaluation. The collective accumulation in the stomach, small intestine and large intestine and thyroid of 99mTc-Phage was negligible, indicating no in vivo reoxidation. The complex presented urinary elimination. Target/non-target ratio (T/NT) was determined both for sterile inflammation and for infection. Values were 2.5 +/- 0.4 and 4.2 +/- 0.3 respectively. These values indicate significant differences between sterile inflammation and infection by Pseudomonas aeruginosa ($p<0.05$ unpaired two sided t-test). Targeted biodistribution profile and good T/NT ratios, indicate that this complex presents enough specificity to discriminate between infection caused by Pseudomonas aeruginosa and sterile inflammation.

Elhadidy, M. and varez-Ordonez, A. Diversity of Survival Patterns among Escherichia coli O157:H7 Genotypes Subjected to Food-Related Stress Conditions. Front Microbiol. 2016; 7:322

ABSTRACT: The purpose of this study was to evaluate the resistance patterns to food-related stresses of Shiga toxin producing Escherichia coli O157:H7 strains belonging to specific genotypes. A total of 33 E. coli O157:H7 strains were exposed to seven different stress conditions acting as potential selective pressures affecting the transmission of E. coli O157:H7 to humans through the food chain. These stress conditions included cold, oxidative, osmotic, acid, heat, freeze-thaw, and starvation stresses. The genotypes used for comparison included lineage-specific polymorphism, Shiga-toxin-encoding bacteriophage insertion sites, clade type, tir (A255T) polymorphism, Shiga toxin 2 subtype, and antiterminator Q gene allele. Bacterial resistance to different stressors was calculated by determining D-values (times required for inactivation of 90% of the bacterial population), which were then subjected to univariate and multivariate analyses. In addition, a relative stress resistance value, integrating resistance values to all tested stressors, was calculated for each bacterial strain and allowed for a ranking-type classification of E. coli O157:H7 strains according to their environmental robustness. Lineage I/II strains were found to be significantly more resistant to acid, cold, and starvation stress than lineage II strains. Similarly, tir (255T) and clade 8 encoding strains were significantly more resistant to acid, heat, cold, and starvation stress than tir (255A) and non-clade 8 strains. Principal component analysis, which allows grouping of strains with similar stress survival characteristics, separated strains of lineage I and I/II from strains of lineage II, which in general showed reduced survival abilities. Results obtained suggest that lineage I/II, tir (255T), and clade 8 strains, which have been previously reported to be more frequently associated with human disease cases, have greater multiple stress resistance than strains of other genotypes. The results from this study provide a better insight into how selective pressures encountered through the food chain may play a role

in the epidemiology of STEC O157:H7 through controlling the transmission of highly adapted strains to humans.

Esteban, P. P., Jenkins, A. T., and Arnot, T. C. Elucidation of the mechanisms of action of Bacteriophage K/nano-emulsion formulations against S. aureus via measurement of particle size and zeta potential. Colloids Surf.B Biointerfaces. 3-1-2016; 139:87-94.

ABSTRACT: In earlier work we have demonstrated the effect that nano-emulsions have on bacterial growth, and most importantly the enhanced bacteriophage infectivity against Staphylococcus aureus in planktonic culture when phage are carried in nano-emulsions. However, the mechanisms of enhancement of the bacteriophage killing effect are not specifically understood. This work focuses on the investigation of the possible interactions between emulsion droplets and bacterial cells, between emulsion droplets and bacteriophages, and finally interactions between all three components: nano-emulsion droplets, bacteria, and bacteriophages. The first approach consists of simple calculations to determine the spatial distribution of the components, based on measurements of particle size. It was found that nano-emulsion droplets are much more numerous than bacteria or bacteriophage, and due to their size and surface area they must be covering the surface of both cells and bacteriophage particles. Stabilisation of bacteriophages due to electrostatic forces and interaction with nano-emulsion droplets is suspected, since bacteriophages may be protected against inactivation due to 'charge shielding'. Zeta potential was measured for the individual components in the system, and for all of them combined. It was concluded that the presence of nano-emulsions could be reducing electrostatic repulsion between bacterial cells and bacteriophage, both of which are very negatively 'charged'. Moreover, nano-emulsions lead to more favourable interaction between bacteriophages and bacteria, enhancing the anti-microbial or killing effect. These findings are relevant since the physicochemical properties of nano-emulsions (i.e. particle size distribution and zeta potential) are key in determining the efficacy of the formulation against infection in the context of responsive burn wound dressings-which is the main target for this work.

Expert round table on acceptance and re-implementation of bacteriophage therapy (2016), Silk route to the acceptance and re-implementation of bacteriophage therapy. Biotechnol.J. 3-23-2016;

ABSTRACT: This multidisciplinary expert panel opinion on bacteriophage therapy has been written in the context of a society that is confronted with an ever-increasing number of antibiotic resistant bacteria. To avoid the return to a pre-antibiotic era, alternative treatments are urgently needed. The authors aim to contribute to the opinion formation of relevant stakeholders on how to potentially develop an infrastructure and legislation that paves the way for the acceptance and re-implementation of bacteriophage therapy.

Fagerquist, C. K. and Zaragoza, W. J. Bacteriophage cell lysis of Shiga toxin-producing Escherichia coli for top-down proteomic identification of Shiga toxins 1 & 2 using matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry. Rapid Commun.Mass Spectrom. 3-30-2016; 30(6):671-680.

ABSTRACT: RATIONAL: Analysis of bacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) often relies upon sample preparation methods that result in cell lysis, e.g. bead-beating. However, Shiga toxin-producing Escherichia coli (STEC) can undergo bacteriophage-induced cell lysis triggered by antibiotic exposure that may allow greater selectivity of the proteins extracted. METHODS: We have developed a sample preparation method for selective extraction of bacteriophage-encoded proteins and specifically Shiga toxins 1 and 2 (Stx1 & 2) expressed from STEC strains induced by DNA-damaging antibiotics. STEC strains were cultured overnight on agar supplemented with ciprofloxacin, mitomycin-C or an iron chelator to induce the bacteriophage lytic cycle with concomitant expression and release of Stx1 and/or Stx2. Sample preparation relied exclusively on bacteriophage lysis for release Stx into the extraction solution. RESULTS: Three clinical STEC strains were analyzed by matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry (MALDI-TOF-TOF-MS/MS) and top-down proteomics analysis: E. coli O157:H7 strain EDL933, E. coli O91:H21 strain B2F1 and E. coli O26:H11 strain ECRC #05.2217. The B-subunit of Stx1a of EDL933 was detected and identified even though it was ~100-fold less abundant than the B-subunit of Stx2a that had been identified previously for this strain. Two bacteriophage-encoded proteins were also identified: L0117 and L0136. The B-subunits of Stx2d of strain B2F1 and Stx1a of strain ECRC #05.2217 were also detected and identified. CONCLUSIONS: Bacteriophage lysis appeared to enhance the detection sensitivity of Stx for these STEC strains compared to previous work using mechanical lysis. Detection/identification of other bacteriophage-encoded proteins (beyond Stx) tends to support the hypothesis of Stx release by bacteriophage cell lysis. Published in 2016. This article is a U.S. Government work and is in the public domain in the USA.

Fauvel, B., Cauchie, H. M., Gantzer, C., and Ogorzaly, L. Contribution of hydrological data to the understanding of the spatio-temporal dynamics of F-specific RNA bacteriophages in river water during rainfall-runoff events. Water Res. 5-1-2016; 94:328-340.

ABSTRACT: Heavy rainfall events were previously reported to bring large amounts of microorganisms in surface water, including viruses. However, little information is available on the origin and transport of viral particles in water during such rain events. In this study, an integrative approach combining microbiological and hydrological measurements was investigated to appreciate the dynamics and origins of F-specific RNA bacteriophage fluxes during two distinct rainfall-runoff events. A high frequency sampling (automatic sampler) was set up to monitor the F-specific RNA bacteriophages fluxes at a fine temporal scale during the whole course of the rainfall-runoff events. A total of 276 rainfall-runoff samples were collected and analysed using both infectivity and RT-qPCR assays. The results highlight an increase of 2.5 log10 and 1.8 log10 of infectious F-specific RNA bacteriophage fluxes in parallel of an increase of the water flow levels for both events. Faecal pollution was characterised as being mainly from anthropic origin with a significant flux of phage particles belonging to the genogroup II. At the temporal scale, two successive distinct waves of phage pollution were established and identified through the hydrological measurements. The first arrival of phages in the water column was likely to be linked to the resuspension of riverbed sediments that was responsible for a high input of genogroup II. Surface runoff contributed further to the second input of phages, and more particularly of genogroup I. In addition, an important contribution of infectious phage particles has been highlighted. These findings imply the existence of a close relationship between the risk for human health and the viral contamination of flood water.

Fiches, G. N., Eyre, N. S., Aloia, A. L., Van Der, Hoek K., Betz-Stablein, B., Luciani, F., Chopra, A., and Beard, M. R. HCV RNA traffic and association with NS5A in living cells. Virology. 3-18-2016; 493:60-74.

ABSTRACT: The spatiotemporal dynamics of Hepatitis C Virus (HCV) RNA localisation are poorly understood. To address this we engineered HCV genomes harbouring MS2 bacteriophage RNA stem-loops within the 3'-untranslated region to allow tracking of HCV RNA via specific interaction with a MS2-Coat-mCherry fusion protein. Despite the impact of these insertions on viral fitness, live imaging revealed that replication of tagged-HCV genomes induced specific redistribution of the mCherry-tagged-MS2-Coat protein to motile and static foci. Further analysis showed that HCV RNA was associated with NS5A in both static and motile structures while a subset of motile NS5A structures was devoid of HCV RNA. Further investigation of viral RNA traffic with respect to lipid droplets (LDs) revealed HCV RNA-positive structures in close association with LDs. These studies provide new insights into the dynamics of HCV RNA traffic with NS5A and LDs and provide a platform for future investigations of HCV replication and assembly.

Fornelos, N., Browning, D. F., and Butala, M. The Use and Abuse of LexA by Mobile Genetic Elements. Trends Microbiol. 3-9-2016;

ABSTRACT: The SOS response is an essential process for responding to DNA damage in bacteria. The expression of SOS genes is under the control of LexA, a global transcription factor that undergoes self-cleavage during stress to allow the expression of DNA repair functions and delay cell division until the damage is rectified. LexA also regulates genes that are not part of this cell rescue program, and the induction of bacteriophages, the movement of pathogenicity islands, and the expression of virulence factors and bacteriocins are all controlled by this important transcription factor. Recently it has emerged that when regulating the expression of genes from mobile genetic elements (MGEs), LexA often does so in concert with a corepressor. This accessory regulator can either be a host-encoded global transcription factor, which responds to various metabolic changes, or a factor that is encoded for by the MGE itself. Thus, the coupling of LexA-mediated regulation to a secondary transcription factor not only detaches LexA from its primary SOS role, but also fine-tunes gene expression from the MGE, enabling it to respond to multiple stresses. Here we discuss the mechanisms of such coordinated regulation and its implications for cells carrying such MGEs.

Forterre, P. To be or not to be alive: How recent discoveries challenge the traditional definitions of viruses and life. Stud.Hist Philos.Biol.Biomed.Sci. 3-17-2016;

ABSTRACT: Three major discoveries have recently profoundly modified our perception of the viral world: molecular ecologists have shown that viral particles are more abundant than cells in natural environments; structural biologists have shown that some viruses from the three domains of life, Bacteria, Eukarya and Archaea, are evolutionarily related, and microbiologists have discovered giant viruses that rival with cells in terms of size and gene content. I discuss here the scientific and philosophical impact of these discoveries on the debates over the definition, nature (living or not), and origin of viruses. I suggest that viruses have often been considered non-living, because they are traditionally assimilated to their virions. However, the term virus describes a biological process and should integrate all aspects of the viral reproduction cycle. It is especially important to focus on

the intracellular part of this cycle, the virocell, when viral information is actively expressed and reproduced, allowing the emergence of new viral genes. The virocell concept theoretically removes roadblocks that prevent defining viruses as living organisms. However, defining a "living organism" remains challenging, as indicated by the case of organelles that evolved from intracellular bacteria. To bypass this problem, I suggest considering that all biological entities that actively participate in the process of life are living.

Frietze, K. M., Roden, R. B., Lee, J. H., Shi, Y., Peabody, D. S., and Chackerian, B. Identification of Anti-CA125 Antibody Responses in Ovarian Cancer Patients by a Novel Deep Sequence-Coupled Biopanning Platform. Cancer Immunol.Res. 2016; 4(2):157-164.

ABSTRACT: High-grade epithelial ovarian cancer kills more women than any other gynecologic cancer and is rarely diagnosed at an early stage. We sought to identify tumor-associated antigens (TAA) as candidate diagnostic and/or immunotherapeutic targets by taking advantage of tumor autoantibody responses in individuals with ovarian cancer. Plasma-derived IgG from a pool of five patients with advanced ovarian cancer was subjected to iterative biopanning using a library of bacteriophage MS2 virus-like particles (MS2-VLPs) displaying diverse short random peptides. After two rounds of biopanning, we analyzed the selectant population of MS2-VLPs by Ion Torrent deep sequencing. One of the top 25 most abundant peptides identified (DISGTNTSRA) had sequence similarity to cancer antigen 125 (CA125/MUC16), a well-known ovarian cancer-associated antigen. Mice immunized with MS2-DISGTNTSRA generated antibodies that cross-reacted with purified soluble CA125 from ovarian cancer cells but not membrane-bound CA125, indicating that the DISGTNTSRA peptide was a CA125/MUC16 peptide mimic of soluble CA125. Preoperative ovarian cancer patient plasma (n = 100) was assessed for anti-DISGTNTSRA, anti-CA125, and CA125. Patients with normal CA125 (<35 IU/mL) at the time of diagnosis had significantly more antibodies to DISGTNTSRA and to CA125 than those patients who had high CA125 (>35 IU/mL). A statistically significant survival advantage was observed for patients who had either normal CA125 and/or higher concentrations of antibodies to CA125 at the time of diagnosis. These data show the feasibility of using deep sequence-coupled biopanning to identify TAA autoantibody responses from cancer patient plasma and suggest a possible antibody-mediated mechanism for low CA125 plasma concentrations in some ovarian cancer patients. Cancer Immunol Res; 4(2); 157-64. (c)2015 AACR.

Furusawa, T., Iwano, H., Higuchi, H., Yokota, H., Usui, M., and Tamura, Y. Bacteriophage can lyse antibiotic-resistant Pseudomonas aeruginosa isolated from canine diseases. J.Vet.Med.Sci. 2-14-2016;

ABSTRACT: Pseudomonas aeruginosa is a pathogen frequently identified as the cause of diverse infections or chronic disease. This microbe has natural resistance to several kinds of antibiotics, because of the species' outer membrane, efflux pumps and growth as a biofilm. This bacterium can acquire increased resistance with specific point mutations. Bacteriophage (phage), however, can lyse these bacteria. Therefore, in the present study, we assessed the host range of phages isolates and their ability to lyse antibiotic-resistant P. aeruginosa. Present phages could lyse many strains of P. aeruginosa (28/39), including strains with high resistance to fluoroquinolones (4/6). In conclusion, application of phages for antibiotic-resistant bacteria is greatly effective. To avoid pervasive

antibiotic-resistant bacteria, further development of phage usage for disease treatment is required.

Gajewski, S., Waddell, M. B., Vaithiyalingam, S., Nourse, A., Li, Z., Woetzel, N., Alexander, N., Meiler, J., and White, S. W. Structure and mechanism of the phage T4 recombination mediator protein UvsY. Proc.Natl.Acad.Sci.U.S.A. 3-22-2016; 113(12):3275-3280.

ABSTRACT: The UvsY recombination mediator protein is critical for efficient homologous recombination in bacteriophage T4 and is the functional analog of the eukaryotic Rad52 protein. During T4 homologous recombination, the UvsX recombinase has to compete with the prebound gp32 single-stranded binding protein for DNA-binding sites and UvsY stimulates this filament nucleation event. We report here the crystal structure of UvsY in four similar open-barrel heptameric assemblies and provide structural and biophysical insights into its function. The UvsY heptamer was confirmed in solution by centrifugation and light scattering, and thermodynamic analyses revealed that the UvsY-ssDNA interaction occurs within the assembly via two distinct binding modes. Using surface plasmon resonance, we also examined the binding of UvsY to both ssDNA and the ssDNA-gp32 complex. These analyses confirmed that ssDNA can bind UvsY and gp32 independently and also as a ternary complex. They also showed that residues located on the rim of the heptamer are required for optimal binding to ssDNA, thus identifying the putative ssDNA-binding surface. We propose a model in which UvsY promotes a helical ssDNA conformation that disfavors the binding of gp32 and initiates the assembly of the ssDNA-UvsX filament.

Gandon, S. Why Be Temperate: Lessons from Bacteriophage lambda. Trends Microbiol. 3-3-2016;

ABSTRACT: Many pathogens have evolved the ability to induce latent infections of their hosts. The bacteriophage lambda is a classical model for exploring the regulation and the evolution of latency. Here, I review recent experimental studies on phage lambda that identify specific conditions promoting the evolution of lysogenic life cycles. In addition, I present specific adaptations of phage lambda that allow this virus to react plastically to variations in the environment and to reactivate its lytic life cycle. All of these different examples are discussed in the light of evolutionary epidemiology theory to disentangle the different evolutionary forces acting on temperate phages. Understanding phage lambda adaptations yield important insights into the evolution of latency in other microbes, including several life-threatening human pathogens.

Gao, S., Zhang, L., and Rao, V. B. Exclusion of small terminase mediated DNA threading models for genome packaging in bacteriophage T4. Nucleic Acids Res. 3-16-2016;

ABSTRACT: Tailed bacteriophages and herpes viruses use powerful molecular machines to package their genomes. The packaging machine consists of three components: portal, motor (large terminase; TerL) and regulator (small terminase; TerS). Portal, a dodecamer, and motor, a pentamer, form two concentric rings at the special five-fold vertex of the icosahedral capsid. Powered by ATPase, the motor ratchets DNA into the capsid through the portal channel. TerS is essential for packaging, particularly for genome recognition, but its mechanism is unknown and controversial. Structures of gear-shaped TerS rings inspired models that invoke DNA threading through the central

channel. Here, we report that mutations of basic residues that line phage T4 TerS (gp16) channel do not disrupt DNA binding. Even deletion of the entire channel helix retained DNA binding and produced progeny phage in vivo. On the other hand, large oligomers of TerS (11-mers/12-mers), but not small oligomers (trimers to hexamers), bind DNA. These results suggest that TerS oligomerization creates a large outer surface, which, but not the interior of the channel, is critical for function, probably to wrap viral genome around the ring during packaging initiation. Hence, models involving TerS-mediated DNA threading may be excluded as an essential mechanism for viral genome packaging.

Garcia, H. G., Brewster, R. C., and Phillips, R. Using synthetic biology to make cells tomorrow's test tubes. Integr.Biol.(Camb.). 3-8-2016;

ABSTRACT: The main tenet of physical biology is that biological phenomena can be subject to the same quantitative and predictive understanding that physics has afforded in the context of inanimate matter. However, the inherent complexity of many of these biological processes often leads to the derivation of complex theoretical descriptions containing a plethora of unknown parameters. Such complex descriptions pose a conceptual challenge to the establishment of a solid basis for predictive biology. In this article, we present various exciting examples of how synthetic biology can be used to simplify biological systems and distill these phenomena down to their essential features as a means to enable their theoretical description. Here, synthetic biology goes beyond previous efforts to engineer nature and becomes a tool to bend nature to understand it. We discuss various recent and classic experiments featuring applications of this synthetic approach to the elucidation of problems ranging from bacteriophage infection, to transcriptional regulation in bacteria and in developing embryos, to evolution. In all of these examples, synthetic biology provides the opportunity to turn cells into the equivalent of a test tube, where biological phenomena can be reconstituted and our theoretical understanding put to test with the same ease that these same phenomena can be studied in the in vitro setting.

Gerstmans, H., Rodriguez-Rubio, L., Lavigne, R., and Briers, Y. From endolysins to Artilysin(R)s: novel enzyme-based approaches to kill drug-resistant bacteria. Biochem.Soc.Trans. 2-15-2016; 44(1):123-128.

ABSTRACT: One of the last untapped reservoirs in nature for the identification of new anti-microbials is bacteriophages, the natural killers of bacteria. Lytic bacteriophages encode peptidoglycan (PG) lytic enzymes able to degrade the PG layer in different steps of their infection cycle. Endolysins degrade the bacterial cell wall at the end of the infection cycle, causing lysis of the host to release the viral progeny. Recombinant endolysins have been successfully applied as anti-bacterial agent against antibiotic-resistant Gram-positive pathogens. This has boosted the study of these enzymes as new anti-microbials in different fields (e.g. medical, food technology). A key example is the recent development of endolysin-based anti-bacterials against Gram-negative pathogens in which the exogenous application of endolysins is hindered by the outer membrane (OM). These novel anti-microbials, termed Artilysin(R)s, are able to pass through the OM and reach the PG where they exert their action. In addition, mycobacteria whose cell wall is structurally different from both Gram-positive and Gram-negative bacteria have also been reported to be inhibited by mycobacteriophage-encoded endolysins. Endolysins and endolysin-based anti-microbials can be considered as ideal candidates for an alternative to antibiotics for several reasons: (1) their unique mode of action and

activity against bacterial persisters (independent of an active host metabolism), (2) their selective activity against both Gram-positive and Gram-negative pathogens (including antibiotic resistant strains) and mycobacteria, (3) the limited resistance development reported so far. The present review summarizes and discusses the potential applications of endolysins as new anti-microbials.

Ghosh, A., Ricke, S. C., Almeida, G., and Gibson, K. E. Combined Application of Essential Oil Compounds and Bacteriophage to Inhibit Growth of Staphylococcus aureus In Vitro. Curr.Microbiol. 2016; 72(4):426-435.

ABSTRACT: Staphylococcus aureus is considered an important human pathogen. This study aimed to investigate the combination of essential oil compounds (EOCs) and bacteriophage as alternative antimicrobials to control S. aureus in vitro. Here, four EOCs (alpha-pinene, 3-carene, (+)-limonene, (1S)-(-)-beta-pinene) were evaluated by disc diffusion assay (DDA) and growth inhibition assay (GIA) to determine inhibitory effects against five strains of S. aureus. Phage adsorption assays were performed with phage K up to 120 h at 6, 13, and 37 degrees C to determine lytic activity. Combinations of phage K and EOCs against S. aureus were also evaluated at 37 degrees C. Alpha-pinene exhibited significantly greater inhibition towards S. aureus strains when compared to other EOCs tested by the DDA. GIAs indicate that all S. aureus strains exhibited significantly reduced growth ($P < 0.006$) over a 48-h period when exposed to EOCs. Phage adsorption assays indicate that phage K has high lytic activity at 37 degrees C with at least a 1.5-log increase in the number of plaque-forming units (PFU) over 6 h when compared to 6 and 13 degrees C. S. aureus strains showed significantly reduced growth ($P < 0.05$) when treated with combined phage K and EOCs. Results from the combined effect of EOC and phage indicate that phage alone inhibits S. aureus in vitro at 37 degrees C as effectively as EOCs alone or in combination with phage with variability between strains. The results from DDA, GIA, and phage adsorption assays indicate that select EOCs and phage K can be used as antimicrobials against S. aureus in vitro with potential application in situ.

Golomidova, A. K., Kulikov, E. E., Prokhorov, N. S., Guerrero-Ferreira Rcapital ES, Cyrillic, Knirel, Y. A., Kostryukova, E. S., Tarasyan, K. K., and Letarov, A. V. Branched Lateral Tail Fiber Organization in T5-Like Bacteriophages DT57C and DT571/2 is Revealed by Genetic and Functional Analysis. Viruses. 2016; 8(1)

ABSTRACT: The T5-like siphoviruses DT57C and DT571/2, isolated from horse feces, are very closely related to each other, and most of their structural proteins are also nearly identical to T5 phage. Their LTFs (L-shaped tail fibers), however, are composed of two proteins, LtfA and LtfB, instead of the single Ltf of bacteriophage T5. In silico and mutant analysis suggests a possible branched structure of DT57C and DT571/2 LTFs, where the LtfB protein is connected to the phage tail via the LtfA protein and with both proteins carrying receptor recognition domains. Such adhesin arrangement has not been previously recognized in siphoviruses. The LtfA proteins of our phages are found to recognize different host O-antigen types: E. coli O22-like for DT57C phage and E. coli O87 for DT571/2. LtfB proteins are identical in both phages and recognize another host receptor, most probably lipopolysaccharide (LPS) of E. coli O81 type. In these two bacteriophages, LTF function is essential to penetrate the shield of the host's O-antigens. We also demonstrate that LTF-mediated adsorption becomes superfluous when the non-specific cell protection by O-antigen is missing, allowing the phages to bind directly to

their common secondary receptor, the outer membrane protein BtuB. The LTF independent adsorption was also demonstrated on an O22-like host mutant missing O-antigen O-acetylation, thus showing the biological value of this O-antigen modification for cell protection against phages.

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ABSTRACT: The T5-like siphoviruses DT57C and DT571/2, isolated from horse feces, are very closely related to each other, and most of their structural proteins are also nearly identical to T5 phage. Their LTFs (L-shaped tail fibers), however, are composed of two proteins, LtfA and LtfB, instead of the single Ltf of bacteriophage T5. In silico and mutant analysis suggests a possible branched structure of DT57C and DT571/2 LTFs, where the LtfB protein is connected to the phage tail via the LtfA protein and with both proteins carrying receptor recognition domains. Such adhesin arrangement has not been previously recognized in siphoviruses. The LtfA proteins of our phages are found to recognize different host O-antigen types: E. coli O22-like for DT57C phage and E. coli O87 for DT571/2. LtfB proteins are identical in both phages and recognize another host receptor, most probably lipopolysaccharide (LPS) of E. coli O81 type. In these two bacteriophages, LTF function is essential to penetrate the shield of the host's O-antigens. We also demonstrate that LTF-mediated adsorption becomes superfluous when the non-specific cell protection by O-antigen is missing, allowing the phages to bind directly to their common secondary receptor, the outer membrane protein BtuB. The LTF independent adsorption was also demonstrated on an O22-like host mutant missing O-antigen O-acetylation, thus showing the biological value of this O-antigen modification for cell protection against phages.

Gong, P., Cheng, M., Li, X., Jiang, H., Yu, C., Kahaer, N., Li, J., Zhang, L., Xia, F., Hu, L., Sun, C., Feng, X., Lei, L., Han, W., and Gu, J. Characterization of Enterococcus faecium bacteriophage IME-EFm5 and its endolysin LysEFm5. Virology. 2-18-2016; 492:11-20.

ABSTRACT: Due to the worldwide prevalence of antibiotic resistant strains, phages therapy has been revitalized recently. In this study, an Enterococcus faecium phage named IME-EFm5 was isolated from hospital sewage. Whole genomic sequence analysis demonstrated that IME-EFm5 belong to the Siphoviridae family, and has a double-stranded genome of 42,265bp (with a 35.51% G+C content) which contains 70 putative coding sequences. LysEFm5, the endolysin of IME-EFm5, contains an amidase domain in its N-terminal and has a wider bactericidal spectrum than its parental phage IME-EFm5, including 7 strains of vancomycin-resistant E. faecium. The mutagenesis analysis revealed that the zinc ion binding residues (H27, H132, and C140), E90, and T138 are required for the catalysis of LysEFm5. However, the antibacterial activity of LysEFm5 is zinc ion independent, which is inconsistent with most of other amidase members. The phage lysin LysEFm5 might be an alternative treatment strategy for infections caused by multidrug-resistant E. faecium.

Gong, Y., Veesler, D., Doerschuk, P. C., and Johnson, J. E. Effect of the viral protease on the dynamics of bacteriophage HK97 maturation intermediates characterized by variance analysis of cryo EM particle ensembles. J.Struct.Biol. 2016; 193(3):188-195.

ABSTRACT: Cryo EM structures of maturation-intermediate Prohead I of bacteriophage HK97 with (PhI(Pro+)) and without (PhI(Pro-)) the viral protease packaged have been reported (Veesler et al., 2014). In spite of PhI(Pro+) containing an additional approximately 100x24kD of protein, the two structures appeared identical although the two particles have substantially different biochemical properties, e.g., PhI(Pro-) is less stable to disassembly conditions such as urea. Here the same cryo EM images are used to characterize the spatial heterogeneity of the particles at 17A resolution by variance analysis and show that PhI(Pro-) has roughly twice the standard deviation of PhI(Pro+). Furthermore, the greatest differences in standard deviation are present in the region where the delta-domain, not seen in X-ray crystallographic structures or fully seen in cryo EM, is expected to be located. Thus presence of the protease appears to stabilize the delta-domain which the protease will eventually digest.

Gorter, F. A., Scanlan, P. D., and Buckling, A. Adaptation to abiotic conditions drives local adaptation in bacteria and viruses coevolving in heterogeneous environments. Biol.Lett. 2016; 12(2)

ABSTRACT: Parasite local adaptation, the greater performance of parasites on their local compared with foreign hosts, has important consequences for the maintenance of diversity and epidemiology. While the abiotic environment may significantly affect local adaptation, most studies to date have failed either to incorporate the effects of the abiotic environment, or to separate them from those of the biotic environment. Here, we tease apart biotic and abiotic components of local adaptation using the bacterium Pseudomonas fluorescens and its viral parasite bacteriophage Phi2. We coevolved replicate populations of bacteria and phages at three different temperatures, and determined their performance against coevolutionary partners from the same and different temperatures. Crucially, we measured performance at different assay temperatures, which allowed us to disentangle adaptation to biotic and abiotic habitat components. Our results show that bacteria and phages are more resistant and infectious, respectively, at the temperature at which they previously coevolved, confirming that local adaptation to abiotic conditions can play a crucial role in determining parasite infectivity and host resistance. Our work underlines the need to assess host-parasite interactions across multiple relevant abiotic environments, and suggests that microbial adaption to local temperatures can create ecological barriers to dispersal across temperature gradients.

Hand, S., Gill, J., and Chu, K. H. Phage-based extraction of polyhydroxybutyrate (PHB) produced from synthetic crude glycerol. Sci.Total Environ. 3-23-2016; 557-558:317-321.

ABSTRACT: Polyhydroxybutyrate (PHB), a biodegradable plastic, is an attractive alternative to traditional petrochemical-derived plastics. However, its production is expensive due to high feedstock and extraction costs. As bacteriophages are natural predators to bacteria and specific to their hosts, bacteriophages offer a new and unique means to release PHB from bacteria via cell lysis. This study examined the feasibility of using bacteriophages as an effective bioextractant to release PHB produced by Pseudomonas oleovorans cultured with glycerol containing common impurities which

are generated from biodiesel production. While bacteria in stationary growth are known to be immune to bacteriophages, a bacteriophage Ke14 - isolated from soil - could lyse the PHB-filled cells effectively when excess nutrients were provided to trigger cell regrowth. The short-term nutrient treatment facilitated cell lysis with a little expense of PHB depolymerization, offering a new way to release PHB from cells without energy/solvent input.

Hardies, S. C., Thomas, J. A., Black, L., Weintraub, S. T., Hwang, C. Y., and Cho, B. C. Identification of structural and morphogenesis genes of Pseudoalteromonas phage phiRIO-1 and placement within the evolutionary history of Podoviridae. Virology. 2016; 489:116-127.

ABSTRACT: The virion proteins of Pseudoalteromonas phage phiRIO-1 were identified and quantitated by mass spectrometry and gel densitometry. Bioinformatic methods customized to deal with extreme divergence defined a phiRIO-1 tail structure homology group of phages, which was further related to T7 tail and internal virion proteins (IVPs). Similarly, homologs of tubular tail components and internal virion proteins were identified in essentially all completely sequenced podoviruses other than those in the subfamily Picovirinae. The podoviruses were subdivided into several tail structure homology groups, in addition to the RIO-1 and T7 groups. Molecular phylogeny indicated that these groups all arose about the same ancient time as the phiRIO-1/T7 split. Hence, the T7-like infection mechanism involving the IVPs was an ancestral property of most podoviruses. The IVPs were found to variably host both tail lysozyme domains and domains destined for the cytoplasm, including the N4 virion RNA polymerase embedded within an IVP-D homolog.

Harprecht, C., Okifo, O., Robbins, K. J., Motwani, T., Alexandrescu, A. T., and Teschke, C. M. Contextual Role of a Salt-Bridge in the Phage P22 Coat Protein I-Domain. J.Biol.Chem. 3-22-2016;

ABSTRACT: The I-domain is a genetic insertion in the phage P22 coat protein that chaperones its folding and stability. Of eleven acidic residues in the I-domain, seven participate in stabilizing electrostatic interactions with basic residues across elements of secondary structure, fastening the beta-barrel fold. A hydrogen-bonded salt-bridge between D302 and H305 is particularly interesting since D302 is the site of a temperature-sensitive-folding mutation. The pKa of H305 is raised to 9.0, indicating the salt-bridge stabilizes the I-domain by ~4 kcal/mol. Consistently, urea denaturation experiments indicate the stability of the WT I-domain decreases by 4 kcal/mol between neutral and basic pH. The mutants D302A and H305A remove the pH-dependence of stability. The D302A substitution destabilizes the I-domain by 4 kcal/mol, while H305A had smaller effects, on the order of 1-2 kcal/mol. The destabilizing effects of D302A are perpetuated in the full-length coat protein as shown by a higher sensitivity to protease digestion, decreased procapsid assembly rates, and impaired phage production in vivo. By contrast, the mutants have only minor effects on capsid expansion or stability in vitro. The effects of the D302-H305 salt-bridge are thus complex and context-dependent. Substitutions that abolish the salt-bridge destabilize coat protein monomers and impair capsid self-assembly, but once capsids are formed the effects of the substitutions are overcome by new quaternary interactions between subunits.

Hassan, S., Keshavarz-Moore, E., and Ward, J. A cell engineering strategy to enhance supercoiled plasmid DNA production for gene therapy. Biotechnol.Bioeng. 3-1-2016;

ABSTRACT: With the recent revival of the promise of plasmid DNA vectors in gene therapy, a novel synthetic biology approach was used to enhance the quantity, (yield), and quality of the plasmid DNA. Quality was measured by percentage supercoiling and supercoiling density, as well as improving segregational stability in fermentation. We examined the hypothesis that adding a Strong Gyrase binding Site (SGS) would increase DNA gyrase-mediated plasmid supercoiling. SGS from 3 different replicons, (the Mu bacteriophage and two plasmids, pSC101 and pBR322) were inserted into the plasmid, pUC57. Different sizes of these variants were transformed into E. coli DH5alpha, and their supercoiling properties and segregational stability measured. A 36% increase in supercoiling density was found in pUC57-SGS, but only when SGS was derived from the Mu phage and was the larger sized version of this fragment. These results were also confirmed at fermentation scale. Total % supercoiled monomer was maintained to 85-90%. A two-fold increase in plasmid yield was also observed for pUC57-SGS in comparison to pUC57. pUC57-SGS displayed greater segregational stability than pUC57-cer and pUC57, demonstrating a further potential advantage of the SGS site. These findings should augment the potential of plasmid DNA vectors in plasmid DNA manufacture. This article is protected by copyright. All rights reserved.

Hatfull, G. F. In the Trenches of Microbial Warfare: Identification of Genes and Pathways Contributing to Bacteriophage Infection by Quantitative Selection Analysis. J.Mol.Biol. 1-29-2016; 428(2 Pt B):413-415.

Abstract Not Available.

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Hill, R. L. and Dokland, T. The Type 2 dUTPase of Bacteriophage varphiNM1 Initiates Mobilization of Staphylococcus aureus Bovine Pathogenicity Island 1. J.Mol.Biol. 1-16-2016; 428(1):142-152.

ABSTRACT: Staphylococcus aureus pathogenicity islands (SaPIs) are genetic elements that are mobilized by specific helper phages. The initial step in mobilization is the derepression of the SaPI by the interaction of a phage protein with the SaPI master repressor Stl. Stl proteins are highly divergent between different SaPIs and respond to different phage-encoded derepressors. One such SaPI, SaPIbov1, is derepressed by the dUTPase (Dut) of bacteriophage 80alpha (Dut80alpha) and its phage varphi11 homolog, Dut11. We previously showed that SaPIbov1 could also be mobilized by phage varphiNM1, even though its dut gene is not homologous with that of 80alpha. Here, we show that varphiNM1 dut encodes a type 2 dUTPase (DutNM1), which has an alpha-helical structure that is distinct from the type 1 trimeric, beta-sheet structure of Dut80alpha. Deletion of dutNM1 abolishes the ability of varphiNM1 to mobilize SaPIbov1. Like Dut80alpha, DutNM1 forms a direct interaction with SaPIbov1 Stl both in vivo and in vitro, leading to inhibition of the dUTPase activity and Stl release from its target DNA. This work provides novel insights into the diverse mechanisms of genetic mobilization in S. aureus.

Hobbs, Z. and Abedon, S. T. Diversity of phage infection types and associated terminology: the problem with 'Lytic or lysogenic'. FEMS Microbiol.Lett. 2016; 363(7)

ABSTRACT: Bacteriophages, or phages, are viruses of members of domain Bacteria. These viruses play numerous roles in shaping the diversity of microbial communities, with impact differing depending on what infection strategies specific phages employ. From an applied perspective, these especially are communities containing undesired or pathogenic bacteria that can be modified through phage-mediated bacterial biocontrol, that is, through phage therapy. Here we seek to categorize phages in terms of their infection strategies as well as review or suggest more descriptive, accurate or distinguishing terminology. Categories can be differentiated in terms of (1) whether or not virion release occurs (productive infections versus lysogeny, pseudolysogeny and/or the phage carrier state), (2) the means of virion release (lytic versus chronic release) and (3) the degree to which phages are genetically equipped to display lysogenic cycles (temperate versus non-temperate phages). We address in particular the use or overuse of what can be a somewhat equivocal phrase, 'Lytic or lysogenic', especially when employed as a means of distinguishing among phages types. We suggest that the implied dichotomy is inconsistent with both modern as well as historical understanding of phage biology. We consider, therefore, less ambiguous terminology for distinguishing between 'Lytic' versus 'Lysogenic' phage types.

Hooton, S. P., Brathwaite, K. J., and Connerton, I. F. The Bacteriophage Carrier State of Features Changes in Host Non-coding RNAs and the Acquisition of New Host-derived CRISPR Spacer Sequences. Front Microbiol. 2016; 7:355

ABSTRACT: Incorporation of self-derived CRISPR DNA protospacers in Campylobacter jejuni PT14 occurs in the presence of bacteriophages encoding a CRISPR-like Cas4 protein. This phenomenon was evident in carrier state infections where both bacteriophages and host are maintained for seemingly indefinite periods as stable populations following serial passage. Carrier state cultures of C. jejuni PT14 have greater aerotolerance in nutrient limited conditions, and may have arisen as an evolutionary response to selective pressures imposed during periods in the extra-intestinal environment. A consequence of this is that bacteriophage and host remain associated and able to survive transition periods where the chances of replicative success are greatly diminished. The majority of the bacteriophage population do not commit to lytic infection, and conversely the bacterial population tolerates low-level bacteriophage replication. We recently examined the effects of Campylobacter bacteriophage/C. jejuni PT14 CRISPR spacer acquisition using deep sequencing strategies of DNA and RNA-Seq to analyze carrier state cultures. This approach identified de novo spacer acquisition in C. jejuni PT14 associated with Class III Campylobacter phages CP8/CP30A but spacer acquisition was oriented toward the capture of host DNA. In the absence of bacteriophage predation the CRISPR spacers in uninfected C. jejuni PT14 cultures remain unchanged. A distinct preference was observed for incorporation of self-derived protospacers into the third spacer position of the C. jejuni PT14 CRISPR array, with the first and second spacers remaining fixed. RNA-Seq also revealed the variation in the synthesis of non-coding RNAs with the potential to bind bacteriophage genes and/or transcript sequences.

Hsieh, S. E., Tseng, Y. H., Lo, H. H., Chen, S. T., and Wu, C. N. Genomic analysis of Staphylococcus phage Stau2 isolated from medical specimen. Virus Genes. 2016; 52(1):107-116.

ABSTRACT: Stau2 is a lytic myophage of Staphylococcus aureus isolated from medical specimen. Exhibiting a broad host range against S. aureus clinical isolates, Stau2 is

potentially useful for topical phage therapy or as an additive in food preservation. In this study, Stau2 was firstly revealed to possess a circularly permuted linear genome of 133,798 bp, with low G + C content, containing 146 open reading frames, but encoding no tRNA. The genome is organized into several modules containing genes for packaging, structural proteins, replication/transcription and host-cell-lysis, with the structural proteins and DNA polymerase modules being organized similarly to that in Twort-like phages of Staphylococcus. With the encoded DNA replication genes, Stau2 can possibly use its own system for replication. In addition, analysis in silico found several introns in seven genes, including those involved in DNA metabolism, packaging, and structure, while one of them (helicase gene) is experimentally confirmed to undergo splicing. Furthermore, phylogenetic analysis suggested Stau2 to be most closely related to Staphylococcus phages SA11 and Remus, members of Twort-like phages. The results of sodium dodecyl sulfate polyacrylamide gel electrophoresis showed 14 structural proteins of Stau2 and N-terminal sequencing identified three of them. Importantly, this phage does not encode any proteins which are known or suspected to be involved in toxicity, pathogenicity, or antibiotic resistance. Therefore, further investigations of feasible therapeutic application of Stau2 are needed.

Huang, X., Qu, Y., Cid, C. A., Finke, C., Hoffmann, M. R., Lim, K., and Jiang, S. C. Electrochemical disinfection of toilet wastewater using wastewater electrolysis cell. Water Res. 4-1-2016; 92:164-172.

ABSTRACT: The paucity of proper sanitation facilities has contributed to the spread of waterborne diseases in many developing countries. The primary goal of this study was to demonstrate the feasibility of using a wastewater electrolysis cell (WEC) for toilet wastewater disinfection. The treated wastewater was designed to reuse for toilet flushing and agricultural irrigation. Laboratory-scale electrochemical (EC) disinfection experiments were performed to investigate the disinfection efficiency of the WEC with four seeded microorganisms (Escherichia coli, Enterococcus, recombinant adenovirus serotype 5, and bacteriophage MS2). In addition, the formation of organic disinfection byproducts (DBPs) trihalomethanes (THMs) and haloacetic acids (HAA5) at the end of the EC treatment was also investigated. The results showed that at an applied cell voltage of +4 V, the WEC achieved 5-log10 reductions of all four seeded microorganisms in real toilet wastewater within 60 min. In contrast, chemical chlorination (CC) disinfection using hypochlorite [NaClO] was only effective for the inactivation of bacteria. Due to the rapid formation of chloramines, less than 0.5-log10 reduction of MS2 was observed in toilet wastewater even at the highest [NaClO] dosage (36 mg/L, as Cl2) over a 1 h reaction. Experiments using laboratory model waters showed that free reactive chlorine generated in situ during EC disinfection process was the main disinfectant responsible for the inactivation of microorganisms. However, the production of hydroxyl radicals [OH], and other reactive oxygen species by the active bismuth-doped TiO2 anode were negligible under the same electrolytic conditions. The formation of THMs and HAA5 were found to increase with higher applied cell voltage. Based on the energy consumption estimates, the WEC system can be operated using solar energy stored in a DC battery as the sole power source.

Huet, A., Duda, R. L., Hendrix, R. W., Boulanger, P., and Conway, J. F. Correct Assembly of the Bacteriophage T5 Procapsid Requires Both the Maturation Protease and the Portal Complex. J.Mol.Biol. 1-16-2016; 428(1):165-181.

ABSTRACT: The 90-nm-diameter capsid of coliphage T5 is organized with T=13 icosahedral geometry and encloses a double-stranded DNA genome that measures 121kbp. Its assembly follows a path similar to that of phage HK97 but yielding a larger structure that includes 775 subunits of the major head protein, 12 subunits of the portal protein and 120 subunits of the decoration protein. As for phage HK97, T5 encodes the scaffold function as an N-terminal extension (-domain) to the major head protein that is cleaved by the maturation protease after assembly of the initial prohead I form and prior to DNA packaging and capsid expansion. Although the major head protein alone is sufficient to assemble capsid-like particles, the yield is poor and includes many deformed structures. Here we explore the role of both the portal and the protease in capsid assembly by generating constructs that include the major head protein and a combination of protease (wild type or an inactive mutant) and portal proteins and overexpressing them in Escherichia coli. Our results show that the inactive protease mutant acts to trigger assembly of the major head protein, probably through binding to the -domain, while the portal protein regulates assembly into the correct T=13 geometry. A cryo-electron microscopy reconstruction of prohead I including inactivated protease reveals density projecting from the prohead interior surface toward its center that is compatible with the -domain, as well as additional internal density that we assign as the inactivated protease. These results reveal complexity in T5 beyond that of the HK97 system.

Hurwitz, B. L., U'Ren, J. M., and Youens-Clark, K. Computational prospecting the great viral unknown. FEMS Microbiol.Lett. 3-29-2016;

ABSTRACT: Bacteriophage play an important role in host-driven biological processes by controlling bacterial population size, horizontally transferring genes between hosts, and expressing host-derived genes to alter host metabolism. Metagenomics provides the genetic basis for understanding the interplay between uncultured bacteria, their phage, and the environment. In particular, viral metagenomes (viromes) are providing new insight into phage-encoded host genes (i.e., auxiliary metabolic genes; AMGs) that reprogram host metabolism during infection. Yet, despite deep sequencing efforts of viral communities, the majority of sequences have no match to known proteins. Reference-independent computational techniques, such as protein clustering, contig spectra, and ecological profiling are overcoming these barriers to examine both the known and unknown components of viromes. As the field of viral metagenomics progresses, a critical assessment of tools is required as the majority of algorithms have been developed for analyzing bacteria. The aim of this paper is to offer an overview of current computational methodologies for virome analysis and to provide an example of reference-independent approaches using human skin viromes. Additionally, we present methods to carefully validate AMGs from host contamination. Despite computational challenges, these new methods offer novel insights into the diversity and functional roles of phage in diverse environments.

Idelevich, E. A., Schaumburg, F., Knaack, D., Scherzinger, A. S., Mutter, W., Peters, G., Peschel, A., and Becker, K. The Recombinant Bacteriophage Endolysin HY-133 Exhibits In Vitro Activity against Different African Clonal Lineages of the Staphylococcus aureus Complex, Including Staphylococcus schweitzeri. Antimicrob.Agents Chemother. 2016; 60(4):2551-2553.

ABSTRACT: HY-133 is a recombinant bacteriophage endolysin with bactericidal activity againstStaphylococcus aureus Here, HY-133 showedin vitroactivity against major African

methicillin-susceptible and methicillin-resistantS. aureuslineages and ceftaroline/ceftobiprole- and borderline oxacillin-resistant isolates. HY-133 was also active againstStaphylococcus schweitzeri, a recently described species of theS. aureuscomplex. The activity of HY-133 on the tested isolates (MIC50, 0.25 mug/ml; MIC90, 0.5 mug/ml; range, 0.125 to 0.5 mug/ml) was independent of the species and strain background or antibiotic resistance.

Inouye, M. The first determination of DNA sequence of a specific gene. Gene. 5-10-2016; 582(1):94-95.

ABSTRACT: How and when the first DNA sequence of a gene was determined? In 1977, F. Sanger came up with an innovative technology to sequence DNA by using chain terminators, and determined the entire DNA sequence of the 5375-base genome of bacteriophage phiX 174 (Sanger et al., 1977). While this Sanger's achievement has been recognized as the first DNA sequencing of genes, we had determined DNA sequence of a gene, albeit a partial sequence, 11years before the Sanger's DNA sequence (Okada et al., 1966).

Jafari, M. R., Lakusta, J., Lundgren, R. J., and Derda, R. Allene Functionalized Azobenzene Linker Enables Rapid and Light-Responsive Peptide Macrocyclization. Bioconjug.Chem. 3-16-2016; 27(3):509-514.

ABSTRACT: In this manuscript, we describe the efficient synthesis of a bis(allenamide) functionalized water-soluble azobenzene reagent (BSBDA) and its application as a new tool for the rapid generation of visible light-responsive macrocyclic peptides and peptide libraries displayed on the surface of bacteriophage. The allenamide functionality promotes cysteine ligation in model peptides and those displayed on phage with rates 2-3 orders of magnitude faster than the established alkyl halide containing azobenzenes.

Jassim, S. A., Limoges, R. G., and El-Cheikh, H. Bacteriophage biocontrol in wastewater treatment. World J.Microbiol.Biotechnol. 2016; 32(4):70

ABSTRACT: Waterborne bacterial pathogens in wastewater remains an important public health concern, not only because of the environmental damage, morbidity and mortality that they cause, but also due to the high cost of disinfecting wastewater by using physical and chemical methods in treatment plants. Bacteriophages are proposed as bacterial pathogen indicators and as an alternative biological method for wastewater treatment. Phage biocontrol in large scale treatment requires adaptive and aggressive phages that are able to overcome the environmental forces that interfere with phage-host interactions while targeting unwanted bacterial pathogens and preventing biofilms and foaming. This review will shed light on aspects of using bacteriophage programming technology in wastewater plants to rapidly target and reduce undesirable bacteria without harming the useful bacteria needed for biodegradation.

Jeon, J., D'Souza, R., Pinto, N., Ryu, C. M., Park, J., Yong, D., and Lee, K. Characterization and complete genome sequence analysis of two Myoviral bacteriophages infecting clinical carbapenem-resistant Acinetobacter baumannii isolates. J.Appl.Microbiol. 3-16-2016;

ABSTRACT: AIMS: The aim of this study was to characterize phenotypical properties, to analyze whole genomes of novel A. baumannii phages infecting carbapenem-resistant A. baumannii (CRAB), and to evaluate their potential as antimicrobial alternatives to control A. baumannii in clinical settings. METHODS AND RESULTS: The A. baumannii phages, Betavarphi-R1215 and Betavarphi-R2315, were isolated from sewage samples. These phages were characterized by transmission electron microscopy, host spectrum, the thermal/pH stability test, the bacterial lysis assay, and the whole genome analysis. Both phages lysed 21 of 45 CRAB hosts, and showed high stability at various pH (pH 4-10) and temperature (25 degrees C-60 degrees C), and were strongly active against host bacteria in vitro. The genomes of Betavarphi-R1215 and Betavarphi-R2315 are linear double-strands of DNA with 44.866 bp and 44.846 bp, respectively. These two genomes revealed high similarity at the DNA level, but the organization and direction of open reading frames (ORFs) were different. CONCLUSIONS: The A. baumannii phages, Betavarphi-R1215 and Betavarphi-R2315, are novel lytic phages lysing CRAB strains which were isolated from respiratory samples of patients. SIGNIFICANCE AND IMPACT OF THE STUDY: In vitro and in silico data showed that these novel A. baumannii phages, Betavarphi-R1215 and Betavarphi-R2315, have potential as antimicrobial alternatives to control CRAB in healthcare settings. This article is protected by copyright. All rights reserved.

Jun, J. W., Kim, H. J., Yun, S. K., Chai, J. Y., Lee, B. C., and Park, S. C. Isolation and Comparative Genomic Analysis of T1-Like Shigella Bacteriophage pSf-2. Curr.Microbiol. 2016; 72(3):235-241.

ABSTRACT: The increasing prevalence of antibiotic-resistant Shigella sp. emphasizes that alternatives to conventional antibiotics are needed. Siphoviridae bacteriophage (phage), pSf-2, infecting S. flexneri ATCC((R)) 12022 was isolated from Geolpocheon stream in Korea. Morphological analysis by transmission electron microscopy revealed that pSf-2 has a head of about 57 +/- 4 nm in diameter with a long tail of 136 +/- 3 nm in length and 15 +/- 2 nm in width. One-step growth analysis revealed that pSf-2 has latent period of 30 min and burst size of 16 PFU/infected cell. The DNA genome of pSf-2 is composed of 50,109 bp with a G+C content of 45.44 %. The genome encodes 83 putative ORFs, 19 putative promoters, and 23 transcriptional terminator regions. Genome sequence analysis of pSf-2 and comparative analysis with the homologous T1-like Shigella phages, Shfl1 and pSf-1, revealed that pSf-2 is a novel T1-like Shigella phage. These results showed that pSf-2 might have a high potential as a biocontrol agent to control shigellosis. Also, the genomic information may lead to further understanding of phage biodiversity, especially T1-like phages.

Jun, J. W., Giri, S. S., Kim, H. J., Yun, S. K., Chi, C., Chai, J. Y., Lee, B. C., and Park, S. C. Bacteriophage application to control the contaminated water with Shigella. Sci.Rep. 2016; 6:22636

ABSTRACT: Shigella is one of the most important waterborne and foodborne pathogens around the world. Emergence of antibiotic-resistant Shigella has made the development of alternatives to conventional antibiotics necessary. In this study, a virulent Myoviridae

bacteriophage, pSs-1 was isolated from environmental water in South Korea and showed infectivity to S. flexneri as well as S. sonnei strains. One-step growth analysis showed that pSs-1 has a short latent period (25 min) and a large burst size (97 PFU/cell). According to the genomic analysis, pSs-1 contains 164,999 bp of genome with a G + C content of 35.54% and it is considered as a member of the T4-like bacteriophage group. These results showed that pSs-1 may have potential as a biocontrol agent instead of conventional antibiotics for shigellosis.

Jung, P., Abdelbary, M. M., Kraushaar, B., Fetsch, A., Geisel, J., Herrmann, M., Witte, W., Cuny, C., and Bischoff, M. Impact of bacteriophage Saint3 carriage on the immune evasion capacity and hemolytic potential of Staphylococcus aureus CC398. Vet.Microbiol. 2-23-2016;

ABSTRACT: Livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) isolates of clonal complex 398 (CC398) are frequently found in Europe, and recent studies highlighted the importance of mobile genetic element (MGE) exchange for host adaptation of this lineage. Of note, one of the MGEs commonly found in human S. aureus isolates, the immune evasion cluster (IEC) harboring bacteriophage Saint3, is very rarely found in LA-MRSA CC398 isolates obtained from farm animals, but more frequently found in LA-MRSA CC398 that were retransmitted to humans. Here, we analyzed with a set of S. aureus CC398 isolates harboring/lacking phiSaint3 how this MGE affects (i) phagocytosis of CC398 isolates by polymorphonuclear neutrophils (PMNs), and (ii) hemolysis of human and livestock-derived erythrocytes. Isolates lacking phiSaint3 were more efficiently phagocytosed by human PMNs in whole blood phagocytosis assays than isolates harboring this bacteriophage, irrespective of their origin. Notably, a similar effect was observed when equine blood was utilized, but not detected with porcine blood. Integration of phiSaint3 into LA-MRSA CC398 strains lacking this MGE confirmed these findings, as phiSaint3-harboring recipients were again less efficiently ingested by PMNs in equine and human blood than their parental strains. Integration of phiSaint3 strongly reduced the hemolytic potential of the culture supernatants against human-derived erythrocytes, and to a smaller extent also against porcine-derived erythrocytes, while phiSaint3 integration only slightly affected the hemolytic capacities against equine-derived red blood cells. The significant protective effect of phiSaint3 against phagocytosis by equine PMNs suggests that the host specificity of the IEC components might be broader than currently assumed.

Kalatzis, P. G., Bastias, R., Kokkari, C., and Katharios, P. Isolation and Characterization of Two Lytic Bacteriophages, phiSt2 and phiGrn1; Phage Therapy Application for Biological Control of Vibrio alginolyticus in Aquaculture Live Feeds. PLoS.One. 2016; 11(3):e0151101

ABSTRACT: Bacterial infections are a serious problem in aquaculture since they can result in massive mortalities in farmed fish and invertebrates. Vibriosis is one of the most common diseases in marine aquaculture hatcheries and its causative agents are bacteria of the genus Vibrio mostly entering larval rearing water through live feeds, such as Artemia and rotifers. The pathogenic Vibrio alginolyticus strain V1, isolated during a vibriosis outbreak in cultured seabream, Sparus aurata, was used as host to isolate and characterize the two novel bacteriophages phiSt2 and phiGrn1 for phage therapy application. In vitro cell lysis experiments were performed against the bacterial host V. alginolyticus strain V1 but also against 12 presumptive Vibrio strains originating from live prey Artemia salina cultures indicating the strong lytic efficacy of the 2 phages. In vivo

administration of the phage cocktail, phiSt2 and phiGrn1, at MOI = 100 directly on live prey A. salina cultures, led to a 93% decrease of presumptive Vibrio population after 4 h of treatment. Current study suggests that administration of phiSt2 and phiGrn1 to live preys could selectively reduce Vibrio load in fish hatcheries. Innovative and environmental friendly solutions against bacterial diseases are more than necessary and phage therapy is one of them.

Kang, Y., Gohlke, U., Engstrom, O., Hamark, C., Scheidt, T., Kunstmann, S., Heinemann, U., Widmalm, G., Santer, M., and Barbirz, S. Bacteriophage tailspikes and bacterial O-antigens as a model system to study weak-affinity protein-polysaccharide interactions. J.Am.Chem.Soc. 4-5-2016;

ABSTRACT: Understanding interactions of bacterial surface polysaccharides with receptor protein scaffolds is important for the development of antibiotic therapies. The corresponding protein recognition domains frequently form low-affinity complexes with polysaccharides that are difficult to address with experimental techniques because of the conformational flexibility of the polysaccharide. In this work, we studied the tailspike protein (TSP) of the bacteriophage Sf6. Sf6TSP binds and hydrolyzes the high-rhamnose, serotype Y O-antigen polysaccharide of the Gram-negative bacterium Shigella flexneri (S. flexneri) as a first step of bacteriophage infection. Spectroscopic analyses and enzymatic cleavage assays confirmed that Sf6TSP binds long stretches of this polysaccharide. Crystal structure analysis and saturation transfer difference (STD) NMR spectroscopy using an enhanced method to interpret the data permitted the detailed description of affinity contributions and flexibility in an Sf6TSP-octasaccharide complex. Dodecasaccharide fragments corresponding to three repeating units of the O-antigen in complex with Sf6TSP were studied computationally by molecular dynamics simulations. They showed that distortion away from the low-energy solution conformation found in the octasaccharide complex is necessary for ligand binding. This is in agreement with a weak-affinity functional polysaccharide-protein contact that facilitates correct placement and thus hydrolysis of the polysaccharide close to the catalytic residues. Our simulations stress that the flexibility of glycan epitopes together with a small number of specific protein contacts provide the driving force for Sf6TSP-polysaccharide complex formation in an overall weak-affinity interaction system.

Karimi, M., Mirshekari, H., Moosavi Basri, S. M., Bahrami, S., Moghoofei, M., and Hamblin, M. R. Bacteriophages and phage-inspired nanocarriers for targeted delivery of therapeutic cargos. Adv.Drug Deliv.Rev. 3-17-2016;

ABSTRACT: The main goal of drug delivery systems is to target therapeutic cargoes to desired cells and to ensure their efficient uptake. Recently a number of studies have focused on designing bio-inspired nanocarriers, such as bacteriophages, and synthetic carriers based on the bacteriophage structure. Bacteriophages are viruses that specifically recognize their bacterial hosts. They can replicate only inside their host cell and can act as natural gene carriers. Each type of phage has a particular shape, a different capacity for loading cargo, a specific production time, and their own mechanisms of supramolecular assembly, that have enabled them to act as tunable carriers. New phage-based technologies have led to the construction of different peptide libraries, and recognition abilities provided by novel targeting ligands. Phage hybridization with non-organic compounds introduces new properties to phages and could be a suitable strategy for construction of bio-inorganic carriers. In this review we try

to cover the major phage species that have been used in drug and gene delivery systems, and the biological application of phages as novel targeting ligands and targeted therapeutics.

Keary, R., Sanz-Gaitero, M., van Raaij, M. J., O'Mahony, J., Fenton, M., McAuliffe, O., Hill, C., Ross, R. P., and Coffey, A. Characterization of a Bacteriophage-Derived Murein Peptidase for Elimination of Antibiotic-Resistant Staphylococcus aureus. Curr.Protein Pept.Sci. 2016; 17(2):183-190.

ABSTRACT: Staphylococcus aureus is a major cause of infection in humans and animals, causing a wide variety of diseases, from local inflammations to fatal sepsis. The bacterium is commonly multi-drug resistant and thus many front-line antibiotics have been rendered ineffective for treating such infections. Research on murein/peptidoglycan hydrolases, derived from bacterial viruses (bacteriophages), has demonstrated that such proteins are attractive candidates for development as novel antibacterial agents for combatting Gram-positive pathogens. Here we review the research produced to-date on the bacteriophage-derived CHAPK murein peptidase. Initially, we sequenced and annotated the genome of anti-staphylococcal bacteriophage K and cloned the gene for the bacteriophage endolysin, a murein hydrolase which plays a role in cell killing during the bacteriophage life cycle. An highly active domain of the enzyme, a cysteine, histidine-dependent amido hydrolase/peptidase (CHAPK), was cloned, overexpressed in E. coli and purified. This CHAPK enzyme was demonstrated to rapidly lyse several strains of methicillin resistant S. aureus and both disrupted and prevented the formation of a staphylococcal biofilm. The staphylolytic activity of the peptidase was demonstrated in vivo using a mouse model, without adverse effects on the animals. The crystal structure of the enzyme was elucidated, revealing a calcium ion close to the active site. Site-directed mutagenesis indicated that this calcium ion is involved in the catalytic mechanism of the enzyme. The crystal structure of this enzyme is a valuable source of information for efficient engineering of this and similar CHAP-domain-containing proteins. Overall, the data collected to date on CHAPK has demonstrated its strong potential as a novel therapeutic candidate for treatment of staphylococcal infections and has provided us with insight into the fundamental enzymatic mechanisms of CHAP domaincontaining peptidoglycan hydrolases.

Keifer, D. Z., Motwani, T., Teschke, C. M., and Jarrold, M. F. Acquiring Structural Information on Virus Particles with Charge Detection Mass Spectrometry. J.Am.Soc.Mass Spectrom. 3-28-2016;

ABSTRACT: Charge detection mass spectrometry (CDMS) is a single-molecule technique particularly well-suited to measuring the mass and charge distributions of heterogeneous, MDa-sized ions. In this work, CDMS has been used to analyze the assembly products of two coat protein variants of bacteriophage P22. The assembly products show broad mass distributions extending from 5 to 15 MDa for A285Y and 5 to 25 MDa for A285T coat protein variants. Because the charge of large ions generated by electrospray ionization depends on their size, the charge can be used to distinguish hollow shells from more compact structures. A285T was found to form T = 4 and T = 7 procapsids, and A285Y makes a small number of T = 3 and T = 4 procapsids. Owing to the decreased stability of the A285Y and A285T particles, chemical cross-linking was required to stabilize them for electrospray CDMS.Graphical Abstract.

Khairnar, K., Chandekar, R., Nair, A., Pal, P., and Paunikar, W. N. Novel application of bacteriophage for controlling foaming in wastewater treatment plant- an eco-friendly approach. Bioengineered. 1-2-2016; 7(1):46-49.

ABSTRACT: This addendum to "Novel application of bacteriophage for controlling foaming in wastewater treatment plant- an eco-friendly approach " includes characteristics of the phages NOC1, NOC2 and NOC3 not discussed in the previous paper. The phage adsorption and host interaction properties, their sensitivity to pH and temperature are inferred. NOC2 is seen to be more temperature resistant while others are not. All the phages show pH sensitivity. There is a variance observed in the behavior of these phages. Also, applicability of the phage based system to large scale reactors is studied and discussed here.

Khakhum, N., Yordpratum, U., Boonmee, A., Tattawasart, U., Rodrigues, J. L., and Sermswan, R. W. Identification of the Burkholderia pseudomallei bacteriophage ST79 lysis gene cassette. J.Appl.Microbiol. 4-2-2016;

ABSTRACT: AIMS: To identify and characterize the lysis gene cassette from the bacteriophage ST79 that lyses Burkholderia pseudomallei. METHODS AND RESULTS: The approximately 1.5 kb of ST79 lysis genes were identified from the phage genome data. It composed of holin, peptidase M15A or endolysin, lysB and lysC. Each gene and combinations were cloned into Escherichia coli and the lytic effects were measured. Co-expression of holin and peptidase M15A showed the highest lysis activity. Expression of holin, lysB/C or holin, peptidase M15A, lysB and lysC lysed the E. coli membrane whereas peptidase M15A alone did not. The predicted transmembrane structures of holin and lysB/C indicated they could insert into the bacterial membrane to form pores, affecting cell permeability and causing lysis. CONCLUSION: This is the first report of an investigation into the lysis genes of B. pseudomallei's lytic phage using E. coli as a model. SIGNIFICANCE AND IMPACT OF THE STUDY: B. pseudomallei, a gram-negative bacterium causing an infectious disease, is intrinsically resistant to several antibiotics and a vaccine is not available. The lysis genes of ST79, the first reported lytic bacteriophage of B. pseudomallei, were characterized. The development of ST79 as an alternative treatment of skin ulceration, for example or to be used as gene cloning tool for B. pseudomallei may be possible with this knowledge. This article is protected by copyright. All rights reserved.

Kim, J., Jo, A., Ding, T., Lee, H. Y., and Ahn, J. Assessment of altered binding specificity of bacteriophage for ciprofloxacin-induced antibiotic-resistant Salmonella Typhimurium. Arch.Microbiol. 3-21-2016;

ABSTRACT: This study describes a new effort toward understanding the interaction mechanisms between antibiotic-resistant Salmonella Typhimurium and phages. The antibiotic susceptibility, beta-lactamase activity, bacterial motility, gene expression, and lytic activity were evaluated in ciprofloxacin-induced antibiotic-sensitive Salmonella Typhimurium (ASSTCIP) and ciprofloxacin-induced antibiotic-resistant S. Typhimurium (ARSTCIP), which were compared to the wild-type strains (ASSTWT and ARSTWT). The MIC values of ampicillin, norfloxacin, chloramphenicol, and tetracycline were significantly increased to > 512, 16, 16, and 256 mug/ml, respectively, in the ARSTCIP. The lowest and

highest extracellular lactamase activities were observed in ASSTWT (6.85 mumol/min/ml) and ARSTCIP (48.83 mumol/min/ml), respectively. The acrA, lpfE, and hilA genes were significantly upregulated by more than tenfold in both ASSTCIP and ARSTCIP. The induction of multiple antibiotic resistance resulted from the increased efflux pump activity (AcrAB-TolC). The highest phage adsorption rates were more than 95 % for ASSTWT, ASSTCIP, and ARSTWT, while the lowest adsorption rate was 52 % for ARSTCIP at 15 min of infection. The least lytic activity of phage was 20 % against the ARSTCIP, followed by ASSTCIP (30 %). The adsorption rate of phage against ARSTCIP was 52 % at 15 min of infection, which resulted in the decrease in lytic activity (12 %). Understanding the interaction of phage and bacteria is essential for the practical application of phage to control and detect antibiotic-resistant bacteria. The results provide useful information for understanding the binding specificity of phages for multiple antibiotic-resistant pathogens.

Kishor, C., Mishra, R. R., Saraf, S. K., Kumar, M., Srivastav, A. K., and Nath, G. Phage therapy of staphylococcal chronic osteomyelitis in experimental animal model. Indian J.Med.Res. 2016; 143(1):87-94.

ABSTRACT: BACKGROUND & OBJECTIVES: Methicillin resistant Staphylococcus aureus (MRSA) are the commonest cause of osteomyelitis. The aim of this study was to evaluate the role of an alternative therapy i.e. application of S. aureus specific bacteriophages in cases of osteomyelitis caused by MRSA in animal model. METHODS: Twenty two rabbits were included in this study. The first two rabbits were used to test the safety of phage cocktail while the remaining 20 rabbits were divided into three groups; group A (n=4) to assess the establishment of osteomyelitis; group B (n=4) osteomyelitis developed but therapy started only after six weeks; and group C (n=12) osteomyelitis developed and therapy started after three weeks. Groups B and C rabbits were treated with four doses of cocktail of seven virulent bacteriophages at the interval of 48 h. Comparison between three groups was made on the basis of observation of clinical, radiological, microbiological, and histopathological examinations. RESULTS: Experimental group rabbits recovered from the illness in the subsequent two weeks of the therapy. Appetite and activity of the rabbits improved, local oedema, erythema and induration subsided. There were minimal changes associated with osteomyelitis in X-ray and histopathology also showed no signs of infection with new bone formation. Control B group rabbits also recovered well from the infection. INTERPRETATION & CONCLUSIONS: The present study shows a potential of phage therapy to treat difficult infections caused by multidrug resistant bacteria.

Korol, N., Van den, Bossche A., Romaniuk, L., Noben, J. P., Lavigne, R., and Tovkach, F. Experimental evidence for proteins constituting virion components and particle morphogenesis of bacteriophage ZF40. FEMS Microbiol.Lett. 2016; 363(6)

ABSTRACT: Bacteriophage ZF40 is the only currently available, temperate Myoviridae phage infecting the potato pathogen Pectobacterium carotovorum subsp. carotovorum. Despite its unusual tail morphology, its major tail sheath and tube proteins remained uncharacterized after the initial genome annotation. Using ESI tandem mass-spectrometry, 24 structural proteins of the ZF40 virion were identified, with a sequence coverage ranging between 15.8% and 87.8%. The putative function of 16 proteins could be elucidated based on secondary structure analysis and conservative domain searches. The experimental annotation of 35% of the encoded gene products within the

structural region of the genome represents a complete view of the virion structure, which can serve as the basis for future structural analysis as a model phage.

Krishnamurthy, S. R., Janowski, A. B., Zhao, G., Barouch, D., and Wang, D. Hyperexpansion of RNA Bacteriophage Diversity. PLoS.Biol. 2016; 14(3):e1002409

ABSTRACT: Bacteriophage modulation of microbial populations impacts critical processes in ocean, soil, and animal ecosystems. However, the role of bacteriophages with RNA genomes (RNA bacteriophages) in these processes is poorly understood, in part because of the limited number of known RNA bacteriophage species. Here, we identify partial genome sequences of 122 RNA bacteriophage phylotypes that are highly divergent from each other and from previously described RNA bacteriophages. These novel RNA bacteriophage sequences were present in samples collected from a range of ecological niches worldwide, including invertebrates and extreme microbial sediment, demonstrating that they are more widely distributed than previously recognized. Genomic analyses of these novel bacteriophages yielded multiple novel genome organizations. Furthermore, one RNA bacteriophage was detected in the transcriptome of a pure culture of Streptomyces avermitilis, suggesting for the first time that the known tropism of RNA bacteriophages may include gram-positive bacteria. Finally, reverse transcription PCR (RT-PCR)-based screening for two specific RNA bacteriophages in stool samples from a longitudinal cohort of macaques suggested that they are generally acutely present rather than persistent.

Kuchinski, K. S., Brimacombe, C. A., Westbye, A. B., Ding, H., and Beatty, J. T. The SOS Response Master Regulator LexA Regulates the Gene Transfer Agent of Rhodobacter capsulatus and Represses Transcription of the Signal Transduction Protein CckA. J.Bacteriol. 2016; 198(7):1137-1148.

ABSTRACT: The gene transfer agent of Rhodobacter capsulatus (RcGTA) is a genetic exchange element that combines central aspects of bacteriophage-mediated transduction and natural transformation. RcGTA particles resemble a small double-stranded DNA bacteriophage, package random approximately 4-kb fragments of the producing cell genome, and are released from a subpopulation (<1%) of cells in a stationary-phase culture. RcGTA particles deliver this DNA to surrounding R. capsulatus cells, and the DNA is integrated into the recipient genome though a process that requires homologs of natural transformation genes and RecA-mediated homologous recombination. Here, we report the identification of the LexA repressor, the master regulator of the SOS response in many bacteria, as a regulator of RcGTA activity. Deletion of the lexA gene resulted in the abolition of detectable RcGTA production and an approximately 10-fold reduction in recipient capability. A search for SOS box sequences in the R. capsulatus genome sequence identified a number of putative binding sites located 5' of typical SOS response coding sequences and also 5' of the RcGTA regulatory gene cckA, which encodes a hybrid histidine kinase homolog. Expression of cckA was increased >5-fold in the lexA mutant, and a lexA cckA double mutant was found to have the same phenotype as a DeltacckA single mutant in terms of RcGTA production. The data indicate that LexA is required for RcGTA production and maximal recipient capability and that the RcGTA-deficient phenotype of the lexA mutant is largely due to the overexpression of cckA. IMPORTANCE: This work describes an unusual phenotype of a lexA mutant of the alphaproteobacterium Rhodobacter capsulatus in respect to the phage transduction-like genetic exchange carried out by

the R. capsulatus gene transfer agent (RcGTA). Instead of the expected SOS response characteristic of prophage induction, this lexA mutation not only abolishes the production of RcGTA particles but also impairs the ability of cells to receive RcGTA-borne genes. The data show that, despite an apparent evolutionary relationship to lambdoid phages, the regulation of RcGTA gene expression differs radically.

Kurabi, A., Pak, K. K., Bernhardt, M., Baird, A., and Ryan, A. F. Discovery of a Biological Mechanism of Active Transport through the Tympanic Membrane to the Middle Ear. Sci.Rep. 2016; 6:22663

ABSTRACT: Otitis media (OM) is a common pediatric disease for which systemic antibiotics are often prescribed. While local treatment would avoid the systemic treatment side-effects, the tympanic membrane (TM) represents an impenetrable barrier unless surgically breached. We hypothesized that the TM might harbor innate biological mechanisms that could mediate trans-TM transport. We used two M13-bacteriophage display biopanning strategies to search for mediators of trans-TM transport. First, aliquots of linear phage library displaying 10(10th) 12mer peptides were applied on the TM of rats with active bacterial OM. The middle ear (ME) contents were then harvested, amplified and the preparation re-applied for additional rounds. Second, the same naive library was sequentially screened for phage exhibiting TM binding, internalization and then transit. Results revealed a novel set of peptides that transit across the TM to the ME in a time and temperature dependent manner. The peptides with highest transport capacities shared sequence similarities. Historically, the TM was viewed as an impermeable barrier. However, our studies reveal that it is possible to translocate peptide-linked small particles across the TM. This is the first comprehensive biopanning for the isolation of TM transiting peptidic ligands. The identified mechanism offers a new drug delivery platform into the ME.

Lagoutte, P., Mignon, C., Donnat, S., Stadthagen, G., Mast, J., Sodoyer, R., Lugari, A., and Werle, B. Scalable chromatography-based purification of virus-like particle carrier for epitope based influenza A vaccine produced in Escherichia coli. J.Virol.Methods. 2016; 232:8-11.

ABSTRACT: Virus-like particles (VLPs) are promising molecular structures for the design and construction of novel vaccines, diagnostic tools, and gene therapy vectors. Size, oligomer assembly and repetitiveness of epitopes are optimal features to induce strong immune responses. Several VLP-based vaccines are currently licensed and commercialized, and many vaccine candidates are now under preclinical and clinical studies. In recent years, the development of genetically engineered recombinant VLPs has accelerated the need for new, improved downstream processes. In particular, a rapid low cost purification process has been identified as a remaining key challenge in manufacturing process development. In the present study we set up a size-exclusion chromatography-based, scalable purification protocol for the purification of a VLP-based influenza A vaccine produced in Escherichia coli. Recombinant VLPs derived from the RNA bacteriophage MS2 displaying an epitope from the ectodomain of Matrix 2 protein from influenza A virus were produced and purified. The 3 steps purification protocol uses a recently developed multimodal size-exclusion chromatography medium (Capto Core 700) in combination with detergent extraction and size-exclusion polishing to reach a 89% VLP purity with a 19% yield. The combination of this downstream strategy following production in E. coli would be suited for production of VLP-based veterinary

vaccines targeting livestock and companion animals where large amounts of doses must be produced at an affordable price.

Lal, T. M., Sano, M., and Ransangan, J. Genome characterization of a novel vibriophage VpKK5 (Siphoviridae) specific to fish pathogenic strain of Vibrio parahaemolyticus. J.Basic Microbiol. 3-9-2016;

ABSTRACT: Vibrio parahaemolyticus has long been known pathogenic to shrimp but only recently it is also reported pathogenic to tropical cultured marine finfish. Traditionally, bacterial diseases in aquaculture are often treated using synthetic antibiotics but concern due to side effects of these chemicals is elevating hence, new control strategies which are both environmental and consumer friendly, are urgently needed. One promising control strategy is the bacteriophage therapy. In this study, we report the isolation and characterization of a novel vibriophage (VpKK5), belonging to the family Siphoviridae that was specific and capable of complete lysing the fish pathogenic strain of V. parahaemolyticus. The VpKK5 exhibited short eclipse and latent periods of 24 and 36 min, respectively, but with a large burst size of 180 pfu/cell. The genome analysis revealed that the VpKK5 is a novel bacteriophage with the estimated genome size of 56,637 bp and has 53.1% G + C content. The vibriophage has about 80 predicted open reading frames consisted of 37 complete coding sequences which did not match to any protein databases. The analysis also found no lysogeny and virulence genes in the genome of VpKK5. With such genome features, we suspected the vibriophage is novel and could be explored for phage therapy against fish pathogenic strains of V. parahaemolyticus in the near future.

Lavergne, T., Lamichhane, R., Malyshev, D. A., Li, Z., Li, L., Sperling, E., Williamson, J. R., Millar, D. P., and Romesberg, F. E. FRET Characterization of Complex Conformational Changes in a Large 16S Ribosomal RNA Fragment Site-Specifically Labeled Using Unnatural Base Pairs. ACS Chem.Biol. 3-4-2016;

ABSTRACT: Ribosome assembly has been studied intensively using Forster resonance energy transfer (FRET) with fluorophore-labeled fragments of RNA produced by chemical synthesis. However, these studies are limited by the size of the accessible RNA fragments. We have developed a replicable unnatural base pair (UBP) formed between (d)5SICS and (d)MMO2 or (d)NaM, which efficiently directs the transcription of RNA containing unnatural nucleotides. We now report the synthesis and evaluation of several of the corresponding ribotriphosphates bearing linkers that enable the chemoselective attachment of different functionalities. We found that the RNA polymerase from T7 bacteriophage does not incorporate NaM derivatives but does efficiently incorporate 5SICSCO, whose linker enables functional group conjugation via Click chemistry, and when combined with the previously identified MMO2A, whose amine side chains permits conjugation via NHS coupling chemistry, enables site-specific double labeling of transcribed RNA. To study ribosome assembly, we transcribed RNA corresponding to a 243-nt fragment of the central domain of Thermus thermophilus 16S rRNA containing 5SICSCO and MMO2A at defined locations and then site-specifically attached the fluorophores Cy3 and Cy5. FRET was characterized using single-molecule total internal reflection fluorescence (smTIRF) microscopy in the presence of various combinations of added ribosomal proteins. We demonstrate that each of the fragment's two three-helix junctions exist in open and closed states, with the latter favored by sequential protein binding. These results elucidate early and previously uncharacterized folding events

underlying ribosome assembly and demonstrate the applicability of UBPs for biochemical, structural, and functional studies of RNAs.

Ledermann, B., Beja, O., and Frankenberg-Dinkel, N. New biosynthetic pathway for pink pigments from uncultured oceanic viruses. Environ.Microbiol. 3-7-2016;

ABSTRACT: The pink open-chain tetrapyrrole pigment phycoerythrobilin (PEB) is employed by marine cyanobacteria, red algae and cryptophytes as a light-harvesting chromophore in phycobiliproteins. Genes encoding biosynthesis proteins for PEB have also been discovered in cyanophages, viruses that infect cyanobacteria, and mimic host pigment biosynthesis with the exception of PebS which combines the enzymatic activities of two host enzymes. In this study we have identified novel members of the PEB biosynthetic enzyme families, heme oxygenases and ferredoxin-dependent bilin reductases. Encoding genes were found in metagenomic datasets and could be traced back to bacteriophage but not cyanophage origin. While the heme oxygenase exhibited standard activity, a new bilin reductase with highest homology to the teal pigment producing enzyme PcyA revealed PEB biosynthetic activity. Although PcyX possesses PebS-like activity both enzymes share only 9% sequence identity and likely catalyze the reaction via two independent mechanisms. Our data point towards the presence of phycobilin biosynthetic genes in phages that probably infect alphaproteobacteria and therefore further support a role of phycobilins outside oxygenic phototrophs. This article is protected by copyright. All rights reserved.

Leung, S. S., Parumasivam, T., Gao, F. G., Carrigy, N. B., Vehring, R., Finlay, W. H., Morales, S., Britton, W. J., Kutter, E., and Chan, H. K. Production of Inhalation Phage Powders Using Spray Freeze Drying and Spray Drying Techniques for Treatment of Respiratory Infections. Pharm.Res. 2-29-2016;

ABSTRACT: PURPOSE: The potential of aerosol phage therapy for treating lung infections has been demonstrated in animal models and clinical studies. This work compared the performance of two dry powder formation techniques, spray freeze drying (SFD) and spray drying (SD), in producing inhalable phage powders. METHOD: A Pseudomonas podoviridae phage, PEV2, was incorporated into multi-component formulation systems consisting of trehalose, mannitol and L-leucine (F1 = 60:20:20 and F2 = 40:40:20). The phage titer loss after the SFD and SD processes and in vitro aerosol performance of the produced powders were assessed. RESULTS: A significant titer loss (~2 log) was noted for droplet generation using an ultrasonic nozzle employed in the SFD method, but the conventional two-fluid nozzle used in the SD method was less destructive for the phage (~0.75 log loss). The phage were more vulnerable during the evaporative drying process (~0.75 log further loss) compared with the freeze drying step, which caused negligible phage loss. In vitro aerosol performance showed that the SFD powders (~80% phage recovery) provided better phage protection than the SD powders (~20% phage recovery) during the aerosolization process. Despite this, higher total lung doses were obtained for the SD formulations (SD-F1 = 13.1 +/- 1.7 x 104 pfu and SD-F2 = 11.0 +/- 1.4 x 104 pfu) than from their counterpart SFD formulations (SFD-F1 = 8.3 +/- 1.8 x 104 pfu and SFD-F2 = 2.1 +/- 0.3 x 104 pfu). CONCLUSION: Overall, the SD method caused less phage reduction during the powder formation process and the resulted powders achieved better aerosol performance for PEV2.

Li, E., Zhao, J., Ma, Y., Wei, X., Li, H., Lin, W., Wang, X., Li, C., Shen, Z., Zhao, R., Jiang, A., Yang, H., Yuan, J., and Zhao, X. Characterization of a novel Achromobacter xylosoxidans specific siphoviruse: phiAxp-1. Sci.Rep. 2016; 6:21943

ABSTRACT: Bacteriophages have recently been considered as an alternative biocontrol tool because of the widespread occurrence of antimicrobial-resistant Achromobacter xylosoxidans. Herein, we isolated a virulent bacteriophage (phiAxp-1) from a water sample of the Bohai sea of China that specifically infects A. xylosoxidans. Transmission electron microscopy revealed that phage phiAxp-1 belongs to the Siphoviridae. We sequenced the genome of phiAxp-1, which comprises 45,045 bp with 64 open reading frames. Most of the proteins encoded by phiAxp-1 have no similarity to sequences in the public databases. Twenty-one proteins with assigned functions share weak homology with those of other dsDNA bacteriophages infecting diverse hosts, such as Burkholderia phage KL1, Pseudomonas phage 73, Pseudomonas phage vB_Pae-Kakheti25, Pseudomonas phage vB_PaeS_SCH_Ab26, Acinetobacter phage IME_AB3 and Achromobacter phage JWX. The genome can be divided into different clusters for the head and tail structure, DNA replication and mazG. The sequence and genomic organization of bacteriophage phiAxp-1 are clearly distinct from other known Siphoviridae phages; therefore, we propose that it is a member of a novel genus of the Siphoviridae family. Furthermore, one-step growth curve and stability studies of the phage were performed, and the specific receptor of phiAxp-1 was identified as the lipopolysaccharide of A. xylosoxidans.

Li, Y., Wang, M., Liu, Q., Song, X., Wang, D., Ma, Y., Shao, H., and Jiang, Y. Complete Genomic Sequence of Bacteriophage H188: A Novel Vibrio kanaloae Phage Isolated from Yellow Sea. Curr.Microbiol. 2-8-2016;

ABSTRACT: Phage H188, a novel Vibrio kanaloae phage, was isolated from the surface water of Yellow Sea. Morphological analysis by transmission electron microscopy reveals that it belongs to the family Myoviridae. Present result suggests that the phage is stable at pH between 4.0 and 12.0. No significant difference in phage titers is noted at temperature 30-50 degrees C. A latent period of approximately 96 mins is indicated by the one-step growth curve. And, the burst size is about three virions per cell. Furthermore, genomic analysis of H188 reveals a genome size of 50364 bp with 43.63 % G+C content, and 76 putative open reading frames. There is no obvious similarity between H188 and other known phages by genomic comparison. Moreover, the H188 genome includes modules for phage structure, phage packaging, DNA replication and regulation, and some additional functions.

Liana, A. E., Chia, E. W., Marquis, C. P., Gunawan, C., Gooding, J. J., and Amal, R. Adsorption of T4 bacteriophages on planar indium tin oxide surface via controlled surface tailoring. J.Colloid Interface Sci. 4-15-2016; 468:192-199.

ABSTRACT: The work investigates the influence of surface physicochemical properties of planar indium tin oxide (ITO) as a model substrate on T4 bacteriophage adsorption. A comparative T4 bacteriophage adsorption study shows a significant difference in bacteriophage adsorption observed on chemically modified planar ITO when compared to similarly modified particulate ITO, which infers that trends observed in virus-particle interaction studies are not necessarily transferrable to predict virus-planar surface adsorption behaviour. We also found that ITO surfaces modified with methyl groups,

(resulting in increased surface roughness and hydrophobicity) remained capable of adsorbing T4 bacteriophage. The adsorption of T4 onto bare, amine and carboxylic functionalised planar ITO suggests the presence of a unique binding behaviour involving specific functional groups on planar ITO surface beyond the non-specific electrostatic interactions that dominate phage to particle interactions. The paper demonstrates the significance of physicochemical properties of surfaces on bacteriophage-surface interactions.

Lim, W. S., Phang, K. K., Tan, A. H., Li, S. F., and Ow, D. S. Small Colony Variants and Single Nucleotide Variations in Pf1 Region of PB1 Phage-Resistant Pseudomonas aeruginosa. Front Microbiol. 2016; 7:282

ABSTRACT: Phage therapy involves the application of lytic bacteriophages for treatment of clinical infections but bacterial resistance may develop over time. Isolated from nosocomial infections, small colony variants (SCVs) are morphologically distinct, highly virulent bacterial strains that are resistant to conventional antibiotics. In this study, SCVs was derived from Pseudomonas aeruginosa exposed to the lytic bacteriophage PB1 and these cells were resistant to subsequent phage infection by PB1. To elucidate the mechanism of the SCV phage resistance, we performed phenotypic assays, DNA microarrays and whole-genome sequencing. Compared with wild-type P. aeruginosa, the SCV isolate showed impaired biofilm formation, decreased twitching motility, reduced elastase and pyocyanin production. The SCV is also more susceptible to the antibiotic ciprofloxacin and exhibited higher syrface hydrophobicity than the wild-type, indicative of changes to cell surface lipopolysaccharide (LPS) composition. Consistent with these results, transcriptomic studies of SCV revealed up-regulation of genes involved in O-specific antigen (OSA) biosynthesis, suggesting the regulation of surface moieties may account for phage resistance. Western blot analysis showed a difference in OSA distribution between the two strains. Simultaneously, genes involved in aromatic and branched chain amino acid catabolism were down-regulated. Whole genome sequencing of the SCV revealed multiple single nucleotide variations within the Pf1 prophage region, a genetic locus known to play a crucial role in biofilm formation and to provide survival advantage via gene transfer to a subpopulation of cells. Insights into phenotypic and genetic changes in SCV gained here should help direct future studies to elucidate mechanisms underpinning phage resistance, leading to novel counter resistance measures.

Lin, X., Ding, H., and Zeng, Q. Transcriptomic response during phage infection of a marine cyanobacterium under phosphorus-limited conditions. Environ.Microbiol. 2016; 18(2):450-460.

ABSTRACT: The transcriptomic responses of bacteria to environmental stresses have been studied extensively, yet we know little about how the stressed cells respond to bacteriophage infection. Here, we conducted the first whole transcriptome sequencing (RNA-seq) study of stressed bacteria to phage infection by infecting the marine picocyanobacterium Prochlorococcus NATL2A with cyanomyovirus P-SSM2 under P limitation, a strong selective force in the oceans. Transcripts of the P-acquisition genes in the uninfected cells were enriched after P limitation, including the high-affinity phosphate-binding protein gene pstS. They were still enriched in the infected cells under P-limited conditions. In contrast, transcripts of adenosine triphosphate (ATP) synthase and ribosomal protein genes were depleted in the uninfected cells after P limitation but were

enriched during phage infection of P-starved cells. Cyanophage P-SSM2 contains pstS, and pstS and its adjacent gene g247 of unknown function were the only phage genes that were enriched under P-limited conditions. We further found that the host pstS transcript number per cell decreased after infection, however, it was still much higher in the P-limited infected cells than that in the nutrient-replete cells. Moreover, phage pstS transcript number per cell was approximately 20 times higher than the host copy, which may help maintain the host phosphate uptake rate during infection.

Liu, Y., Li, C., Li, Z., Chan, S. D., Eto, D., Wu, W., Zhang, J. P., Chien, R. L., Wada, H. G., Greenstein, M., and Satomura, S. On-chip quantitative PCR using integrated real-time detection by capillary electrophoresis. Electrophoresis. 2016; 37(3):545-552.

ABSTRACT: Quantitative PCR (qPCR) has been widely used for the detection and monitoring of a variety of infectious diseases. PCR and CE were integrated into a microfluidic chip that was designed to achieve rapid real-time amplicon sampling, separation, and quantitation without requiring various probes. A novel chip design allows the overlapped execution of PCR and CE, minimizing the time required for CE analysis after each PCR cycle. The performance of the on-chip qPCR method was demonstrated using a 45-minutes model assay protocol for the phiX174 bacteriophage, and the multiplexing capability of the method was demonstrated by adding a second target, E. coli genomic DNA, to the model assay. The results indicate good sensitivity, reproducibility, and linearity over the tested assay range, 50 to 2 x 10(4) copies/25 muL reaction. Based on this performance, the on-chip qPCR method should be applicable to a wide variety of infectious disease detection and monitoring assays with the addition of suitable sample preparation protocols.

Luzar, J., Molek, P., Silar, M., Korosec, P., Kosnik, M., Strukelj, B., and Lunder, M. Identification and characterization of major cat allergen Fel d 1 mimotopes on filamentous phage carriers. Mol.Immunol. 2016; 71:176-183.

ABSTRACT: Cat allergy is one of the most prevalent allergies worldwide and can lead to the development of rhinitis and asthma. Thus far, only allergen extracts from natural sources have been used for allergen-specific immunotherapy. However, extracts and whole allergens in immunotherapy present an anaphylaxis risk. Identification of allergen epitopes or mimotopes has an important role in development of safe and effective allergen-specific immunotherapy. Moreover, with a suitable immunogenic carrier, the absence of sufficient immune response elicited by short peptides could be surmounted. In this study, we identified five structural mimotopes of the major cat allergen Fel d 1 by immunoscreening with random peptide phage libraries. The mimotopes were computationally mapped to the allergen surface, and their IgE reactivity was confirmed using sera from cat-allergic patients. Importantly, the mimotopes showed no basophil activation of the corresponding cat-allergic patients, which makes them good candidates for the development of hypoallergenic vaccine. As bacteriophage particles are becoming increasingly recognized as immunogenic carriers, we constructed bacteriophage particles displaying multiple copies of each selected mimotope on major phage coat protein. These constructed phages elicited T cell-mediated immune response, which was predominated by the type 1 T cell response. Mimotopes alone contributed to the type 1 T cell response by promoting IL-2 production. Fel d 1

mimotopes, as well as their filamentous phage immunogenic carriers, represent promising candidates in the development of hypoallergenic vaccine against cat allergy.

Mao, H., Saha, M., Reyes-Aldrete, E., Sherman, M. B., Woodson, M., Atz, R., Grimes, S., Jardine, P. J., and Morais, M. C. Structural and Molecular Basis for Coordination in a Viral DNA Packaging Motor. Cell Rep. 3-1-2016; 14(8):2017-2029.

ABSTRACT: Ring NTPases are a class of ubiquitous molecular motors involved in basic biological partitioning processes. dsDNA viruses encode ring ATPases that translocate their genomes to near-crystalline densities within pre-assembled viral capsids. Here, X-ray crystallography, cryoEM, and biochemical analyses of the dsDNA packaging motor in bacteriophage phi29 show how individual subunits are arranged in a pentameric ATPase ring and suggest how their activities are coordinated to translocate dsDNA. The resulting pseudo-atomic structure of the motor and accompanying functional analyses show how ATP is bound in the ATPase active site; identify two DNA contacts, including a potential DNA translocating loop; demonstrate that a trans-acting arginine finger is involved in coordinating hydrolysis around the ring; and suggest a functional coupling between the arginine finger and the DNA translocating loop. The ability to visualize the motor in action illuminates how the different motor components interact with each other and with their DNA substrate.

Marsh, A., Casey-Green, K., Probert, F., Withall, D., Mitchell, D. A., Dilly, S. J., James, S., Dimitri, W., Ladwa, S. R., Taylor, P. C., and Singer, D. R. Simvastatin Sodium Salt and Fluvastatin Interact with Human Gap Junction Gamma-3 Protein. PLoS.One. 2016; 11(2):e0148266

ABSTRACT: Finding pleiomorphic targets for drugs allows new indications or warnings for treatment to be identified. As test of concept, we applied a new chemical genomics approach to uncover additional targets for the widely prescribed lipid-lowering pro-drug simvastatin. We used mRNA extracted from internal mammary artery from patients undergoing coronary artery surgery to prepare a viral cardiovascular protein library, using T7 bacteriophage. We then studied interactions of clones of the bacteriophage, each expressing a different cardiovascular polypeptide, with surface-bound simvastatin in 96-well plates. To maximise likelihood of identifying meaningful interactions between simvastatin and vascular peptides, we used a validated photo-immobilisation method to apply a series of different chemical linkers to bind simvastatin so as to present multiple orientations of its constituent components to potential targets. Three rounds of biopanning identified consistent interaction with the clone expressing part of the gene GJC3, which maps to Homo sapiens chromosome 7, and codes for gap junction gamma-3 protein, also known as connexin 30.2/31.3 (mouse connexin Cx29). Further analysis indicated the binding site to be for the N-terminal domain putatively 'regulating' connexin hemichannel and gap junction pores. Using immunohistochemistry we found connexin 30.2/31.3 to be present in samples of artery similar to those used to prepare the bacteriophage library. Surface plasmon resonance revealed that a 25 amino acid synthetic peptide representing the discovered N-terminus did not interact with simvastatin lactone, but did bind to the hydrolysed HMG CoA inhibitor, simvastatin acid. This interaction was also seen for fluvastatin. The gap junction blockers carbenoxolone and flufenamic acid also interacted with the same peptide providing insight into potential site of binding. These findings raise key questions about the functional significance of GJC3 transcripts in the vasculature and other tissues, and this connexin's role in therapeutic and adverse effects of statins in a range of disease states.

Massad, T., Papadopolos, E., Stenmark, P., and Damberg, P. The C repressor of the P2 bacteriophage. J.Biomol.NMR. 2016; 64(2):175-180.

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Abstract Not Available.

Merrill, J. R., Krajewski, K., Yuan, H., Frank, J. E., Lalush, D. S., Patterson, C., and Veleva, A. N. Synthesis and comparative evaluation of novel (64)Cu-labeled high affinity cell-specific peptides for positron emission tomography imaging of tumor vasculature. Biomaterials. 2016; 84:241-249.

ABSTRACT: Tumor angiogenesis, the formation of new tumor blood supply, has been recognized as a hallmark of cancer and represents an important target for clinical management of various angiogenesis-dependent solid tumors. Previously, by screening a bacteriophage peptide library we have discovered the FHT-peptide sequence that binds specifically to bone marrow-derived tumor vasculature with high affinity. Here in an effort to determine the potential of the FHT-peptide for in vivo positron emission tomography (PET) imaging of aggressive tumor vasculature we studied four FHT-derivatives: NOTA-FHT, NOTA-(FHT)2, NOTA-PEG-FHT, and NOTA-PEG-(FHT)2. These peptide analogs were synthesized, labeled with the PET radionuclide (64)Cu, and characterized side-by-side with small animal PET and computed tomography imaging (microPET/CT) at 1 h, 4 h, and 24 h post injection in a subcutaneous Lewis lung carcinoma (LLC) tumor model. Because of its excellent in vivo kinetic properties and high tumor-to-background ratio, the (64)Cu-NOTA-FHT radiopeptide was selected for more detailed evaluation. Blocking studies with excess of unlabeled peptide showed specific and peptide mediated (64)Cu-NOTA-FHT tumor uptake. Biodistribution experiments in the same tumor model confirmed microPET/CT imaging results. Human radiation absorbed dose extrapolated from rodent biodistribution of (64)Cu-NOTA-FHT revealed favorable dosimetry profile. The findings from this investigation warrant further development of (64)Cu-NOTA-FHT as a potential targeted diagnostic radiopharmaceutical for PET imaging of aggressive tumor vasculature.

Messing, J. Phage M13 for the treatment of Alzheimer and Parkinson disease. Gene. 6-1-2016; 583(2):85-89.

ABSTRACT: The studies of microbes have been instrumental in combatting infectious diseases, but they have also led to great insights into basic biological mechanism like DNA replication, transcription, and translation of mRNA. In particular, the studies of bacterial viruses, also called bacteriophage, have been quite useful to study specific cellular processes because of the ease to isolate their DNA, mRNA, and proteins. Here, I review the recent discovery of how properties of the filamentous phage M13 emerge as a novel approach to combat neurodegenerative diseases.

Meyer, J., Brissac, T., Frapy, E., Omer, H., Euphrasie, D., Bonavita, A., Nassif, X., and Bille, E. Characterization of MDAPhi, a temperate filamentous bacteriophage of Neisseria meningitidis. Microbiology. 2016; 162(2):268-282.

ABSTRACT: The mechanism by which Neisseria meningitidis becomes invasive is not well understood. Comparative genomics identified the presence of an 8 kb island in strains belonging to invasive clonal complexes. This island was designated MDA for meningococcal disease associated. MDA is highly conserved among meningococcal isolates and its analysis revealed a genomic organization similar to that of a filamentous prophage such as CTXPhi of Vibrio cholerae. Subsequent molecular investigations showed that the MDA island has indeed the characteristics of a filamentous prophage, which can enter into a productive cycle and is secreted using the type IV pilus (tfp) secretin PilQ. At least three genes of the prophage are necessary for the formation of the replicative cytoplasmic form (orf1, orf2 and orf9). Immunolabelling of the phage with antibodies against the major capsid protein, ORF4, confirmed that filamentous particles, about 1200 nm long, covered with ORF4 are present at the bacterial surface forming bundles in some places and interacting with pili. The MDA bacteriophage is able to infect different N. meningitidis strains, using the type IV pili as a receptor via an interaction with the adsorption protein ORF6. Altogether, these data demonstrate that the MDA island encodes a functional prophage able to produce infectious filamentous phage particles.

Millard, A. D., Pearce, D., and Zwirglmaier, K. Biogeography of bacteriophages at four hydrothermal vent sites in the Antarctic based on g23 sequence diversity. FEMS Microbiol.Lett. 2016; 363(7)

ABSTRACT: In this study, which was carried out within the ChEsSO consortium project (Chemosynthetically driven ecosystems south of the Polar Front), we sampled two hydrothermal vent sites on the East Scotia Ridge, Scotia Sea, one in the Kemp Caldera, South Sandwich Arc and one in the Bransfield Strait, north-west of the Antarctic Peninsula, which exhibit strong differences in their chemical characteristics. We compared a subset of their bacteriophage population by Sanger- and 454-sequencing of g23, which codes for the major capsid protein of T4likeviruses. We found that the sites differ vastly in their bacteriophage diversity, which reflects the differences in the chemical conditions and therefore putatively the differences in microbial hosts living at these sites. Comparing phage diversity in the vent samples to other aquatic samples, the vent samples formed a distinct separate cluster, which also included the non-vent control samples that were taken several hundred meters above the vent chimneys. This indicates that the influence of the vents on the microbial population and therefore also the bacteriophage population extends much further than anticipated.

Minoshima, M., Lu, Y., Kimura, T., Nakano, R., Ishiguro, H., Kubota, Y., Hashimoto, K., and Sunada, K. Comparison of the antiviral effect of solid-state copper and silver compounds. J.Hazard.Mater. 3-9-2016; 312:1-7.

ABSTRACT: Antiviral activities of insoluble solid-state and soluble ionic copper and silver compounds were evaluated against influenza A virus (A/PR8/H1N1) possessing a viral envelope and bacteriophage Qbeta lacking an envelope. The viral solutions were exposed on glass samples uniformly loaded with copper and silver compounds. Exposure to solid-state cuprous oxide (Cu2O) efficiently inactivated both influenza A virus and bacteriophage Qbeta, whereas solid-state cupric oxide (CuO) and silver sulfide (Ag2S) showed little antiviral activity. Copper ions from copper chloride (CuCl2) had little effect on the activity of bacteriophage Qbeta in spite of the fact that copper ions strongly inactivate influenza A in previous studies. Silver ions from silver nitrate (AgNO3) and

silver(I) oxide (Ag2O) in solution showed strong inactivation of influenza A and weak inactivation of bacteriophage Qbeta. We also investigated the influence of the compounds on the function of two influenza viral proteins, hemagglutinin and neuraminidase. Silver ions from AgNO3 and Ag2O remarkably decreased enzymatic activity of neuraminidase through the breakage of disulfide (SS) bonds, corresponding to the selective inactivation of influenza A virus. By contrast, exposure to Cu2O markedly reduced the activity of hemagglutinin rather than neuraminidase. These findings suggest that solid-state Cu2O disrupts host cell recognition by denaturing protein structures on viral surfaces, leading to the inactivation of viruses regardless of the presence of a viral envelope.

Molugu, S. K., Hildenbrand, Z. L., Morgan, D. G., Sherman, M. B., He, L., Georgopoulos, C., Sernova, N. V., Kurochkina, L. P., Mesyanzhinov, V. V., Miroshnikov, K. A., and Bernal, R. A. Ring Separation Highlights the Protein-Folding Mechanism Used by the Phage EL-Encoded Chaperonin. Structure. 3-16-2016;

ABSTRACT: Chaperonins are ubiquitous, ATP-dependent protein-folding molecular machines that are essential for all forms of life. Bacteriophage phiEL encodes its own chaperonin to presumably fold exceedingly large viral proteins via profoundly different nucleotide-binding conformations. Our structural investigations indicate that ATP likely binds to both rings simultaneously and that a misfolded substrate acts as the trigger for ATP hydrolysis. More importantly, the phiEL complex dissociates into two single rings resulting from an evolutionarily altered residue in the highly conserved ATP-binding pocket. Conformational changes also more than double the volume of the single-ring internal chamber such that larger viral proteins are accommodated. This is illustrated by the fact that phiEL is capable of folding beta-galactosidase, a 116-kDa protein. Collectively, the architecture and protein-folding mechanism of the phiEL chaperonin are significantly different from those observed in group I and II chaperonins.

Moon, B. Y., Park, J. Y., Robinson, D. A., Thomas, J. C., Park, Y. H., Thornton, J. A., and Seo, K. S. Mobilization of Genomic Islands of Staphylococcus aureus by Temperate Bacteriophage. PLoS.One. 2016; 11(3):e0151409

ABSTRACT: The virulence of Staphylococcus aureus, in both human and animal hosts, is largely influenced by the acquisition of mobile genetic elements (MGEs). Most S. aureus strains carry a variety of MGEs, including three genomic islands (nuSaalpha, nuSabeta, nuSagamma) that are diverse in virulence gene content but conserved within strain lineages. Although the mobilization of pathogenicity islands, phages and plasmids has been well studied, the mobilization of genomic islands is poorly understood. We previously demonstrated the mobilization of nuSabeta by the adjacent temperate bacteriophage varphiSaBov from strain RF122. In this study, we demonstrate that varphiSaBov mediates the mobilization of nuSaalpha and nuSagamma, which are located remotely from varphiSaBov, mostly to recipient strains belonging to ST151. Phage DNA sequence analysis revealed that chromosomal DNA excision events from RF122 were highly specific to MGEs, suggesting sequence-specific DNA excision and packaging events rather than generalized transduction by a temperate phage. Disruption of the int gene in varphiSaBov did not affect phage DNA excision, packaging, and integration events. However, disruption of the terL gene completely abolished phage DNA packing events, suggesting that the primary function of temperate phage in the transfer of genomic islands is to allow for phage DNA packaging by TerL and that

transducing phage particles are the actual vehicle for transfer. These results extend our understanding of the important role of bacteriophage in the horizontal transfer and evolution of genomic islands in S. aureus.

Morra, R., Shankar, J., Robinson, C. J., Halliwell, S., Butler, L., Upton, M., Hay, S., Micklefield, J., and Dixon, N. Dual transcriptional-translational cascade permits cellular level tuneable expression control. Nucleic Acids Res. 2-18-2016; 44(3):e21

ABSTRACT: The ability to induce gene expression in a small molecule dependent manner has led to many applications in target discovery, functional elucidation and bio-production. To date these applications have relied on a limited set of protein-based control mechanisms operating at the level of transcription initiation. The discovery, design and reengineering of riboswitches offer an alternative means by which to control gene expression. Here we report the development and characterization of a novel tunable recombinant expression system, termed RiboTite, which operates at both the transcriptional and translational level. Using standard inducible promoters and orthogonal riboswitches, a multi-layered modular genetic control circuit was developed to control the expression of both bacteriophage T7 RNA polymerase and recombinant gene(s) of interest. The system was benchmarked against a number of commonly used E. coli expression systems, and shows tight basal control, precise analogue tunability of gene expression at the cellular level, dose-dependent regulation of protein production rates over extended growth periods and enhanced cell viability. This novel system expands the number of E. coli expression systems for use in recombinant protein production and represents a major performance enhancement over and above the most widely used expression systems.

Mortusewicz, O., Evers, B., and Helleday, T. PC4 promotes genome stability and DNA repair through binding of ssDNA at DNA damage sites. Oncogene. 2-11-2016; 35(6):761-770.

ABSTRACT: The transcriptional cofactor PC4 is an ancient single-strand DNA (ssDNA)-binding protein that has a homologue in bacteriophage T5 where it is likely the elusive replicative ssDNA-binding protein. We hypothesize that human PC4 has retained functions in ssDNA binding to stabilize replication forks and prevent genome instability in mammalian cells. Here we demonstrate that PC4 is recruited to hydroxyurea (HU)-stalled replication forks, which is dependent on active transcription and its ssDNA-binding ability. Interestingly, we demonstrate that ssDNA binding by PC4 is critical to suppress spontaneous DNA damage and promote cellular survival. PC4 accumulation co-localizes with replication protein A (RPA) at stalled forks and is increased upon RPA depletion, demonstrating compensatory functions in ssDNA binding. Depletion of PC4 not only results in defective resolution of HU-induced DNA damage but also significantly reduces homologous recombination repair efficiency. Altogether, our results indicate that PC4 has similar functions to RPA in binding ssDNA to promote genome stability, especially at sites of replication-transcription collisions.

Motlagh, A. M., Bhattacharjee, A. S., and Goel, R. Biofilm control with natural and genetically-modified phages. World J.Microbiol.Biotechnol. 2016; 32(4):67

ABSTRACT: Bacteriophages, as the most dominant and diverse entities in the universe, have the potential to be one of the most promising therapeutic agents. The emergence

of multidrug-resistant bacteria and the antibiotic crisis in the last few decades have resulted in a renewed interest in phage therapy. Furthermore, bacteriophages, with the capacity to rapidly infect and overcome bacterial resistance, have demonstrated a sustainable approach against bacterial pathogens-particularly in biofilm. Biofilm, as complex microbial communities located at interphases embedded in a matrix of bacterial extracellular polysaccharide substances (EPS), is involved in health issues such as infections associated with the use of biomaterials and chronic infections by multidrug resistant bacteria, as well as industrial issues such as biofilm formation on stainless steel surfaces in food industry and membrane biofouling in water and wastewater treatment processes. In this paper, the most recent studies on the potential of phage therapy using natural and genetically-modified lytic phages and their associated enzymes in fighting biofilm development in various fields including engineering, industry, and medical applications are reviewed. Phage-mediated prevention approaches as an indirect phage therapy strategy are also explored in this review. In addition, the limitations of these approaches and suggestions to overcome these constraints are discussed to enhance the efficiency of phage therapy process. Finally, future perspectives and directions for further research towards a better understanding of phage therapy to control biofilm are recommended.

Munns, K. D., Zaheer, R., Xu, Y., Stanford, K., Laing, C. R., Gannon, V. P., Selinger, L. B., and McAllister, T. A. Comparative Genomic Analysis of Escherichia coli O157:H7 Isolated from Super-Shedder and Low-Shedder Cattle. PLoS.One. 2016; 11(3):e0151673

ABSTRACT: Cattle are the primary reservoir of the foodborne pathogen Escherichia coli O157:H7, with the concentration and frequency of E. coli O157:H7 shedding varying substantially among individual hosts. The term "super-shedder" has been applied to cattle that shed >/=104 cfu E. coli O157:H7/g of feces. Super-shedders have been reported to be responsible for the majority of E. coli O157:H7 shed into the environment. The objective of this study was to determine if there are phenotypic and/or genotypic differences between E. coli O157:H7 isolates obtained from super-shedder compared to low-shedder cattle. From a total of 784 isolates, four were selected from low-shedder steers and six isolates from super-shedder steers (4.01-8.45 log cfu/g feces) for whole genome sequencing. Isolates were phage and clade typed, screened for substrate utilization, pH sensitivity, virulence gene profiles and Stx bacteriophage insertion (SBI) sites. A range of 89-2473 total single nucleotide polymorphisms (SNPs) were identified when sequenced strains were compared to E. coli O157:H7 strain Sakai. More non-synonymous SNP mutations were observed in low-shedder isolates. Pan-genomic and SNPs comparisons did not identify genetic segregation between super-shedder or low-shedder isolates. All super-shedder isolates and 3 of 4 of low-shedder isolates were typed as phage type 14a, SBI cluster 3 and SNP clade 2. Super-shedder isolates displayed increased utilization of galactitol, thymidine and 3-O-beta-D-galactopyranosyl-D-arabinose when compared to low-shedder isolates, but no differences in SNPs were observed in genes encoding for proteins involved in the metabolism of these substrates. While genetic traits specific to super-shedder isolates were not identified in this study, differences in the level of gene expression or genes of unknown function may still contribute to some strains of E. coli O157:H7 reaching high densities within bovine feces.

Murphy, J., Bottacini, F., Mahony, J., Kelleher, P., Neve, H., Zomer, A., Nauta, A., and van, Sinderen D. Comparative genomics and functional analysis of the 936 group of lactococcal Siphoviridae phages. Sci.Rep. 2016; 6:21345

ABSTRACT: Genome sequencing and comparative analysis of bacteriophage collections has greatly enhanced our understanding regarding their prevalence, phage-host interactions as well as the overall biodiversity of their genomes. This knowledge is very relevant to phages infecting Lactococcus lactis, since they constitute a significant risk factor for dairy fermentations. Of the eighty four lactococcal phage genomes currently available, fifty five belong to the so-called 936 group, the most prevalent of the ten currently recognized lactococcal phage groups. Here, we report the genetic characteristics of a new collection of 936 group phages. By combining these genomes to those sequenced previously we determined the core and variable elements of the 936 genome. Genomic variation occurs across the 936 phage genome, such as genetic elements that (i) lead to a +1 translational frameshift resulting in the formation of additional structures on the phage tail, (ii) specify a double neck passage structure, and (iii) encode packaging module-associated methylases. Hierarchical clustering of the gene complement of the 936 group phages and nucleotide alignments allowed grouping of the ninety 936 group phages into distinct clusters, which in general appear to correspond with their geographical origin.

Nagayoshi, Y., Kumagae, K., Mori, K., Tashiro, K., Nakamura, A., Fujino, Y., Hiromasa, Y., Iwamoto, T., Kuhara, S., Ohshima, T., and Doi, K. Physiological Properties and Genome Structure of the Hyperthermophilic Filamentous Phage phiOH3 Which Infects Thermus thermophilus HB8. Front Microbiol. 2016; 7:50

ABSTRACT: A filamentous bacteriophage, phiOH3, was isolated from hot spring sediment in Obama hot spring in Japan with the hyperthermophilic bacterium Thermus thermophilus HB8 as its host. Phage phiOH3, which was classified into the Inoviridae family, consists of a flexible filamentous particle 830 nm long and 8 nm wide. phiOH3 was stable at temperatures ranging from 70 to 90 degrees C and at pHs ranging from 6 to 9. A one-step growth curve of the phage showed a 60-min latent period beginning immediately postinfection, followed by intracellular virus particle production during the subsequent 40 min. The released virion number of phiOH3 was 109. During the latent period, both single stranded DNA (ssDNA) and the replicative form (RF) of phage DNA were multiplied from min 40 onward. During the release period, the copy numbers of both ssDNA and RF DNA increased sharply. The size of the phiOH3 genome is 5688 bp, and eight putative open reading frames (ORFs) were annotated. These ORFs were encoded on the plus strand of RF DNA and showed no significant homology with any known phage genes, except ORF 5, which showed 60% identity with the gene VIII product of the Thermus filamentous phage PH75. All the ORFs were similar to predicted genes annotated in the Thermus aquaticus Y51MC23 and Meiothermus timidus DSM 17022 genomes at the amino acid sequence level. This is the first report of the whole genome structure and DNA multiplication of a filamentous T. thermophilus phage within its host cell.

Ogaki, M. B., Rocha, K. R., Terra, M. R., Furlaneto, M. C., and Maia, L. F. Screening of the enterocin-encoding genes and antimicrobial activity in Enterococcus species. J.Microbiol.Biotechnol. 2-24-2016;

ABSTRACT: In the current study, a total of 135 enterococci strains from different sources were searched for the presence of enterocin-encoding genes entA, entP, entB, entL50A and entL50B. Enterocin genes occurred at different frequencies where entA was the

most frequent, followed by entP and entB; entL50A and L50B were not detected. The frequency of genes occurring singly was higher than in multiple combinations. The 80 isolates that harbor at least one enterocin-encoding gene (denoted "Gene+ strains") were screened for antimicrobial activity. Some 82.5% of the Gene+ strains inhibited at least one of the indicator strains, besides, the isolates harboring multiple enterocin-encoding genes inhibited a larger number of indicators strains than isolates harboring a single gene. The indicator strains that exhibited growth inhibition include Staphylococcus aureus ATCC 25923, S. aureus ATCC 29213, S. aureus ATCC 6538, Listeria innocua CLIP 12612, Listeria monocytogenes CDC 4555, Enterococcus faecalis ATCC 29212 and Salmonella enteritidis ATCC 13076. Occcurrence of inhibition due to either bacteriophage lysis or cytolysin activity was excluded. Inhibition assay of positive anti-listerial Gene+ strains was further tested under different culture conditions. Among distinct culture media, the MRS (Man, Rogosa, Sharpe) agar medium supplemented with 2% (w/v) of yeast extract was the better solidified-medium for enterocin production. Our findings extend current knowledge of enterocin-producing enterococci and may have potential application as biopreservatives in food industry, due to their capability of controlling spoilage food pathogens.

Ogata, N., Sakasegawa, M., Miura, T., Shibata, T., Takigawa, Y., Taura, K., Taguchi, K., Matsubara, K., Nakahara, K., Kato, D., Sogawa, K., and Oka, H. Inactivation of Airborne Bacteria and Viruses Using Extremely Low Concentrations of Chlorine Dioxide Gas. Pharmacology. 2016; 97(5-6):301-306.

ABSTRACT: Infectious airborne microbes, including many pathological microbes that cause respiratory infections, are commonly found in medical facilities and constitute a serious threat to human health. Thus, an effective method for reducing the number of microbes floating in the air will aid in the minimization of the incidence of respiratory infectious diseases. Here, we demonstrate that chlorine dioxide (ClO2) gas at extremely low concentrations, which has no detrimental effects on human health, elicits a strong effect to inactivate bacteria and viruses and significantly reduces the number of viable airborne microbes in a hospital operating room. In one set of experiments, a suspension of Staphylococcus aureus, bacteriophage MS2, and bacteriophage PhiX174 were released into an exposure chamber. When ClO2 gas at 0.01 or 0.02 parts per million (ppm, volume/volume) was present in the chamber, the numbers of surviving microbes in the air were markedly reduced after 120 min. The reductions were markedly greater than the natural reductions of the microbes in the chamber. In another experiment, the numbers of viable airborne bacteria in the operating room of a hospital collected over a 24-hour period in the presence or absence of 0.03 ppm ClO2 gas were found to be 10.9 +/- 6.7 and 66.8 +/- 31.2 colony-forming units/m3 ($n = 9$, $p < 0.001$), respectively. Taken together, we conclude that ClO2 gas at extremely low concentrations (</=0.03 ppm) can reduce the number of viable microbes floating in the air in a room. These results strongly support the potential use of ClO2 gas at a non-toxic level to reduce infections caused by the inhalation of pathogenic microbes in nursing homes and medical facilities.

Otsuka, Y. Prokaryotic toxin-antitoxin systems: novel regulations of the toxins. Curr.Genet. 1-16-2016;

ABSTRACT: Toxin-antitoxin (TA) systems are widely conserved in prokaryotic plasmids and chromosomes and are linked to many roles in cell physiology, including plasmid

maintenance, stress response, persistence and protection from phage infection. A TA system is composed of a stable toxin and a labile antitoxin that inhibits a harmful effect of the cognate toxin. When gene expression from the TA loci is repressed under certain conditions such as nutrient starvation, the toxin is freed from the rapidly degrading antitoxin and obstructs an essential cellular process, such as DNA replication, translation and peptidoglycan synthesis, which subsequently causes growth arrest. TA systems are classified into five types according to the nature and the function of antitoxins, and the activity of toxins is tightly regulated in a variety of ways. This short-review highlights several novel regulatory mechanisms for Escherichia coli toxins that we recently discovered.

Otsuka, Y. Prokaryotic toxin-antitoxin systems: novel regulations of the toxins. Curr.Genet. 1-16-2016;

ABSTRACT: Toxin-antitoxin (TA) systems are widely conserved in prokaryotic plasmids and chromosomes and are linked to many roles in cell physiology, including plasmid maintenance, stress response, persistence and protection from phage infection. A TA system is composed of a stable toxin and a labile antitoxin that inhibits a harmful effect of the cognate toxin. When gene expression from the TA loci is repressed under certain conditions such as nutrient starvation, the toxin is freed from the rapidly degrading antitoxin and obstructs an essential cellular process, such as DNA replication, translation and peptidoglycan synthesis, which subsequently causes growth arrest. TA systems are classified into five types according to the nature and the function of antitoxins, and the activity of toxins is tightly regulated in a variety of ways. This short-review highlights several novel regulatory mechanisms for Escherichia coli toxins that we recently discovered.

Pan, X., Cui, X., Zhang, F., He, Y., Li, L., and Yang, H. Genetic Evidence for O-Specific Antigen as Receptor of Pseudomonas aeruginosa Phage K8 and Its Genomic Analysis. Front Microbiol. 2016; 7:252

ABSTRACT: Phage therapy requires the comprehensive understanding of the mechanisms underlying the host-phage interactions. In this work, to identify the genes related to Pseudomonas aeruginosa phage K8 receptor synthesis, 16 phage-resistant mutants were selected from a Tn5G transposon mutant library of strain PAK. The disrupted genetic loci were identified and they were related to O-specific antigen (OSA) synthesis, including gene wbpR, ssg, wbpV, wbpO, and Y880_RS05480, which encoded a putative O-antigen polymerase Wzy. The Lipopolysaccharide profile of the Y880_RS05480 mutant was analyzed and shown to lack the O-antigen. Therefore, the data from characterization of Y880_RS05480 by TMHMM and SDS-PAGE silver staining analysis suggest that this locus might encode Wzy. The complete phage K8 genome was characterized as 93879 bp in length and contained identical 1188-bp terminal direct repeats. Comparative genomic analysis showed that phage K8 was highly homologous to members of the genus PaP1-like phages. On the basis of our genetic findings, OSA of P. aeruginosa PAK is proven to be the receptor of phage K8. The highly conserved structural proteins among the genetic closely related phages suggest that they may recognize the same receptor.

Peltomaa, R., Lopez-Perolio, I., ito-Pena, E., Barderas, R., and Moreno-Bondi, M. C. Application of bacteriophages in sensor development. Anal.Bioanal.Chem. 2016; 408(7):1805-1828.

ABSTRACT: Bacteriophage-based bioassays are a promising alternative to traditional antibody-based immunoassays. Bacteriophages, shortened to phages, can be easily conjugated or genetically engineered. Phages are robust, ubiquitous in nature, and harmless to humans. Notably, phages do not usually require inoculation and killing of animals; and thus, the production of phages is simple and economical. In recent years, phage-based biosensors have been developed featuring excellent robustness, sensitivity, and selectivity in combination with the ease of integration into transduction devices. This review provides a critical overview of phage-based bioassays and biosensors developed in the last few years using different interrogation methods such as colorimetric, enzymatic, fluorescence, surface plasmon resonance, quartz crystal microbalance, magnetoelastic, Raman, or electrochemical techniques.

Pintilie, G., Chen, D. H., Haase-Pettingell, C. A., King, J. A., and Chiu, W. Resolution and Probabilistic Models of Components in CryoEM Maps of Mature P22 Bacteriophage. Biophys.J. 2-23-2016; 110(4):827-839.

ABSTRACT: CryoEM continues to produce density maps of larger and more complex assemblies with multiple protein components of mixed symmetries. Resolution is not always uniform throughout a cryoEM map, and it can be useful to estimate the resolution in specific molecular components of a large assembly. In this study, we present procedures to 1) estimate the resolution in subcomponents by gold-standard Fourier shell correlation (FSC); 2) validate modeling procedures, particularly at medium resolutions, which can include loop modeling and flexible fitting; and 3) build probabilistic models that combine high-accuracy priors (such as crystallographic structures) with medium-resolution cryoEM densities. As an example, we apply these methods to new cryoEM maps of the mature bacteriophage P22, reconstructed without imposing icosahedral symmetry. Resolution estimates based on gold-standard FSC show the highest resolution in the coat region (7.6 A), whereas other components are at slightly lower resolutions: portal (9.2 A), hub (8.5 A), tailspike (10.9 A), and needle (10.5 A). These differences are indicative of inherent structural heterogeneity and/or reconstruction accuracy in different subcomponents of the map. Probabilistic models for these subcomponents provide new insights, to our knowledge, and structural information when taking into account uncertainty given the limitations of the observed density.

Pourcel, C., Midoux, C., Bourkaltseva, M., Pleteneva, E., and Krylov, V. Complete Genome Sequence of PM105, a New Pseudomonas aeruginosa B3-Like Transposable Phage. Genome Announc. 2016; 4(2)

ABSTRACT: The complete genome of the Pseudomonas aeruginosa bacteriophage PM105 is 39,593 bp long. The phage belongs to the B3 family of transposable Mu-like phages, as confirmed by the presence of bacterial DNA joined to the phage genome ends. PM105, together with other B3-like phages, form a newly arising species.

Qazi, S., Miettinen, H. M., Wilkinson, R. A., McCoy, K., Douglas, T., and Wiedenheft, B. Programmed Self-Assembly of an Active P22-Cas9 Nanocarrier System. Mol.Pharm. 3-7-2016; 13(3):1191-1196.

ABSTRACT: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) RNA-guided endonucleases are powerful new tools for targeted genome engineering. These

nucleases provide an efficient and precise method for manipulating eukaryotic genomes; however, delivery of these reagents to specific cell-types remains challenging. Virus-like particles (VLPs) derived from bacteriophage P22, are robust supramolecular protein cage structures with demonstrated utility for cell type-specific delivery of encapsulated cargos. Here, we genetically fuse Cas9 to a truncated form of the P22 scaffold protein, which acts as a template for capsid assembly as well as a specific encapsulation signal for Cas9. Our results indicate that Cas9 and a single-guide RNA are packaged inside the P22 VLP, and activity assays indicate that this RNA-guided endonuclease is functional for sequence-specific cleavage of dsDNA targets. This work demonstrates the potential for developing P22 as a delivery vehicle for cell specific targeting of Cas9.

Qin, K., Cheng, B., Zhang, S., Wang, N., Fang, Y., Zhang, Q., Kuang, A., Lin, L., Ji, X., and Wei, Y. Complete genome sequence of the cold-active bacteriophage VMY22 from Bacillus cereus. Virus Genes. 3-3-2016;

ABSTRACT: The cold-active bacteriophage VMY22, belonging to the Podoviridae family, was isolated from Mingyong Glacier in China. Sequence analysis revealed that the genome is 18,609 bp long, with an overall G + C content of 36.4 mol%, and 25 open reading frames (ORFs). The sequence contains 46 potential promoters, 6 transcription terminators, and no tRNAs. Most of the ORFs show a high degree of similarity to B103 (NC_004165). Two noteworthy findings were made. First, one of the predicted proteins, ORF 19, shows high sequence similarity to the bacteriocin biosynthesis protein from Bacillus cereus. From this information, we propose that the VMY22 phage is at an intermediate phase in its coevolution with its bacterial host. Second, seven of the hypothetical proteins appear to be unique to this cold-active B. cereus phage (i.e., not found in temperate-active B. cereus phages). These observations add to our current knowledge about the coevolution of bacteriophages and their hosts. The identification of a novel group of gene and protein structures and functions will lead to a better understanding of cold-adaptation mechanisms in bacteria and their bacteriophages.

Rao, C., Guyard, C., Pelaz, C., Wasserscheid, J., Bondy-Denomy, J., Dewar, K., and Ensminger, A. W. Active and Adaptive Legionella CRISPR-Cas reveals a recurrent challenge to the pathogen. Cell Microbiol. 3-3-2016;

ABSTRACT: CRISPR-Cas systems are widely recognized as critical genome defense systems that protect microbes from external threats such as bacteriophage infection. Several isolates of the intracellular pathogen Legionella pneumophila possess multiple CRISPR-Cas systems (Type I-C, Type I-F, and Type II-B), yet the targets of these systems remain unknown. With the recent observation that at least one of these systems (II-B) plays a non-canonical role in supporting intracellular replication, the possibility remained that these systems are vestigial genome defense systems co-opted for other purposes. Our data indicate this is not the case. Using an established plasmid transformation assay, we demonstrate Type I-C, I-F, and II-B CRISPR-Cas provide protection against spacer targets. We observe efficient laboratory acquisition of new spacers under " priming" conditions, in which initially incomplete target elimination leads to the generation of new spacers and ultimate loss of the invasive DNA. Critically, we identify the first known target of L. pneumophila CRISPR-Cas: a 30 kilobase episome of unknown function whose interbacterial transfer is guarded against by CRISPR-Cas. We provide evidence that the element can subvert CRISPR-Cas by mutating its targeted sequences - but that primed

spacer acquisition may limit this mechanism of escape. Rather than generally impinging on bacterial fitness, this element drives a host-specialization event - with improved fitness in Acanthamoeba but a reduced ability to replicate in other hosts and conditions. These observations add to a growing body of evidence that host-range restriction can serve as an existential threat to L. pneumophila in the wild.

Rhaiem, R. B. and Houimel, M. Targeting Leishmania major parasite with peptides derived from a combinatorial phage display library. Acta Trop. 3-17-2016; 159:11-19.

ABSTRACT: Cutaneous leishmaniasis (CL) is a global problem caused by intracellular protozoan pathogens of the genus Leishmania for which there are no suitable vaccine or chemotherapy options. Thus, de novo identification of small molecules binding to the Leishmania parasites by direct screening is a promising and appropriate alternative strategy for the development of new drugs. In this study, we used a random linear hexapeptide library fused to the gene III protein of M13 filamentous bacteriophage to select binding peptides to metacyclic promastigotes from a highly virulent strain of Leishmania major (Zymodeme MON-25; MHOM/TN/94/GLC94). After four rounds of stringent selection and amplification, polyclonal and monoclonal phage-peptides directed against L. major metacyclic promastigotes were assessed by ELISA, and the optimal phage-peptides were grown individually and characterized for binding to L. major by monoclonal phage ELISA. The DNA of 42 phage-peptides clones was amplified by PCR, sequenced, and their amino acid sequences deduced. Six different peptide sequences were obtained with frequencies of occurrence ranging from 2.3% to 85.7%. The biological effect of the peptides was assessed in vitro on human monocytes infected with L. major metacyclic promastigotes, and in vivo on susceptible parasite-infected BALB/c mice. The development of cutaneous lesions in the right hind footpads of infected mice after 13 weeks post-infection showed a protection rate of 81.94% with the injected peptide P2. Moreover, Western blots revealed that the P2 peptide interacted with the major surface protease gp63, a protein of 63kDa molecular weight. Moreover, bioinformatics were used to predict the interaction between peptides and the major surface molecule of the L. major. The molecular docking showed that the P2 peptide has the minimum interaction energy and maximum shape complimentarity with the L. major gp63 active site. Our study demonstrated that the P2 peptide occurs at high frequency during the screening procedure, best inhibits L. major growth kinetics in vitro, and reduces cutaneous lesions in BALB/c mice, thus showing great promise in the development of new therapeutic molecules.

Roces, C., Campelo, A. B., Escobedo, S., Wegmann, U., Garcia, P., Rodriguez, A., and Martinez, B. Reduced Binding of the Endolysin LysTP712 to Lactococcus lactis DeltaftsH Contributes to Phage Resistance. Front Microbiol. 2016; 7:138

ABSTRACT: Absence of the membrane protease FtsH in Lactococcus lactis hinders release of the bacteriophage TP712. In this work we have analyzed the mechanism responsible for the non-lytic phenotype of L. lactis DeltaftsH after phage infection. The lytic cassette of TP712 contains a putative antiholin-pinholin system and a modular endolysin (LysTP712). Inducible expression of the holin gene demonstrated the presence of a dual start motif which is functional in both wildtype and L. lactis DeltaftsH cells. Moreover, simulating holin activity with ionophores accelerated lysis of wildtype cells but not L. lactis DeltaftsH cells, suggesting inhibition of the endolysin rather than a role of FtsH in holin activation. However, zymograms revealed the synthesis of an active endolysin in

both wildtype and L. lactis DeltaftsH TP712 lysogens. A reporter protein was generated by fusing the cell wall binding domain of LysTP712 to the fluorescent mCherry protein. Binding of this reporter protein took place at the septa of both wildtype and L. lactis DeltaftsH cells as shown by fluorescence microscopy. Nonetheless, fluorescence spectroscopy demonstrated that mutant cells bound 40% less protein. In conclusion, the non-lytic phenotype of L. lactis DeltaftsH is not due to direct action of the FtsH protease on the phage lytic proteins but rather to a putative function of FtsH in modulating the architecture of the L. lactis cell envelope that results in a lower affinity of the phage endolysin to its substrate.

Rodriguez-Rubio, L., Gutierrez, D., Donovan, D. M., Martinez, B., Rodriguez, A., and Garcia, P. Phage lytic proteins: biotechnological applications beyond clinical antimicrobials. Crit Rev.Biotechnol. 2016; 36(3):542-552.

ABSTRACT: Most bacteriophages encode two types of cell wall lytic proteins: endolysins (lysins) and virion-associated peptidoglycan hydrolases. Both enzymes have the ability to degrade the peptidoglycan of Gram-positive bacteria resulting in cell lysis when they are applied externally. Bacteriophage lytic proteins have a demonstrated potential in treating animal models of infectious diseases. There has also been an increase in the study of these lytic proteins for their application in areas such as food safety, pathogen detection/diagnosis, surfaces disinfection, vaccine development and nanotechnology. This review summarizes the more recent developments, outlines the full potential of these proteins to develop new biotechnological tools and discusses the feasibility of these proteins to develop new biotechnological tools and discusses the feasibility of these proposals.

Rolfsson, O., Middleton, S., Manfield, I. W., White, S. J., Fan, B., Vaughan, R., Ranson, N. A., Dykeman, E., Twarock, R., Ford, J., Cheng, Kao C., and Stockley, P. G. Direct Evidence for Packaging Signal-Mediated Assembly of Bacteriophage MS2. J.Mol.Biol. 1-29-2016; 428(2 Pt B):431-448.

ABSTRACT: Using cross-linking coupled to matrix-assisted laser desorption/ionization mass spectrometry and CLIP-Seq sequencing, we determined the peptide and oligonucleotide sequences at the interfaces between the capsid proteins and the genomic RNA of bacteriophage MS2. The results suggest that the same coat protein (CP)-RNA and maturation protein (MP)-RNA interfaces are used in every viral particle. The portions of the viral RNA in contact with CP subunits span the genome, consistent with a large number of discrete and similar contacts within each particle. Many of these sites match previous predictions of the locations of multiple, dispersed and degenerate RNA sites with cognate CP affinity termed packaging signals (PSs). Chemical RNA footprinting was used to compare the secondary structures of protein-free genomic fragments and the RNA in the virion. Some PSs are partially present in protein-free RNA but others would need to refold from their dominant solution conformations to form the contacts identified in the virion. The RNA-binding peptides within the MP map to two sections of the N-terminal half of the protein. Comparison of MP sequences from related phages suggests a similar arrangement of RNA-binding sites, although these N-terminal regions have only limited sequence conservation. In contrast, the sequences of the C-termini are highly conserved, consistent with them encompassing pilin-binding domains required for initial contact with host cells. These results provide independent and unambiguous support for the assembly of MS2 virions via a PS-mediated mechanism involving a series of induced-fit viral protein interactions with RNA.

Romanienko, P. J., Giacalone, J., Ingenito, J., Wang, Y., Isaka, M., Johnson, T., You, Y., and Mark, W. H. A Vector with a Single Promoter for In Vitro Transcription and Mammalian Cell Expression of CRISPR gRNAs. PLoS.One. 2016; 11(2):e0148362

ABSTRACT: The genomes of more than 50 organisms have now been manipulated due to rapid advancement of gene editing technology. One way to perform gene editing in the mouse using the CRISPR/CAS system, guide RNA (gRNA) and CAS9 mRNA transcribed in vitro are microinjected into fertilized eggs that are then allowed to develop to term. As a rule, gRNAs are tested first in tissue culture cells and the one with the highest locus-specific cleavage activity is chosen for microinjection. For cell transfections, gRNAs are typically expressed using the human U6 promoter (hU6). However, gRNAs for microinjection into zygotes are obtained by in vitro transcription from a T7 bacteriophage promoter in a separate plasmid vector. Here, we describe the design and construction of a combined U6T7 hybrid promoter from which the same gRNA sequence can be expressed. An expression vector containing such a hybrid promoter can now be used to generate gRNA for testing in mammalian cells as well as for microinjection purposes. The gRNAs expressed and transcribed from this vector are found to be functional in cells as well as in mice.

Rombouts, S., Volckaert, A., Venneman, S., Declercq, B., Vandenheuvel, D., Allonsius, C. N., Van, Malderghem C., Jang, H. B., Briers, Y., Noben, J. P., Klumpp, J., Van, Vaerenbergh J., Maes, M., and Lavigne, R. Characterization of Novel Bacteriophages for Biocontrol of Bacterial Blight in Leek Caused by Pseudomonas syringae pv. porri. Front Microbiol. 2016; 7:279

ABSTRACT: Pseudomonas syringae pv. porri, the causative agent of bacterial blight in leek (Allium porrum), is increasingly frequent causing problems in leek cultivation. Because of the current lack of control measures, novel bacteriophages were isolated to control this pathogen using phage therapy. Five novel phages were isolated from infected fields in Flanders (vB_PsyM_KIL1, vB_PsyM_KIL2, vB_PsyM_KIL3, vB_PsyM_KIL4, and vB_PsyM_KIL5), and were complemented with one selected host range mutant phage (vB_PsyM_KIL3b). Genome analysis of the phages revealed genome sizes between 90 and 94 kb and an average GC-content of 44.8%. Phylogenomic networking classified them into a novel clade, named the "KIL-like viruses," related to the Felixounalikevirus genus, together with phage phiPsa374 from P. syringae pv. actinidiae. In vitro characterization demonstrated the stability and lytic potential of these phages. Host range analysis confirmed heterogeneity within P. syringae pv. porri, leading to the development of a phage cocktail with a range that covers the entire set of 41 strains tested. Specific bio-assays demonstrated the in planta efficacy of phages vB_PsyM_KIL1, vB_PsyM_KIL2, vB_PsyM_KIL3, and vB_PsyM_KIL3b. In addition, two parallel field trial experiments on three locations using a phage cocktail of the six phages showed variable results. In one trial, symptom development was attenuated. These data suggest some potential for phage therapy in controlling bacterial blight of leek, pending optimization of formulation and application methods.

Ruff, Z., Nathan, S. H., Unwin, R. R., Zupkauskas, M., Joshi, D., Salmond, G. P., Grey, C. P., and Eiser, E. Designing disordered materials using DNA-coated colloids of bacteriophage fd and gold. Faraday Discuss. 2-11-2016;

ABSTRACT: DNA has emerged as an exciting binding agent for programmable colloidal self-assembly. Its popularity derives from its unique properties: it provides highly specific short-ranged interactions and at the same time it acts as a steric stabilizer against non-specific van der Waals and Coulomb interactions. Because complementary DNA strands are linked only via hydrogen bonds, DNA-mediated binding is thermally reversible: it provides an effective attraction that can be switched off by raising the temperature only by a few degrees. In this article we introduce a new binary system made of DNA-functionalized filamentous fd viruses of approximately 880 nm length with an aspect ratio of approximately 100, and 50 nm gold nanoparticles (gold NPs) coated with the complementary DNA strands. When quenching mixtures below the melt temperature Tm, at which the attraction is switched on, we observe aggregation. Conversely, above Tm the system melts into a homogenous particulate 'gas'. We present the aggregation behavior of three different gold NP to virus ratios and compare them to a gel made solely of gold NPs. In particular, we have investigated the aggregate structures as a function of cooling rate and determine how they evolve as function of time for given quench depths, employing fluorescence microscopy. Structural information was extracted in the form of an effective structure factor and chord length distributions. Rapid cooling rates lead to open aggregates, while slower controlled cooling rates closer to equilibrium DNA hybridization lead to more fine-stranded gels. Despite the different structures we find that for both cooling rates the quench into the two-phase region leads to initial spinodal decomposition, which becomes arrested. Surprisingly, although the fine-stranded gel is disordered, the overall structure and the corresponding length scale distributions in the system are remarkably reproducible. Such highly porous systems can be developed into new functional materials.

Russell, D. A. and Hatfull, G. F. Complete Genome Sequence of Arthrobacter sp. ATCC 21022, a Host for Bacteriophage Discovery. Genome Announc. 2016; 4(2)

ABSTRACT: We report the complete genome sequence ofArthrobactersp. ATCC 21022, a strain maintained by ATCC and a commonly used host for bacteriophage isolation and genomic analysis. The strain is prophage-free and CRISPR-free but codes for two predicted restriction-modification systems.

Saboo, S., Tumban, E., Peabody, J., Wafula, D., Peabody, D. S., Chackerian, B., and Muttil, P. An optimized formulation of a thermostable spray-dried virus-like particles vaccine against human papillomavirus. Mol.Pharm. 3-28-2016;

ABSTRACT: Existing vaccines against human papillomavirus (HPV) require continuous cold-chain storage. Previously, we developed a bacteriophage virus-like particle (VLP) based vaccine for Human Papillomavirus (HPV) infection, which elicits broadly neutralizing antibodies against diverse HPV types. Here, we formulated these VLPs into a thermostable dry powder using a multi-component excipient system and by optimizing the spray drying parameters using a half-factorial design approach. Dry powder VLPs were stable after spray drying and after long-term storage at elevated temperatures. Immunization of mice with a single dose of reconstituted dry powder VLPs that were stored at 37 degrees C for more than a year elicited high anti-L2 IgG antibody titers. Spray dried thermostable, broadly protective L2 bacteriophage VLPs vaccine could be accessible to remote regions of the world (where ~84% of cervical cancer patients reside) by eliminating the cold-chain requirement during transportation and storage.

Sagona, A. P., Grigonyte, A. M., MacDonald, P. R., and Jaramillo, A. Genetically modified bacteriophages. Integr.Biol.(Camb.). 2-24-2016;

ABSTRACT: Phages or bacteriophages, viruses that infect and replicate inside bacteria, are the most abundant microorganisms on earth. The realization that antibiotic resistance poses a substantial risk to the world's health and global economy is revitalizing phage therapy as a potential solution. The increasing ease by which phage genomes can be modified, owing to the influx of new technologies, has led to an expansion of their natural capabilities, and a reduced dependence on phage isolation from environmental sources. This review will discuss the way synthetic biology has accelerated the construction of genetically modified phages and will describe the wide range of their applications. It will further provide insight into the societal and economic benefits that derive from the use of recombinant phages in various sectors, from health to biodetection, biocontrol and the food industry.

Sarker, S. A., Sultana, S., Reuteler, G., Moine, D., Descombes, P., Charton, F., Bourdin, G., McCallin, S., Ngom-Bru, C., Neville, T., Akter, M., Huq, S., Qadri, F., Talukdar, K., Kassam, M., Delley, M., Loiseau, C., Deng, Y., El, Aidy S., Berger, B., and Brussow, H. Oral Phage Therapy of Acute Bacterial Diarrhea With Two Coliphage Preparations: A Randomized Trial in Children From Bangladesh. EBioMedicine. 2016; 4:124-137.

ABSTRACT: BACKGROUND: Antibiotic resistance is rising in important bacterial pathogens. Phage therapy (PT), the use of bacterial viruses infecting the pathogen in a species-specific way, is a potential alternative. METHOD: T4-like coliphages or a commercial Russian coliphage product or placebo was orally given over 4 days to Bangladeshi children hospitalized with acute bacterial diarrhea. Safety of oral phage was assessed clinically and by functional tests; coliphage and Escherichia coli titers and enteropathogens were determined in stool and quantitative diarrhea parameters (stool output, stool frequency) were measured. Stool microbiota was studied by 16S rRNA gene sequencing; the genomes of four fecal Streptococcus isolates were sequenced. FINDINGS: No adverse events attributable to oral phage application were observed (primary safety outcome). Fecal coliphage was increased in treated over control children, but the titers did not show substantial intestinal phage replication (secondary microbiology outcome). 60% of the children suffered from a microbiologically proven E. coli diarrhea; the most frequent diagnosis was ETEC infections. Bacterial co-pathogens were also detected. Half of the patients contained phage-susceptible E. coli colonies in the stool. E. coli represented less than 5% of fecal bacteria. Stool ETEC titers showed only a short-lived peak and were otherwise close to the replication threshold determined for T4 phage in vitro. An interim analysis after the enrollment of 120 patients showed no amelioration in quantitative diarrhea parameter by PT over standard care (tertiary clinical outcome). Stool microbiota was characterized by an overgrowth with Streptococcus belonging to the Streptococcus gallolyticus and Streptococcus salivarius species groups, their abundance correlated with quantitative diarrhea outcome, but genome sequencing did not identify virulence genes. INTERPRETATION: Oral coliphages showed a safe gut transit in children, but failed to achieve intestinal amplification and to improve diarrhea outcome, possibly due to insufficient phage coverage and too low E. coli pathogen titers requiring higher oral phage doses. More knowledge is needed on in vivo phage-bacterium interaction and the role of E. coli in childhood diarrhea for successful PT. FUNDING: The study was supported by a grant from Nestle Nutrition and

Nestle Health Science. The trial was registered with Identifier NCT00937274 at ClinicalTrials.gov.

Schmelcher, M. and Loessner, M. J. Bacteriophage endolysins: applications for food safety. Curr.Opin.Biotechnol. 2016; 37:76-87.

ABSTRACT: Bacteriophage endolysins (peptidoglycan hydrolases) have emerged as a new class of antimicrobial agents useful for controlling bacterial infection or other unwanted contaminations in various fields, particularly in the light of the worldwide increasing frequency of drug-resistant pathogens. This review summarizes and discusses recent developments regarding the use of endolysins for food safety. Besides the use of native and engineered endolysins for controlling bacterial contamination at different points within the food production chain, this also includes the application of high-affinity endolysin-derived cell wall binding domains for rapid detection of pathogenic bacteria. Novel approaches to extend the lytic action of endolysins towards Gram-negative cells will also be highlighted.

Sellers, D. L., Bergen, J. M., Johnson, R. N., Back, H., Ravits, J. M., Horner, P. J., and Pun, S. H. Targeted axonal import (TAxI) peptide delivers functional proteins into spinal cord motor neurons after peripheral administration. Proc.Natl.Acad.Sci.U.S.A. 3-1-2016; 113(9):2514-2519.

BSTRACT: A significant unmet need in treating neurodegenerative disease is effective methods for delivery of biologic drugs, such as peptides, proteins, or nucleic acids into the central nervous system (CNS). To date, there are no operative technologies for the delivery of macromolecular drugs to the CNS via peripheral administration routes. Using an in vivo phage-display screen, we identify a peptide, targeted axonal import (TAxI), that enriched recombinant bacteriophage accumulation and delivered protein cargo into spinal cord motor neurons after intramuscular injection. In animals with transected peripheral nerve roots, TAxI delivery into motor neurons after peripheral administration was inhibited, suggesting a retrograde axonal transport mechanism for delivery into the CNS. Notably, TAxI-Cre recombinase fusion proteins induced selective recombination and tdTomato-reporter expression in motor neurons after intramuscular injections. Furthermore, TAxI peptide was shown to label motor neurons in the human tissue. The demonstration of a nonviral-mediated delivery of functional proteins into the spinal cord establishes the clinical potential of this technology for minimally invasive administration of CNS-targeted therapeutics.

Sepulveda, L. A., Xu, H., Zhang, J., Wang, M., and Golding, I. Measurement of gene regulation in individual cells reveals rapid switching between promoter states. Science. 3-11-2016; 351(6278):1218-1222.

ABSTRACT: In vivo mapping of transcription-factor binding to the transcriptional output of the regulated gene is hindered by probabilistic promoter occupancy, the presence of multiple gene copies, and cell-to-cell variability. We demonstrate how to overcome these obstacles in the lysogeny maintenance promoter of bacteriophage lambda, P(RM). We simultaneously measured the concentration of the lambda repressor CI and the number of messenger RNAs (mRNAs) from P(RM) in individual Escherichia coli cells, and used a theoretical model to identify the stochastic activity corresponding to

different CI binding configurations. We found that switching between promoter configurations is faster than mRNA lifetime and that individual gene copies within the same cell act independently. The simultaneous quantification of transcription factor and promoter activity, followed by stochastic theoretical analysis, provides a tool that can be applied to other genetic circuits.

Sharma, A., Haque, F., Pi, F., Shlyakhtenko, L. S., Evers, B. M., and Guo, P. Controllable self-assembly of RNA dendrimers. Nanomedicine. 2016; 12(3):835-844.

ABSTRACT: We report programmable self-assembly of branched, 3D globular, monodisperse and nanoscale sized dendrimers using RNA as building blocks. The central core and repeating units of the RNA dendrimer are derivatives of the ultrastable three-way junction (3WJ) motif from the bacteriophage phi29 motor pRNA. RNA dendrimers were constructed by step-wise self-assembly of modular 3WJ building blocks initiating with a single 3WJ core (Generation-0) with overhanging sticky end and proceeding in a radial manner in layers up to Generation-4. The final constructs were generated under control without any structural defects in high yield and purity, as demonstrated by gel electrophoresis and AFM imaging. Upon incorporation of folate on the peripheral branches of the RNA dendrimers, the resulting constructs showed high binding and internalization into cancer cells. RNA dendrimers are envisioned to have a major impact in targeting, disease therapy, molecular diagnostics and bioelectronics in the near future. FROM THE CLINICAL EDITOR: Dendrimers are gaining importance as a carrier platform for diagnosis and therapeutics. The authors here reported building of their dendrimer molecules using RNA as building blocks. The addition of folate also allowed recognition and subsequent binding to tumor cells. This new construct may prove to be useful in many clinical settings.

Shen, Y., Barros, M., Vennemann, T., Gallagher, D. T., Yin, Y., Linden, S. B., Heselpoth, R. D., Spencer, D. J., Donovan, D. M., Moult, J., Fischetti, V. A., Heinrich, F., Losche, M., and Nelson, D. C. A bacteriophage endolysin that eliminates intracellular streptococci. Elife. 3-15-2016; 5

ABSTRACT: PlyC, a bacteriophage-encoded endolysin, lyses Streptococcus pyogenes (Spy) on contact. Here, we demonstrate that PlyC is a potent agent for controlling intracellular Spy that often underlies refractory infections. We show that the PlyC holoenzyme, mediated by its PlyCB subunit, crosses epithelial cell membranes and clears intracellular Spy in a dose-dependent manner. Quantitative studies using model membranes establish that PlyCB interacts strongly with phosphatidylserine (PS) whereas its interaction with other lipids is weak, suggesting specificity for PS as its cellular receptor. Neutron reflection further substantiates that PlyC penetrates bilayers above a PS threshold concentration. Crystallography and docking studies identify key residues that mediate PlyCB-PS interactions, which are validated by site-directed mutagenesis. This is the first report that a native endolysin can translocate epithelial membranes, thus substantiating the potential of PlyC as an antimicrobial for Spy in the extra- and intracellular milieu and as a scaffold for engineering other functionalities.

Silva, J. B., Storms, Z., and Sauvageau, D. Host receptors for bacteriophage adsorption. FEMS Microbiol.Lett. 1-10-2016;

ABSTRACT: The adsorption of bacteriophages (phages) onto host cells is, in all but a few rare cases, a sine qua non condition for the onset of the infection process. Understanding the mechanisms involved and the factors affecting it is, thus, crucial for the investigation of host-phage interactions. This review provides a survey of the phage host receptors involved in recognition and adsorption and their interactions during attachment. Comprehension of the whole infection process, starting with the adsorption step, can enable and accelerate our understanding of phage ecology and the development of phage-based technologies. To assist in this effort we have established an open-access resource - the Phage Receptor Database (PhReD) - to serve as a repository for information on known and newly identified phage receptors.

Solis-Sanchez, A., Hernandez-Chinas, U., Navarro-Ocana, A., De la, Mora J., Xicohtencatl-Cortes, J., and Eslava-Campos, C. Genetic characterization of OVC8 lytic phage for Vibrio cholerae O1. Virol.J. 2016; 13(1):47

ABSTRACT: BACKGROUND: Epidemics and pandemics of cholera, a diarrheal disease, are attributed to Vibrio cholerae serogroups O1 and O139. In recent years, specific lytic phages of V. cholerae have been proposed to be important factors in the cyclic occurrence of cholera in endemic areas. However, the role and potential participation of lytic phages during long interepidemic periods of cholera in non-endemic regions have not yet been described. The purpose of this study was to isolate and characterize specific lytic phages of V. cholerae O1 strains. METHODS: Sixteen phages were isolated from wastewater samples collected at the Endho Dam in Hidalgo State, Mexico, concentrated with PEG/NaCl, and purified by density gradient. The lytic activity of the purified phages was tested using different V. cholerae O1 and O139 strains. Phage morphology was visualized by transmission electron microscopy (TEM), and phage genome sequencing was performed using the Genome Analyzer IIx System. Genome assembly and bioinformatics analysis were performed using a set of high-throughput programs. Phage structural proteins were analyzed by mass spectrometry. RESULTS: Sixteen phages with lytic and lysogenic activity were isolated; only phage OVC8 showed specific lytic activity against V. cholerae O1 strains. TEM images of OVC8 revealed a phage with a short tail and an isometric head. The OVC8 genome comprises linear double-stranded DNA of 39,422 bp with 50.8 % G + C. Of the 48 annotated ORFs, 16 exhibit homology with sequences of known function and several conserved domains. Bioinformatics analysis showed multiple conserved domains, including an Ig domain, suggesting that OVC8 might adhere to different mucus substrates such as the human intestinal epithelium. The results suggest that OVC8 genome utilize the "single-stranded cohesive ends" packaging strategy of the lambda-like group. The two structural proteins sequenced and analyzed are proteins of known function. CONCLUSIONS: OVC8 is a lytic phage with specific activity against V. cholerae O1 strains and is grouped as a member of the VP2-like phage subfamily. The encoding of an Ig domain by OVC8 makes this phage a good candidate for use in phage therapy and an alternative tool for monitoring V. cholerae populations.

Song, J., Xia, F., Jiang, H., Li, X., Hu, L., Gong, P., Lei, L., Feng, X., Sun, C., Gu, J., and Han, W. Identi fi cation and characterization of HolGH15, the holin of Staphylococcus aureus bacteriophage GH15. J.Gen.Virol. 2-12-2016;

ABSTRACT: Holins are phage-encoded hydrophobic membrane proteins that spontaneously and non-specifically accumulate and form lesions in the cytoplasmic

membrane. The ORF72 gene (also designated HolGH15) derived from the genome of the Staphylococcus aureus phage GH15 was predicted to encode a membrane protein. An analysis indicated that the protein encoded by HolGH15 potentially consisted of two hydrophobic transmembrane helices. This protein exhibited the structural characteristics of class II holins and belonged to the phage_holin_1 superfamily. Expression of HolGH15 in Escherichia coli BL21 cells resulted in growth retardation of the host cells, which was triggered prematurely by the addition of 2,4-dinitrophenol (DNP). The expression of HolGH15 caused morphological alterations in engineered E. coli cells, including the loss of the cell wall and cytoplasmic membrane integrity and the release of intracellular components, which were visualized by transmission electron microscopy (TEM). HolGH15 exerted efficient antibacterial activity at 37 degrees C and pH 5.2. Mutation analysis indicated that the two transmembrane domains of HolGH15 were indispensable for the activity of the full-length protein. HolGH15 showed a broad antibacterial range; it not only inhibited S. aureus but also demonstrated antibacterial activity against other species, including Listeria monocytogenes, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia and E. coli. At the minimal inhibitory concentration (MIC), HolGH15 evoked the release of cellular contents and resulted in the shrinkage and death of S. aureus and L. monocytogenes cells. To the best of our knowledge, this study is the first report of an S. aureus phage holin that exerts antibacterial activity against heterogeneous pathogens.

Spears, P. A., Havell, E. A., Hamrick, T. S., Goforth, J. B., Levine, A. L., Thomas, Abraham S., Heiss, C., Azadi, P., and Orndorff, P. E. Listeria monocytogenes Wall Teichoic Acid Decoration in Virulence and Cell-to-Cell Spread. Mol.Microbiol. 2-12-2016;

ABSTRACT: Wall teichoic acid (WTA) comprises a class of glycopolymers covalently attached to the peptidoglycan of gram positive bacteria. In Listeria monocytogenes, mutations that prevent addition of certain WTA decorating sugars are attenuating. However, the steps required for decoration and the pathogenic process interrupted are not well described. We systematically examined the requirement for WTA galactosylation in a mouse oral-virulent strain by first creating mutations in four genes whose products conferred resistance to a WTA-binding bacteriophage. WTA biochemical and structural studies indicated that galactosylated WTA was directly required for bacteriophage adsorption and that mutant WTA lacked appreciable galactose in all except one mutant-which retained a level ca. 7% of the parent. All mutants were profoundly attenuated in orally infected mice and were impaired in cell-to-cell spread in vitro. Confocal microscopy of cytosolic mutants revealed that all expressed ActA on their cell surface and formed actin tails with a frequency similar to the parent. However, the mutant tails were significantly shorter-suggesting a defect in actin based motility. Roles for the gene products in WTA galactosylation are proposed. Identification and interruption of WTA decoration pathways may provide a general strategy to discover non-antibiotic therapeutics for gram positive infections. This article is protected by copyright. All rights reserved.

Sukumaran, A. T., Nannapaneni, R., Kiess, A., and Sharma, C. S. Reduction of Salmonella on chicken breast fillets stored under aerobic or modified atmosphere packaging by the application of lytic bacteriophage preparation SalmoFreshTM. Poult.Sci. 2016; 95(3):668-675.

ABSTRACT: The present study evaluated the efficacy of recently approved Salmonella lytic bacteriophage preparation (SalmoFresh) in reducing Salmonella on chicken breast fillets, as a surface and dip application. The effectiveness of phage in combination with modified atmosphere packaging (MAP) and the ability of phage preparation in reducing Salmonella on chicken breast fillets at room temperature was also evaluated. Chicken breast fillets inoculated with a cocktail of Salmonella Typhimurium, S. Heidelberg, and S. Enteritidis were treated with bacteriophage (10(9) PFU/mL) as either a dip or surface treatment. The dip-treated samples were stored at 4 degrees C aerobically and the surface-treated samples were stored under aerobic and MAP conditions (95% CO2/5% O2) at 4 degrees C for 7 d. Immersion of Salmonella-inoculated chicken breast fillets in bacteriophage solution reduced Salmonella ($P < 0.05$) by 0.7 and 0.9 log CFU/g on d 0 and d 1 of storage, respectively. Surface treatment with phage significantly ($P < 0.05$) reduced Salmonella by 0.8, 0.8, and 1 log CFU/g on d 0, 1, and 7 of storage, respectively, under aerobic conditions. Higher reductions in Salmonella counts were achieved on chicken breast fillets when the samples were surface treated with phage and stored under MAP conditions. The Salmonella counts were reduced by 1.2, 1.1, and 1.2 log CFU/g on d 0, 1, and 7 of storage, respectively. Bacteriophage surface application on chicken breast fillets stored at room temperature reduced the Salmonella counts by 0.8, 0.9, and 0.4 log CFU/g after 0, 4, and 8 h, respectively, compared to the untreated positive control. These findings indicate that lytic phage preparation was effective in reducing Salmonella on chicken breast fillets stored under aerobic and modified atmosphere conditions.

Sun, P., Tyree, C., and Huang, C. H. Inactivation of Escherichia coli, Bacteriophage MS2, and Bacillus Spores under UV/HO and UV/Peroxydisulfate Advanced Disinfection Conditions. Environ.Sci.Technol. 4-1-2016;

ABSTRACT: Ultraviolet light (UV) combined with peroxy chemicals, such as H2O2 and peroxydisulfate (PDS), have been considered potentially highly effective disinfection processes. This study investigated the inactivation of Escherichia coli, bacteriophage MS2, and Bacillus subtilis spores as surrogates for pathogens under UV/H2O2 and UV/PDS conditions, with the aim to provide further understanding of UV-based advanced disinfection processes (ADPs). Results showed that one additional log of inactivation of E. coli was achieved with 0.3 mM H2O2 or PDS at 5.2 x 10-5 Einstein.L-1 photo fluence (at 254 nm) compared with UV irradiation alone. Addition of H2O2 and PDS greatly enhanced the inactivation rate of MS2 by around 15 folds and 3 folds, respectively, whereas the inactivation of B. subtilis spores was slightly enhanced. Reactive species responsible for the inactivation were identified to be *OH, SO4.-, and CO3.- based on manipulation of solution conditions. The CT value of each reactive species was calculated with respect to each microbial surrogate, which showed that the disinfection efficacy ranked as *OH > SO4.- > CO3.- >> O2.-/HO2.. A comprehensive dynamic model was developed and successfully predicted the inactivation of the microbial surrogates in surface water and wastewater matrices. The concepts of UV-efficiency and EE/O were employed to provide a cost-effective evaluation for UV-based ADPs. Overall, the present study suggests that it will be beneficial to upgrade UV disinfection to UV/H2O2 ADP for the inactivation of viral pathogens.

Temmam, S., Davoust, B., Chaber, A. L., Lignereux, Y., Michelle, C., Monteil-Bouchard, S., Raoult, D., and Desnues, C. Screening for Viral Pathogens in African Simian Bushmeat Seized at A French Airport. Transbound.Emerg.Dis. 2-14-2016;

ABSTRACT: Illegal bushmeat traffic is an important threat to biodiversity conservation of several endangered species and may contribute to the emergence and spread of infectious diseases in humans. The hunting, manipulation and consumption of wildlife-based products, especially those of primate origin, may be a threat to human health; however, few studies have investigated the role of bushmeat trade and consumption as a potential source of human infections to date. In this study, we report the screening of viral pathogens in African simian game seized by French customs at Toulouse Blagnac Airport. Epifluorescence microscopy revealed the presence of virus-like particles in the samples, and further metagenomic sequencing of the DNA and RNA viromes confirmed the presence of sequences related to the Siphoviridae, Myoviridae and Podoviridae bacteriophage families; some of them infecting bacterial hosts that could be potentially pathogenic for humans. To increase the sensitivity of detection, twelve pan-generic PCRs targeting several viral zoonoses were performed, but no positive signal was detected. A large-scale inventory of bacteria, viruses and parasites is urgently needed to globally assess the risk for human health of the trade, manipulation and consumption of wildlife-related bushmeat.

Thandar, M., Lood, R., Winer, B. Y., Deutsch, D. R., Euler, C. W., and Fischetti, V. A. Novel engineered peptides of a phage lysin as effective antimicrobials against multidrug resistant Acinetobacter baumannii. Antimicrob.Agents Chemother. 2-8-2016;

ABSTRACT: Acinetobacter baumannii is a Gram-negative bacterial pathogen responsible for a range of nosocomial infections. The recent rise and spread of multidrug resistant A. baumannii clones has fueled a search for alternative therapies, including bacteriophage endolysins with potent antibacterial activities. A common feature of these lysins is the presence of a highly positively charged C-terminal domain with a likely role in promoting outer membrane penetration. In the current study, we show that the C-terminal amino acid 108-138 of phage lysin PlyF307, named P307, alone was sufficient to kill A. baumannii (>3-logs). Furthermore, P307 could be engineered for improved activity, the most active derivative being P307SQ-8C (>5-log kill). Both P307 and P307SQ-8C showed high in vitro activity against A. baumannii in biofilms. Moreover, P307SQ-8C exhibited MICs comparable to levofloxacin and ceftazidime and acted synergistically with polymyxin B. While the peptides were shown to kill by disrupting the bacterial cytoplasmic membrane, they did not lyse human red blood cells or B cells; however, serum was found to be inhibitory to lytic activity. In a murine model of A. baumannii skin infection, P307SQ-8C reduced the bacterial burden by approximately 2-logs in 2 h. This study demonstrates the prospect of using peptide derivatives from bacteriophage lysins to treat topical infections and remove biofilms caused by Gram-negative pathogens.

Thiaville, J. J., Kellner, S. M., Yuan, Y., Hutinet, G., Thiaville, P. C., Jumpathong, W., Mohapatra, S., Brochier-Armanet, C., Letarov, A. V., Hillebrand, R., Malik, C. K., Rizzo, C. J., Dedon, P. C., and de Crecy-Lagard, V. Novel genomic island modifies DNA with 7-deazaguanine derivatives. Proc.Natl.Acad.Sci.U.S.A. 2-29-2016;

ABSTRACT: The discovery of approximately 20-kb gene clusters containing a family of paralogs of tRNA guanosine transglycosylase genes, called tgtA5, alongside 7-cyano-7-deazaguanine (preQ0) synthesis and DNA metabolism genes, led to the hypothesis that 7-deazaguanine derivatives are inserted in DNA. This was established by detecting 2'-deoxy-preQ0 and 2'-deoxy-7-amido-7-deazaguanosine in enzymatic hydrolysates of

DNA extracted from the pathogenic, Gram-negative bacteria Salmonella enterica serovar Montevideo. These modifications were absent in the closely related S. enterica serovar Typhimurium LT2 and from a mutant of S. Montevideo, each lacking the gene cluster. This led us to rename the genes of the S. Montevideo cluster as dpdA-K for 7-deazapurine in DNA. Similar gene clusters were analyzed in approximately 150 phylogenetically diverse bacteria, and the modifications were detected in DNA from other organisms containing these clusters, including Kineococcus radiotolerans, Comamonas testosteroni, and Sphingopyxis alaskensis. Comparative genomic analysis shows that, in Enterobacteriaceae, the cluster is a genomic island integrated at the leuX locus, and the phylogenetic analysis of the TgtA5 family is consistent with widespread horizontal gene transfer. Comparison of transformation efficiencies of modified or unmodified plasmids into isogenic S. Montevideo strains containing or lacking the cluster strongly suggests a restriction-modification role for the cluster in Enterobacteriaceae. Another preQ0 derivative, 2'-deoxy-7-formamidino-7-deazaguanosine, was found in the Escherichia coli bacteriophage 9g, as predicted from the presence of homologs of genes involved in the synthesis of the archaeosine tRNA modification. These results illustrate a deep and unexpected evolutionary connection between DNA and tRNA metabolism.

Tomas, M. E., Cadnum, J. L., Mana, T. S., Jencson, A. L., Koganti, S., Alhmidi, H., Kundrapu, S., Sunkesula, V. C., and Donskey, C. J. Utility of a Novel Reflective Marker Visualized by Flash Photography for Assessment of Personnel Contamination During Removal of Personal Protective Equipment. Infect.Control Hosp.Epidemiol. 3-15-2016; 1-3.

ABSTRACT: In an experimental study, the frequency of contamination of healthcare personnel during removal of contaminated personal protective equipment (PPE) was similar for bacteriophage MS2 and a novel reflective marker visualized using flash photography. The reflective marker could be a useful tool to visualize and document personnel contamination during PPE removal. Infect. Control Hosp. Epidemiol. 2016;1-3.

Tsai, Y. C., Conlan, S., Deming, C., Segre, J. A., Kong, H. H., Korlach, J., and Oh, J. Resolving the Complexity of Human Skin Metagenomes Using Single-Molecule Sequencing. MBio. 2016; 7(1)

ABSTRACT: Deep metagenomic shotgun sequencing has emerged as a powerful tool to interrogate composition and function of complex microbial communities. Computational approaches to assemble genome fragments have been demonstrated to be an effective tool for de novo reconstruction of genomes from these communities. However, the resultant "genomes" are typically fragmented and incomplete due to the limited ability of short-read sequence data to assemble complex or low-coverage regions. Here, we use single-molecule, real-time (SMRT) sequencing to reconstruct a high-quality, closed genome of a previously uncharacterized Corynebacterium simulans and its companion bacteriophage from a skin metagenomic sample. Considerable improvement in assembly quality occurs in hybrid approaches incorporating short-read data, with even relatively small amounts of long-read data being sufficient to improve metagenome reconstruction. Using short-read data to evaluate strain variation of this C. simulans in its skin community at single-nucleotide resolution, we observed a dominant C. simulans strain with moderate allelic heterozygosity throughout the population. We demonstrate the utility of SMRT sequencing and hybrid approaches in metagenome quantitation, reconstruction, and annotation. IMPORTANCE: The species comprising a

microbial community are often difficult to deconvolute due to technical limitations inherent to most short-read sequencing technologies. Here, we leverage new advances in sequencing technology, single-molecule sequencing, to significantly improve reconstruction of a complex human skin microbial community. With this long-read technology, we were able to reconstruct and annotate a closed, high-quality genome of a previously uncharacterized skin species. We demonstrate that hybrid approaches with short-read technology are sufficiently powerful to reconstruct even single-nucleotide polymorphism level variation of species in this a community.

Utt, A., Quirin, T., Saul, S., Hellstrom, K., Ahola, T., and Merits, A. Versatile Trans-Replication Systems for Chikungunya Virus Allow Functional Analysis and Tagging of Every Replicase Protein. PLoS.One. 2016; 11(3):e0151616

ABSTRACT: Chikungunya virus (CHIKV; genus Alphavirus, family Togaviridae) has recently caused several major outbreaks affecting millions of people. There are no licensed vaccines or antivirals, and the knowledge of the molecular biology of CHIKV, crucial for development of efficient antiviral strategies, remains fragmentary. CHIKV has a 12 kb positive-strand RNA genome, which is translated to yield a nonstructural (ns) or replicase polyprotein. CHIKV structural proteins are expressed from a subgenomic RNA synthesized in infected cells. Here we have developed CHIKV trans-replication systems, where replicase expression and RNA replication are uncoupled. Bacteriophage T7 RNA polymerase or cellular RNA polymerase II were used for production of mRNAs for CHIKV ns polyprotein and template RNAs, which are recognized by CHIKV replicase and encode for reporter proteins. CHIKV replicase efficiently amplified such RNA templates and synthesized large amounts of subgenomic RNA in several cell lines. This system was used to create tagged versions of ns proteins including nsP1 fused with enhanced green fluorescent protein and nsP4 with an immunological tag. Analysis of these constructs and a matching set of replicon vectors revealed that the replicases containing tagged ns proteins were functional and maintained their subcellular localizations. When cells were co-transfected with constructs expressing template RNA and wild type or tagged versions of CHIKV replicases, formation of characteristic replicase complexes (spherules) was observed. Analysis of mutations associated with noncytotoxic phenotype in CHIKV replicons showed that a low level of RNA replication is not a pre-requisite for reduced cytotoxicity. The CHIKV trans-replicase does not suffer from genetic instability and represents an efficient, sensitive and reliable tool for studies of different aspects of CHIKV RNA replication process.

Van Tassell, M. L., Angela, Daum M., Kim, J. S., and Miller, M. J. Creative lysins: Listeria and the engineering of antimicrobial enzymes. Curr.Opin.Biotechnol. 2016; 37:88-96.

ABSTRACT: Cell wall lytic enzymes have been of increasing interest as antimicrobials for targeting Gram-positive spoilage and pathogenic bacteria, largely due to the development of strains resistant to antibiotics and bacteriophage therapy. Such lysins show considerable promise against Listeria monocytogenes, a primary concern in food-processing environments, but there is room for improvement via protein engineering. Advances in antilisterial applications could benefit from recent developments in lysin biotechnology that have largely targeted other organisms. Herein we present various considerations for the future development of lysins, including environmental factors, cell physiology concerns, and dynamics of protein architecture. Our goal is to review key developments in lysin biotechnology to provide a contextual framework for the current

models of lysin-cell interactions and highlight key considerations for the characterization and design of novel lytic enzymes.

van Zyl, L. J., Taylor, M. P., and Trindade, M. Engineering resistance to phage GVE3 in Geobacillus thermoglucosidasius. Appl.Microbiol.Biotechnol. 2016; 100(4):1833-1841.

ABSTRACT: Geobacillus thermoglucosidasius is a promising platform organism for the production of biofuels and other metabolites of interest. G. thermoglucosidasius fermentations could be subject to bacteriophage-related failure and financial loss. We develop two strains resistant to a recently described G. thermoglucosidasius-infecting phage GVE3. The phage-encoded immunity gene, imm, was overexpressed in the host leading to phage resistance. A phage-resistant mutant was isolated following expression of a putative anti-repressor-like protein and phage challenge. A point mutation was identified in the polysaccharide pyruvyl transferase, csaB. A double crossover knockout mutation of csaB confirmed its role in the phage resistance phenotype. These resistance mechanisms appear to prevent phage DNA injection and/or lysogenic conversion rather than just reducing efficiency of plating, as no phage DNA could be detected in resistant bacteria challenged with GVE3 and no plaques observed even at high phage titers. Not only do the strains developed here shed light on the biological relationship between the GVE3 phage and its host, they could be employed by those looking to make use of this organism for metabolite production, with reduced occurrence of GVE3-related failure.

Varga, M., Pantu, cek R., Ru, zickova, V, and Doskar, J. Molecular characterization of a new efficiently transducing bacteriophage identified in meticillin-resistant Staphylococcus aureus. J.Gen.Virol. 2016; 97(1):258-268.

ABSTRACT: In Staphylococcus aureus, generalized transduction mediated by temperate bacteriophages represents a highly efficient way of transferring antibiotic resistance genes between strains. In the present study, we identified and characterized in detail a new efficiently transducing bacteriophage of the family Siphoviridae, designated varphiJB, which resides as a prophage in the meticillin-resistant S. aureus (MRSA) strain Jevons B. Whole-genome sequencing followed by detailed in silico analysis uncovered a linear dsDNA genome consisting of 43 012 bp and comprising 70 ORFs, of which approximately 40 encoded proteins with unknown function. A global genome alignment of varphiJB and other efficiently transducing phages varphi11, varphi53, varphi80, varphi80alpha and varphiNM4 showed a high degree of homology with varphiNM4 and substantial differences with regard to other phages. Using a model transduction system with a well-defined donor and recipient, varphiJB transferred the tetracycline resistance plasmid pT181 and a penicillinase plasmid with outstanding frequencies, beating most of the above-mentioned phages by an order of magnitude. Moreover, varphiJB demonstrated high frequencies of transferring antibiotic resistance plasmids even upon induction from a lysogenic donor strain. Considering such transducing potential, varphiJB and related bacteriophages may serve as a suitable tool for elucidating the nature of transduction and its contribution to the spread of antibiotic resistance genes in naturally occurring MRSA populations.

Wang, N., Jia, S., Xu, H., Liu, Y., and Huang, D. Multiple Horizontal Transfers of Bacteriophage WO and Host Wolbachia in Fig Wasps in a Closed Community. Front Microbiol. 2016; 7:136

ABSTRACT: Wolbachia-bacteriophage WO is a good model system for studying interactions between bacteria and viruses. Previous surveys of insect hosts have been conducted via sampling from open or semi-open communities; however, no studies have reported the infection patterns of phage WO of insects living in a closed community. Figs and fig wasps form a peculiar closed community in which the Ficus tree provides a compact syconium habitat for a variety of fig wasp. Therefore, in this study, we performed a thorough survey of Wolbachia and bacteriophage WO infection patterns in a total of 1406 individuals from 23 fig wasps species living on three different fig tree species. The infection rates of Wolbachia and phage WO were 82.6% (19/23) and 39.1% (9/23), respectively. Additionally, phage WO from fig wasps showed strong insect host specificity based on orf7 sequences from fig wasps and 21 other insect species. Probably due to the physical barrier of fig syconium, most phage WO from fig wasps form a specific clade. Phylogenetic analysis showed the absence of congruence between WO and host Wolbachia, WO and insect host, as well as Wolbachia and fig wasps, suggesting that both Wolbachia and phage WO exchanged frequently and independently within the closed syconium. Thus, the infection pattern of bacteriophage WO from fig wasps appeared quite different from that in other insects living outside, although the effect and the transfer routes of phage WO are unclear, which need to be investigated in the future.

Wang, Y., Mi, Z., Niu, W., An, X., Yuan, X., Liu, H., Li, P., Liu, Y., Feng, Y., Huang, Y., Zhang, X., Zhang, Z., Fan, H., Peng, F., Tong, Y., and Bai, C. Intranasal treatment with bacteriophage rescues mice from Acinetobacter baumannii-mediated pneumonia. Future.Microbiol. 2-29-2016;

ABSTRACT: AIM: With the emergence of drug-resistant bacteria, finding alternative agents to treat antibiotic-resistant bacterial infections is imperative. MATERIALS & METHODS: A mouse pneumonia model was developed by combining cyclophosphamide pretreatment and Acinetobacter baumannii challenge, and a lytic bacteriophage was evaluated for its therapeutic efficacy in this model by examining the survival rate, bacterial load in the lung and lung pathology. RESULTS: Intranasal instillation with bacteriophage rescued 100% of mice following lethal challenge with A. baumannii. Phage treatment reduced bacterial load in the lung. Microcomputed tomography indicated a reduction in lung inflammation in mice given phage. CONCLUSION: This research demonstrates that intranasal application of bacteriophage is viable, and could provide complete protection from pneumonia caused by A. baumannii.

Wei, Y., Gao, Z., Zhang, H., and Dong, Y. Structural characterizations of phage antitoxin Dmd and its interactions with bacterial toxin RnlA. Biochem.Biophys.Res.Commun. 4-15-2016; 472(4):592-597.

ABSTRACT: Toxin-antitoxin (TA) loci are widespread in bacteria plasmids and chromosomes, and target various cellular functions to regulate cell growth and death. A type II TA system RnlA-RnlB from Escherichia coli is associated with phage-resistance. After the infection of bacteriophage T4 with Dmd defection, RnlA is activated by the disappearance of RnlB, resulting in the rapid degradation of T4 mRNAs. Dmd can bind to RnlA directly and neutralize RnlA toxicity to allow phage reproduction. Dmd represent a heterogenous antitoxin of RnlA replacing antitoxin RnlB. Here, we reported two structures of Dmd from T4 phage and RB69 phage. Both Dmd structures are high similar with a compacted domain composed of a four-stranded anti-parallel beta-sheet and an

alpha-helix. Chromatography and SAXS suggest Dmd forms a dimer in solution consistent with that in crystal. Structure-based mutagenesis of Dmd reveals key residues involved in RnlA-binding. Possibility cavities in Dmd used for compounds design were modeled. Our structural study revealed the recognition and inhibition mechanism of RnlA by Dmd and providing a potential laboratory phage prevention target for drug design.

Wells, C. D., Deighan, P., Brigham, M., and Hochschild, A. Nascent RNA length dictates opposing effects of NusA on antitermination. Nucleic Acids Res. 3-28-2016;

ABSTRACT: The NusA protein is a universally conserved bacterial transcription elongation factor that binds RNA polymerase (RNAP). When functioning independently, NusA enhances intrinsic termination. Paradoxically, NusA stimulates the function of the N and Q antiterminator proteins of bacteriophage lambda. The mechanistic basis for NusA's functional plasticity is poorly understood. Here we uncover an effect of nascent RNA length on the ability of NusA to collaborate with Q. Ordinarily, Q engages RNAP during early elongation when it is paused at a specific site just downstream of the phage late-gene promoter. NusA facilitates this engagement process and both proteins remain associated with the transcription elongation complex (TEC) as it escapes the pause and transcribes the late genes. We show that the lambda-related phage 82 Q protein (82Q) can also engage RNAP that is paused at a promoter-distal position and thus contains a nascent RNA longer than that associated with the natively positioned TEC. However, the effect of NusA in this context is antagonistic rather than stimulatory. Moreover, cleaving the long RNA associated with the promoter-distal TEC restores NusA's stimulatory effect. Our findings reveal a critical role for nascent RNA in modulating NusA's effect on 82Q-mediated antitermination, with implications for understanding NusA's functional plasticity.

Westbye, A. B., Kuchinski, K., Yip, C. K., and Beatty, J. T. The Gene Transfer Agent RcGTA Contains Head Spikes Needed for Binding to the Rhodobacter capsulatus Polysaccharide Cell Capsule. J.Mol.Biol. 1-29-2016; 428(2 Pt B):477-491.

ABSTRACT: Viruses and bacteriophages recognize cell surface proteins using receptor-binding proteins. In most tailed bacteriophages, receptor-binding proteins are located on the bacteriophage tail. The gene transfer agent of Rhodobacter capsulatus, RcGTA, morphologically resembles a tailed bacteriophage and binds to a capsular polysaccharide covering R. capsulatus cells. Here, we report that the RcGTA capsid (head) is decorated by spikes that are needed for binding to the capsule. The triangular spikes measured ~12nm and appeared to be attached at the capsid vertices. Head spike production required the putative carbohydrate-binding protein ghsB (rcc01080) previously thought to encode a side tail fiber protein. We found that ghsB is likely co-transcribed with ghsA (rcc01079) and that ghsA/ghsB is regulated by the CckA-ChpT-CtrA phosphorelay homologues and a quorum-sensing system. GhsA and GhsB were found to be CckA-dependent RcGTA maturation factors, as GhsA- and GhsB-deficient particles were found to have altered native-gel electrophoresis migration. Additionally, we provide electron microscopy images showing that RcGTA contains side tail fibers and a baseplate-like structure near the tip of the tail, which are independent of ghsB.

Xie, X., Bahnemann, J., Wang, S., Yang, Y., and Hoffmann, M. R. "Nanofiltration" Enabled by Super-Absorbent Polymer Beads for Concentrating Microorganisms in Water Samples. Sci.Rep. 2016; 6:20516

ABSTRACT: Detection and quantification of pathogens in water is critical for the protection of human health and for drinking water safety and security. When the pathogen concentrations are low, large sample volumes (several liters) are needed to achieve reliable quantitative results. However, most microbial identification methods utilize relatively small sample volumes. As a consequence, a concentration step is often required to detect pathogens in natural waters. Herein, we introduce a novel water sample concentration method based on superabsorbent polymer (SAP) beads. When SAP beads swell with water, small molecules can be sorbed within the beads, but larger particles are excluded and, thus, concentrated in the residual non-sorbed water. To illustrate this approach, millimeter-sized poly(acrylamide-co-itaconic acid) (P(AM-co-IA)) beads are synthesized and successfully applied to concentrate water samples containing two model microorganisms: Escherichia coli and bacteriophage MS2. Experimental results indicate that the size of the water channel within water swollen P(AM-co-IA) hydrogel beads is on the order of several nanometers. The millimeter size coupled with a negative surface charge of the beads are shown to be critical in order to achieve high levels of concentration. This new concentration procedure is very fast, effective, scalable, and low-cost with no need for complex instrumentation.

Xu, Y., Ma, Y., Yao, S., Jiang, Z., Pei, J., and Cheng, C. Characterization, Genome Sequence, and Analysis of Escherichia Phage CICC 80001, a Bacteriophage Infecting an Efficient L-Aspartic Acid Producing Escherichia coli. Food Environ.Virol. 2016; 8(1):18-26.

ABSTRACT: Escherichia phage CICC 80001 was isolated from the bacteriophage contaminated medium of an Escherichia coli strain HY-05C (CICC 11022S) which could produce L-aspartic acid. The phage had a head diameter of 45-50 nm and a tail of about 10 nm. The one-step growth curve showed a latent period of 10 min and a rise period of about 20 min. The average burst size was about 198 phage particles per infected cell. Tests were conducted on the plaques, multiplicity of infection, and host range. The genome of CICC 80001 was sequenced with a length of 38,810 bp, and annotated. The key proteins leading to host-cell lysis were phylogenetically analyzed. One protein belonged to class II holin, and the other two belonged to the endopeptidase family and N-acetylmuramoyl-L-alanine amidase family, respectively. The genome showed the sequence identity of 82.7 % with that of Enterobacteria phage T7, and carried ten unique open reading frames. The bacteriophage resistant E. coli strain designated CICC 11021S was breeding and its L-aspartase activity was 84.4 % of that of CICC 11022S.

Yang, J. A., Kang, I., Moon, M., Ryu, U. C., Kwon, K. K., Cho, J. C., and Oh, H. M. Complete genome sequence of Celeribacter marinus IMCC12053(T), the host strain of marine bacteriophage P12053L. Mar.Genomics. 2016; 26:5-7.

ABSTRACT: Isolated from coastal seawater from Yellow Sea of Korea, Celeribacter marinus IMCC12053 was used as the host bacterium for bacteriophage P12053L. Here we report the complete genome sequence of strain IMCC12053 for further study of the marine bacteriophage P12053L functional genes. Single molecule real-time technology (PacBio RSII) was used for the single circular chromosome that is 3,096,705 base pairs in length and the GC content is 56.24%. It contains 3155 ORFs with 45 tRNAs and 6 rRNAs genes. N(6)-methyladenosine patterns were also investigated for 32 unmethylated genes and intergenic regions that covered many regulators and phage genes as well as

ribosomal RNA genes and tRNA genes. Cryptic N(4)-methylcytosine pattern was investigated to speculate GpC methylase activity throughout the genome. Comparative genomics with other Celeribacter genomes were carried out for polyaromatic hydrocarbon degradation, but there were no aromatic ring oxygenases in IMCC12053 when compared to Celeribacter indicus P73.

Yap, M. L., Klose, T., Arisaka, F., Speir, J. A., Veesler, D., Fokine, A., and Rossmann, M. G. Role of bacteriophage T4 baseplate in regulating assembly and infection. Proc.Natl.Acad.Sci.U.S.A. 3-8-2016; 113(10):2654-2659.

ABSTRACT: Bacteriophage T4 consists of a head for protecting its genome and a sheathed tail for inserting its genome into a host. The tail terminates with a multiprotein baseplate that changes its conformation from a "high-energy" dome-shaped to a "low-energy" star-shaped structure during infection. Although these two structures represent different minima in the total energy landscape of the baseplate assembly, as the dome-shaped structure readily changes to the star-shaped structure when the virus infects a host bacterium, the dome-shaped structure must have more energy than the star-shaped structure. Here we describe the electron microscopy structure of a 3.3-MDa in vitro-assembled star-shaped baseplate with a resolution of 3.8 A. This structure, together with other genetic and structural data, shows why the high-energy baseplate is formed in the presence of the central hub and how the baseplate changes to the low-energy structure, via two steps during infection. Thus, the presence of the central hub is required to initiate the assembly of metastable, high-energy structures. If the high-energy structure is formed and stabilized faster than the low-energy structure, there will be insufficient components to assemble the low-energy structure.

Yu, J. G., Lim, J. A., Song, Y. R., Heu, S., Kim, G. H., Koh, Y. J., and Oh, C. S. Isolation and Characterization of Bacteriophages Against Pseudomonas syringae pv. actinidiae Causing Bacterial Canker Disease in Kiwifruit. J.Microbiol.Biotechnol. 2-28-2016; 26(2):385-393.

ABSTRACT: Pseudomonas syringae pv. actinidiae causes bacterial canker disease in kiwifruit. Owing to the prohibition of agricultural antibiotic use in major kiwifruit-cultivating countries, alternative methods need to be developed to manage this disease. Bacteriophages are viruses that specifically infect target bacteria and have recently been reconsidered as potential biological control agents for bacterial pathogens owing to their specificity in terms of host range. In this study, we isolated bacteriophages against P. syringae pv. actinidiae from soils collected from kiwifruit orchards in Korea and selected seven bacteriophages for further characterization based on restriction enzyme digestion patterns of genomic DNA. Among the studied bacteriophages, two belong to the Myoviridae family and three belong to the Podoviridae family, based on morphology observed by transmission electron microscopy. The host range of the selected bacteriophages was confirmed using 18 strains of P. syringae pv. actinidiae, including the Psa2 and Psa3 groups, and some were also effective against other P. syringae pathovars. Lytic activity of the selected bacteriophages was sustained in vitro until 80 h, and their activity remained stable up to 50 degrees C, at pH 11, and under UV-B light. These results indicate that the isolated bacteriophages are specific to P. syringae species and are resistant to various environmental factors, implying their potential use in control of bacterial canker disease in kiwifruits.

Yuksel, D., Bianco, P. R., and Kumar, K. De novo design of protein mimics of B-DNA. Mol.Biosyst. 2016; 12(1):169-177.

ABSTRACT: Structural mimicry of DNA is utilized in nature as a strategy to evade molecular defences mounted by host organisms. One such example is the protein Ocr - the first translation product to be expressed as the bacteriophage T7 infects E. coli. The structure of Ocr reveals an intricate and deliberate arrangement of negative charges that endows it with the ability to mimic approximately 24 base pair stretches of B-DNA. This uncanny resemblance to DNA enables Ocr to compete in binding the type I restriction modification (R/M) system, and neutralizes the threat of hydrolytic cleavage of viral genomic material. Here, we report the de novo design and biophysical characterization of DNA mimicking peptides, and describe the inhibitory action of the designed helical bundles on a type I R/M enzyme, EcoR124I. This work validates the use of charge patterning as a design principle for creation of protein mimics of DNA, and serves as a starting point for development of therapeutic peptide inhibitors against human pathogens that employ molecular camouflage as part of their invasion stratagem.

Zebrowska, J., Zolnierkiewicz, O., Skowron, M. A., Zylicz-Stachula, A., Jezewska-Frackowiak, J., and Skowron, P. M. A putative Type IIS restriction endonuclease GeoICI from Geobacillus sp. - A robust, thermostable alternative to mezophilic prototype BbvI. J.Biosci. 2016; 41(1):27-38.

ABSTRACT: Screening of extreme environments in search for novel microorganisms may lead to the discovery of robust enzymes with either new substrate specificities or thermostable equivalents of those already found in mesophiles, better suited for biotechnology applications. Isolates from Iceland geysers' biofilms, exposed to a broad range of temperatures, from ambient to close to water boiling point, were analysed for the presence of DNA-interacting proteins, including restriction endonucleases (REases). GeoICI, a member of atypical Type IIS REases, is the most thermostable isoschizomer of the prototype BbvI, recognizing/cleaving 5'-GCAGC(N8/12)-3'DNA sequences. As opposed to the unstable prototype, which cleaves DNA at 30 degrees C, GeoICI is highly active at elevated temperatures, up to 73 degrees C and over a very wide salt concentration range. Recognition/cleavage sites were determined by: (i) digestion of plasmid and bacteriophage lambda DNA (Lambda); (ii) cleavage of custom PCR substrates, (iii) run-off sequencing of GeoICI cleavage products and (iv) shotgun cloning and sequencing of Lambda DNA fragmented with GeoICI. Geobacillus sp. genomic DNA was PCR-screened for the presence of other specialized REases-MTases and as a result, another putative REase- MTase, GeoICII, related to the Thermus sp. family of bifunctional REases-methyltransferases (MTases) was detected.

Zhao, H., Speir, J. A., Matsui, T., Lin, Z., Liang, L., Lynn, A. Y., Varnado, B., Weiss, T. M., and Tang, L. Structure of a Bacterial Virus DNA-Injection Protein Complex Reveals a Decameric Assembly with a Constricted Molecular Channel. PLoS.One. 2016; 11(2):e0149337

ABSTRACT: The multi-layered cell envelope structure of Gram-negative bacteria represents significant physical and chemical barriers for short-tailed phages to inject phage DNA into the host cytoplasm. Here we show that a DNA-injection protein of bacteriophage Sf6, gp12, forms a 465-kDa, decameric assembly in vitro. The electron microscopic structure of the gp12 assembly shows a ~150-A, mushroom-like architecture

consisting of a crown domain and a tube-like domain, which embraces a 25-A-wide channel that could precisely accommodate dsDNA. The constricted channel suggests that gp12 mediates rapid, uni-directional injection of phage DNA into host cells by providing a molecular conduit for DNA translocation. The assembly exhibits a 10-fold symmetry, which may be a common feature among DNA-injection proteins of P22-like phages and may suggest a symmetry mismatch with respect to the 6-fold symmetric phage tail. The gp12 monomer is highly flexible in solution, supporting a mechanism for translocation of the protein through the conduit of the phage tail toward the host cell envelope, where it assembles into a DNA-injection device.

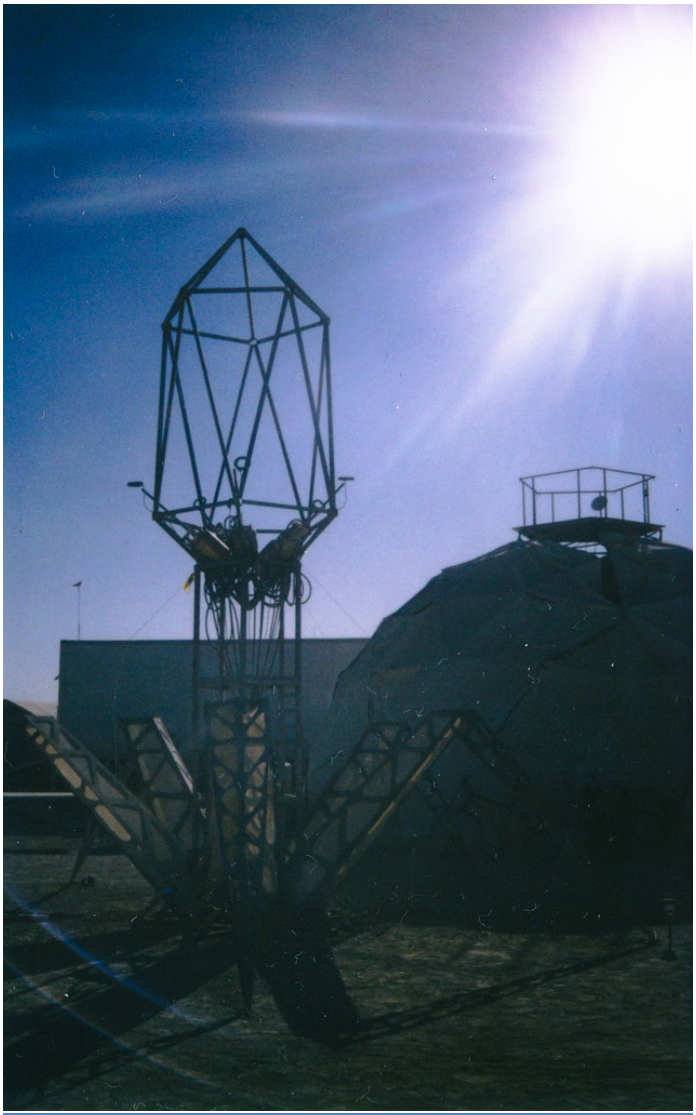
Zhao, X., Huang, S., Zhao, J., He, X., Li, E., Li, H., Liu, W., Zou, D., Wei, X., Wang, X., Dong, D., Yang, Z., Yan, X., Shen, Z., and Yuan, J. Characterization of phiCFP-1, a virulent bacteriophage specific for Citrobacter freundii. J.Med.Virol. 2016; 88(5):895-905.

ABSTRACT: Citrobacter freundii, a Gram-negative bacterium, causes many opportunistic infections. Bacteriophage phiCFP-1 was isolated and characterized by its ability to lyse the multidrug-resistant clinical C. freundii strain P10159. Transmission electron microscopy showed that the phage has an icosahedral head and a short tail, making it a Podoviridae family member. In a single-step growth experiment, phiCFP-1 exhibited an eclipse period of 20 min and a burst size of 100 particles per cell. Its genome assembled as a circular molecule when genomic sequencing was completed. However, based on genome content and organization, it was categorized as a classic T7-related phage, and such phages are known to have linear genomes with direct terminal repeats. With the quick and simple method established herein, the 38,625-bp linear double-stranded DNA with 229-bp direct terminal repeats was accurately identified. The genome contained 43 putative open reading frames and no tRNA genes. Using a proteomics-based approach, seven viral and two host proteins from purified phiCFP-1 particles were identified. Comparative genomics and recombination analyzes revealed close genetic relatedness among phiCFP-1, phiYeO3-12/vB_YenP_AP5 (from Yersinia enterocolitica O3), and phiSG-JL2 (from Salmonella enterica). J. Med. Virol. 88:895-905, 2016. (c) 2015 Wiley Periodicals, Inc.

Zhao, Y., Liang, Y., Liu, Y., Zhang, X., Hu, X., Tu, S., Wu, A., Zhang, C., Zhong, J., Zhao, S., Liu, X., and Tu, K. Isolation of broad-specificity domain antibody from phage library for development of pyrethroid immunoassay. Anal.Biochem. 3-7-2016;

ABSTRACT: An antibody to phenoxybenzoic acid (PBA), the conserved chemical region of pyrethroids, was developed using a domain antibody (DAB) library to enable pyrethroid detection in agricultural products. The DAB library, constructed without animal immunization and based on a human VH framework, displayed repertoires on filamentous bacteriophage. After four rounds of panning, we obtained five domain antibodies that are capable of binding to PBA. Antibody A3 has strong identification capability to cypermethrin, beta-cypermethrin, and fenvalerate. The antibody A3 was used to develop an enzyme-linked immunosorbent assay (ELISA). The IC50 values were 2.586, 1.814, and 2.251 mug/ml for cypermethrin, beta-cypermethrin, and fenvalerate, respectively. The assay shows weak competition with flucythrinate but shows no competition with fenpropathrin, deltamethrin, and permethrin. The developed ELISA process was successfully applied to fortified Chinese cabbage samples, with the recoveries of cypermethrin, beta-cypermethrin, and fenvalerate ranging from 84.4 to 112.3%. We developed an immunoassay to detect pyrethroids depending on the

domain antibody library, which overcomes the limitation of requiring protein antigen to immunize animals raising antibody.



Ian Norman, Phage Camp, September 7 2011, Creative Commons License

H. Probiotics & Bacteriotherapy

Abdulkadir, B., Nelson, A., Skeath, T., Marrs, E. C., Perry, J. D., Cummings, S. P., Embleton, N. D., Berrington, J. E., and Stewart, C. J. Routine Use of Probiotics in Preterm Infants: Longitudinal Impact on the Microbiome and Metabolome. Neonatology. 2-10-2016; 109(4):239-247.

ABSTRACT: BACKGROUND: Probiotics are live microbial supplements that colonize the gut and potentially exert health benefit to the host. OBJECTIVES: We aimed to determine the impact of a probiotic (Infloran(R): Lactobacillus acidophilus-NCIMB701748 and Bifidobacterium bifidum-ATCC15696) on the bacterial and metabolic function of the preterm gut while in the neonatal intensive care unit (NICU) and following discharge. METHODS: Stool samples (n = 88) were collected before, during, and after probiotic intake from 7 patients, along with time-matched controls from 3 patients. Samples were also collected following discharge home from the NICU. Samples underwent bacterial profiling analysis by 16S rRNA gene sequencing and quantitative PCR (qPCR), as well as metabolomic profiling using liquid chromatography mass spectrometry. RESULTS: Bacterial profiling showed greater Bifidobacterium (15.1%) and Lactobacillus (4.2%) during supplementation compared to the control group (4.0% and 0%, respectively). While Lactobacillus became reduced after the probiotic had been stopped, Bifidobacterium remained high following discharge, suggestive of successful colonisation. qPCR analysis showed a significant increase (p </= 0.01) in B. bifidum in infants who received probiotic treatment compared to controls, but no significant increase was observed for L. acidophilus (p = 0.153). Metabolite profiling showed clustering based on receiving probiotic or matched controls, with distinct metabolites associated with probiotic administration. CONCLUSIONS: Probiotic species successfully colonise the preterm gut, reducing the relative abundance of potentially pathogenic bacteria, and effecting gut functioning. Bifidobacterium (but not Lactobacillus) colonised the gut in the long term, suggesting the possibility that therapeutically administered probiotics may continue to exert important functional effects on gut microbial communities in early infancy.

Africa, J. A., Newton, K. P., and Schwimmer, J. B. Lifestyle Interventions Including Nutrition, Exercise, and Supplements for Nonalcoholic Fatty Liver Disease in Children. Dig.Dis.Sci. 4-4-2016;

ABSTRACT: Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease among children. Lifestyle interventions, such as diet and exercise, are frequently recommended. Children with NAFLD have a distinct physiology that is different from obesity alone and has the potential to influence lifestyle treatments. Studies of diet alone in the treatment of pediatric NAFLD have focused on sugar and carbohydrate, but did not indicate any one dietary approach that was superior to another. For children who are obese and have NAFLD, weight loss may have a beneficial effect regardless of the diet used. Exercise is widely believed to improve NAFLD because a sedentary lifestyle, poor aerobic fitness, and low muscle mass are all risk factors for NAFLD. However, there have been no randomized controlled trials of exercise as a treatment for children with NAFLD. Studies of the combination of diet and exercise suggest a potential for improvement in serum alanine aminotransferase activity and/or magnetic resonance

imaging liver fat fraction with intervention. There is also enthusiasm for the use of dietary supplements; however, studies in children have shown inconsistent effects of vitamin E, fish oil, and probiotics. This review presents the available data from studies of lifestyle intervention and dietary supplements published to date and highlights challenges that must be addressed in order to advance the evidence base for the treatment of pediatric NAFLD.

Akar, M., Eras, Z., Oncel, M. Y., Arayici, S., Guzoglu, N., Canpolat, F. E., Uras, N., and Oguz, S. S. Impact of oral probiotics on neurodevelopmental outcomes in preterm infants. J.Matern.Fetal Neonatal Med. 4-5-2016; 1-19.

ABSTRACT: OBJECTIVE: The aim of the study was to evaluate the neurodevelopment outcomes of very low birth weight (VLBW) preterm infants supplemented with oral probiotics for the prevention of necrotizing enterocolitis (NEC). METHODS: A prospective follow-up study was performed in a cohort of VLBW preterm infants enrolled in a single center randomized controlled clinical trial to evaluate the efficacy of oral probiotics for the prevention of NEC. Cognitive and neuromotor development were assessed by using the Bayley Scales of Infant Development II. Sensory and neurological performance was evaluated by standard techniques. The primary outcome was neurodevelopmental impairment at 18 to 24 months' corrected age. RESULTS: A total of 400 infants completed the trial protocol. Of the 370 infants eligible for follow up, 249 infants (124 in the probiotics group and 125 in the control group) were evaluated. There was no significant difference in any of the neurodevelopmental and sensory outcomes between the two groups. CONCLUSION: Oral probiotic given to VLBW infants to reduce the incidense and severity of NEC started with the first feed did not affect neuromotor, neurosensory, and cognitive outcomes at 18 to 24 months' corrected age.

Akkasheh, G., Kashani-Poor, Z., Tajabadi-Ebrahimi, M., Jafari, P., Akbari, H., Taghizadeh, M., Memarzadeh, M. R., Asemi, Z., and Esmaillzadeh, A. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial. Nutrition. 2016; 32(3):315-320.

ABSTRACT: OBJECTIVE: We are aware of no study examining the effects of probiotic supplementation on symptoms of depression, metabolic profiles, serum high-sensitivity C-reactive protein (hs-CRP), and biomarkers of oxidative stress in patients with major depressive disorder (MDD). The present study was designed to determine the effects of probiotic intake on symptoms of depression and metabolic status in patients with MDD. METHODS: This randomized, double-blind, placebo-controlled clinical trial included 40 patients with a diagnosis of MDD based on DSM-IV criteria whose age ranged between 20 and 55 y. Patients were randomly allocated into two groups to receive either probiotic supplements (n = 20) or placebo (n = 20) for 8 wk. Probiotic capsule consisted of three viable and freeze-dried strains: Lactobacillus acidophilus (2 x 10(9) CFU/g), Lactobacillus casei (2 x 10(9) CFU/g), and Bifidobacterium bifidum (2 x 10(9) CFU/g). Fasting blood samples were taken at the beginning and end of the trial to quantify the relevant variables. All participants provided three dietary records (two weekdays and one weekend) and three physical activity records during the intervention. RESULTS: Dietary intake of study participants was not significantly different between the two groups. After 8 wk of intervention, patients who received probiotic supplements had significantly decreased Beck Depression Inventory total scores (-5.7 +/- 6.4 vs. -1.5 +/- 4.8, P = 0.001) compared with the placebo. In addition, significant decreases in serum insulin levels (-2.3

+/- 4.1 vs. 2.6 +/- 9.3 mulU/mL, P = 0.03), homeostasis model assessment of insulin resistance (-0.6 +/- 1.2 vs. 0.6 +/- 2.1, P = 0.03), and serum hs-CRP concentrations (-1138.7 +/- 2274.9 vs. 188.4 +/- 1455.5 ng/mL, P = 0.03) were observed after the probiotic supplementation compared with the placebo. Additionally, taking probiotics resulted in a significant rise in plasma total glutathione levels (1.8 +/- 83.1 vs. -106.8 +/- 190.7 mumol/L, P = 0.02) compared with the placebo. We did not find any significant change in fasting plasma glucose, homeostatic model assessment of beta cell function, quantitative insulin sensitivity check index, lipid profiles, and total antioxidant capacity levels. CONCLUSIONS: Probiotic administration in patients with MDD for 8 wk had beneficial effects on Beck Depression Inventory, insulin, homeostasis model assessment of insulin resistance, hs-CRP concentrations, and glutathione concentrations, but did not influence fasting plasma glucose, homeostatic model assessment of beta cell function, quantitative insulin sensitivity check index, lipid profiles, and total antioxidant capacity levels.

Al-Dughaishi, T., Nikolic, D., Al-Hashmi, K., Al-Waili, K., Rizzo, M., and Al-Rasadi, K. Nutraceuticals as Lipid-Lowering Treatment in Pregnancy and Their Effects on the Metabolic Syndrome. Curr.Pharm.Biotechnol. 4-1-2016;

ABSTRACT: Maternal nutrition and lifestyle before and during pregnancy influence both mother and offspring's health and can be correlated with the metabolic syndrome in later life. Findings from animal and human studies indicate that nutrition during pregnancy has an important role in microbiological, metabolic, physiologic and immunologic development and homeostasis. A low nutritional intake in early pregnancy may represent a risk for adverse effects during pregnancy as well as on birth outcome. It seems that dietary supplementation with probiotics in perinatal period may represent safe and practical approach in dealing with the most common adverse pregnancy outcomes such as obesity and gestational diabetes. The SPRING (Study of Probiotics in the prevention of Gestational diabetes) will give important answers about potential benefits of probiotics in pregnant women who are obese and overweight and otherwise at the high risk for complications during pregnancy. Fish oil supplementation during the last trimester of pregnancy showed no effects on plasma lipids and lipoproteins in offspring, as well as on their adiposity. The effect of hypercholesterolemia during pregnancy on both mothers and child needs to be further investigated as it could have a biological role. The guidelines for the eventual clinical approach currently do not exist. Potential benefits of nutraceuticals on several metabolic parameters have been suggested. Limited evidence does not allow to draw final conclusions on preventive health strategies and dietary patterns that should be promoted during pregnancy. Further prospective and intervention studies are needed to establish it. Healthy lifestyle and dietary advice with appropriate supplements usage should be considered.

Amadori, M. and Zanotti, C. Immunoprophylaxis in intensive farming systems: the way forward. Vet.Immunol.Immunopathol. 2-20-2016;

ABSTRACT: High levels of production in intensive farming systems are associated with increased replacement rates as a result of multifactorial diseases. The so-called "production diseases" may include low-grade infection reducing profitability without increased morbidity. Such infections are sustained by low pathogenic viral and bacterial agents which give rise to full-blown disease in association with poor environmental conditions. In these farms, the results of vaccination may be disappointing. Therefore,

fundamental issues should be dealt with toward successful immunoprophylaxis. High lean meat and milk production are associated with chronic inflammation and activation of the innate immune system vis-a-vis cellular stress. This may negatively affect adaptive immune responses. A negative modulation of the host microbiome by farm management practices and drug treatments is a further risk factor. The immune response to stressed cells questions the usual correlates of protection investigated after vaccination. In particular, there is evidence that specific and non-specific immune responses may overlap in vitro as a result of a high level of innate immune responses to Damage-Associated Molecular Patterns (DAMPS) and stress antigens. A vigorous adaptive immune response to microbial agents may be sometimes counterproductive, as suggested in porcine reproductive and respiratory syndrome virus (PRRSV) infection. Alternative outcomes should be sometimes pursued: a better homeostatic control of the inflammatory response, effective and self-limiting innate immune responses, and even tolerance induction. On the whole, successful immunoprophylaxis in intensive farming systems demands co-ordinated and multi-disciplinary efforts in terms of animal breeding, farm management and hygiene, correct choice and harmonization of the prophylactic tools (vaccines, immunomodulators, pre- and probiotics). Finally, there is evidence that disease-predicting parameters of the innate immune response may greatly ease the identification of herds and animals at risk, and contribute to reduced antibiotic usage on farm.

Arai, T., Obuchi, S., Eguchi, K., and Seto, Y. In vitro investigation of molecules involved in Lactobacillus gasseri SBT2055 adhesion to host intestinal tract components. J.Appl.Microbiol. 3-21-2016;

ABSTRACT: AIMS: The adhesion ability of Lactobacillus gasseri SBT2055 was investigated in vitro by searching for its adhesion molecules. METHODS AND RESULTS: L. gasseri SBT2055 showed adherence to host components, including two commercially available mucins, Caco-2 epithelial-like cells, and the extracellular matrix molecule fibronectin (Fn). Its adhesion rates to host components were generally higher than those of other Lactobacillus strains. We examined sortase-dependent proteins (SDPs) anchored by a sortase enzyme encoded by srtA1. The adhesion rates of an srtA1 disruptant were lower than those of L. gasseri SBT2055, and the relative adherences were as follows: two mucins, 43% and 40%; Caco-2, 66%; and Fn, 28%. Seven additional gene disruptants were generated to determine the precise SDPs that contribute to adhesion to each component. CONCLUSIONS: The adhesion ability of L. gasseri SBT2055 was superior to those of other Lactobacillus strains. Additionally, four adhesion molecules were newly identified from candidate SDPs. SIGNIFICANCE AND IMPACT OF STUDY: Although the contribution of SDPs to adhesion has been reported using sortase gene disruptants, this is the first report to identify the precise SDPs that act as adhesion molecules. Our results will contribute to achieving better understanding of probiotic bacterial adherence. This article is protected by copyright. All rights reserved.

Arasu, M. V., Al-Dhabi, N. A., Ilavenil, S., Choi, K. C., and Srigopalram, S. In vitro importance of probiotic Lactobacillus plantarum related to medical field. Saudi.J.Biol.Sci. 2016; 23(1):S6-S10.

ABSTRACT: Lactobacillus plantarum is a Gram positive lactic acid bacterium commonly found in fermented food and in the gastro intestinal tract and is commonly used in the food industry as a potential starter probiotic. Recently, the consumption of food together

with probiotics has tremendously increased. Among the lactic acid bacteria, L. plantarum attracted many researchers because of its wide applications in the medical field with antioxidant, anticancer, anti-inflammatory, antiproliferative, anti-obesity and antidiabetic properties. The present study aimed to investigate the in vitro importance of L. plantarum toward medical applications. Moreover, this report short listed various reports related to the applications of this promising strain. In conclusion, this study would attract the researchers in commercializing this strain toward the welfare of humans related to medical needs.

Arboleya, S., Stanton, C., Ryan, C. A., Dempsey, E., and Ross, P. R. Bosom Buddies: The Symbiotic Relationship Between Infants and Bifidobacterium longum ssp. longum and ssp. infantis. Genetic and Probiotic Features. Annu.Rev.Food Sci.Technol. 2016; 7:1-21.

ABSTRACT: The intestinal microbiota is a complex community that plays an important role in human health from the initial steps of its establishment. Its microbial composition has been suggested to result from selective pressures imposed by the host and is modulated by competition among its members. Bifidobacterium longum is one of the most abundant species of the Bifidobacterium genus in the gut microbiota of healthy breast-fed infants and adults. The recent advancements of 'omics techniques have facilitated the genetic and functional studies of different gut microbiota members. They have revealed the complex genetic pathways used to metabolize different compounds that likely contribute to the competitiveness and persistence of B. longum in the colon. The discovery of a genomic island in B. longum ssp. infantis that encodes specific enzymes for the metabolism of human milk oligosaccharides suggests a specific ecological adaptation. Moreover, B. longum is widely used as probiotic, and beneficial effects in infant health have been reported in several studies.

Arruda, P. H., Madson, D. M., Ramirez, A., Rowe, E. W., and Songer, J. G. Bacterial probiotics as an aid in the control of Clostridium difficile disease in neonatal pigs. Can.Vet.J. 2016; 57(2):183-188.

ABSTRACT: Although Clostridium difficile infection (CDI) is a common disease in swine, there is a lack of prevention strategies. The objectives of this study were to evaluate: i) the effectiveness of Lactobacillus spp. and ii) non-toxigenic C. difficile (NTCD) as prevention for the development of CDI in piglets. Cesarean-derived piglets (N = 150) were randomly assigned to 6 groups: GROUP 1 - negative control (n = 10); GROUP 2 - NTCD only (n = 13); GROUP 3 - Lactobacillus spp. only (n = 14); GROUP 4 - positive control (challenged with toxigenic C. difficile strain) (n = 35); GROUP 5 - NTCD and challenged with the toxigenic C. difficile strain (n = 34); and GROUP 6 - Lactobacillus spp. and challenged with the toxigenic C. difficile strain (n = 44). Piglets which received NTCD showed lower prevalence of toxin-positive feces, mesocolonic edema, and microscopic lesions compared with positive control piglets. Administration of Lactobacillus spp. did not reveal clear benefits.

Asrani, V. M., Yoon, H. D., Megill, R. D., Windsor, J. A., and Petrov, M. S. Interventions That Affect Gastrointestinal Motility in Hospitalized Adult Patients: A Systematic Review and Meta-Analysis of Double-Blind Placebo-Controlled Randomized Trials. Medicine (Baltimore). 2016; 95(5):e2463

ABSTRACT: Gastrointestinal (GI) dysmotility is a common complication in acute, critically ill, postoperative, and chronic patients that may lead to impaired nutrient delivery, poor clinical, and patient-reported outcomes. Several pharmacological and nonpharmacological interventions to treat GI dysmotility were investigated in dozens of clinical studies. However, they often yielded conflicting results, at least in part, because various (nonstandardized) definitions of GI dysmotility were used and methodological quality of studies was poor. While a universally accepted definition of GI dysmotility is yet to be developed, a systematic analysis of data derived from double-blind placebo-controlled randomized trials may provide robust data on absolute and relative effectiveness of various interventions as the study outcome (GI motility) was assessed in the least biased manner.To systematically review data from double-blind placebo-controlled randomized trials to determine and compare the effectiveness of interventions that affect GI motility.Three electronic databases (MEDLINE, SCOPUS, and EMBASE) were searched. A random effects model was used for meta-analysis. The summary estimates were reported as mean difference (MD) with the corresponding 95% confidence interval (CI).A total of 38 double-blind placebo-controlled randomized trials involving 2371 patients were eligible for inclusion in the systematic review. These studies investigated a total of 20 different interventions, of which 6 interventions were meta-analyzed. Of them, the use of dopamine receptor antagonists (MD, -8.99; 95% CI, -17.72 to -0.27; P = 0.04) and macrolides (MD, -26.04; 95% CI, -51.25 to -0.82; P = 0.04) significantly improved GI motility compared with the placebo group. The use of botulism toxin significantly impaired GI motility compared with the placebo group (MD, 5.31; 95% CI, -0.04 to 10.67; P = 0.05). Other interventions (dietary factors, probiotics, hormones) did not affect GI motility.Based on the best available data and taking into account the safety profile of each class of intervention, dopamine receptor antagonists and macrolides significantly improve GI motility and are medications of choice in treating GI dysmotility.

Awasti, N., Tomar, S. K., Pophaly, S. D., Poonam, Lule, V. K., Singh, T. P., and Anand, S. Probiotic and Functional Characterization of Bifidobacteria of Indian Human Origin. J.Appl.Microbiol. 2-5-2016;

ABSTRACT: AIMS: To identify and characterize the probiotic and functional attributes of bifidobacteria isolated from human sources by in vitro methods. METHODS AND RESULTS: Twelve isolates of bifidobacteria were obtained from different human sources viz; human milk, infant and adult feces. The preliminary identification of isolates was done using genus specific PCR followed by species level identification using 16S rRNA sequencing. All the isolates invariably showed potential probiotic characteristics. Finally, three most promising isolates were subjected to safety evaluation and were found to be safe. These were further evaluated for their potential functional characteristics like, antioxidative, antimutagenic and cholesterol assimilation. The isolates viz; NBIF-5 (73.55 +/- 0.03%) and NBIF-7 (64.06 +/- 0.03%) assimilated significantly higher cholesterol than NBIF-2. The maximum antioxidative activity was observed in NBIF-2 (56.56 +/- 0.28% of radical inhibition). High percentage antimutagenicity scores of 52.41 +/- 2.25% and 53.68 +/- 1.98% against sodium azide (NaN3) were shown by NBIF-2 and NBIF-7 respectively. CONCLUSIONS: Three strains (NBIF-2, NBIF-5 and NBIF-7) were found to be endowed with appreciable probiotic and functional activities. SIGNIFICANCE AND IMPACT OF STUDY: With growing demands for probioitcs and bifidobacteria being prominent ones, new strains of diversified origin have the potential to be explored for commercial and functional applications and thus can be added to the existing commercially available strains of this genera. This article is protected by copyright. All rights reserved.

Baglatzi, L., Gavrili, S., Stamouli, K., Zachaki, S., Favre, L., Pecquet, S., Benyacoub, J., and Costalos, C. Effect of Infant Formula Containing a Low Dose of the Probiotic Bifidobacterium lactis CNCM I-3446 on Immune and Gut Functions in C-Section Delivered Babies: A Pilot Study. Clin.Med.Insights.Pediatr. 2016; 10:11-19.

ABSTRACT: BACKGROUND: In the absence of breast-feeding and its immunomodulatory factors, supplementation of starter infant formula (IF) with probiotics is currently used to support immune functions and gut development. AIM: To assess whether immune-related beneficial effects of regular dose (10(7) CFU/g of powder) of the probiotic Bifidobacterium lactis CNCM I-3446 (hereafter named B. lactis) in starter IF supplementation can be maintained with starter IF containing a low dose (10(4) CFU/g of powder) of B. lactis. METHOD: This trial was designed as a pilot, prospective, double-blind, randomized, single-center clinical trial of two parallel groups (n = 77 infants/group) of C-section delivered infants receiving a starter IF containing either low dose or regular dose of the probiotic B. lactis from birth to six months of age. In addition, a reference group of infants breast-fed for a minimum of four months (n = 44 infants), also born by C-section, were included. All groups were then provided follow-up formula without B. lactis up to 12 months of age. Occurrence of diarrhea, immune and gut maturation, responses to vaccinations, and growth were assessed from birth to 12 months. The effect of low-dose B. lactis formula was compared to regular-dose B. lactis formula, considered as reference for IF with probiotics, and both were further compared to breast-feeding as a physiological reference. RESULTS: Data showed that feeding low-dose B. lactis IF provides similar effects as feeding regular-dose B. lactis IF or breast milk. No consistent statistical differences regarding early life protection against gastrointestinal infections, immune and gut maturation, microbiota establishment, and growth were observed between randomized formula-fed groups as well as with the breast-fed reference group. CONCLUSION: This pilot study suggests that supplementing C-section born neonates with low-dose B. lactis-containing starter formula may impact immune as well as gut maturation similarly to regular-dose B. lactis, close to the breast-feeding reference.

Bayat, A., zizi-Soleiman, F., Heidari-Beni, M., Feizi, A., Iraj, B., Ghiasvand, R., and Askari, G. Effect of Cucurbita ficifolia and Probiotic Yogurt Consumption on Blood Glucose, Lipid Profile, and Inflammatory Marker in Type 2 Diabetes. Int.J.Prev.Med. 2016; 7:30

ABSTRACT: BACKGROUND: Control of blood sugar, hypertension, and dyslipidemia are key factors in diabetes management. Cucurbita ficifolia (pumpkin) is a vegetable which has been used traditionally as a remedy for diabetes in Iran. In addition, consumption of probiotics may have beneficial effects on people with Type 2 diabetes. The aim of this study was an investigation of the effects of C. ficifolia and probiotic yogurt consumption alone or at the same time on blood glucose and serum lipids in diabetic patients. METHODS: Eighty eligible participants randomly were assigned to four groups: 1 - green C. ficifolia (100 g); 2 - probiotic yogurt (150 g); 3 - C. ficifolia plus probiotic yogurt (100 g C. ficifolia plus 150 g yogurt); and 4 -control (dietary advice) for 8 weeks. Blood pressure, glycemic response, lipid profile, and high-sensitive C-reactive protein (hsCRP) were measured before and after the intervention. RESULTS: Total cholesterol (TC) decreased significantly in yogurt and yogurt plus C. ficifolia groups (within groups P = 0.010, and P < 0.001, respectively). C. ficifolia plus yogurt consumption resulted in a decrease in triglyceride (TG) and an increase in high-density lipoprotein cholesterol (HDL-C) (within groups P < 0.001 and P = 0.001, respectively). All interventions led to a significant decrease in blood sugar, hemoglobin A1c (HbA1c), hsCRP, and low-density lipoprotein

cholesterol (LDL-C) level within groups. Blood pressure decreased significantly in Cucurbita group and yogurt group (within groups P < 0.001, and P = 0.001 for systolic blood pressure [SBP] and P < 0.001, and P = 0.004 for diastolic blood pressure [DBP], respectively). All variables changed between groups significantly except LDL-C level. CONCLUSIONS: Variables including TG, HDL-C, TC, fasting blood sugar, HbA1c, SBP, DBP, and hsCRP changed beneficially between groups. It seems that consumption of C. ficifolia and probiotic yogurt may help treatment of diabetic patients.

Bennett, W. E., Jr. Quantitative Risk-Benefit Analysis of Probiotic Use for Irritable Bowel Syndrome and Inflammatory Bowel Disease. Drug Saf. 2016; 39(4):295-305.

ABSTRACT: Probiotics have seen widespread use for a variety of gastrointestinal problems, especially in two common disorders: irritable bowel syndrome and inflammatory bowel disease. Since a wide variety of probiotic preparations has been used, and despite a large number of studies performed, a great deal of heterogeneity exists among them. Straightforward evidence-based recommendations for the use of probiotics in irritable bowel syndrome and inflammatory bowel disease have thus been difficult to formulate. In an effort to improve understanding of the risk-benefit balance of probiotics in these conditions, this study (1) queried the US FDA Adverse Event Reporting System (FAERS) database for all reported adverse drug events related to probiotics in 2013, and (2) constructed risk-benefit planes for both irritable bowel syndrome and inflammatory bowel disease using a geometric approximation of the confidence region between risk and benefit. The results show that adverse events from probiotics vary widely by disease, and when they occur, they are mild and may be difficult to distinguish from the natural history of the underlying disorders they are used to treat. The risk-benefit plane for irritable bowel syndrome straddles the risk-benefit threshold, so patients can expect a balance between a low chance of risk and also a low chance of benefit. The risk-benefit plane for inflammatory bowel disease largely lies above the risk-benefit threshold, so patients may expect more benefit than risk in most cases. More standardized and high-quality research is needed to improve our understanding of risk and benefit for these complex biopharmaceuticals.

Bhandari, P., Rishi, P., and Prabha, V. Positive effect of probiotic Lactobacillus plantarum in reversing the LPS induced infertility in mouse model. J.Med.Microbiol. 2-12-2016;

ABSTRACT: Bacterial infections have high incidence among the female population at reproductive age and are widely known to cause infertility due to inflammation. The purpose of the present work was to investigate the effect of the inflammatory agent, LPS, on fertility outcome and to evaluate the ability of Lactobacillus plantarum in ameliorating the LPS mediated inflammation induced infertility. Female BALB/c mice infused intravaginally with a single dose of 20microl sterile normal saline containing 5, 10 or 20microg LPS were divided into two sets for evaluation of tissue histology and pregnancy outcome. In set I, aimed at observing changes in tissue histology, inflammation was observed in vaginal sections of mice instilled with a single dose of 20microg LPS, which were sacrificed on day 2 and 5. In set II, aimed at evaluating pregnancy outcome, female mice administered with 20microg LPS were rendered infertile upon mating on day 2, 5 and 8. In another experiment, normal histology of the vaginal sections was observed in set I, administered with single dose of 20microg LPS followed by 108 CFU/20 microl of L. plantarum, till the mice were sacrificed on day 2, 5 and 8. Upon similar treatment to mice in set II, female mice, when mated with proven

male breeder mice on day 2, 5 and 8 retained fertility and delivered pups. These results were further confirmed by the downregulation of pro-inflammatory cytokines and increase in anti-inflammatory cytokines on treatment with L. plantarum revealing the role of probiotics in ameliorating inflammation induced infertility.

Boursier, J., Mueller, O., Barret, M., Machado, M., Fizanne, L., raujo-Perez, F., Guy, C. D., Seed, P. C., Rawls, J. F., David, L. A., Hunault, G., Oberti, F., Cales, P., and Diehl, A. M. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. Hepatology. 2016; 63(3):764-775.

ABSTRACT: Several animal studies have emphasized the role of gut microbiota in nonalcoholic fatty liver disease (NAFLD). However, data about gut dysbiosis in human NAFLD remain scarce in the literature, especially studies including the whole spectrum of NAFLD lesions. We aimed to evaluate the association between gut dysbiosis and severe NAFLD lesions, that is, nonalcoholic steatohepatitis (NASH) and fibrosis, in a well-characterized population of adult NAFLD. Fifty-seven patients with biopsy-proven NAFLD were enrolled. Taxonomic composition of gut microbiota was determined using 16S ribosomal RNA gene sequencing of stool samples. Thirty patients had F0/F1 fibrosis stage at liver biopsy (10 with NASH), and 27 patients had significant F>/=2 fibrosis (25 with NASH). Bacteroides abundance was significantly increased in NASH and F>/=2 patients, whereas Prevotella abundance was decreased. Ruminococcus abundance was significantly higher in F>/=2 patients. By multivariate analysis, Bacteroides abundance was independently associated with NASH and Ruminococcus with F>/=2 fibrosis. Stratification according to the abundance of these two bacteria generated three patient subgroups with increasing severity of NAFLD lesions. Based on imputed metagenomic profiles, Kyoto Encyclopedia of Genes and Genomes pathways significantly related to NASH and fibrosis F>/=2 were mostly related to carbohydrate, lipid, and amino acid metabolism. CONCLUSION: NAFLD severity associates with gut dysbiosis and a shift in metabolic function of the gut microbiota. We identified Bacteroides as independently associated with NASH and Ruminococcus with significant fibrosis. Thus, gut microbiota analysis adds information to classical predictors of NAFLD severity and suggests novel metabolic targets for pre-/probiotics therapies. (Hepatology 2016;63:764-775).

Briskey, D., Tucker, P., Johnson, D. W., and Coombes, J. S. The role of the gastrointestinal tract and microbiota on uremic toxins and chronic kidney disease development. Clin.Exp.Nephrol. 3-10-2016;

ABSTRACT: It is well-established that uremic toxins are positively correlated with the risk of developing chronic kidney disease and cardiovascular disease. In addition, emerging data suggest that gut bacteria exert an influence over both the production of uremic toxins and the development of chronic kidney disease. As such, modifying the gut microbiota may have the potential as a treatment for chronic kidney disease. This is supported by data that suggest that rescuing microbiota dysbiosis may: reduce uremic toxin production; prevent toxins and pathogens from crossing the intestinal barrier; and, reduce gastrointestinal tract transit time allowing nutrients to reach the microbiota in the distal portion of the gastrointestinal tract. Despite emerging literature, the gut-kidney axis has yet to be fully explored. A special focus should be placed on examining clinically translatable strategies that might encourage improvements to the microbiome, thereby potentially reducing the risk of the development of chronic kidney disease. This review

aims to present an overview of literature linking changes to the gastrointestinal tract with microbiota dysbiosis and the development and progression of chronic kidney disease.

Budzynski, J., Wisniewska, J., Ciecierski, M., and Kedzia, A. Association between Bacterial Infection and Peripheral Vascular Disease: A Review. Int.J.Angiol. 2016; 25(1):3-13.

ABSTRACT: There are an increasing number of data showing a clinically important association between bacterial infection and peripheral artery disease (PAD). Bacteria suspected of being involved in PAD pathogenesis are: periodontal bacteria, gut microbiota, Helicobacter pylori, and Chlamydia pneumoniae. Infectious agents may be involved in the pathogenesis of atherosclerosis via activation of a systemic or local host immunological response to contamination of extravascular tissues or the vascular wall, respectively. A systemic immunological reaction may damage vascular walls in the course of autoimmunological cross-reactions between anti-pathogen antibodies and host vascular antigens (immunological mimicry), pathogen burden mechanisms (nonspecific activation of inflammatory processes in the vascular wall), and neuroendocrine-immune cross-talk. Besides activating the inflammatory pathway, bacterial infection may trigger PAD progression or exacerbation by enhancement of platelet reactivity, by a stimulatory effect on von Willebrand factor binding, factor VIII, fibrinogen, P-selectin activation, disturbances in plasma lipids, increase in oxidative stress, and resistance to insulin. Local inflammatory host reaction and induction of atherosclerotic plaque progression and/or instability result mainly from atherosclerotic plaque colonization by microorganisms. Despite these premises, the role of bacterial infection in PAD pathogenesis should still be recognized as controversial, and randomized, controlled trials are required to evaluate the outcome of periodontal or gut bacteria modification (through diet, prebiotics, and probiotics) or eradication (using antibiotics) in hard and surrogate cardiovascular endpoints.

Bultman, S. J. The microbiome and its potential as a cancer preventive intervention. Semin.Oncol. 2016; 43(1):97-106.

ABSTRACT: It is becoming increasingly clear that microbiota inhabiting our bodies influence cancer predisposition and etiology. In addition to pathogens with oncogenic properties, commensal and symbiotic microbiota have tumor-suppressive properties. Diet and other environmental factors can modulate the abundance of certain members of microbial communities within the gastrointestinal tract and at other anatomical sites. Furthermore, some dietary factors are metabolized by commensal/symbiotic gut microbiota into bioactive food components believed to prevent cancer. For example, dietary fiber undergoes bacterial fermentation in the colon to yield butyrate, which is a short-chain fatty acid and histone deacetylase (HDAC) inhibitor that suppresses the viability and growth of colorectal cancer cell lines. A recent study using gnotobiotic mouse models demonstrates that fiber can protect against colorectal tumorigenesis in a microbiota- and butyrate-dependent manner that involves the Warburg effect. This and other examples suggest that some of the inter-individual variation observed in epidemiology and intervention studies that have investigated associations between diet and cancer risk might be explained by differences in microbiota among the participants. Data from basic research studies also support the idea that probiotics and prebiotics could be plausible chemoprevention strategies that may be utilized to a greater extent in the future.

Caggianiello, G., Kleerebezem, M., and Spano, G. Exopolysaccharides produced by lactic acid bacteria: from health-promoting benefits to stress tolerance mechanisms. Appl.Microbiol.Biotechnol. 3-28-2016;

ABSTRACT: A wide range of lactic acid bacteria (LAB) is able to produce capsular or extracellular polysaccharides, with various chemical compositions and properties. Polysaccharides produced by LAB alter the rheological properties of the matrix in which they are dispersed, leading to typically viscous and "ropy" products. Polysaccharides are involved in several mechanisms such as prebiosis and probiosis, tolerance to stress associated to food process, and technological properties of food. In this paper, we summarize the beneficial properties of exopolysaccharides (EPS) produced by LAB with particular attention to prebiotic properties and to the effect of exopolysaccharides on the LAB-host interaction mechanisms, such as bacterial tolerance to gastrointestinal tract conditions, ability of ESP-producing probiotics to adhere to intestinal epithelium, their immune-modulatory activity, and their role in biofilm formation. The pro-technological aspect of exopolysaccharides is discussed, focusing on advantageous applications of EPS in the food industry, i.e., yogurt and gluten-free bakery products, since it was found that these microbial biopolymers positively affect the texture of foods. Finally, the involvement of EPS in tolerance to stress conditions that are commonly encountered in fermented beverages such as wine is discussed.

Celebioglu, H. U., Ejby, M., Majumder, A., Kobler, C., Jun, Goh Y., Thorsen, K., Schmidt, B., O'Flaherty, S., Abou, Hachem M., Lahtinen, S. J., Jacobsen, S., Klaenhammer, T. R., Brix, S., Molhave, K., and Svensson, B. Differential proteome and cellular adhesion analyses of the probiotic bacterium Lactobacillus acidophilus NCFM grown on raffinose - an emerging prebiotic. Proteomics. 3-9-2016;

ABSTRACT: Whole cell and surface proteomes were analyzed together with adhesive properties of the probiotic bacterium Lactobacillus acidophilus NCFM (NCFM) grown on the emerging prebiotic raffinose, exemplifying a synbiotic. Adhesion of NCFM to mucin and intestinal HT-29 cells increased three-fold after culture with raffinose versus glucose, as also visualized by scanning electron microscopy. Comparative proteomics using 2D-DIGE showed 43 unique proteins to change in relative abundance in whole cell lysates from NCFM grown on raffinose compared to glucose. Furthermore, 14 unique proteins in 18 spots of the surface subproteome underwent changes identified by differential 2DE, including elongation factor G, thermostable pullulanase, and phosphate starvation inducible stress-related protein increasing in a range of +2.1 - +4.7 fold. By contrast five known moonlighting proteins decreased in relative abundance by up to -2.4 fold. Enzymes involved in raffinose catabolism were elevated in the whole cell proteome; alpha-galactosidase (+13.9 fold); sucrose phosphorylase (+5.4 fold) together with metabolic enzymes from the Leloir pathway for galactose utilization and the glycolysis; beta-galactosidase (+5.7 fold); galactose (+2.9/+3.1 fold) and fructose (+2.8 fold) kinases. The insights at the molecular and cellular levels contributed to the understanding of the interplay of a synbiotic composed of NCFM and raffinose with the host. This article is protected by copyright. All rights reserved.

Cerezuela, R., Guardiola, F. A., Cuesta, A., and Esteban, M. A. Enrichment of gilthead seabream (Sparus aurata L.) diet with palm fruit extracts and probiotics: Effects on skin mucosal immunity. Fish.Shellfish.Immunol. 2016; 49:100-109.

ABSTRACT: Fish skin mucus contains numerous immune substances still poorly studied. To date, there are no studies regarding the possible influence of dietary supplements on such important substances. In the present work, a commercial diet used as control diet was enriched with: 1) probiotic Shewanella putrefaciens (Pdp11 diet, 10(9) cfu g(-1)); 2) probiotic Bacillus sp. (Bacillus diet, 10(9) ufc g(-1)); 3) aqueous date palm fruits extracts (DPE diet, 4%), and 4) a combination of Pdp11 + Bacillus sp + aqueous DPE (Mix diet). After 2 and 4 weeks of the feeding trial, enzymatic activities (proteases, antiproteases and peroxidases), IgM levels and terminal carbohydrates abundance were determined in skin mucus. In addition, the expression of certain immune related genes was evaluated in the skin. Our results demonstrated the significant alteration of the terminal carbohydrate abundance in skin mucus. Carbohydrates more affected by experimental diets were N-acetyl-galactosamine, N-acetyl-glucosamine, galactose, mannose, glucose and fucose. IgM, peroxidase activity and protease were also significantly higher in fish fed enriched diets. For last, an important up-regulation on the immune related gene studied on the skin was also detected. Present findings provide robust evidence that fish skin mucosal immunity can be improved by the diet.

Chen, Y. P., Hsu, C. A., Hung, W. T., and Chen, M. J. Effects of Lactobacillus paracasei 01 fermented milk beverage on protection of intestinal epithelial cell in vitro. J.Sci.Food Agric. 2016; 96(6):2154-2160.

ABSTRACT: BACKGROUND: Intestinal protection is an important function of probiotics. However, there is no evidence that the potential probiotic Lactobacillus paracasei 01 is able to improve intestinal function. In the present study, the protective effect and underlying mechanisms of L. paracasei 01 on intestinal epithelial cell in vitro were investigated. RESULTS: A fermented milk beverage containing L. paracasei 01 at 3 x 10(8) CFU mL(-1) was made from skim milk powder, glucose, sucrose and isomaltooligosaccharide. Results indicated that L. paracasei 01 fermented milk beverage could reduce macromolecule permeability caused by dextran sodium sulfate-induced damage. Besides, 50 mL L(-1) fermented milk beverage with live L. paracasei 01 in the cell medium could increase intestinal epithelial cell Caco-2 growth, decrease lipopolysaccharide/tumor necrosis factor-alpha (TNF-alpha)/interferon-gamma (IFN-gamma)-induced Caco-2 cell death and chemokine CCL-20 production. The protecting mechanisms involve promoting intestinal epithelial cell growth and intestinal epithelial integrity to strengthen the intestinal barrier against chemical and inflammatory stimuli-induced damage. Inhibition of inflammatory cytokines and chemokine such as IFN-gamma, TNF-alpha and CCL-20 also contributes to the beneficial effects of this product on epithelial function. CONCLUSION: Lactobacillus paracasei 01 fermented milk beverage may be a new functional food with intestinal protecting effects in vitro. (c) 2015 Society of Chemical Industry.

Cherdyntseva, T. A., Kotova, I. B., and Netrusov, A. I. The Isolation, Identification and Analyses of Lactobacillus Genus Bacteria with Probiotic Potential. Adv.Exp.Med.Biol. 2016; 897:103-111.

ABSTRACT: 103 strains of lactic acid bacteria of Lactobacillus genus were isolated from natural sources and identified for genus and species level with API tests and 16S rRNA sequencing. However, only 27 strains from isolated cultures demonstrated a high stability to gastric stress and from that - only 15 strains were highly resistant to intestinal stress.

Results indicated that only some isolated cultures of lactobacilli possessed potential probiotical properties and could serve as new probiotics for dairy industry with high resistance to gastro-intestinal stresses.

Choi, I. D., Kim, S. H., Jeong, J. W., Lee, D. E., Huh, C. S., Hong, S. S., Sim, J. H., and Ahn, Y. T. Triglyceride-Lowering Effects of Two Probiotics, Lactobacillus plantarum KY1032 and Lactobacillus curvatus HY7601, in a Rat Model of High-Fat Diet-Induced Hypertriglyceridemia. J.Microbiol.Biotechnol. 3-28-2016; 26(3):483-487.

ABSTRACT: The triglyceride-lowering effect of probiotics Lactobacillus plantarum KY1032 and Lactobacillus curvatus HY7601 were investigated. Male SD Wistar rats were randomly divided into three groups and fed high-fat diet (HFD), HFD and probiotics (5 X 10(9) CFU/day of L. plantarum KY1032 and 5 X 10(9) CFU/day of L. curvatus HY7601), or normal diet for 6 weeks. Probiotic treatment significantly lowered the elevated plasma triglyceride and increased plasma free fatty acid, glycerol, and plasma apolipoprotein A-V (ApoA-V) levels. The probiotic-treated group showed elevated hepatic mRNA expression of PPARalpha, bile acid receptor (FXR), and ApoA-V. These results demonstrate that L. plantarum KY1032 and L. curvatus HY7601 lower triglycerides in hypertriglyceridemic rats by upregulating ApoA-V, PPARalpha, and FXR.

Chuah, L. O., Effarizah, M. E., Goni, A. M., and Rusul, G. Antibiotic Application and Emergence of Multiple Antibiotic Resistance (MAR) in Global Catfish Aquaculture. Curr.Environ.Health Rep. 4-1-2016;

ABSTRACT: Catfish is one of the most cultivated species worldwide. Antibiotics are usually used in catfish farming as therapeutic and prophylactic agents. In the USA, only oxytetracycline, a combination of sulfadimethoxine and ormetoprim, and florfenicol are approved by the Food Drug Administration for specific fish species (e.g., catfish and salmonids) and their specific diseases. Misuse of antibiotics as prophylactic agents in disease prevention, however, is common and contributes in the development of antibiotic resistance. Various studies had reported on antibiotic residues and/or resistance in farmed species, feral fish, water column, sediments, and, in a lesser content, among farm workers. Ninety percent of the world aquaculture production is carried out in developing countries, which lack regulations and enforcement on the use of antibiotics. Hence, efforts are needed to promote the development and enforcement of such a regulatory structure. Alternatives to antibiotics such as antibacterial vaccines, bacteriophages and their lysins, and probiotics have been applied to curtail the increasing emergence of antibiotic-resistant bacteria due to the imprudent application of antibiotics in aquaculture.

Ciani, O., Arendsen, E., Romancik, M., Lunik, R., Costantini, E., Di, Biase M., Morgia, G., Fragala, E., Roman, T., Bernat, M., Guazzoni, G., Tarricone, R., and Lazzeri, M. Intravesical administration of combined hyaluronic acid (HA) and chondroitin sulfate (CS) for the treatment of female recurrent urinary tract infections: a European multicentre nested case-control study. BMJ Open. 2016; 6(3):e009669

ABSTRACT: OBJECTIVES: To compare the clinical effectiveness of the intravesical administration of combined hyaluronic acid and chondroitin sulfate (HA+CS) versus current standard management in adult women with recurrent urinary tract infections

(RUTIs). SETTING: A European Union-based multicentre, retrospective nested case-control study. PARTICIPANTS: 276 adult women treated for RUTIs starting from 2009 to 2013. INTERVENTIONS: Patients treated with either intravesical administration of HA+CS or standard of care (antimicrobial/immunoactive prophylaxis/probiotics/cranberry). PRIMARY AND SECONDARY OUTCOME MEASURES: The primary outcome was occurrence of bacteriologically confirmed recurrence within 12 months. Secondary outcomes were time to recurrence, total number of recurrences, health-related quality of life and healthcare resource consumption. Crude and adjusted results for unbalanced characteristics are presented. RESULTS: 181 patients treated with HA+CS and 95 patients treated with standard of care from 7 centres were included. The crude and adjusted ORs (95% CI) for the primary end point were 0.77 (0.46 to 1.28) and 0.51 (0.27 to 0.96), respectively. However, no evidence of improvement in terms of total number of recurrences (incidence rate ratio (95% CI), 0.99 (0.69 to 1.43)) or time to first recurrence was seen (HR (95% CI), 0.99 (0.61 to 1.61)). The benefit of intravesical HA+CS therapy improves when the number of instillations is >/=5. CONCLUSIONS: Our results show that bladder instillations of combined HA+CS reduce the risk of bacteriologically confirmed recurrences compared with the current standard management of RUTIs. Total incidence rates and hazard rates were instead non-significantly different between the 2 groups after adjusting for unbalanced factors. In contrast to what happens with antibiotic prophylaxis, the effectiveness of the HA+CS reinstatement therapy improves over time. TRIAL REGISTRATION NUMBER: NCT02016118.

Cordero, H., Morcillo, P., Meseguer, J., Cuesta, A., and Esteban, M. A. Effects of Shewanella putrefaciens on innate immunity and cytokine expression profile upon high stocking density of gilthead seabream specimens. Fish.Shellfish.Immunol. 2016; 51:33-40.

ABSTRACT: High stocking density increases the number of emerging diseases triggering economic losses worldwide. Probiotics provide an effective and natural solution for preventing some diseases through an improvement of innate immune system among others. In the present work dietary administration of the probiotic Shewanella putrefaciens (known as Pdp11) was evaluated under stress by high stocking density after 2 and 4 weeks of administration to gilthead seabream (Sparus aurata) specimens. Results showed an increase in cellular peroxidase and respiratory burst activity as well as a modulation of cytokine profile when Pdp11 was administered to fish reared at high stocking density. Overall, our results showed how Pdp11 is not only able to improve to some extent the cellular and humoral immunity but also to increase the gene expression profile of pro-inflammatory cytokines such as il1b or il6 in response to high stocking density in gilthead seabream. These findings may support the potential use of this probiotic as functional feed against stress in fish farms.

Costeloe, K., Hardy, P., Juszczak, E., Wilks, M., and Millar, M. R. Bifidobacterium breve BBG-001 in very preterm infants: a randomised controlled phase 3 trial. Lancet. 2-13-2016; 387(10019):649-660.

ABSTRACT: BACKGROUND: Probiotics may reduce necrotising enterocolitis and late-onset sepsis after preterm birth. However, there has been concern about the rigour and generalisability of some trials and there is no agreement about whether or not they should be used routinely. We aimed to test the effectiveness of the probiotic Bifidobacterium breve BBG-001 to reduce necrotising enterocolitis, late-onset sepsis, and death in preterm infants. METHODS: In this multicentre, randomised controlled phase 3

study (the PiPS trial), we recruited infants born between 23 and 30 weeks' gestational age within 48 h of birth from 24 hospitals in southeast England. Infants were randomly assigned (1:1) to probiotic or placebo via a minimisation algorithm randomisation programme. The probiotic intervention was B breve BBG-001 suspended in dilute elemental infant formula given enterally in a daily dose of 8.2 to 9.2 log10 CFU; the placebo was dilute infant formula alone. Clinicians and families were masked to allocation. The primary outcomes were necrotising enterocolitis (Bell stage 2 or 3), blood culture positive sepsis more than 72 h after birth; and death before discharge from hospital. All primary analyses were by intention to treat. This trial is registered with ISRCTN, number 05511098 and EudraCT, number 2006-003445-17. FINDINGS: Between July 1, 2010, and July 31, 2013, 1315 infants were recruited; of whom 654 were allocated to probiotic and 661 to placebo. Five infants had consent withdrawn after randomisation, thus 650 were analysed in the probiotic group and 660 in the placebo group. Rates of the primary outcomes did not differ significantly between the probiotic and placebo groups. 61 infants (9%) in the probiotic group had necrotising enterocolitis compared with 66 (10%) in the placebo group (adjusted risk ratio 0.93 (95% CI 0.68-1.27); 73 (11%) infants in the probiotics group had sepsis compared with 77 (12%) in the placebo group (0.97 (0.73-1.29); and 54 (8%) deaths occurred before discharge home in the probiotic group compared with 56 (9%) in the placebo group (0.93 [0.67-1.30]). No probiotic-associated adverse events were reported. INTERPRETATION: There is no evidence of benefit for this intervention in this population; this result does not support the routine use of B breve BBG-001 for prevention of necrotising enterocolitis and late-onset sepis in very preterm infants. FUNDING: UK National Institute for Health Research Health Technology Assessment programme.

Cui, S., Zhao, J., Liu, X., Chen, Y. Q., Zhang, H., and Chen, W. Maximum-biomass prediction of homofermentative Lactobacillus. J.Biosci.Bioeng. 2-17-2016;

ABSTRACT: Fed-batch and pH-controlled cultures have been widely used for industrial production of probiotics. The aim of this study was to systematically investigate the relationship between the maximum biomass of different homofermentative Lactobacillus and lactate accumulation, and to develop a prediction equation for the maximum biomass concentration in such cultures. The accumulation of the end products and the depletion of nutrients by various strains were evaluated. In addition, the minimum inhibitory concentrations (MICs) of acid anions for various strains at pH 7.0 were examined. The lactate concentration at the point of complete inhibition was not significantly different from the MIC of lactate for all of the strains, although the inhibition mechanism of lactate and acetate on Lactobacillus rhamnosus was different from the other strains which were inhibited by the osmotic pressure caused by acid anions at pH 7.0. When the lactate concentration accumulated to the MIC, the strains stopped growing. The maximum biomass was closely related to the biomass yield per unit of lactate produced (YX/P) and the MIC (C) of lactate for different homofermentative Lactobacillus. Based on the experimental data obtained using different homofermentative Lactobacillus, a prediction equation was established as follows: Xmax - X0 = (0.59 +/- 0.02).YX/P.C.

Culpepper, T., Christman, M. C., Nieves, C., Jr., Specht, G. J., Rowe, C. C., Spaiser, S. J., Ford, A. L., Dahl, W. J., Girard, S. A., and Langkamp-Henken, B. Bifidobacterium bifidum R0071 decreases stress-associated diarrhoea-related symptoms and self-reported stress: a secondary analysis of a randomised trial. Benef.Microbes. 2-3-2016; 1-10.

ABSTRACT: Psychological stress is associated with gastrointestinal (GI) distress. This secondary analysis from a randomised, double-blind, placebo-controlled study examined whether three different probiotics could normalise self-reported stress-associated GI discomfort and reduce overall self-reported stress. Undergraduate students (n=581) received Lactobacillus helveticus R0052, Bifidobacterium longum ssp. infantis R0033, Bifidobacterium bifidum R0071, or placebo. Participants self-reported 2 outcomes for a 6-week period, which included final academic exams: daily level of stress (0=no stress to 10=extremely stressed) and weekly three diarrhoea-related symptoms (DS, 1=no discomfort to 7=severe discomfort) using the GI Symptom Rating Scale. Self-reported stress was positively related to DS (P=0.0068). Mean DS scores were lower with B. bifidum versus placebo at week 2 at the average level of stress and the average body mass index (BMI). DS scores were lower with B. bifidum at week 5 versus week 0 and 1 and with B. infantis R0033 at week 6 versus week 0. DS scores were higher when antibiotics were used in the prior week with placebo (P=0.0092). DS were not different with or without antibiotic use with the probiotics. Only B. bifidum had an effect on self-reported stress scores (P=0.0086). The self-reported stress score was also dependent on hours of sleep per day where it decreased by 0.13 for each additional hour of sleep. During a stressful period, B. bifidum R0071 decreases DS and self-reported stress scores. This trial was registered at clinicaltrials.gov as NCT01709825.

Dani, C., Coviello, C. C., Corsini, I., I, Arena, F., Antonelli, A., and Rossolini, G. M. Lactobacillus Sepsis and Probiotic Therapy in Newborns: Two New Cases and Literature Review. AJP Rep. 2016; 6(1):e25-e29.

ABSTRACT: Many term and preterm infants are commonly supplemented with probiotics to prevent adverse effects of antibiotic administration and necrotizing enterocolitis and they are believed to be safe. However, the supplementation with Lactobacillus rhamnosus GG has been associated with the development of sepsis with a cause-effect relationship in six newborns and children. In this study, we report two further cases and discuss the emerging issue of probiotic supplementation safety in neonates. We conclude that physicians must be aware that supplementation with L. rhamnosus GG can cause sepsis in high-risk patients on rare occasions.

Das, J. K., Mahapatra, R., Patro, S., Goswami, C., and Suar, M. Lactobacillus acidophilus binds to MUC3 component of cultured intestinal epithelial cells with highest affinity. FEMS Microbiol.Lett. 3-4-2016;

ABSTRACT: Lactobacillus strains have been shown to adhere to the mucosal components of intestinal epithelial cells. However, established in-vitro adhesion assays have several drawbacks in assessing the adhesion of new Lactobacillus strains. The present study aimed to compare the adhesion of four different Lactobacillus strains and select the most adherent microbe, based on in-silico approach supported by in-vitro results. The mucus binding proteins in Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus brevis and Lactobacillus fermentum were identified and their capacities to interact with intestinal mucin were compared by molecular docking analysis. Lactobacillus acidophilus had the maximal affinity of binding to mucin with predicted free energy of -6.066 kcal/mol. Further, in-vitro experimental assay of adhesion was performed to validate the in-silico results. The adhesion of Lactobacillus acidophilus to mucous secreting colon epithelial HT-29 MTX cells was highest at 12% and it formed biofilm with maximum depth (Z = 84mum). Lactobacillus acidophilus was determined to

be the most adherent strain in the study. All the Lactobacillus strains tested in this study, displayed maximum affinity of binding to MUC3 component of mucus as compared to other gastrointestinal mucins. These findings may have importance in the design of probiotics and health care management.

De, Angelis M., Calasso, M., Cavallo, N., Di, Cagno R., and Gobbetti, M. Functional proteomics within the genus Lactobacillus. Proteomics. 2016; 16(6):946-962.

ABSTRACT: Lactobacillus are mainly used for the manufacture of fermented dairy, sourdough, meat, and vegetable foods or used as probiotics. Under optimal processing conditions, Lactobacillus strains contribute to food functionality through their enzyme portfolio and the release of metabolites. An extensive genomic diversity analysis was conducted to elucidate the core features of the genus Lactobacillus, and to provide a better comprehension of niche adaptation of the strains. However, proteomics is an indispensable "omics" science to elucidate the proteome diversity, and the mechanisms of regulation and adaptation of Lactobacillus strains. This review focuses on the novel and comprehensive knowledge of functional proteomics and metaproteomics of Lactobacillus species. A large list of proteomic case studies of different Lactobacillus species is provided to illustrate the adaptability of the main metabolic pathways (e.g., carbohydrate transport and metabolism, pyruvate metabolism, proteolytic system, amino acid metabolism, and protein synthesis) to various life conditions. These investigations have highlighted that lactobacilli modulate the level of a complex panel of proteins to growth/survive in different ecological niches. In addition to the general regulation and stress response, specific metabolic pathways can be switched on and off, modifying the behavior of the strains.

Delcaru, C., Alexandru, I., Podgoreanu, P., Cristea, V. C., Bleotu, C., Chifiriuc, M. C., Bezirtzoglou, E., and Lazar, V. Antagonistic activities of some Bifidobacterium sp. strains isolated from resident infant gastrointestinal microbiota on Gram-negative enteric pathogens. Anaerobe. 2-26-2016; 39:39-44.

ABSTRACT: The gastrointestinal microbiota contributes to the consolidation of the anti-infectious barrier against enteric pathogens. The purpose of this study was to investigate the influence of Bifidobacterium sp. strains, recently isolated from infant gastrointestinal microbiota on the in vitro growth and virulence features expression of enteropathogenic bacterial strains. The antibacterial activity of twelve Bifidobacterium sp. strains isolated from human feces was examined in vitro against a wide range of Gram negative pathogenic strains isolated from 30 infant patients (3 days to 5 years old) with diarrhea. Both potential probiotic strains (Bifidobacterium longum, Bifidobacterium pseudocatenulatum, Bifidobacterium catenulatum, Bifidobacterium breve, Bifidobacterium ruminantium) and enteropathogenic strains (EPEC, EIEC, Klebsiella pneumoniae, Salmonella sp., Yersinia enterocolitica, Pseudomonas aeruginosa) were identified by MALDI-TOF and confirmed serologically when needed. The bactericidal activity, growth curve, adherence to the cellular HEp-2 substratum and production of soluble virulence factors have been assessed in the presence of different Bifidobacterium sp. cultures and fractions (whole culture and free-cell supernatants). Among the twelve Bifidobacterium sp. strains, the largest spectrum of antimicrobial activity against 9 of the 18 enteropathogenic strains was revealed for a B. breve strain recently isolated from infant intestinal feces. The whole culture and free-cell supernatant of B. breve culture decreased the multiplication rate, shortened the log phase and the total duration of the

growth curve, with an earlier entrance in the decline phase and inhibited the adherence capacity to a cellular substratum and the swimming/swarming motility too. These results indicate the significant probiotic potential of the B. breve strain.

Dini, C., Bolla, P. A., and de Urraza, P. J. Treatment of in vitro enterohemorrhagic E. coli infection using phage and probiotics. J.Appl.Microbiol. 3-11-2016;

ABSTRACT: Alternative therapies to antibiotics are receiving increased attention, mainly due to the proliferation of multi-resistant pathogens. Antibiotics have had poor outcomes in the treatment of bacterial infections such as with EHEC. AIMS: The combination of phage and probiotics against EHEC was assayed in vitro on infected Hep-2 cells. METHODS AND RESULTS: Phage and probiotics treatments on EHEC O157:H7 infected Hep-2 cells were assayed individually or combined. The effect of freeze-drying on phage and probiotics antimicrobial activity was also studied. While treatment with phage alone increased cells detachment caused by EHEC infection, the treatments with MM alone or in combination with phage proved to effectively diminish cell damage caused by EHEC infection. Combined treatment showed a decrease in apoptotic cells count of 57.3% and a reduction in EHEC adhesion to cell monolayer of 1.2 log CFU. The simultaneous use of phage and probiotics showed no antagonistic effect, and freeze-drying did not affect their antipathogenic activity. CONCLUSIONS: The combination of phage and probiotics has great potential for reducing the number of pathogens adhered to epithelial cells during EHEC O157:H7 infection and attenuating the cytotoxic effect derived from it. Further in vivo assays are needed for assessing the actual effectiveness of the treatment. SIGNIFICANCE AND IMPACT OF STUDY: This study presents a freeze-dried formulation of phage and probiotics capable of controlling EHEC infections and reducing epithelial cell damage in vitro. This article is protected by copyright. All rights reserved.

Distrutti, E., Monaldi, L., Ricci, P., and Fiorucci, S. Gut microbiota role in irritable bowel syndrome: New therapeutic strategies. World J.Gastroenterol. 2-21-2016; 22(7):2219-2241.

ABSTRACT: In the last decade the impressive expansion of our knowledge of the vast microbial community that resides in the human intestine, the gut microbiota, has provided support to the concept that a disturbed intestinal ecology might promote development and maintenance of symptoms in irritable bowel syndrome (IBS). As a correlate, manipulation of gut microbiota represents a new strategy for the treatment of this multifactorial disease. A number of attempts have been made to modulate the gut bacterial composition, following the idea that expansion of bacterial species considered as beneficial (Lactobacilli and Bifidobacteria) associated with the reduction of those considered harmful (Clostridium, Escherichia coli, Salmonella, Shigella and Pseudomonas) should attenuate IBS symptoms. In this conceptual framework, probiotics appear an attractive option in terms of both efficacy and safety, while prebiotics, synbiotics and antibiotics still need confirmation. Fecal transplant is an old treatment translated from the cure of intestinal infective pathologies that has recently gained a new life as therapeutic option for those patients with a disturbed gut ecosystem, but data on IBS are scanty and randomized, placebo-controlled studies are required.

Djanic, M., Pavlovic, N., Stanimirov, B., Stojancevic, T., Golocorbin-Kon, S., Bojic, G., and Mikov, M. Docking-based preliminary study on the interactions of bile acids with drugs at the transporter level in intestinal bacteria. Eur.Rev.Med.Pharmacol.Sci. 2016; 20(3):553-560.

ABSTRACT: OBJECTIVE: The aim of this study was to estimate the binding-affinities of different bile acids towards drug transporters in Lactobacillus acidophilus and Bifidobacterium longum in order to predict the influence of bile acids and probiotics interactions on drug pharmacokinetics. MATERIALS AND METHODS: In order to study interactions of bile acids with transporters of intestinal bacteria, molecular-docking step was performed, using SwissDock web-service. For the purpose of comparison, two natural bile acids, cholic acid (CA) and deoxycholic acid (DCA), and one semi-synthetic bile acid, 12-monoketocholic acid (MKC), were studied in parallel. The free-binding energy was used as the main criterion for ranking ligands. RESULTS: Studied bile acids exhibited different binding affinities towards bacterial transporters with MKC showing the most prominent effect. For the majority of studied transporters, the estimated affinities of bile acids decreased in the following order: MKC-CA-DCA. Namely, 38.7% of examined transport proteins gave the lowest free-binding energy with MKC. The weak inverse relationship between number of hydrogen bonds and estimated free-binding energies was revealed. CONCLUSIONS: The predominant effect of MKC for the majority of studied transport proteins suggests that keto group at carbon 12 of the steroid core has a significant influence on the properties of MKC and consequently, on interactions with membrane transporters. Present findings might have a role in the prediction of potential influence of bile acids and probiotics on drug pharmacokinetics.

Doan, T. N., Kong, D. C., Marshall, C., Kirkpatrick, C. M., and McBryde, E. S. Modeling the impact of interventions against Acinetobacter baumannii transmission in intensive care units. Virulence. 2-17-2016; 7(2):141-152.

ABSTRACT: The efficacy of infection control interventions against Acinetobacter baumannii remains unclear, despite such information being critical for effective prevention of the transmission of this pathogen. Mathematical modeling offers an alternative to clinical trials, which may be prohibitively expensive, unfeasible or unethical, in predicting the impact of interventions. Furthermore, it allows the ability to ask key "what if" questions to evaluate which interventions have the most impact. We constructed a transmission dynamic model to quantify the effects of interventions on reducing A. baumannii prevalence and the basic reproduction ratio (R0) in intensive care units (ICUs). We distinguished between colonization and infection, and incorporated antibiotic exposure and transmission from free-living bacteria in the environment. Under the assumptions and parameterization in our model, 25% and 18% of patients are colonized and infected with A. baumannii, respectively; and R0 is 1.4. Improved compliance with hand hygiene (>/=87%), enhanced environmental cleaning, reduced length of ICU stay of colonized patients (</= 10 days), shorter durations of antibiotic treatment of A. baumannii (</=6 days), and isolation of infected patients combined with cleaning of isolation rooms are effective, reducing R0 to below unity. In contrast, expediting the recovery of the intestinal microbiota (e.g. use of probiotics) is not effective. This study represents a biologically realistic model of the transmission dynamics of A. baumannii, and the most comprehensive analysis of the effectiveness of interventions against this pathogen. Our study provides important data for designing effective infection control interventions.

Drissi, F., Raoult, D., and Merhej, V. Metabolic role of lactobacilli in weight modification in humans and animals. Microb.Pathog. 3-23-2016;

ABSTRACT: Obesity represents a worldwide public health crisis. Trials suggested that intestinal microbiota may contribute to the development of obesity and highlighted the involvement of bacteria, including Lactobacillus spp., in changes to the host metabolism. Several experiments have shown significant effects of Lactobacillus probiotics on weight modification. Lactobacillus spp. are involved in the digestion of complex carbohydrates not digested by the host in the colon and also participate in the degradation of lipids and simple sugars in the duodenum and jejunum. Moreover, Lactobacillus species survive throughout the gastrointestinal tract, as they are able to survive in the presence of bile and low pH, and produce an antimicrobial agent, allowing them to reduce the number of bacteria in the gut. Hence, Lactobacillus spp. can have a significant impact on microbiota and, consequently, on weight change. Here, we review current studies of Lactobacillus spp. involved in weight change and discuss the different mechanisms of action by which Lactobacillus spp. acts on host digestion and appears to influence weight.

Durchschein, F., Petritsch, W., and Hammer, H. F. Diet therapy for inflammatory bowel diseases: The established and the new. World J.Gastroenterol. 2-21-2016; 22(7):2179-2194.

ABSTRACT: Although patients with inflammatory bowel diseases (IBD) have a strong interest in dietary modifications as part of their therapeutic management, dietary advice plays only a minor part in published guidelines. The scientific literature shows that dietary factors might influence the risk of developing IBD, that dysbiosis induced by nutrition contributes to the pathogenesis of IBD, and that diet may serve as a symptomatic treatment for irritable bowel syndrome-like symptoms in IBD. The role of nutrition in IBD is underscored by the effect of various dietary therapies. In paediatric patients with Crohn's disease (CD) enteral nutrition (EN) reaches remission rates similar to steroids. In adult patients, however, EN is inferior to corticosteroids. EN is not effective in ulcerative colitis (UC). Total parenteral nutrition in IBD is not superior to steroids or EN. The use of specific probiotics in patients with IBD can be recommended only in special clinical situations. There is no evidence for efficacy of probiotics in CD. By contrast, studies in UC have shown a beneficial effect in selected patients. For patients with pouchitis, antibiotic treatment followed by probiotics, like VSL#3 or Lactobacillus GG, is effective. When probiotics are used, the risk of bacterial translocation and subsequent bacteremia has to be considered. More understanding of the normal intestinal microflora, and better characterization of probiotic strains at the phenotypic and genomic levels is needed as well as clarification of the mechanisms of action in different clinical settings. A FODMAP reduced diet may improve symptoms in IBD.

Ehrhardt, S., Guo, N., Hinz, R., Schoppen, S., May, J., Reiser, M., Schroeder, M. P., Schmiedel, S., Keuchel, M., Reisinger, E. C., Langeheinecke, A., de, Weerth A., Schuchmann, M., Schaberg, T., Ligges, S., Eveslage, M., Hagen, R. M., Burchard, G. D., and Lohse, A. W. Saccharomyces boulardii to Prevent Antibiotic-Associated Diarrhea: A Randomized, Double-Masked, Placebo-Controlled Trial. Open.Forum Infect.Dis. 2016; 3(1):ofw011

ABSTRACT: Background. Antibiotic-associated diarrhea (AAD) and Clostridium difficile-associated diarrhea (CDAD) are common complications of antibiotic use. Data on the efficacy of probiotics to prevent AAD and CDAD are unclear. We aimed to evaluate the efficacy of Saccharomyces boulardii to prevent AAD and CDAD in hospitalized adult patients. Methods. We conducted a multicenter, phase III, double-masked, randomized, placebo-controlled trial in hospitalized patients who received systemic antibiotic

treatment in 15 hospitals in Germany between July 2010 and October 2012. Participants received Perenterol forte 250 mg capsules or matching placebo twice per day within 24 hours of initiating antibiotic treatment, continued treatment for 7 days after antibiotic discontinuation, and were then observed for 6 weeks. Results. Two thousand four hundred forty-four patients were screened. The trial was stopped early for futility after inclusion of 477 participants. Two hundred forty-six patients aged 60.1 +/- 16.5 years and 231 patients aged 56.5 +/- 17.8 were randomized to the S boulardii group and the placebo group, respectively, with 21 and 19 AADs in the respective groups (P = .87). The hazard ratio of AAD in the S boulardii group compared with the placebo group was 1.02 (95% confidence interval, .55-1.90; P = .94). Clostridium difficile-associated diarrhea occurred in 0.8% of participants (4 of 477). Nine serious adverse events were recorded in the S boulardii group, and 3 serious adverse events were recorded in the placebo group. None were related to study participation. Conclusions. We found no evidence for an effect of S boulardii in preventing AAD or CDAD in a population of hospitalized patients without particular risk factors apart from systemic antibiotic treatment. ClinicalTrials.gov Identifier. NCT01143272.

El, Enshasy H., Malik, K., Malek, R. A., Othman, N. Z., Elsayed, E. A., and Wadaan, M. Anaerobic Probiotics: The Key Microbes for Human Health. Adv.Biochem.Eng Biotechnol. 2-24-2016;

ABSTRACT: Human gastrointestinal microbiota (HGIM) incorporate a large number of microbes from different species. Anaerobic bacteria are the dominant organisms in this microbial consortium and play a crucial role in human health. In addition to their functional role as the main source of many essential metabolites for human health, they are considered as biotherapeutic agents in the regulation of different human metabolites. They are also important in the prevention and in the treatment of different physical and mental diseases. Bifidobacteria are the dominant anaerobic bacteria in HGIM and are widely used in the development of probiotic products for infants, children and adults. To develop bifidobacteria-based bioproducts, therefore, it is necessary to develop a large-scale biomass production platform based on a good understanding of the ideal medium and bioprocessing parameters for their growth and viability. In addition, high cell viability should be maintained during downstream processing and storage of probiotic cell powder or the final formulated product. In this work we review the latest information about the biology, therapeutic activities, cultivation and industrial production of bifidobacteria.

Elgin, T. G., Kern, S. L., and McElroy, S. J. Development of the Neonatal Intestinal Microbiome and Its Association With Necrotizing Enterocolitis. Clin.Ther. 2-3-2016;

ABSTRACT: PURPOSE: Neonatal necrotizing enterocolitis (NEC) remains the most devastating gastrointestinal disease for premature infants. In the United States alone, NEC affects >4000 premature infants yearly, has a mortality rate of nearly 33%, and costs the health care system >$1 billion annually. Although NEC has been actively researched for several decades, its pathophysiology remains elusive. One potential mechanism suggests that disruption of the normal neonatal intestinal bacterial flora induces a proinflammatory state, allowing translocation of pathogens across the intestinal epithelia. Disruption of the normal intestinal flora (dysbiosis) is associated with many human diseases. Thus, it is a reasonable hypothesis that dysbiosis may play an important role in the development of NEC. This hypothesis is supported by evidence that probiotic use in premature infants can prevent the development of NEC. Although the role of probiotics

and NEC is covered in other reviews, this review instead focuses on normal bacterial colonization in both term and preterm infants and on the association of dysbiosis and the development of NEC. METHODS: PubMed was queried with the use of the following key search terms: NEC, neonatal microbiome, fetal microbiome, maternal microbiome, neonatal dysbiosis, and microbiome ontogeny. Relevant literature was reviewed and selected for inclusion in accordance with the objectives of the article according to the authors' discretion. Articles that made key salient points in review articles were further pulled from PubMed. FINDINGS: Although the onset of NEC is thought to involve bacteria, the mechanisms behind their involvement remain unclear. Research to date has failed to identify a single causative organism, and current theories and data now indicate that a disruption of the host intestinal flora is associated with the onset of disease. Recent reports have found that a bloom of Proteobacteria, specifically Enterobacteriacae species, occurs just before the diagnosis of NEC. Whether this is a causative event or merely a marker of intestinal disease is still unclear. IMPLICATIONS: Because of the complexity of these interactions, it is vital that we continue to investigate the host-bacterial axis in the developing intestine in both humans and in animal models.

Embleton, N. D., Zalewski, S., and Berrington, J. E. Probiotics for prevention of necrotizing enterocolitis and sepsis in preterm infants. Curr.Opin.Infect.Dis. 3-26-2016;

ABSTRACT: PURPOSE OF REVIEW: Few areas in neonatal medicine have generated as much discussion and controversy as the use of prophylactic probiotics for the prevention of necrotizing enterocolitis. We summarize recent studies from the last 1-2 years. RECENT FINDINGS: Systematic reviews show that probiotics reduce the risk of necrotizing enterocolitis but there are methodological limitations to all the published trials, and the largest trial to date is at odds with the conclusions of the meta-analyses. Trials have used a range of commercially available products with differing species, and administered these at different times to heterogeneous populations of preterm babies. Although there is strong evidence to show that 'probiotics' are likely to represent a major advance for neonatal care, it is increasingly clear that not all species have beneficial effects in preterm infants. This makes interpretation of meta-analyses complex, and the determination of a single 'risk reduction' potentially flawed. SUMMARY: Despite current uncertainties, it is difficult for clinicians to ignore the current data, and increasing numbers now use commercially available products. It remains a matter of concern that many products lack the robust quality control most clinicians and parents would consider important for use in vulnerable populations. Head-to-head trials are needed.

Espinosa-Martos, I., Jimenez, E., de, Andres J., Rodriguez-Alcala, L. M., Tavarez, S., Manzano, S., Fernandez, L., Alonso, E., Fontecha, J., and Rodriguez, J. M. Milk and blood biomarkers associated to the clinical efficacy of a probiotic for the treatment of infectious mastitis. Benef.Microbes. 3-1-2016; 1-14.

ABSTRACT: Previous studies have shown the efficacy of oral administration of selected lactobacilli strains to treat mastitis. The objective of this study was to find microbiological, biochemical and/or immunological biomarkers of the probiotic effect. Women with (n=23) and without (n=8) symptoms of mastitis received three daily doses (109 cfu) of Lactobacillus salivarius PS2 for 21 days. Samples of milk, blood and urine were collected before and after the probiotic intervention, and screened for a wide spectrum of microbiological, biochemical and immunological parameters. In the mastitis group, L. salivarius PS2 intake led to a reduction in milk bacterial counts, milk and blood leukocyte

counts and interleukin (IL)-8 level in milk, an increase in those of immunoglobulin (Ig)E, IgG3, epidermal growth factor and IL-7, a modification of the milk electrolyte profile, and a reduction of some oxidative stress biomarkers. Such biomarkers will be useful in future clinical studies involving a larger cohort.

Etyemez, M. and Balcazar, J. L. Isolation and characterization of bacteria with antibacterial properties from Nile tilapia (Oreochromis niloticus). Res.Vet.Sci. 2016; 105:62-64.

ABSTRACT: One hundred and twenty bacterial isolates were obtained from the intestinal mucus of Nile tilapia (Oreochromis niloticus) and screened for antagonistic activity and adherence abilities. Based on in vitro antagonism against two pathogens (Streptococcus iniae and Edwardsiella piscicida), five isolates were selected and identified by 16S rRNA gene sequence analysis. All antagonistic isolates were affiliated to the genus Bacillus, which showed inhibitory activity against S. iniae. Only the isolate B191 (closely related to Bacillus mojavensis) inhibited the growth of both pathogens. Moreover, isolate B191 adhered significantly better to fish intestinal mucus than other antagonistic isolates. According to our results, these bacterial isolates, particularly isolate B191, should be further studied to explore their probiotic effects under in vivo conditions.

Ewe, J. A. and Loo, S. Y. Effect of cream fermentation on microbiological, physicochemical and rheological properties of L. helveticus-butter. Food Chem. 6-15-2016; 201:29-36.

ABSTRACT: The primary objective of this study was to evaluate the physicochemical and rheological properties of butter produced by Lactobacillus helveticus fermented cream. The incorporation of putative probiotic - the L. helveticus, to ferment cream prior to butter production was anticipated to alter the nutritional composition of butter. Changes in crude macronutrients and the resultant modification relating to textural properties of butter induced upon metabolic activities of L. helveticus in cream were focused in this research. Fermented butter (LH-butter) was produced by churning the cream that was fermented by lactobacilli at 37 degrees C for 24h. Physicochemical analysis, proximate analysis and rheology properties of LH-butter were compared with butter produced using unfermented cream (control). LH-butter showed a significantly ($P<0.05$) higher fat content and acid value; lower moisture and ash; and was softer than the control. Cream fermentation modified nutritional and textural properties of butter in which LH-butter contained higher health beneficial unsaturated fatty acids than the control and thus rendered the product softer. Its enrichment with probiotics could thus further enhance its functional property.

Fernandez, L., Cardenas, N., Arroyo, R., Manzano, S., Jimenez, E., Martin, V., and Rodriguez, J. M. Prevention of Infectious Mastitis by Oral Administration of Lactobacillus salivarius PS2 During Late Pregnancy. Clin.Infect.Dis. 3-1-2016; 62(5):568-573.

ABSTRACT: BACKGROUND: Previous studies have shown that oral administration of lactobacilli can be an efficient approach to treat lactational infectious mastitis. In this trial, we have evaluated the potential of Lactobacillus salivarius PS2 to prevent this condition when orally administered during late pregnancy to women who had experienced infectious mastitis after previous pregnancies. METHODS: In this study, 108 pregnant women were randomly assigned to one of 2 groups. Those in the probiotic group (n = 55) ingested daily 9 log10 colony-forming units of L. salivarius PS2 from

approximately week 30 of pregnancy until delivery, whereas those in the placebo group (n = 53) received a placebo. The occurrence of mastitis was evaluated during the first 3 months after delivery. RESULTS: Globally, 44 of 108 women (41%) developed mastitis; however, the percentage of women with mastitis in the probiotic group (25% [n = 14]) was significantly lower than in the control group (57% [n = 30]). When mastitis occurred, the milk bacterial counts in the probiotic group were significantly lower than those obtained in the placebo group. CONCLUSIONS: Oral administration of L. salivarius PS2 during late pregnancy appears to be an efficient method to prevent infectious mastitis in a susceptible population. CLINICAL TRIALS REGISTRATION: NCT01505361.

Fibi, S., Klose, V., Mohnl, M., Weber, B., Haslberger, A. G., and Sattler, V. A. Suppression subtractive hybridisation and real-time PCR for strain-specific quantification of the probiotic Bifidobacterium animalis BAN in broiler feed. J.Microbiol.Methods. 2016; 123:94-100.

ABSTRACT: To ensure quality management during the production processes of probiotics and for efficacy testing in vivo, accurate tools are needed for the identification and quantification of probiotic strains. In this study, a strain-specific qPCR assay based on Suppression Subtractive Hybridisation (SSH) for identifying unique sequences, was developed to quantify the strain Bifidobacterium animalis BAN in broiler feed. Seventy potential BAN specific sequences were obtained after SSH of the BAN genome, with a pool of closely related strain genomes and subsequent differential screening by dot blot hybridisation. Primers were designed for 30 sequences which showed no match with any sequence database entry, using BLAST and FASTA. Primer specificity was assessed by qPCR using 45 non-target strains and species in a stepwise approach. Primer T39_S2 was the only primer pair without any unspecific binding properties and it showed a PCR efficiency of 80% with a Cq value of 17.32 for 20ng BAN DNA. Optimised feed-matrix dependent calibration curve for the quantification of BAN was generated, ranging from 6.28x10(3)cfug(-1) to 1.61x10(6)cfug(-1). Limit of detection of the qPCR assay was 2x10(1)cfug(-1) BAN. Applicability of the strain-specific qPCR assay was confirmed in a spiking experiment which added BAN to the feed in two concentrations, 2x10(6)cfug(-1) and 2x10(4)cfug(-1). Results showed BAN mean recovery rates in feed of 1.44x10(6)+/-4.39x10(5)cfug(-1) and 1.59x10(4)+/-1.69x10(4)cfug(-1), respectively. The presented BAN-specific qPCR assay can be applied in animal feeding trials, in order to control the correct inclusion rates of the probiotic to the feed, and it could further be adapted, to monitor the uptake of the probiotic into the gastrointestinal tract of broiler chickens.

Firouzi, S., Majid, H. A., Ismail, A., Kamaruddin, N. A., and Barakatun-Nisak, M. Y. Effect of multi-strain probiotics (multi-strain microbial cell preparation) on glycemic control and other diabetes-related outcomes in people with type 2 diabetes: a randomized controlled trial. Eur.J.Nutr. 3-17-2016;

ABSTRACT: AIM: Evidence of a possible connection between gut microbiota and several physiological processes linked to type 2 diabetes is increasing. However, the effect of multi-strain probiotics in people with type 2 diabetes remains unclear. This study investigated the effect of multi-strain microbial cell preparation-also refers to multi-strain probiotics-on glycemic control and other diabetes-related outcomes in people with type 2 diabetes. DESIGN: A randomized, double-blind, parallel-group, controlled clinical trial. SETTING: Diabetes clinic of a teaching hospital in Kuala Lumpur, Malaysia. PARTICIPANTS: A total of 136 participants with type 2 diabetes, aged 30-70 years, were recruited and

randomly assigned to receive either probiotics (n = 68) or placebo (n = 68) for 12 weeks. OUTCOMES: Primary outcomes were glycemic control-related parameters, and secondary outcomes were anthropomorphic variables, lipid profile, blood pressure and high-sensitivity C-reactive protein. The Lactobacillus and Bifidobacterium quantities were measured before and after intervention as an indicator of successful passage of the supplement through gastrointestinal tract. STATISTICAL ANALYSIS: Intention-to-treat (ITT) analysis was performed on all participants, while per-protocol (PP) analysis was performed on those participants who had successfully completed the trial with good compliance rate. RESULTS: With respect to primary outcomes, glycated hemoglobin decreased by 0.14 % in the probiotics and increased by 0.02 % in the placebo group in PP analysis ($p < 0.05$, small effect size of 0.050), while these changes were not significant in ITT analysis. Fasting insulin increased by 1.8 microU/mL in placebo group and decreased by 2.9 microU/mL in probiotics group in PP analysis. These changes were significant between groups at both analyses ($p < 0.05$, medium effect size of 0.062 in PP analysis and small effect size of 0.033 in ITT analysis). Secondary outcomes did not change significantly. Probiotics successfully passed through the gastrointestinal tract. CONCLUSION: Probiotics modestly improved HbA1c and fasting insulin in people with type 2 diabetes.

Fuchs-Tarlovsky, V., Marquez-Barba, M. F., and Sriram, K. Probiotics in dermatologic practice. Nutrition. 2016; 32(3):289-295.

ABSTRACT: OBJECTIVE: Probiotics are live microorganisms that beneficially affect the host when administered in adequate amounts. They have an excellent safety profile. Probiotics have been used as immunomodulators in inflammatory skin conditions, such as atopic dermatitis. The aim of this study was to summarize the available evidence concerning the use of different strains of probiotics in dermatology practice. METHODS: We conducted a literature review of English and Spanish publications listed in standard databases (PubMed, Ovid, Google Scholar, Medline, and EBSCO), between 1994 and 2015 using the words "probiotics" and "dermatology." We found approximately 70 studies containing these criteria and selected 42 in which probiotics were used for dermatologic purposes. RESULTS: We found enough evidence to recommend the use of probiotics in specific conditions in dermatology practice, especially in children with atopic dermatitis. CONCLUSIONS: Further well-designed, large population based trials are needed to validate the use of probiotics in dermatology practice, including innovative therapies to rebuild skin barrier defects, protection against microbial colonization, and restoration of immunologic balance.

Gao, M., Song, H., Zheng, H., Ren, Y., Li, S., Liu, X., Yu, W., and Ma, X. Culture of low density E. coli cells in alginate-chitosan microcapsules facilitates stress resistance by up-regulating luxS/AI-2 system. Carbohydr.Polym. 5-5-2016; 141:160-165.

ABSTRACT: Entrapped low density cells with culture (ELDCwc) have been proved as a more effective way than direct entrapped high density cells (dEHDC) and free cells to protect probiotics from harsh environment, that is, to improve their stress resistance. The aim of this study was to investigate whether bacterial quorum sensing (QS) facilitated the stress resistance of Escherichia coli in microcapsules by detecting the expression of luxS/AI-2 system. As a result, both the expression of luxS gene and the concentration of autoinducer-2 (AI-2, QS signal molecule) have been discovered higher in ELDCwc than in

dEHDC and free cells. Besides that, the luxS mutant E. coli strain was used as a negative control of QS to verify the influence of QS on bacterial stress resistance in microcapsules. The significantly decreased viability of luxS mutant strain in simulated gastric fluid also indicated that the QS played a critical role in protecting microorganisms from severe environment.

Gao, X., Zhu, Y., Wen, Y., Liu, G., and Wan, C. Efficacy of probiotics in nonalcoholic fatty liver disease in adult and children: A meta-analysis of randomized controlled trials. Hepatol.Res. 2-11-2016;

ABSTRACT: OBJECTIVE: To evaluate the efficacy of probiotics in the treatment of adult and childhood Nonalcoholic Fatty Liver Disease (NAFLD). METHODS: Randomized controlled trials (RCTs) on the efficacy of probiotics in the treatment of adult and childhood NAFLD published before July 2015 were searched in multiple databases, including Cochrane library, PubMed/MEDLINE, EBSCO, OVID, SCI, CNKI, and VIP. Literature identification and data extraction were performed by 2 reviewers based on the inclusion and exclusion criteria. RevMan 5.3 software was used for Meta-analysis. RESULTS: A total of 9 RCTs with a total of 535 cases of NAFLD were included. Statistical differences of homeostasis model assessment (HOMA), total cholesterol (T-chol), high density lipoprotein (HDL), Triglyceride (TG), and Tumor necrosis factor-alpha (TNF- alpha) were detected between the probiotics and control groups with variations in different populations. No significant difference of body mass index (BMI), glucose and insulin were detected between the two groups. Statistically differences of low density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate transaminase (AST), and BMI were detected between the two childhood groups (P </= 0.05). CONCLUSION: Probiotics provided improvements in the outcomes of HOMA, Total-chol, HDL, TNF-alpha in any NAFLD patients, TG in Italian and Spanish patients, but no improvement in the outcomes of BMI, glucose, insulin in adult NAFLD patients; The currently available data are not sufficient to compare the the effects of probiotics between adult and childhood NAFLD patients. This article is protected by copyright. All rights reserved.

Ghomrassi, H., Ben, Braiek O., Choiset, Y., Haertle, T., Hani, K., Chobert, J. M., and Ghrairi, T. Evaluation of marine bacteriocinogenic enterococci strains with inhibitory activity against fish-pathogenic Gram-negative bacteria. Dis.Aquat.Organ. 2-11-2016; 118(1):31-43.

ABSTRACT: Use of lactic acid bacteria (LAB) as probiotics may provide an alternative to the use of antibiotics in aquaculture. LAB strains isolated from wild fish viscera and skin were evaluated for bacteriocin production and safety aspects (lack of antibiotic resistance, production of virulence factors). 16S rRNA gene sequences revealed the presence of Enterococcus faecium (13 isolates) and Lactococcus lactis (3 isolates) from fish samples. Pulsed-field gel electrophoresis analyses of the 13 enterococci isolates showed that they were all clustered, with greater than 95% similarity. However, RAPD analysis revealed significant molecular diversity between enterococci strains. Six enterococci strains were chosen and evaluated for their antibacterial activities. These strains produced a bacteriocin-like substance and exhibited a broad spectrum of inhibition against pathogenic bacteria isolated from diseased fish, including Streptococcus parauberis, Vagococcus spp., and Carnobacterium maltaromaticum, and in particular against the Gram-negative bacteria Flavobacterium frigidarium, Vibrio pectenicida, V. penaeicida, and Photobacterium damselae. The inhibition activity

towards bacterial indicator strains was at a maximum when bacteria were grown at 37 degrees C. However, bacteriocin production was observed at 15 degrees C after 12 h of incubation. Only structural genes of enterocins A and B were detected by PCR in the 6 enterococci strains, suggesting the production of these enterocins. In addition, these strains did not harbor any virulence factors or any significant antibiotic resistance, and they tolerated bile. Our results suggest that enterococci are an important part of the bacterial flora of fish and that some strains have the potential to be used as probiotics.

Ghoneim, M. A. and Moselhy, S. S. Antioxidant status and hormonal profile reflected by experimental feeding of probiotics. Toxicol.Ind.Health. 2016; 32(4):741-750.

ABSTRACT: Excessive production of free radicals can result in tissue damage, which mainly involves generation of hydroxyl radical and other oxidants. Such free radical-induced cell damage appears to play a major role in the pathogenesis of many diseases. Probiotics have been used therapeutically to modulate immunity, improve digestive processes, lower cholesterol, treat rheumatoid arthritis, and prevent cancer. The proposed research was designed to evaluate the changes in oxidative and antioxidative profile in addition to metabolic-related hormones of living animal model, which may generally affect the health status. Two groups of rabbits (10 animals each) were allocated in hygienic cages of controlled animal house. Control group received standard diet, and the other group received the same diet containing one probiotic for 30 days. Lactate dehydrogenase (LDH) activity in leukocytes, blood glucose, reduced glutathione (GSH), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were estimated in different tissues. Malondialdehyde (MDA) and total proteins were also determined in different tissues. Certain hormones related to metabolism and growth were also evaluated. Leukocytic LDH activity was significantly increased along with nonsignificant increase of blood glucose in probiotics-fed animals. Results showed significant decreases in the levels of triiodothyronine and thyroid-stimulating hormone but showed significant elevations in thyroxine, insulin, growth hormone, and testosterone levels in animals fed with probiotics. Total proteins content was highly significantly elevated in liver, kidneys, and muscles of probiotic-administered animals. Microsomal GSH level was significantly decreased only in skeletal muscles of probiotic-treated animals. MDA was significantly lowered in animal tissues fed with probiotics. GSH-Px activity was elevated in hepatic and muscular microsomes of probiotic-supplemented animals while it was nonsignificantly increased in renal microsomes. Microsomal SOD activity was elevated in liver, kidneys, and skeletal muscles of probiotics-administrated animals. It is concluded that supplementation of probiotic may enhance antioxidant efficacy and scavenge free radicals and thus may be used as a preventive measure for protection against free radicals-induced disorders.

Giddings, S. L., Stevens, A. M., and Leung, D. T. Traveler's Diarrhea. Med.Clin.North Am. 2016; 100(2):317-330.

ABSTRACT: Traveler's diarrhea (TD) is the most common travel-related illness, and it can have a significant impact on the traveler. Pretravel consultation provides an excellent opportunity for the clinician to counsel the traveler and discuss strategies such as food and water hygiene, vaccinations, and medications for prophylaxis or self-treatment that may decrease the incidence and impact of TD. Postinfectious sequelae, such as postinfectious irritable bowel syndrome, reactive arthritis, and Guillain-Barre syndrome, may develop weeks or months after return.

Gleeson, M. Immunological aspects of sport nutrition. Immunol.Cell Biol. 2016; 94(2):117-123.

ABSTRACT: Prolonged bouts of exercise and heavy training regimens are associated with depression of immune system functions that can increase the risk of picking up opportunistic infections such as the common cold and influenza. Some common sport nutrition practices including high-carbohydrate diets and carbohydrate ingestion during exercise, training with low-glycogen stores, intentional dieting for weight loss, ingestion of high-dose antioxidant supplements and protein ingestion post exercise may influence immune system status in athletes. In order to maintain robust immunity, athletes need to consume a well-balanced diet that is sufficient to meet their requirements for energy, carbohydrate, protein and micronutrients. Dietary deficiencies of protein and specific micronutrients are well known to be potential causes of immune dysfunction and an adequate intake of some essential minerals including iron and zinc and the vitamins A, D, E, B6 and B12 are important to maintain a healthy immune function. Vitamin D may be a particular concern as recent studies have emphasised its importance in limiting infection episode incidence and duration in both the general population and in athletes and many individuals exhibit inadequate vitamin D status during the winter months. There is only limited evidence that individual amino acids, beta-glucans, herbal extracts and zinc are capable of boosting immunity or reducing infection risk in athletes. The ingestion of carbohydrate during exercise and daily consumption of probiotics, vitamin D3, bovine colostrum and plant polyphenol containing supplements or foodstuffs currently offer the best chance of success, particularly for those individuals who are prone to illness.

Gleeson, M. and Pyne, D. B. Respiratory inflammation and infections in high-performance athletes. Immunol.Cell Biol. 2016; 94(2):124-131.

ABSTRACT: Upper respiratory illness is the most common reason for non-injury-related presentation to a sports medicine clinic, accounting for 35-65% of illness presentations. Recurrent or persistent respiratory illness can have a negative impact on health and performance of athletes undertaking high levels of strenuous exercise. The cause of upper respiratory symptoms (URS) in athletes can be uncertain but the majority of cases are related to common respiratory viruses, viral reactivation, allergic responses to aeroallergens and exercise-related trauma to the integrity of respiratory epithelial membranes. Bacterial respiratory infections are uncommon in athletes. Undiagnosed or inappropriately treated asthma and/or allergy are common findings in clinical assessments of elite athletes experiencing recurrent URS. High-performance athletes with recurrent episodes of URS should undergo a thorough clinical assessment to exclude underlying treatable conditions of respiratory inflammation. Identifying athletes at risk of recurrent URS is important in order to prescribe preventative clinical, training and lifestyle strategies. Monitoring secretion rates and falling concentrations of salivary IgA can identify athletes at risk of URS. Therapeutic interventions are limited by the uncertainty of the underlying cause of inflammation. Topical anti-inflammatory sprays can be beneficial for some athletes. Dietary supplementation with bovine colostrum, probiotics and selected antioxidants can reduce the incidence or severity of URS in some athletes. Preliminary studies on athletes prone to URS indicate a genetic predisposition to a pro-inflammatory response and a dysregulated anti-inflammatory cytokine response to intense exercise as a possible mechanism of respiratory inflammation. This review focuses on respiratory infections and inflammation in elite/professional athletes.

Gong, Y., Li, T., Li, S., Jiang, Z., Yang, Y., Huang, J., Liu, Z., and Sun, H. Achieving High Yield of Lactic Acid for Antimicrobial Characterization in Cephalosporin Resistance Lactobacillus by the Co-expression of the Phosphofructokinase and Glucokinase. J.Microbiol.Biotechnol. 3-14-2016;

ABSTRACT: Lactobacilli are universally recognized as probiotics that are widely used in the adjuvant treatment of inflammatory diseases, such as vaginitis and enteritis. With the overuse of antibiotics in recent years, the Lactobacilli in the human body are killed, which could disrupt the micro ecological balance in the human body and affect health adversely. In this work, cephalosporin-resistant Lactobacillus casei RL20 was obtained successfully from the feces of healthy volunteers which possessed a stable genetic set. However, the shortage of lactic acid (72.0 g/L at 48 h) by fermentation did not meet the requirement for its use in medicine.To increase the production of lactic acid, the functional genes pfk and glk were introduced into the wild strain, thereby obtaining a yield of 144.2 g/L lactic acid obtained in the transgenic L. casei RL20-2 after fermentation for 48 h in 1 L of the basic fermentation medium with an initial glucose concentration of 100 g/L and increasing antibacterial activity. These data suggested that L. casei RL20-2 exhibited a high yield of lactic acid that may be a potential probiotic that inhibits the spread of bacterial infectious diseases and may be used for vaginitis therapy.

Gonzalez-Rascon, A. and Mata-Haro, V. MicroRNAs: Regulators of TLR2-Mediated Probiotic Immune Responses. Microrna. 2016; 4(3):168-174.

ABSTRACT: MicroRNAs (miRNAs) are similar in importance to transcription factors and critical to confer accuracy and robustness in gene expression programs and consequently, have emerged as controllers of the immune response. On the other hand, probiotic influence over immune responses against a wide spectrum of health conditions are widely studied but the mechanism for this modulation has not yet completely elucidated. One proposed mechanism involves the receptor-mediated interaction of dendritic cells with components of the cellular membrane and/or secreted peptides of probiotics ending with the production of cytokines. However, the cytokine response elicited by dendritic cells is largely strain-dependent, and not to the same extent. The signaling pathway involved must be tightly regulated in order to be precise and effective; proteins as TNF receptor-associated factor 6 (TRAF6), Interleukin-1 receptor-associated kinase 4 (IRAK4) and Interleukin-1 receptor-associated kinase 3 (IRAK3 or IRAKM) participate in an important way in the nuclear factor kappa B (NF-kappaB) signaling pathway, which is the main cascade activated in response to probiotics. These proteins can be regulated by miRNAs and alter the immune outcome. This review discusses the current understanding on the participation of miRNAs modulating the TLR2/NF-kappaB pathway in the innate immune response mediated by probiotics.

Gordon, S. Elie Metchnikoff, the Man and the Myth. J.Innate.Immun. 2-3-2016;

ABSTRACT: The year 2016 marks the centenary of the death of Elie Metchnikoff, the father of innate immunity and discoverer of the significance of phagocytosis in development, homeostasis and disease. Through a series of intravital experiments on invertebrates and vertebrates, he described the role of specialised phagocytic cells, macrophages and microphages, subsequently renamed neutrophils and polymorphonuclear leucocytes, in

the host response to injury, inflammation, infection and tissue repair. As a vigorous proponent of cellular immunity, he championed its importance versus humoral immunity in the so-called antibody wars. By 1908, when the Nobel Prize was awarded to Elie Metchnikoff and Paul Ehrlich, this debate was not yet resolved. Even earlier, Metchnikoff had turned his research interests to the process of ageing and the possible link to intestinal auto-intoxication, giving rise to the current interest in the microbiome of the gut and the use of probiotics to promote health and longevity. During the past century, Metchnikoff's reputation has waxed and waned, as lymphocyte heterogeneity, specificity and memory began to dominate the field of adaptive immunity, yet his benign visage continues to provide an iconic presence for specialists in innate immunology, whose studies have made a striking comeback in the past decade. In this review, I shall consider the nature of his studies and the person as well as the legendary description of his Eureka experience in Messina in 1882, a story loved by students and investigators alike, that marked, in his own words, his transformation from zoologist to pathologist.

Gorska, S., Buda, B., Brzozowska, E., Schwarzer, M., Srutkova, D., Kozakova, H., and Gamian, A. Identification of Lactobacillus proteins with different recognition patterns between immune rabbit sera and nonimmune mice or human sera. BMC.Microbiol. 2016; 16(1):17

ABSTRACT: BACKGROUND: The genus Lactobacillus belongs to a large heterogeneous group of low G + C Gram-positive anaerobic bacteria, which are frequently used as probiotics. The health-beneficial effects, in particular the immunomodulation effect, of probiotics depend on the strain and dose used. Strain variations may be related to diversity of the cell surface architecture of bacteria and the ability to express specific antigens or secrete compounds. The use of Lactobacillus as probiotic requires a comprehensive understanding of its effect on host immune system. To evaluate the potential immunoreactive properties of proteins isolated from four Lactobacillus strains: L. johnsonii 142 and L. johnsonii 151, L. rhamnosus LOCK 0900 and L. casei LOCK 0919, the polyclonal sera obtained from mouse and human have been tested as well as with sera from rabbits immunized with whole lactobacilli cells. RESULTS: The reactivity of isolated proteins detected by SDS-PAGE and Western blotting was heterogeneous and varied between different serum samples. The proteins with the highest immunoreactivity were isolated, purified and sequenced, in particular the fractions were identified as phosphoglycerate kinase (L. johnsonii 142), glyceraldehyde 3-phosphate dehydrogenase (L. johnosnii 142, L. rhamnosus LOCK 0900), hypothetic protein JDM1_1307 (L. johnsonii 151) and fructose/tagatose-bisphosphate-aldolase (L. casei LOCK 0919). CONCLUSION: The different prevalence of reactions against tested antigens in rabbit, mouse and human sera may indicate significant differences in immune system and commensal cross-talk in these groups. The identification of immunoreactive lactobacilli proteins opens the possibility to use them as an antigens for development of vaccines.

Gracia, M. I., Millan, C., Sanchez, J., Guyard-Nicodeme, M., Mayot, J., Carre, Y., Csorbai, A., Chemaly, M., and Medel, P. Efficacy of feed additives against Campylobacter in live broilers during the entire rearing period: Part B. Poult.Sci. 2016; 95(4):886-892.

ABSTRACT: A total of 636 day-of-hatch Ross 308 broilers chicks were used in 4 independent trials carried out to screen the effect of 12 feed additives on reducing cecal colonization ofCampylobacterin broilers. The tested additives were probiotics based onB. subtilis and S. cerevisae, a garlic extract, a blend of herbal substances and

essential oils, two different combinations of essential oils and organic acids (OA), two mixtures of flavoring compounds, medium chain fatty acids (MCFA), monoglycerides (MG) of MCFA and MG-MCFA+OA. At 14 days of age, all the birds were orally infected with 0.1 mL of a bacterial suspension ofC. jejuniST-45 diluted at 10(5)cfu/mL in tryptone salt broth. In each trial, there was a positive control group and 2 (Trials 1 and 2) or 4 (Trials 3 and 4) additional treatment groups supplemented with additives, which were added to feed or water only to the finisher (21 to 42 d) diet (Trials 1 and 2) or to the starter (0 to 21 d) and finisher diets (Trials 3 and 4). Feed and water were available ad libitum. On days 35 and 42 of age in Trials 1 and 2, and on days 21, 35 and 42 of age in Trials 3 and 4, 10 (Trials 1 and 2) or 12 birds (Trials 3 and 4) per group were euthanized for cecal sampling. In Trial 1, birds fed with MCFA and MG-MCFA had a significant ($P< 0.05$) reduction in cecalCampylobactercolonization compared to control at 35 d, but only the group treated with MG-MCFA maintained the reduction at 42 d. In Trials 2 to 4, no significant differences ($P> 0.05$) in cecalCampylobactercounts were found between the treated and control animals. In conclusion, although none of the treatments were able to completely prevent the colonization of chickens withC. jejuni, MCFA and MG-MCFAs could reduce the pathogen counts when supplemented from 21 days onwards.

Grazul, H., Kanda, L. L., and Gondek, D. Impact of probiotic supplements on microbiome diversity following antibiotic treatment of mice. Gut Microbes. 3-10-2016; 1-14.

ABSTRACT: Shifts in microbial populations of the intestinal tract have been associated with a multitude of nutritional, autoimmune, and infectious diseases. The limited diversity following antibiotic treatments creates a window for opportunistic pathogens, diarrhea, and inflammation as the microbiome repopulates. Depending on the antibiotics used, microbial diversity can take weeks to months to recover. To alleviate this loss of diversity in the intestinal microbiota, supplementation with probiotics has become increasingly popular. However, our understanding of the purported health benefits of these probiotic bacteria and their ability to shape the microbiome is significantly lacking. This study examined the impact of probiotics concurrent with antibiotic treatment or during the recovery phase following antibiotic treatment of mice. We found that probiotics did not appear to colonize the intestine themselves or shift the overall diversity of the intestinal microbiota. However, the probiotic supplementation did significantly change the types of bacteria which were present. In particular, during the recovery phase the probiotic caused a suppression of Enterobacteriaceae outgrowth (Shigella and Escherichia) while promoting a blooming of Firmicutes, particularly from the Anaerotruncus genus. These results indicate that probiotics have a significant capacity to remodel the microbiome of an individual recovering from antibiotic therapy.

Grotkjaer, T., tzon-Tilia, M., D'Alvise, P., Dourala, N., Nielsen, K. F., and Gram, L. Isolation of TDA-producing Phaeobacter strains from sea bass larval rearing units and their probiotic effect against pathogenic Vibrio spp. in Artemia cultures. Syst.Appl.Microbiol. 2-12-2016;

ABSTRACT: Fish-pathogenic Vibrio can cause large-scale crashes in marine larval rearing units and, since the use of antibiotics can result in bacterial antibiotic resistance, new strategies for disease prevention are needed. Roseobacter-clade bacteria from turbot larval rearing facilities can antagonize Vibrio anguillarum and reduce mortality in V. anguillarum-infected cod and turbot larvae. In this study, it was demonstrated that antagonistic Roseobacter-clade bacteria could be isolated from sea bass larval rearing units. In addition, it was shown that they not only antagonized V. anguillarum but also V.

harveyi, which is the major bacterial pathogen in crustaceans and Mediterranean sea bass larvae cultures. Concomitantly, they significantly improved survival of V. harveyi-infected brine shrimp. 16S rRNA gene sequence homology identified the antagonists as Phaeobacter sp., and in silico DNA-DNA hybridization indicated that they could belong to a new species. The genomes contained genes involved in synthesis of the antibacterial compound tropodithietic acid (TDA), and its production was confirmed by UHPLC-TOFMS. The new Phaeobacter colonized live feed (Artemia) cultures and reduced Vibrio counts significantly, since they reached only 104CFUmL-1, as opposed to 108CFUmL-1 in non-Phaeobacter treated controls. Survival of V. anguillarum-challenged Artemia nauplii was enhanced by the presence of wild type Phaeobacter compared to challenged control cultures (89+/-1.0% vs 8+/-3.2%). In conclusion, TDA-producing Phaeobacter isolated from Mediterranean marine larviculture are promising probiotic bacteria against pathogenic Vibrio in crustacean live-feed cultures for marine fish larvae.

Gruner, D., Paris, S., and Schwendicke, F. Probiotics for managing caries and periodontitis: Systematic review and meta-analysis. J.Dent. 3-8-2016;

ABSTRACT: OBJECTIVES: Probiotics might be beneficial to prevent or treat caries, gingivitis or periodontitis. We aimed to appraise trials assessing probiotics for managing caries and periodontal disease. DATA: We included randomized controlled trials comparing the efficacy of probiotics versus (placebo) control with regards to Streptococcus mutans [SM], lactobacilli [LB], periodontal pathogens numbers, gingivitis, oral hygiene, caries incidence/experience increment, or periodontitis. Meta-analysis and trial-sequential-analysis were performed. SOURCES: Three electronic databases (Medline, Embase, Central) were screened. STUDY SELECTION: 50 studies (3247 participants) were included. Studies were mainly performed in children and used lactobacilli (45); bifidobacteria (12) or other genus (3). Probiotics significantly increased the chance of reducing SM (OR: 2.20, 95% CI: 1.23/3.92) or LB (OR: 2.84; 1.34/6.03)<104CFU/ml. Such reduction was confirmed for SM counts (standardized mean differences: -1.18, 95% CI: -1.64/-0.72), but not LB (SMD: 0.33; 0.15/0.52). For periodontal pathogens, no significant difference was found. Probiotics significantly reduced bleeding-on-probing (SMD: -1.15; -1.68/-0.62) and gingival index (SMD: -0.86; -1.52/-0.20), but not plaque index (SMD: -0.34; -0.89/0.21). Caries incidence was not significantly reduced (OR: 0.60; 0.35/1.04), neither was caries experience (SMD: -0.26; -0.55/0.03) or CAL (SMD: -0.46; -0.84/0.08). In contrast, probing-pocket depths (SMD: -0.86; -1.55/-0.17) were significantly reduced. Data was quantitatively insufficient for conclusive findings, and risk of bias was high. CONCLUSION: Current evidence is insufficient for recommending probiotics for managing dental caries, but supportive towards managing gingivitis or periodontitis. Future studies should only record bacterial numbers alongside accepted disease markers or indicators. CLINICAL SIGNIFICANCE: Probiotic therapy could be used for managing periodontal diseases. For caries, further studies should ascertain both efficacy and safety.

Guardiola, F. A., Porcino, C., Cerezuela, R., Cuesta, A., Faggio, C., and Esteban, M. A. Impact of date palm fruits extracts and probiotic enriched diet onantioxidant status, innate immune response and immune-related geneexpression of European seabass (Dicentrarchuslabrax). Fish.Shellfish.Immunol. 3-23-2016;

ABSTRACT: The application of additives in the diet as plants or extracts of plants as natural and innocuous compounds has potential in aquaculture as an alternative to antibiotics and immunoprophylactics. The aim of the current study was to evaluate the

potential effects of dietary supplementation of date palm fruit extracts alone or in combination with Pdp11 probiotic on serum antioxidant status, on the humoral and cellular innate immune status, as well as, on the expression levels of some immune-related genes in head-kidney and gut of European sea bass (Dicentrarchus labrax) after 2 and 4 weeks of administration.This study showed for the first time in European sea bass an immunostimulation in several of the parameters evaluated in fish fed with date palm fruits extracts enriched diet or fed with this substance in combination with Pdp 11 probiotic, mainly after 4 weeks of treatment. In the same way, dietary supplementation of mixture diet has positive effects on the expression levels of immune-related genes, chiefly in head-kidney of D. labrax. Therefore, the combination of both could be considered of great interest as potential additives for farmed fish.

Gueimonde, L., Vesterlund, S., Garcia-Pola, M. J., Gueimonde, M., Soderling, E., and Salminen, S. Supplementation of xylitol-containing chewing gum with probiotics: a double blind, randomised pilot study focusing on saliva flow and saliva properties. Food Funct. 3-16-2016; 7(3):1601-1609.

ABSTRACT: The aim of this study was to investigate the impact of daily chewing, for 12 weeks, of 2 different probiotic gums compared with placebo on saliva flow rate, saliva IgA levels and saliva pH. The intervention study included 54 adult volunteers with hyposalivation in a double-blind, randomised and placebo-controlled design with three parallel groups. Volunteers were randomly assigned to 3 different groups: subjects in group A (n = 19) were given placebo chewing gum, group B (n = 17) received Bifidobacterium animalis ssp. lactis Bb12 (ATCC 27536) and group C (n = 18) received Lactobacillus rhamnosus LGG (ATCC 53103), Bifidobacterium longum 46 (DSM 14583) and Bifidobacterium longum 2C (DSM 14579) gums, during 3 months. Two volunteers from group B left the study for personal reasons leaving 19, 15 and 18 volunteers, respectively, for analyses. Clinical examinations, personal interviews, sialometries and saliva sampling were conducted at baseline and after 1, 2, 3 and 4 months. No statistically significant differences were found between probiotic and placebo groups for any of the parameters analysed. No side effects of probiotic or placebo chewing gums were observed. Chewing gum, with and without probiotics, had a positive impact on salivary flow rate and saliva pH and IgA levels.

Guo, X., Chen, D. D., Peng, K. S., Cui, Z. W., Zhang, X. J., Li, S., and Zhang, Y. A. Identification and characterization of Bacillus subtilis from grass carp (Ctenopharynodon idellus) for use as probiotic additives in aquatic feed. Fish.Shellfish.Immunol. 3-15-2016; 52:74-84.

ABSTRACT: Bacillus subtilis is widely used as probiotic species in aquaculture for water quality control, growth promoting, or immunity enhancing. The aim of this study is to find novel B. subtilis strains from fish as potential probiotics for aquaculture. Eleven B. subtilis isolates derived from the intestinal tract of grass carp were identified by gene sequencing and biochemical tests. These isolates were classified into 4 groups, and the representatives (GC-5, GC-6, GC-21 and GC-22) of each group were further investigated for antibiotic susceptibility, sporulation rate, biofilm formation, activity against pathogenic bacteria, resistance to stress conditions of intestinal tract (high percentage of bile and low pH) and high temperature, which are important for probiotics to be used as feed additives. Additionally, the adhesion properties of the 4 characterized strains were assessed using Caco-2 cell and gut mucus models. The results showed that the 4 strains differed in their capacities to adhere to intestinal epithelial cells and mucus.

Furthermore, the strains GC-21 and GC-22 up-regulated the expression levels of IL-10 and TGF-beta but down-regulated IL-1beta, suggesting their potential anti-inflammatory abilities. Based on physiological properties of the 4 characterized B. subtilis strains, one or more strains may have potential to be used as probiotics in aquaculture.

Guthmann, F., rlettaz Mieth, R. P., Bucher, H. U., and Buhrer, C. Short courses of dual-strain probiotics appear to be effective in reducing necrotising enterocolitis. Acta Paediatr. 2016; 105(3):255-259.

ABSTRACT: AIM: Prophylactic probiotics to reduce necrotising enterocolitis (NEC) are mostly given for at least 28 days or until discharge. We describe the effects of a shorter duration dosing strategy. METHODS: Retrospective cohort study of neonates (birthweight 400-1500 g) in three neonatal intensive care units in Switzerland and Germany that embarked on probiotic prophylaxis given for 10 or 14 days, employing a fixed combination (Lactobacillus acidophilus plus Bifidobacterium infantis, each 10(9) CFU/day) licensed as a drug in Switzerland. Probiotics were initiated upon discontinuation of antibiotics, or on day 1-3 in infants without antibiotics. Repeat probiotic courses were given whenever antibiotics had been instituted and were discontinued. RESULTS: Birthweight and gestational age were similar in the two 24-month pre- and postimplementation cohorts. NEC rates fell from 33 of 633 (5.2%) to 8 of 591 infants alive at three days (1.4%; risk ratio (RR) 0.26, 95% confidence interval (CI) 0.12-0.55). The drop in NEC was significant both for infants of 400-999 g (6.4% to 2.5%) and 1000-1500 g birthweight (4.4% to 0.6%). Mortality was 5.1% (32/633) without, as opposed to 3.5% (21/591) with probiotics, respectively (RR 0.69, 95% CI 0.41-1.19). CONCLUSION: Short courses of a dual-strain probiotics appear to be effective in reducing NEC.

Gutierrez, S., Martinez-Blanco, H., Rodriguez-Aparicio, L. B., and Ferrero, M. A. Effect of fermented broth from lactic acid bacteria on pathogenic bacteria proliferation. J.Dairy Sci. 2016; 99(4):2654-2665.

ABSTRACT: In this study, the effect that 5 fermented broths of lactic acid bacteria (LAB) strains have on the viability or proliferation and adhesion of 7 potentially pathogenic microorganisms was tested. The fermented broth from Lactococcus lactis C660 had a growth inhibitory effect on Escherichia coli K92 that reached of 31%, 19% to Pseudomonas fluorescens, and 76% to Staphylococcus epidermidis. The growth of Staph. epidermidis was negatively affected to 90% by Lc. lactis 11454 broth, whereas the growth of P. fluorescens (25%) and both species of Staphylococcus (35% to Staphylococcus aureus and 76% to Staph. epidermidis) were inhibited when they were incubated in the presence of Lactobacillus casei 393 broth. Finally, the fermented broth of Lactobacillus rhamnosus showed an inhibitory effect on growth of E. coli K92, Listeria innocua, and Staph. epidermidis reached values of 12, 28, and 76%, respectively. Staphylococcus epidermidis was the most affected strain because the effect was detected from the early stages of growth and it was completely abolished. The results of bacterial adhesion revealed that broths from Lc. lactis strains, Lactobacillus paracasei, and Lb. rhamnosus caused a loss of E. coli K92 adhesion. Bacillus cereus showed a decreased of adhesion in the presence of the broths of Lc. lactis strains and Lb. paracasei. Listeria innocua adhesion inhibition was observed in the presence of Lb. paracasei broth, and the greatest inhibitory effect was registered when this pathogenic bacterium was incubated in presence of Lc. lactis 11454 broth. With respect to the 2 Pseudomonas, we observed a slight adhesion inhibition showed by Lactobacillus rhamnosus broth against

Pseudomonas putida. These results confirm that the effect caused by the different LAB assayed is also broth- and species-specific and reveal that the broth from LAB tested can be used as functional bioactive compounds to regulate the adhesion and biofilm synthesis and ultimately lead to preventing food and clinical contamination and colonization of E. coli K92, B. cereus, and Ls. innocua.

Guyard-Nicodeme, M., Keita, A., Quesne, S., Amelot, M., Poezevara, T., Le, Berre B., Sanchez, J., Vesseur, P., Martin, A., Medel, P., and Chemaly, M. Efficacy of feed additives against Campylobacter in live broilers during the entire rearing period. Poult.Sci. 2016; 95(2):298-305.

ABSTRACT: Poultry meat is the major source of human campylobacteriosis, the most frequently reported zoonosis in the EU. The prevalence of Campylobacter colonization in European broiler flocks is 71%. Despite considerable efforts, there is still no effective strategy available to prevent or reduce Campylobacter colonization in broilers.This study tested a wide variety of feed additives to reduce Campylobacter shedding in primary poultry production. Twelve additives containing organic or fatty acids, monoglycerides, plant extracts, prebiotics, or probiotics were tested. For each additive, broilers contaminated with Campylobacter jejuni were fed with an additive free diet (control group) or with a supplemented diet (treated group) and Campylobacter loads compared at three sampling times.No treatment was able to prevent broiler colonization by Campylobacter, and there was a high degree of variation in contamination among the birds. At 14 d of age, eight treatments significantly decreased the colonization level compared to the control group by a maximum of 2 log10 CFU/g. At 35 d of age, three of these treatments still had a significant effect with a maximum reduction of 1.88 log10 CFU/g for a probiotic. At 42 d of age, only one short-chain fatty acid was still significantly efficient with a mean reduction over 2 log10 CFU/g. In addition, a probiotic and a prebiotic-like compound significantly decreased the contamination by a maximum of 3 log10 CFU/g, only at the 42-d sampling period.This study gives promising results regarding the use of feed additives to reduce Campylobacter infection in flocks. Nevertheless, a global approach, combining intervention measures at the different steps of the broiler meat production chain could have a greater impact on the reduction of public health risk.

Ha, E. M. Escherichia coli-derived uracil increases the antibacterial activity and growth rate of Lactobacillus plantarum. J.Microbiol.Biotechnol. 3-24-2016;

ABSTRACT: Lactobacillus plantarum (L. plantarum) is a representative probiotic. In particular, L. plantarum is the first commensal bacterium to colonize the intestine of infants. For this reason, the initial settlement of L. plantarum can play an important role in determining an infant's health as well as their eventual health status as an adult. In addition, L. plantarum combats pathogenic infections (such as Escherichia coli [E. coli], one of the early pathogenic colonizers in an unhealthy infant gut) by secreting antimicrobial substances. The aim of this research was to determine how L. plantarum combats E. coli infection and why it is a representative probiotic in the intestine. Consequently, this research observed that E. coli releases uracil. L. plantarum specifically recognizes E. coli-derived uracil, which increases the growth rate and production of antimicrobial substance of L. plantarum. In addition, through the inhibitory activity test, this study postulates that the antimicrobial substance is a protein and can be considered a bacteriocin-like substance. Therefore, this research assumes that L. plantarum exerts its

antibacterial ability by recognizing E. coli and increasing its growth rate as a result, and this phenomenon could be one of the reasons for L. plantarum settling in the intestine of infants as a beneficial bacterium.

Haileselassie, Y., Navis, M., Vu, N., Qazi, K. R., Rethi, B., and Sverremark-Ekstrom, E. Postbiotic Modulation of Retinoic Acid Imprinted Mucosal-like Dendritic Cells by Probiotic Lactobacillus reuteri 17938 In Vitro. Front Immunol. 2016; 7:96

ABSTRACT: Lactobacilli are widely used as probiotics with beneficial effects on infection-associated diarrhea, but also used in clinical trials of e.g., necrotizing enterocolitis and inflammatory bowel diseases. The possibility of using probiotic metabolic products, so-called postbiotics, is desirable as it could prevent possible side effects of live bacteria in individuals with a disturbed gut epithelial barrier. Here, we studied how Lactobacillus reuteri DSM 17938 cell-free supernatant (L. reuteri-CFS) influenced retinoic acid (RA)-driven mucosal-like dendritic cells (DC) and their subsequent effect on T regulatory cells (Treg) in vitro. RA clearly imprinted a mucosal-like DC phenotype with higher IL10 production, increased CD103 and CD1d expression, and a downregulated mRNA expression of several inflammatory-associated genes (NFkappaB1, RELB, and TNF). Treatment with L. reuteri-CFS further influenced the tolerogenic phenotype of RA-DC by downregulating most genes involved in antigen uptake, antigen presentation, and signal transduction as well as several chemokine receptors, while upregulating IL10 production. L. reuteri-CFS also augmented CCR7 expression on RA-DC. In cocultures, RA-DC increased IL10 and FOXP3 expression in Treg, but pre-treatment with L. reuteri-CFS did not further influence the Treg phenotype. In conclusion, L. reuteri-CFS modulates the phenotype and function of mucosal-like DC, implicating its potential application as postbiotic.

Hakobyan, L., Harutyunyan, K., Harutyunyan, N., Melik-Andreasyan, G., and Trchounian, A. Adhesive Properties and Acid-Forming Activity of Lactobacilli and Streptococci Under Inhibitory Substances, Such as Nitrates. Curr.Microbiol. 3-4-2016;

ABSTRACT: One of the main requirements for probiotics is their ability to survive during passage through gastrointestinal tract and to maintain their activity at different adverse conditions. The aim of the study was to look for the strains of lactobacilli and streptococci with high adhesive properties even affected by inhibitory substances, such as nitrates (NO3 -). To study the adhesion properties hemagglutination reaction of bacterial cells with red blood cells of different animals and humans was used. The acid formation ability of bacteria was determined by the method of titration after 7 days of incubation in the sterile milk. These properties were investigated at different concentrations of NO3 -. The high concentration (mostly >/=2.0 %).

Halpern, R. and Coelho, R. Excessive crying in infants. J.Pediatr.(Rio J.). 3-17-2016;

ABSTRACT: OBJECTIVE: Review the literature on excessive crying in young infants, also known as infantile colic, and its effects on family dynamics, its pathophysiology, and new treatment interventions. DATA SOURCE: The literature review was carried out in the Medline, PsycINFO, LILACS, SciELO, and Cochrane Library databases, using the terms "excessive crying," and "infantile colic," as well technical books and technical reports on child development, selecting the most relevant articles on the subject, with emphasis on

recent literature published in the last five years. SUMMARY OF THE FINDINGS: Excessive crying is a common symptom in the first 3 months of life and leads to approximately 20% of pediatric consultations. Different prevalence rates of excessive crying have been reported, ranging from 14% to approximately 30% in infants up to 3 months of age. There is evidence linking excessive crying early in life with adaptive problems in the preschool period, as well as with early weaning, maternal anxiety and depression, attention deficit hyperactivity disorder, and other behavioral problems. Several pathophysiological mechanisms can explain these symptoms, such as circadian rhythm alterations, central nervous system immaturity, and alterations in the intestinal microbiota. Several treatment alternatives have been described, including behavioral measures, manipulation techniques, use of medication, and acupuncture, with controversial results and effectiveness. CONCLUSION: Excessive crying in the early months is a prevalent symptom; the pediatrician's attention is necessary to understand and adequately manage the problem and offer support to exhausted parents. The prescription of drugs of questionable action and with potential side effects is not a recommended treatment, except in extreme situations. The effectiveness of dietary treatments and use of probiotics still require confirmation. There is incomplete evidence regarding alternative treatments such as manipulation techniques, acupuncture, and use of the herbal supplements and behavioral interventions.

Hamza, A., Fdhila, K., Zouiten, D., and Masmoudi, A. S. Virgibacillus proomii and Bacillus mojavensis as probiotics in sea bass (Dicentrarchus labrax) larvae: effects on growth performance and digestive enzyme activities. Fish.Physiol Biochem. 2016; 42(2):495-507.

ABSTRACT: This study examined the effects of two probiotics (Virgibacillus proomii and Bacillus mojavensis) on the digestive enzyme activity, survival and growth of Dicentrarchus labrax at various ontogenetic stages in three separate experiments. These probiotics were incorporated as single or mixed into fish feed for a period of 60 days. The growth parameters, proximate composition of whole body, digestive enzymes and gut microbiology were monitored at regular. The increments in length and weight and the survival were significantly higher ($P < 0.05$), and the values of food conversions were significantly lower ($P < 0.05$) in fishes fed the probiotic. The administration of V. proomii and B. mojavensis in diet resulted in an increase ($P > 0.05$) in body ash and protein content and in the specific activity of phosphatase alkaline and amylase in the digestive tract of all the fishes. V. proomii and B. mojavensis persisted in the fish intestine and in the feed in high numbers during the feeding period (group 1: 5.8 x 10(4) CFU/ml, group 2: 9.6 x 10(4) CFU/ml, and group 3: 9.8 x 10(4) CFU/ml day 60). The two probiotics V. proomii and B. mojavensis were adequate for improved growth performance and survival and for healthy gut microenvironment of the host.

Hashemi, A., Villa, C. R., and Comelli, E. M. Probiotics in early life: a preventative and treatment approach. Food Funct. 3-16-2016;

ABSTRACT: Microbial colonization of the infant gut plays a key role in immunological and metabolic pathways impacting human health. Since the maturation of the gut microbiota coincides with early life development, failure to develop a health compatible microbiota composition may result in pathology and disease in later life. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Maternal transfer of microorganisms is possible during pregnancy and lactation, and the mother's diet and microbiota can influence that of her offspring.

Furthermore, pre-term birth, Caesarean section birth, formula feeding, antibiotic use, and malnutrition have been linked to dysbiosis, which in turn is associated with several pathologies such as necrotizing enterocolitis, inflammatory bowel diseases, antibiotic associated diarrhea, colic, and allergies. Thus, early life should represent a preferred stage of life for probiotic interventions. In this context, they could be regarded as a means to 'program' the individual for health maintenance, in order to prevent pathologies associated with dysbiosis. In order to elucidate the mechanisms underlying the benefits of probiotic administration, pre-clinical studies have been conducted and found an array of positive results such as improved microbial composition, intestinal maturation, decreased pathogenic load and infections, and improved immune response. Moreover, specific probiotic strains administered during the perinatal period have shown promise in attenuating severity of necrotizing enterocolitis. The mechanisms elucidated suggest that probiotic interventions in early life can be envisaged for disease prevention in both healthy offspring and offspring at risk of chronic disease.

Hayakawa, T., Masuda, T., Kurosawa, D., and Tsukahara, T. Dietary administration of probiotics to sows and/or their neonates improves the reproductive performance, incidence of post-weaning diarrhea and histopathological parameters in the intestine of weaned piglets. Anim Sci.J. 3-28-2016;

ABSTRACT: Probiotics have gained considerable attention with respect to their beneficial effects on livestock performance and health. The most significant effects of probiotics on the gut microbiota and the host animals take place when they are included in diets during particularly stressful periods such as weaning and/or at the beginning of the lactation period. The probiotics Bacillus mesentericus strain TO-A at 1 x 108 colony forming units (CFU)/g, Clostridium butyricum strain TO-A at 1 x 108 CFU/g and Enterococcus faecalis strain T-100 at 1 x 109 CFU/g were used. Litter weight at delivery and ratio of return to estrous improved significantly (17% and 24% improvement, respectively) by probiotic administration to sows (0.2% (w/w)). Furthermore, the feed intake of the probiotics-administered sows was greater than that of the control sows during the late lactation period. Post-weaning diarrheal incidence and growth performance was improved by probiotics administration to neonates (0.02% (w/w)), while the combined use of probiotics in sows and their neonates induced the enlargement of villous height and prevented muscle layer thinning in the small intestine of weaning piglets. The administration of probiotics of three species of live bacteria improved the porcine reproductive performance around stressful periods of sows (farrowing) and piglets (weaning).

Hendaus, M. A., Jomha, F. A., and Ehlayel, M. Allergic diseases among children: nutritional prevention and intervention. Ther.Clin.Risk Manag. 2016; 12:361-372.

ABSTRACT: Allergic diseases comprise a genetically heterogeneous group of chronic, immunomediated diseases. It has been clearly reported that the prevalence of these diseases has been on the rise for the last few decades, but at different rates, in various areas of the world. This paper discusses the epidemiology of allergic diseases among children and their negative impact on affected patients, their families, and societies. These effects include the adverse effects on quality of life and economic costs. Medical interest has shifted from tertiary or secondary prevention to primary prevention of these chronic diseases among high-risk infants in early life. Being simple, practical, and cost-effective are mandatory features for any candidate methods delivering these strategies.

Dietary therapy fits this model well, as it is simple, practical, and cost-effective, and involves diverse methods. The highest priority strategy is feeding these infants breast milk. For those who are not breast-fed, there should be a strategy to maintain beneficial gut flora that positively influences intestinal immunity. We review the current use of probiotics, prebiotics, and synbiotics, and safety and adverse effects. Other dietary modalities of possible potential in achieving this primary prevention, such as a Mediterranean diet, use of milk formula with modified (hydrolyzed) proteins, and the role of micronutrients, are also explored. Breast-feeding is effective in reducing the risk of asthma, allergic rhinitis, and atopic eczema among children. In addition, breast milk constitutes a major source of support for gut microbe colonization, due to its bifidobacteria and galactooligosaccharide content. The literature lacks consensus in recommending the addition of probiotics to foods for prevention and treatment of allergic diseases, while prebiotics may prove to be effective in reducing atopy in healthy children. There is insufficient evidence to support soy formulas or amino acid formulas for prevention of allergic disease. A healthy diet, such as the Mediterranean diet, may have a protective effect on the development of asthma and atopy in children. In children with asthma and allergic diseases, vitamin D deficiency correlates strongly with asthma, allergic rhinitis, and wheezing.

Hernandez-Rocha, C., Pidal, P., Ajenjo, M. C., Quera, R., Quintanilla, M., Lubascher, J., Jemenao, M. I., Ibanez, P., varez-Lobos, M., Diomedi, A., Marcotti, A., Acuna, M., Arab, J. P., Riquelme, A., Candia, R., and Carvajal, S. [Chilean consensus of prevention, diagnosis and treatment of Clostridium difficile-associated diarrhea]. Rev.Chilena.Infectol. 2016; 33(1):98-118.

ABSTRACT: BACKGROUND: Clostridium dijfficile-associated diarrhea (CDAD) has become very important due to the increase in its incidence, severity, recurrence and the associated economic burden. Having a national consensus guideline is essential to improve its management. OBJECTIVE: To build a multidisciplinary and evidence-based consensus in prevention, diagnosis and treatment of CDAD. METHODS: We convened a panel of experts in the field of infectious diseases, gastroenterology, evidence-based medicine and consensus methodology. The panel conducted a structured review of published literature in CDAD evaluating evidence levels and recommendation degree according to the methodology proposed by the GRADE working-group. A modified three-round Delphi technique was used to reach a consensus among the experts. RESULTS: A group of 16 experts was established, 12 of them answered 18 clinically relevant questions. The levels of agreement achieved by the panel of 16 experts were 79% in the first round and 100% in the second and third round. The main consensus recommendations in prevention are: restricting the use of proton-pump inhibitors, primary prophylaxis with probiotics in antibiotics users, education of health personnel, isolation for patients hospitalized with CDAD, and cleaning the rooms exposed to C. difficile with products based in chlorine or hydrogen peroxide. In the diagnosis: use of biology molecular-based techniques is preferred and if not available, glutamate dehydrogenase-based algorithms may be recommended. With regard to treatment: the use of oral metronidazole in mild-moderate CDAD and oral vancomycin in severe CDAD are recommended. Treat the first recurrence with the same antibiotics according to severity. In the case of second and subsequent recurrences consider prolonged therapy with vancomycin, rifaximin or fecal microbiota transplant. CONCLUSION: The first Chilean consensus on prevention, diagnosis and treatment of CDAD is presented, which is a major step in improving national standards in the management of this disease.

Hossain, N., Kanwar, P., and Mohanty, S. R. A Comprehensive Updated Review of Pharmaceutical and Nonpharmaceutical Treatment for NAFLD. Gastroenterol.Res.Pract. 2016; 2016:7109270

ABSTRACT: Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in the western world with prevalence of 20-33%. NAFLD comprises a pathological spectrum. Nonalcoholic fatty liver (NAFL) is at one end and consists of simple hepatic steatosis. On the contrary, nonalcoholic steatohepatitis (NASH) consists of steatosis, inflammation, and ballooning degeneration and can progress to cirrhosis. Despite the rising incidence, definitive treatment for NAFLD, specifically NASH, has not yet been established. Lifestyle modification with dietary changes combined with regular aerobic exercise, along with multidisciplinary approach including cognitive behavior therapy, has been shown to be an effective therapeutic option, even without a significant weight loss. Pioglitazone and vitamin E have shown to be most effective in NASH patients. Surgery and weight loss medication are effective means of weight loss but can potentially worsen NASH related fibrosis. Other agents such as n-3 polyunsaturated fatty acids, probiotics, and pentoxifylline along with herbal agent such as milk thistle as well as daily intake of coffee have shown potential benefits, but further well organized studies are definitely warranted. This review focuses on the available evidence on pharmaceutical and nonpharmaceutical therapy in the treatment and the prevention of NAFLD, primarily NASH.

Hoy-Schulz, Y. E., Jannat, K., Roberts, T., Zaidi, S. H., Unicomb, L., Luby, S., and Parsonnet, J. Safety and acceptability of Lactobacillus reuteri DSM 17938 and Bifidobacterium longum subspecies infantis 35624 in Bangladeshi infants: a phase I randomized clinical trial. BMC.Complement Altern.Med. 2016; 16(1):44

ABSTRACT: BACKGROUND: Probiotics have rarely been studied in young healthy infants from low-income countries. This phase I study investigated the safety and acceptability of two probiotics in Bangladesh. METHODS: Healthy infants aged four to twelve weeks from urban slums in Bangladesh were randomized to one of three different intervention dosing arms (daily, weekly, biweekly - once every two weeks) of Lactobacillus reuteri DSM 17938 and Bifidobacterium longum subspecies infantis 35624 over one month or to a fourth arm that received no probiotics. All subjects were followed for two additional months. Reported gastrointestinal and respiratory symptoms as well as breastfeeding rates, hospitalizations, differential withdrawals, and caretakers' perception of probiotic use were compared among arms. RESULTS: In total, 160 infants were randomized (40 to each arm) with 137 (Daily n = 35, Weekly n = 35, Biweekly n = 35, Control n = 32) followed up for a median of twelve weeks; 113 completed the study. Illness and breastfeeding rates were similar across all arms. Ten hospitalizations unrelated to probiotic use occurred. Forty eight percent of the caretakers of infants in intervention arms believed that probiotics improved their baby's health. CONCLUSIONS: These two commonly used probiotics appeared safe and well-accepted by Bangladeshi families. TRIAL REGISTRATION: ClinicalTrials.gov NCT01899378 . Registered July 10, 2013.

Hsieh, S. C., Liu, J. M., Pua, X. H., Ting, Y., Hsu, R. J., and Cheng, K. C. Optimization of Lactobacillus acidophilus cultivation using taro waste and evaluation of its biological activity. Appl.Microbiol.Biotechnol. 2016; 100(6):2629-2639.

ABSTRACT: In this study, taro waste (TW) was utilized for Lactobacillus acidophilus BCRC 14079 cultivation and the anti-tumor and immune-modulatory properties of heat-killed cells (HKCs), cytoplasmic fraction (CF), and exopolysaccharide (EPS) were evaluated. The optimum liquefaction enzyme dosage, temperature, and time determined by Box-Behnken design response surface methodology (BBD-RSM) were 9 mL/L of alpha-amylase, 79.2 degrees C, and 5 h of reaction, respectively. The optimum temperature and reaction time for saccharification were determined as 60 degrees C and 3 h. The optimum medium, CGMY1 medium, constitutes of TW hydrolysate containing 37 g/L of glucose, 25 g/L of corn gluten meal (CGM), and 1 g/L of yeast extract (YE). Results of MTT assay showed that HKCs and EPS from CGM medium exhibited the highest anti-proliferative in HT-29 (IC50 of HKCs, 467.25 mug/mL; EPS, 716.10 mug/mL) and in Caco-2 cells (IC50 of EPS, 741.60 mug/mL). Luciferase-based NF-KappaB and COX-2 systems indicated HKCs from CGM medium stimulated the highest expression of luciferin in both systems. The luciferase activities by using 100 and 500 mug/mL of HKCs from CGM were 24.30- and 45.83-fold in NF-KappaB system and 11.54- and 4.93-fold in COX-2 system higher than the control. In conclusion, this study demonstrated the potential of TW medium for L. acidophilus cultivation and the production of non-viable probiotics with enhanced biological activities.

Hu, X., Zhang, H., Lu, H., Qian, G., Lv, L., Zhang, C., Guo, J., Jiang, H., Zheng, B., Yang, F., Gu, S., Chen, Y., Bao, Q., Yu, L., Jiang, X., Hu, Q., Shi, H., Gao, H., and Li, L. The Effect of Probiotic Treatment on Patients Infected with the H7N9 Influenza Virus. PLoS.One. 2016; 11(3):e0151976

ABSTRACT: BACKGROUND: A novel avian-origin influenza A (H7N9) virus emerged and spread among humans in Eastern China in 2013. Prophylactic treatment with antibiotics and probiotics for secondary infection is as important as antiviral treatment. This study aims to assess the ability of probiotic treatment to restore internal homeostasis under antibiotic pressure and to reduce/ameliorate the risk of secondary infections resulting from infection with the H7N9 virus. METHODS: This is a retrospective study in archival samples. Between April 1 and May 10, 2013, 113 stool, sputum, and blood specimens were collected and analyzed by denaturing gradient gel electrophoresis (DGGE) to determine the composition of the patient microbiomes. Microbial diversity was calculated using Gel-Pro analyzer and Past software. Cluster analysis of DGGE pattern profiles was employed to create a phylogenetic tree for each patient, and multidimensional scaling (MDS) and principal component analysis (PCA) were performed to visualize relationships between individual lanes. RESULTS: Five patients had secondary infections, including Klebsiella pneumonia, Acinetobacter baumanii and Candida albicans infection. The DGGE profiles of fecal samples obtained at different time points from the same individual were clearly different, particularly for patients with secondary infections. Shannon's diversity index and evenness index were lower in all infected groups compared to the control group. After B. subtilis and E. faecium or C. butyricum administration, the fecal bacterial profiles of patients who had not been treated with antibiotics displayed a trend of increasing diversity and evenness. C. butyricum failed to reduce/ameliorate secondary infection in H7N9-infected patients, but administration of B. subtilis and E. faecium appeared to reduce/ameliorate secondary infection in one patient. CONCLUSION: H7N9 infection might decrease intestinal microbial diversity and species richness in humans. C. butyricum failed to reduce/ameliorate secondary infection in H7N9-infected patients. B. subtilis and E. faecium may also play a role in reducing/ameliorating secondary infection in these patients.

Hu, Y., Gui, L., Chang, J., Liu, J., Xu, S., Deng, C., Yu, F., Ma, Z., Wang, G., and Zhang, C. The incidence of infants with rotavirus enteritis combined with lactose intolerance. Pak.J.Pharm.Sci. 2016; 29(1 Suppl):321-323.

ABSTRACT: This study was to research the incidence of infants with rotavirus enteritis combined with lactose intolerance and the clinical effect of low lactose milk powder for infantile rotavirus enteritis with lactose intolerance. The control groups were 126 cases of infants with diarrhea randomly collected from our hospital at the same period, which their rotavirus detection was negative. The observation group was 185 cases of infants with rotavirus, which was tested to be positive. Through the urine galactose determination, 62 cases of the control group were positive and 124 cases of the observation group were positive. Then 124 cases of infants with rotavirus combined with lactose intolerance were randomly divided into two groups. 60 cases in the control group were given rehydration, correction of acidosis, oral smecta, Intestinal probiotics and other conventional treatment, then continued to the original feeding method. While, 64 cases in the treatment group, on the basis of routine treatment, applied the low lactose milk feeding. To observe the total effective rate for the two groups. The incidence of lactose intolerance in children with rotavirus enteritis (67.03%) was significantly higher than that of children with diarrhea (49.2%), which was tested to be negative. And the difference was statistically significant ($p<0.5$). In the aspect of reducing the frequency of diarrhea, and diarrhea stool forming time, the treatment group has the obvious superiority. The total effective rate was 95.4% for treatment group, which was higher than that in the control group (76.7%), the difference was statistically significant ($P<0.05$). CONCLUSION: Infants with rotavirus enteritis was easier to merge with lactose intolerance. The low lactose milk powder could improve the therapeutic effectively and could reduce the duration of disease, and restored to normal diet for 2 weeks feeding time.

Hutsko, S. L., Meizlisch, K., Wick, M., and Lilburn, M. S. Early intestinal development and mucin transcription in the young poult with probiotic and mannan oligosaccharide prebiotic supplementation. Poult.Sci. 3-4-2016;

ABSTRACT: Alternative and adjunctive approaches to decreasing the use of dietary antibiotics are becoming popular areas of study. Supplemental probiotics (commensal microbes) and prebiotics (indigestible complex carbohydrates) are 2 dietary approaches to facilitating the intestinal colonization of beneficial bacteria to compete with potential pathogens, thus creating a healthy mucosal environment. The intestinal mucosa is composed of mucin glycoproteins, which play a key role in preventing the attachment of pathogenic bacteria. At hatch, the neonatal turkey intestine is relatively aseptic and vulnerable to bacterial colonization by both commensal and pathogenic microbes. In the current study, we determined the transcription of MUC2, the primary mucin protein produced by goblet cells within the small intestine, and we also measured intestinal morphology immediately post-hatch through d 11. Poults were fed a conventional starter diet, the starter diet supplemented with one of 2 commercial probiotics (A, B), or a commercial mannan oligosaccharide. MUC2 transcription increased from d zero to d 4 post-hatch ($P < 0.05$), but there was no effect of probiotic or prebiotic supplementation. Villus height and villus area both increased with Probiotic B and mannan oligosaccharide supplementation ($P < 0.05$) and there was a significant d X treatment interaction effect for crypt depth ($P = 0.007$). These results suggest that

probiotic and prebiotic supplementation can positively alter the intestinal microenvironment.

Islam, S. U. Clinical Uses of Probiotics. Medicine (Baltimore). 2016; 95(5):e2658

ABSTRACT: Probiotics are live nonpathogenic microorganisms. Many of these microorganisms are part of the normal human gut flora, where they live in a symbiotic relationship. Probiotics have been used to treat gastrointestinal (GI) and non-GI medical conditions. However, the data supporting their use are often conflicting, especially for non-GI-associated illnesses. The strongest evidence supporting the use of probiotics is related to the treatment of acute diarrhea and pouchitis. Atopic eczema in children and genitourinary infections are the only non-GI-related medical conditions where probiotics may have some beneficial effects. Product selection and dosing are not the same in all conditions, and the beneficial effects of each probiotic strain cannot be generalized.The purpose of this article is to provide most recent information about probiotics and its uses. In contrast with previously published reviews on probiotics, we also discuss the composition of various products (Table 1), indications for their use (Table 2), product selection, and dosing of probiotics.Probiotics are safe and appear to exert some beneficial effects in GI-related illnesses. The use of probiotics in non-GI illnesses is not sufficiently supported by current data.

Iweala, O. I. and Burks, A. W. Food Allergy: Our Evolving Understanding of Its Pathogenesis, Prevention, and Treatment. Curr.Allergy Asthma Rep. 2016; 16(5):37

ABSTRACT: Food allergy is defined as an IgE-mediated hypersensitivity response to ingested food with allergic symptoms ranging from urticaria to life-threatening anaphylaxis. Food allergy is thought to develop because of (1) failed induction of tolerance upon initial exposure to food antigen or (2) breakdown of established tolerance to food antigen. We review current understanding of the pathogenesis, epidemiology, and natural history of food allergy, including the unconventional IgE-mediated food allergy to mammalian meat known as alpha-gal food allergy. We highlight emerging data on food allergy treatment and prevention, emphasizing the growing appeal of manipulating the gut microenvironment using probiotics and helminth products to blunt systemic allergic responses to food.

James, K. M., MacDonald, K. W., Chanyi, R. M., Cadieux, P. A., and Burton, J. P. Inhibition of Candida albicans biofilm formation and modulation of gene expression by probiotic cells and supernatant. J.Med.Microbiol. 2016; 65(4):328-336.

ABSTRACT: Oral candidiasis is a disease caused by opportunistic species of Candida that normally reside on human mucosal surfaces. The transition of Candida from budding yeast to filamentous hyphae allows for covalent attachment to oral epithelial cells, followed by biofilm formation, invasion and tissue damage. In this study, combinations of Lactobacillus plantarum SD5870, Lactobacillus helveticus CBS N116411 and Streptococcus salivarius DSM 14685 were assessed for their ability to inhibit the formation of and disrupt Candida albicans biofilms. Co-incubation with probiotic supernatants under hyphae-inducing conditions reduced C. albicans biofilm formation by >75 % in all treatment groups. Likewise, combinations of live probiotics reduced biofilm formation of C. albicans by >67 %. When live probiotics or their supernatants were overlaid on

preformed C. albicans biofilms, biofilm size was reduced by >63 and >65 % respectively. Quantitative real-time PCR results indicated that the combined supernatants of SD5870 and CBS N116411 significantly reduced the expression of several C. albicans genes involved in the yeast-hyphae transition: ALS3 (adhesin/invasin) by 70 % (P < 0.0001), EFG1 (hyphae-specific gene activator) by 47 % (P = 0.0061), SAP5 (secreted protease) by 49 % (P < 0.0001) and HWP1 (hyphal wall protein critical to biofilm formation) by >99 % (P < 0.0001). These findings suggest the combination of L. plantarum SD5870, L. helveticus CBS N116411 and S. salivarius DSM 14685 is effective at both preventing the formation of and removing preformed C. albicans biofilms. Our novel results point to the downregulation of several Candida genes critical to the yeast-hyphae transition, biofilm formation, tissue invasion and cellular damage.

Jiang, Y., Yang, G., Meng, F., Yang, W., Hu, J., Ye, L., Shi, C., and Wang, C. Immunological mechanisms involved in probiotic-mediated protection against Citrobacter rodentium-induced colitis. Benef.Microbes. 3-1-2016; 1-12.

ABSTRACT: Inflammatory bowel disease is a group of chronic, incurable inflammatory disorders of the gastrointestinal tract that cause severe diarrhoea, intestinal inflammation, pain, fatigue and weight loss. In this study, we first developed a model of Citrobacter rodentium-induced colitis and then evaluated the protective effects of selected probiotics on inflammation. The results showed that administration of a combination of probiotics including Lactobacillus rhamnosus ATCC 53103, Lactobacillus acidophilus ATCC 4356 and Lactobacillus plantarum A significantly increased the production of CD11c+ dendritic cells in the spleen (3.62% vs phosphate buffered saline (PBS)-treated control, P<0.01) and mesenteric lymph nodes (MLNs). In addition, the presence of probiotics significantly up-regulated the development of CD4+/CD25+/Foxp3+ regulatory T cells in MLNs by approximately 2.07% compared to the effect observed in the PBS-treated control (P<0.01) and down-regulated the expression of inflammatory cytokines, including interleukin-17, tumour necrosis factor-alpha and interferon-gamma, by 0.11, 0.11 and 0.15%, respectively, compared to the effect observed in the PBS-treated control (P<0.01).These effects conferred protection against colitis, as shown by histopathological analyses.

Jiang, Y., Ren, F., Liu, S., Zhao, L., Guo, H., and Hou, C. Enhanced Acid Tolerance in Bifidobacterium longum by Adaptive Evolution: Comparison of the Genes between the Acid-Resistant Variant and Wild-Type Strain. J.Microbiol.Biotechnol. 3-28-2016; 26(3):452-460.

ABSTRACT: Acid stress can affect the viability of probiotics, especially Bifidobacterium. This study aimed to improve the acid tolerance of Bifidobacterium longum BBMN68 using adaptive evolution. The stress response, and genomic differences of the parental strain and the variant strain were compared by acid stress. The highest acid-resistant mutant strain (BBMN68m) was isolated from more than 100 asexual lines, which were adaptive to the acid stress for 10(th), 20(th), 30(th), 40(th), and 50(th) repeats, respectively. The variant strain showed a significant increase in acid tolerance under conditions of pH 2.5 for 2 h (from 7.92 to 4.44 log CFU/ml) compared with the wildtype strain (WT, from 7.87 to 0 log CFU/ml). The surface of the variant strain was also smoother. Comparative whole-genome analysis showed that the galactosyl transferase D gene (cpsD, bbmn68_1012), a key gene involved in exopolysaccharide (EPS) synthesis, was altered by two nucleotides in the mutant, causing alteration in amino acids, pI (from 8.94 to 9.19), and predicted

protein structure. Meanwhile, cpsD expression and EPS production were also reduced in the variant strain ($p < 0.05$) compared with WT, and the exogenous WT-EPS in the variant strain reduced its acid-resistant ability. These results suggested EPS was related to acid responses of BBMN68.

Karimi, S., Ahl, D., Vagesjo, E., Holm, L., Phillipson, M., Jonsson, H., and Roos, S. In Vivo and In Vitro Detection of Luminescent and Fluorescent Lactobacillus reuteri and Application of Red Fluorescent mCherry for Assessing Plasmid Persistence. PLoS.One. 2016; 11(3):e0151969

ABSTRACT: Lactobacillus reuteri is a symbiont that inhabits the gastrointestinal (GI) tract of mammals, and several strains are used as probiotics. After introduction of probiotic strains in a complex ecosystem like the GI tract, keeping track of them is a challenge. The main objectives of this study were to introduce reporter proteins that would enable in vivo and in vitro detection of L. reuteri and increase knowledge about its interactions with the host. We describe for the first time cloning of codon-optimized reporter genes encoding click beetle red luciferase (CBRluc) and red fluorescent protein mCherry in L. reuteri strains ATCC PTA 6475 and R2LC. The plasmid persistence of mCherry-expressing lactobacilli was evaluated by both flow cytometry (FCM) and conventional plate count (PC), and the plasmid loss rates measured by FCM were lower overall than those determined by PC. Neutralization of pH and longer induction duration significantly improved the mCherry signal. The persistency, dose-dependent signal intensity and localization of the recombinant bacteria in the GI tract of mice were studied with an in vivo imaging system (IVIS), which allowed us to detect fluorescence from 6475-CBRluc-mCherry given at a dose of 1x1010 CFU and luminescence signals at doses ranging from 1x105 to 1x1010 CFU. Both 6475-CBRluc-mCherry and R2LC-CBRluc were localized in the colon 1 and 2 h after ingestion, but the majority of the latter were still found in the stomach, possibly reflecting niche specificity for R2LC. Finally, an in vitro experiment showed that mCherry-producing R2LC adhered efficiently to the intra cellular junctions of cultured IPEC-J2 cells. In conclusion, the two reporter genes CBRluc and mCherry were shown to be suitable markers for biophotonic imaging (BPI) of L. reuteri and may provide useful tools for future studies of in vivo and in vitro interactions between the bacteria and the host.

Karpen, H. E. Nutrition in the Cardiac Newborns: Evidence-based Nutrition Guidelines for Cardiac Newborns. Clin.Perinatol. 2016; 43(1):131-145.

ABSTRACT: Both protein and energy malnutrition are common in neonates and infants with congenital heart disease (CHD). Neonates with CHD are at increased risk of developing necrotizing enterocolitis (NEC), particularly the preterm population. Mortality in patients with CHD and NEC is higher than for either disease process alone. Standardized feeding protocols may affect both incidence of NEC and growth failure in infants with CHD. The roles of human milk and probiotics have not yet been explored in this patient population.

Kato-Kataoka, A., Nishida, K., Takada, M., Suda, K., Kawai, M., Shimizu, K., Kushiro, A., Hoshi, R., Watanabe, O., Igarashi, T., Miyazaki, K., Kuwano, Y., and Rokutan, K. Fermented milk containing Lactobacillus casei strain Shirota prevents the onset of physical symptoms in medical students under academic examination stress. Benef.Microbes. 3-11-2016; 7(2):153-156.

ABSTRACT: This pilot study investigated the effects of the probiotic Lactobacillus casei strain Shirota (LcS) on psychological, physiological, and physical stress responses in medical students undertaking an authorised nationwide examination for promotion. In a double-blind, placebo-controlled trial, 24 and 23 healthy medical students consumed a fermented milk containing LcS and a placebo milk, respectively, once a day for 8 weeks until the day before the examination. Psychophysical state, salivary cortisol, faecal serotonin, and plasma L-tryptophan were analysed on 5 different sampling days (8 weeks before, 2 weeks before, 1 day before, immediately after, and 2 weeks after the examination). Physical symptoms were also recorded in a diary by subjects during the intervention period for 8 weeks. In association with a significant elevation of anxiety at 1 day before the examination, salivary cortisol and plasma L-tryptophan levels were significantly increased in only the placebo group ($P<0.05$). Two weeks after the examination, the LcS group had significantly higher faecal serotonin levels ($P<0.05$) than the placebo group. Moreover, the rate of subjects experiencing common abdominal and cold symptoms and total number of days experiencing these physical symptoms per subject were significantly lower in the LcS group than in the placebo group during the pre-examination period at 5-6 weeks (each $P<0.05$) and 7-8 weeks (each $P<0.01$) during the intervention period. Our results suggest that the daily consumption of fermented milk containing LcS may exert beneficial effects preventing the onset of physical symptoms in healthy subjects exposed to stressful situations.

Kiess, A. S., Hirai, J. H., Triplett, M. D., Parker, H. M., and McDaniel, C. D. Impact of oral Lactobacillus acidophilus gavage on rooster seminal and cloacal Lactobacilli concentrations. Poult.Sci. 4-2-2016;

ABSTRACT: The use of antibiotics in poultry is being heavily scrutinized, therefore alternatives such as probiotics are being investigated.Lactobacillispp. are a commonly used bacteria in formulating probiotics, and the addition ofLactobacillito broiler diets has demonstrated increased growth rates, stimulated immune systems, and reduced pathogen loads in the gastro-intestinal tract (GI:) tract. However, previous research has shown that when rooster semen is directly exposed toLactobacillus acidophilus(L. acidophilus) sperm quality is reduced. Therefore, the objective of the current study was to determine if oral administration ofL. acidophilusincreases the concentration ofLactobacilliin semen as well as the cloaca. A total of 30 roosters were used: 15 roosters were gavaged with 1X PBS (Control) and 15 roosters were gavaged with 107cfu/mL ofL. acidophilus(Treated). All roosters were gavaged for 14 consecutive days. Semen was collected on a 3 d interval, and cloacal swabs were collected on a 2 d interval, beginning on the first day prior to oral administration. Semen and cloacal swabs were serial diluted, and 100 muL of each dilution was then plated on Man, Rogosa, Sharpe (MRS:) agar plates. All plates were incubated for 48 h at 37 degrees C under anaerobic conditions and counted. AllLactobacillicounts were first log transformed, then log transformed (day 0) pre-counts were subtracted from the log transformed day counts providing log differences for the analysis. SeminalLactobacillicounts were not altered by treatments. However, the main effect of treatment (P= 0.026) for cloacal counts indicated that roosters gavaged withLactobacilliyielded higher counts than the controls. Additionally, cloaca samples also demonstrated a treatment by day interaction trend (P= 0.082), whereLactobacilliwas higher in theL. acidophilusgavaged roosters than the controls only on days 3, 5, 13, and 15. In conclusion, the addition ofL. acidophilusto the male breeder diet over extended periods may increase concentrations ofLactobacilliin the cloaca even higher than the concentrations observed in this study.

IfLactobacillireaches high enough concentrations in the cloaca, then sperm quality may be impacted which could lead to poor fertility within the breeder flock.

Kim, B., Park, K. Y., Ji, Y., Park, S., Holzapfel, W., and Hyun, C. K. Protective effects of Lactobacillus rhamnosus GG against dyslipidemia in high-fat diet-induced obese mice. Biochem.Biophys.Res.Commun. 3-23-2016;

ABSTRACT: Recent reports suggest that gut microbiota can be a major determinant of dyslipidemia and non-alcoholic fatty liver disease (NAFLD) and its modulation by treating probiotics is a valid strategy to exert a protective effect. In this study, high-fat diet (HFD)-fed mice were orally administrated with Lactobacillus rhamnosus GG (LGG) for 13 weeks. Significant reductions in the weights of the liver, mesenteric and subcutaneous adipose tissues were observed in LGG-treated HFD-fed mice compared to LGG-non-treated controls. The serum levels of triglyceride and cholesterol were also significantly reduced in LGG-treated mice. Gut microbial composition analysis showed that shifts in the diversity of dominant gut bacteria were caused by HFD and restored by LGG treatment. A remarkable decrease of hepatic fat content was also observed in LGG-treated mice, accompanied by downregulated expressions of lipogenic and pro-inflammatory genes in the liver. LGG-treated mice had lower expression levels of genes involved in cholesterol synthesis, but conversely, higher expression levels of cholesterol efflux-related genes compared to LGG-non-treated controls. The cholesterol-lowering effect of LGG was also found to be mediated by suppression of FXR and FGF15 signaling, resulting in the upregulation of hepatic CYP7A1. Our findings confirm a therapeutic potential of probiotics for ameliorating dyslipidemia and NAFLD.

Kim, H. W., Yan, F. F., Hu, J. Y., Cheng, H. W., and Kim, Y. H. Effects of probiotics feeding on meat quality of chicken breast during postmortem storage. Poult.Sci. 3-4-2016;

ABSTRACT: This study evaluated the effects of dietary probiotic supplement and postmortem storage on meat quality of chicken breast during retail display. A total of 35 birds were randomly obtained from 3 feeding groups (control without probiotic supplement, 250 ppm Sporulin, and 500 ppm PoultryStar). The probiotic supplement had no influence on feed conversion ratio and body weight gain, as well as body weight at 29 and 44 d ($P > 0.05$). After slaughter, each side of the breast muscles (M. Pectoralis major) was assigned to either one d or 5 d of postmortem storage. Probiotic supplement had no influence on the rate of pH decline of chicken breast muscles during the initial 6 h postmortem ($P > 0.05$). No interactions between probiotic supplement and postmortem storage on meat quality were found ($P > 0.05$). Postmortem storage decreased drip loss from 25.30 to 18.05% ($P < 0.05$). Probiotics-fed chicken groups, particularly PoultryStar treatment, had a higher myofibrillar fragmentation index than the control group ($P < 0.05$). However, shear force values were not affected by the probiotic treatments. Decreases in color and lipid stabilities of breast muscles were found during display ($P < 0.05$), but were not affected by the probiotic supplement ($P > 0.05$). Our result suggests that probiotic supplement had no adverse impacts on proteolysis and oxidative changes during 5 d postmortem display storage.

Kim, J. H., Jeun, E. J., Hong, C. P., Kim, S. H., Jang, M. S., Lee, E. J., Moon, S. J., Yun, C. H., Im, S. H., Jeong, S. G., Park, B. Y., Kim, K. T., Seoh, J. Y., Kim, Y. K., Oh, S. J., Ham, J. S., Yang, B. G., and Jang, M. H. Extracellular vesicle-derived protein from Bifidobacterium longum

alleviates food allergy through mast cell suppression. J.Allergy Clin.Immunol. 2016; 137(2):507-516.

ABSTRACT: BACKGROUND: The incidence of food allergies has increased dramatically during the last decade. Recently, probiotics have been studied for the prevention and treatment of allergic disease. OBJECTIVE: We examined whether Bifidobacterium longum KACC 91563 and Enterococcus faecalis KACC 91532 have the capacity to suppress food allergies. METHODS: B longum KACC 91563 and E faecalis KACC 91532 were administered to BALB/c wild-type mice, in which food allergy was induced by using ovalbumin and alum. Food allergy symptoms and various immune responses were assessed. RESULTS: B longum KACC 91563, but not E faecalis KACC 91532, alleviated food allergy symptoms. Extracellular vesicles of B longum KACC 91563 bound specifically to mast cells and induced apoptosis without affecting T-cell immune responses. Furthermore, injection of family 5 extracellular solute-binding protein, a main component of extracellular vesicles, into mice markedly reduced the occurrence of diarrhea in a mouse food allergy model. CONCLUSION: B longum KACC 91563 induces apoptosis of mast cells specifically and alleviates food allergy symptoms. Accordingly, B longum KACC 91563 and family 5 extracellular solute-binding protein exhibit potential as therapeutic approaches for food allergies.

Klu, Y. A. and Chen, J. Influence of probiotics, included in peanut butter, on the fate of selected Salmonella and Listeria strains under simulated gastrointestinal conditions. J.Appl.Microbiol. 2016; 120(4):1052-1060.

ABSTRACT: AIMS: This study observed the behaviour of probiotics and selected bacterial pathogens co-inoculated into peanut butter during gastrointestinal simulation. METHODS AND RESULTS: Peanut butter homogenates co-inoculated with Salmonella/Listeria strains (5 log CFU ml(-1)) and lyophilized or cultured probiotics (9 log CFU ml(-1)) were exposed to simulated gastrointestinal conditions for 24 h at 37 degrees C. Sample pH, titratable acidity and pathogen populations were determined. Agar diffusion assay was performed to assess the inhibitory effect of probiotic culture supernatants with either natural (3.80 (Lactobacillus), 3.78 (Bifidobacteirum) and 5.17 (Streptococcus/Lactococcus)) or neutralized (6.0) pH. Antibacterial effect of crude bacteriocin extracts were also evaluated against the pathogens. After 24 h, samples with probiotics had lower pH and higher titratable acidity than those without probiotics. The presence of probiotics caused a significant reduction ($P < 0.05$) in pathogen populations. Supernatants of Bifidobacterium and Lactobacillus cultures inhibited pathogen growth; however, the elevation of pH diminished their antibacterial activities. Crude bacteriocin extracts had a strain-specific inhibitory effect only towards Listeria monocytogenes. CONCLUSION: Probiotics in 'peanut butter' survived simulated gastrointestinal conditions and inhibited the growth of Salmonella/Listeria. SIGNIFICANCE AND IMPACT OF THE STUDY: Peanut butter is a plausible carrier to deliver probiotics to improve the gastrointestinal health of children in developing countries.

Kobyliak, N., Falalyeyeva, T., Virchenko, O., Mykhalchyshyn, G., Bodnar, P., Spivak, M., Yankovsky, D., Beregova, T., and Ostapchenko, L. Comparative experimental investigation on the efficacy of mono- and multiprobiotic strains in non-alcoholic fatty liver disease prevention. BMC.Gastroenterol. 2016; 16(1):34

ABSTRACT: BACKGROUND: To investigate the efficacy of different probiotic strains, their combinations and forms (alive or lyophilized) in nonalcoholic fatty liver disease (NAFLD) prevention. METHODS: In this study, 70 rats have been used divided into 7 groups of 10 animals in each: I - intact rats, II-.

Kobyliak, N., Conte, C., Cammarota, G., Haley, A. P., Styriak, I., Gaspar, L., Fusek, J., Rodrigo, L., and Kruzliak, P. Probiotics in prevention and treatment of obesity: a critical view. Nutr.Metab (Lond). 2016; 13:14

ABSTRACT: The worldwide prevalence of obesity more than doubled between 1980 and 2014. The obesity pandemic is tightly linked to an increase in energy availability, sedentariness and greater control of ambient temperature that have paralleled the socioeconomic development of the past decades. The most frequent cause which leads to the obesity development is a dysbalance between energy intake and energy expenditure. The gut microbiota as an environmental factor which influence whole-body metabolism by affecting energy balance but also inflammation and gut barrier function, integrate peripheral and central food intake regulatory signals and thereby increase body weight. Probiotics have physiologic functions that contribute to the health of gut microbiota, can affect food intake and appetite, body weight and composition and metabolic functions through gastrointestinal pathways and modulation of the gut bacterial community.

Kocijancic, D., Felgner, S., Frahm, M., Komoll, R. M., Iljazovic, A., Pawar, V., Rohde, M., Heise, U., Zimmermann, K., Gunzer, F., Hammer, J., Crull, K., Leschner, S., and Weiss, S. Therapy of solid tumors using probiotic Symbioflor-2 - restraints and potential. Oncotarget. 3-10-2016;

ABSTRACT: To date, virulent bacteria remain the basis of most bacteria mediated cancer therapies. For clinical application attenuation is required. However, this might result in a drastically lowered therapeutic capacity. Herein we argue that the E. coli probiotic Symbioflor-2, with a history of safe application may constitute a viable tumor therapeutic candidate. We demonstrate that Symbioflor-2 displays a highly specific tumor targeting ability as determined in murine CT26 and RenCa tumor models. The excellent specificity was ascribed to reduced levels of adverse colonization. A high safety standard was demonstrated in WT and Rag1-/- mice. Thus, Symbioflor-2 may represent an ideal tumor targeting delivery system for therapeutic molecules. Moreover, Symbioflor-2 was capable of inducing CT26 tumor clearance as result of an adjuvant effect on tumor specific CD8+ T cells analogous to the Salmonella variant SL7207. However, lower therapeutic efficacy against RenCa tumors suggested a generally reduced therapeutic potency for probiotics. Interestingly, concurrent depletion of Gr-1+ or Ly6G+ cells installed therapeutic efficacy equal to SL7207, thus highlighting the role of innate effector cells in restraining the anti-tumor effects of Symbioflor-2. Collectively, our findings argue for a strategy of safe strain application and a more sustainable use of bacteria as a delivery system for therapeutic molecules.

Komatsu, S., Sakamoto, E., Norimizu, S., Shingu, Y., Asahara, T., Nomoto, K., and Nagino, M. Efficacy of perioperative synbiotics treatment for the prevention of surgical site infection after laparoscopic colorectal surgery: a randomized controlled trial. Surg.Today. 2016; 46(4):479-490.

ABSTRACT: PURPOSE: The aim of this study was to assess the effect of perioperative oral administration of synbiotics on the surgical outcome in patients undergoing laparoscopic colorectal resection. METHODS: In this single-center randomized, controlled trial, patients scheduled to undergo elective laparoscopic colorectal surgery were eligible to participate and randomly assigned to a synbiotics group or a control group. The primary study outcome was the development of infectious complications, particularly surgical site infection (SSI), within 30 days of surgery. RESULTS: In this study, 379 patients were enrolled and randomly assigned (173 to the synbiotics group and 206 to the control group), of whom 362 patients (168 to the synbiotics group and 194 to the control group) were eligible for this study. SSI occurred in 29 (17.3 %) patients in the synbiotics group and 44 (22.7 %) patients in the control group (OR: 0.761, 95 % CI 0.50-1.16; p = 0.20). Overall, the rate of postoperative complications, including anastomotic leakage, did not differ significantly between the two groups. Synbiotics treatment reversed the changes in fecal bacteria and organic acids after surgery and suppressed the increases in potentially pathogenic species, such as Clostridium difficile. CONCLUSION: The efficacy of perioperative administration of synbiotics was not validated as a treatment for reducing the incidence of infectious complications after laparoscopic colorectal resection. However, the microbial imbalance, in addition to the reduction in organic acids, could be improved by perioperative synbiotics treatment.

Korada, S. K., Yarla, N. S., Bishayee, A., Aliev, G., Aruna, Lakshmi K., Arunasree, M. K., Dananajaya, B. L., and Mishra, V. Can Probiotics Cure Inflammatory Bowel Diseases? Curr.Pharm.Des. 2016; 22(7):904-917.

ABSTRACT: Gastrointestinal (GI) disorders, especially microbial dysbiosis play role in several GI ailments such as irritable bowel syndrome, colorectal cancer, inflammatory bowel diseases, and antibiotic-associated diarrhoea. Role of inflammatory bowel disease (IBD) is multifactorial as it involves loss of maintaining intestinal epithelial barrier integrity, increased release of pro-inflammatory molecules, and microbial dysbiosis in gut microflora. Some specific pathogens also play a key role in the IBD development. The origin and causation are still in unfathomable condition and the exact root cause is unknown. Recently probiotic studies have been gaining importance because of their positive responses in their IBD experimental results. According to joint Food and Agricultural Organisation/World Health Organisation working group, probiotics are defined as live microorganisms which when administered in adequate amount confer health benefit on the host. These live beneficial microorganisms are considered helpful in improving gut colonization and perseverance thereby improves prophylactic effect. In the direction of IBD research, a number of studies are needed to standardize its methodology and its applicability on human usage. The particular review presents an overview of gut microflora and its impact on host health, types of IBD and existing therapies to treat this disorder, mechanism of several probiotic actions, role of probiotics in IBD prevention with their supporting evidences.

Lacy, B. E. Diagnosis and treatment of diarrhea-predominant irritable bowel syndrome. Int.J.Gen.Med. 2016; 9:7-17.

ABSTRACT: Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders worldwide. The economic impact of IBS on the health care system is substantial,

as is the personal impact on patients. Patients with diarrhea-predominant IBS (IBS-D) comprise a substantial proportion of the overall IBS population. Primary care providers are often the first point of contact for patients with IBS-D and can accurately diagnose IBS after a careful history and examination without extensive diagnostic tests. Several pharmacologic treatments (eg, loperamide, alosetron, and antidepressants) and non-pharmacologic treatments (eg, dietary modification and probiotics) are available for IBS-D, but restrictions on use (eg, alosetron) or the lack of controlled trial data showing reductions in both global and individual IBS-D symptoms (eg, bloating, pain and stool frequency) emphasize the need for alternative treatment options. Two newer medications (eluxadoline and rifaximin) were approved in May 2015 for the treatment of IBS-D, and represent new treatment options for this common gastrointestinal condition.

Ladas, E. J., Bhatia, M., Chen, L., Sandler, E., Petrovic, A., Berman, D. M., Hamblin, F., Gates, M., Hawks, R., Sung, L., and Nieder, M. The safety and feasibility of probiotics in children and adolescents undergoing hematopoietic cell transplantation. Bone Marrow Transplant. 2016; 51(2):262-266.

ABSTRACT: Hematopoietic cell transplantation (HCT) has become a standard treatment for many adult and pediatric conditions. Emerging evidence suggests that perturbations in the microbiota diversity increase recipients' susceptibilities to gut-mediated conditions such as diarrhea, infection and acute GvHD. Probiotics preserve the microbiota and may minimize the risk of developing a gut-mediated condition; however, their safety has not been evaluated in the setting of HCT. We evaluated the safety and feasibility of the probiotic, Lactobacillus plantarum (LBP), in children and adolescents undergoing allogeneic HCT. Participants received once-daily supplementation with LBP beginning on day -8 or -7 and continued until day +14. Outcomes were compliance with daily administration and incidence of LBP bacteremia. Administration of LBP was feasible with 97% (30/31, 95% confidence interval (CI) 83-100%) of children receiving at least 50% of the probiotic dose (median 97%; range 50-100%). We did not observe any case of LBP bacteremia (0% (0/30) with 95% CI 0-12%). There were not any unexpected adverse events related to LBP. Our study provides preliminary evidence that administration of LBP is safe and feasible in children and adolescents undergoing HCT. Future steps include the conduct of an approved randomized, controlled trial through Children's Oncology Group.

Lahner, E., Bellisario, C., Hassan, C., Zullo, A., Esposito, G., and Annibale, B. Probiotics in the Treatment of Diverticular Disease. A Systematic Review. J.Gastrointestin.Liver Dis. 2016; 25(1):79-86.

ABSTRACT: BACKGROUND AND AIMS: Diverticular disease is a common gastrointestinal condition. Low-grade inflammation and altered intestinal microbiota have been identified as factors contributing to abdominal symptoms. Probiotics may lead to symptoms improvement by modifying the gut microbiota and are promising treatments for diverticular disease. The aim of this study was to systematically review the efficacy of probiotics in diverticular disease in terms of remission of abdominal symptoms and prevention of acute diverticulitis. METHODS: According to PRISMA, we identified studies on diverticular disease patients treated with probiotics (Pubmed, Embase, Cochrane). The quality of these studies was evaluated by the Jadad scale. Main outcomes measures were remission of abdominal symptoms and prevention of acute diverticulitis. RESULTS: 11 studies (2 double-blind randomized placebo-controlled, 5 open randomized, 4 non-

randomized open studies) were eligible. Overall, diverticular disease patients were 764 (55.1% females, age 58-75 years). Three studies included patients with symptomatic uncomplicated diverticular disease, 4 studies with symptomatic uncomplicated diverticular disease in remission, 4 studies with complicated or acute diverticulitis. Mainly (72.7%) single probiotic strains had been used, most frequently Lactobacilli. Follow-up ranged from 1 to 24 months. Interventions were variable: in 8 studies the probiotic was administered together with antibiotic or anti-inflammatory agents and compared with the efficacy of the drug alone; in 3 studies the probiotic was compared with a high-fibre diet or used together with phytoextracts. As an outcome measure, 4 studies evaluated the occurrence rate of acute diverticulitis, 6 studies the reduction of abdominal symptoms, and 6 studies the recurrence of abdominal symptoms. Meta-analysis on the efficacy of probiotics in diverticular disease could not be performed due to the poor quality of retrieved studies. CONCLUSION: This systematic review showed that high-quality data on the efficacy of probiotics in diverticular disease are scant: the available data do not permit conclusions. Further investigation is required to understand how probiotics can be employed in this condition.

Lambaek, I. D., Fonnest, G., Gormsen, M., Brok, J., and Greisen, G. Probiotics to prevent necrotising enterocolitis in very preterm infants. Dan.Med.J. 2016; 63(3)

ABSTRACT: INTRODUCTION: Meta-analyses of randomised trials have shown that probiotics reduce the risk of necrotising enterocolitis (NEC) in preterm infants. However, the generalisability of these results, particularly for the most preterm infants, remains unresolved. Hence, we wanted to evaluate the benefit of implementing prophylactic use of probiotics as standard care in infants younger than 30 weeks of gestation. METHODS: Two three-year periods were compared. The first period was prior to a policy change. In this period no probiotics were used. The second period featured routine administration of probiotics (bifidobacillus and lactobacillus) once daily by nasogastric tube from the third day of life. The main outcome: NEC grades 2 and 3 were assessed in a blinded fashion from a clinical abstract and available X-rays. RESULTS: A total of 381 infants treated before the change of policy were compared with 333 infants treated after the policy change had been introduced. There was no statistically significant change in NEC (odds ratio (OR) = 0.75, p = 0.34, 95% confidence interval (CI): 0.43-1.30). The OR for death was 0.92 (p = 0.55, 95% CI: 0.62-1.40). Unexpectedly, symptoms of NEC appeared earlier in the latter period (median six versus 14 days, p = 0.004). No side effects and no blood cultures with lactobacillus or bifidobacterium were observed. CONCLUSIONS: This historically controlled study did not indicate that probiotics had a significant effect on NEC. We continue our practice, but larger cohort studies or meta-analyses of such studies are needed to confirm previous beneficial findings in randomised trials. FUNDING: none. TRIAL REGISTRATION: Clinicaltrials.gov NCT01670916.

Landete, J. M. A review of food-grade vectors in lactic acid bacteria: from the laboratory to their application. Crit Rev.Biotechnol. 2-26-2016; 1-13.

ABSTRACT: Lactic acid bacteria (LAB) have a long history of use in fermented foods and as probiotics. Genetic manipulation of these microorganisms has great potential for new applications in food safety, as well as in the development of improved food products and in health. While genetic engineering of LAB could have a major positive impact on the food and pharmaceutical industries, progress could be prevented by legal issues related to the controversy surrounding this technology. The safe use of genetically

modified LAB requires the development of food-grade cloning systems containing only the DNA from homologous hosts or generally considered as safe organisms, and not dependent antibiotic markers. The rationale for the development of cloning vectors derived from cryptic LAB plasmids is the need for new genetic engineering tools, therefore a vision from cryptic plasmids to applications in food-grade vectors for LAB plasmids is shown in this review. Replicative and integrative vectors for the construction of food-grade vectors, and the relationship between resistance mechanism and expression systems, will be treated in depth in this paper. Finally, we will discuss the limited use of these vectors, and the problems arising from their use.

Lau, C. S. and Chamberlain, R. S. Probiotics are effective at preventing Clostridium difficile-associated diarrhea: a systematic review and meta-analysis. Int.J.Gen.Med. 2016; 9:27-37.

ABSTRACT: INTRODUCTION: Clostridium difficile infection (CDI) is the leading cause of antibiotic-associated diarrhea. CDI has increased in incidence and severity over the past decade, and is a growing worldwide health problem associated with substantial health care costs and significant morbidity and mortality. This meta-analysis examines the impact of probiotics on the incidence of Clostridium difficile-associated diarrhea (CDAD) among children and adults, in both hospital and outpatient settings. METHODS: A comprehensive literature search of all published randomized control trials (RCTs) assessing the use of probiotics in the prevention of CDAD in patients receiving antibiotic therapy was conducted, and the incidence of CDAD was analyzed. RESULTS: Twenty-six RCTs involving 7,957 patients were analyzed. Probiotic use significantly reduced the risk of developing CDAD by 60.5% (relative risk [RR] =0.395; 95% confidence interval [CI], 0.294-0.531; P<0.001). Probiotics proved beneficial in both adults and children (59.5% and 65.9% reduction), especially among hospitalized patients. Lactobacillus, Saccharomyces, and a mixture of probiotics were all beneficial in reducing the risk of developing CDAD (63.7%, 58.5%, and 58.2% reduction). CONCLUSION: Probiotic supplementation is associated with a significant reduction in the risk of developing CDAD in patients receiving antibiotics. Additional studies are required to determine the optimal dose and strain of probiotic.

Lewis, Z. T., Shani, G., Masarweh, C. F., Popovic, M., Frese, S. A., Sela, D. A., Underwood, M. A., and Mills, D. A. Validating bifidobacterial species and subspecies identity in commercial probiotic products. Pediatr.Res. 2016; 79(3):445-452.

ABSTRACT: BACKGROUND: The ingestion of probiotics to attempt to improve health is increasingly common; however, quality control of some commercial products can be limited. Clinical practice is shifting toward the routine use of probiotics to aid in prevention of necrotizing enterocolitis in premature infants, and probiotic administration to term infants is increasingly common to treat colic and/or prevent atopic disease. Since bifidobacteria dominate the feces of healthy breast-fed infants, they are often included in infant-targeted probiotics. METHODS: We evaluated 16 probiotic products to determine how well their label claims describe the species of detectable bifidobacteria in the product. Recently developed DNA-based methods were used as a primary means of identification, and were confirmed using culture-based techniques. RESULTS: We found that the contents of many bifidobacterial probiotic products differ from the ingredient list, sometimes at a subspecies level. Only 1 of the 16 probiotics perfectly matched its bifidobacterial label claims in all samples tested, and both pill-to-pill and lot-to-lot

variation were observed. CONCLUSION: Given the known differences between various bifidobacterial species and subspecies in metabolic capacity and colonization abilities, the prevalence of misidentified bifidobacteria in these products is cause for concern for those involved in clinical trials and consumers of probiotic products.

Li, F., Duan, K., Wang, C., McClain, C., and Feng, W. Probiotics and Alcoholic Liver Disease: Treatment and Potential Mechanisms. Gastroenterol.Res.Pract. 2016; 2016:5491465

ABSTRACT: Despite extensive research, alcohol remains one of the most common causes of liver disease in the United States. Alcoholic liver disease (ALD) encompasses a broad spectrum of disorders, including steatosis, steatohepatitis, and cirrhosis. Although many agents and approaches have been tested in patients with ALD and in animals with experimental ALD in the past, there is still no FDA (Food and Drug Administration) approved therapy for any stage of ALD. With the increasing recognition of the importance of gut microbiota in the onset and development of a variety of diseases, the potential use of probiotics in ALD is receiving increasing investigative and clinical attention. In this review, we summarize recent studies on probiotic intervention in the prevention and treatment of ALD in experimental animal models and patients. Potential mechanisms underlying the probiotic function are also discussed.

Li, J., Sung, C. Y., Lee, N., Ni, Y., Pihlajamaki, J., Panagiotou, G., and El-Nezami, H. Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice. Proc.Natl.Acad.Sci.U.S.A. 3-1-2016; 113(9):E1306-E1315.

ABSTRACT: The beneficial roles of probiotics in lowering the gastrointestinal inflammation and preventing colorectal cancer have been frequently demonstrated, but their immunomodulatory effects and mechanism in suppressing the growth of extraintestinal tumors remain unexplored. Here, we adopted a mouse model and metagenome sequencing to investigate the efficacy of probiotic feeding in controlling s.c. hepatocellular carcinoma (HCC) and the underlying mechanism suppressing the tumor progression. Our result demonstrated that Prohep, a novel probiotic mixture, slows down the tumor growth significantly and reduces the tumor size and weight by 40% compared with the control. From a mechanistic point of view the down-regulated IL-17 cytokine and its major producer Th17 cells, whose levels decreased drastically, played critical roles in tumor reduction upon probiotics feeding. Cell staining illustrated that the reduced Th17 cells in the tumor of the probiotic-treated group is mainly caused by the reduced frequency of migratory Th17 cells from the intestine and peripheral blood. In addition, shotgun-metagenome sequencing revealed the crosstalk between gut microbial metabolites and the HCC development. Probiotics shifted the gut microbial community toward certain beneficial bacteria, including Prevotella and Oscillibacter, that are known producers of antiinflammatory metabolites, which subsequently reduced the Th17 polarization and promoted the differentiation of antiinflammatory Treg/Tr1 cells in the gut. Overall, our study offers novel insights into the mechanism by which probiotic treatment modulates the microbiota and influences the regulation of the T-cell differentiation in the gut, which in turn alters the level of the proinflammatory cytokines in the extraintestinal tumor microenvironment.

Li, Q. and Zhou, J. M. The microbiota-gut-brain axis and its potential therapeutic role in autism spectrum disorder. Neuroscience. 3-8-2016; 324:131-139.

ABSTRACT: Autism spectrum disorder (ASD) is a series of neurodevelopmental disorders that are characterized by deficits in both social and cognitive functions. Although the exact etiology and pathology of ASD remain unclear, a disorder of the microbiota-gut-brain axis is emerging as a prominent factor in the generation of autistic behaviors. Clinical studies have shown that gastrointestinal symptoms and compositional changes in the gut microbiota frequently accompany cerebral disorders in patients with ASD. A disturbance in the gut microbiota, which is usually induced by a bacterial infection or chronic antibiotic exposure, has been implicated as a potential contributor to ASD. The bidirectional microbiota-gut-brain axis acts mainly through neuroendocrine, neuroimmune, and autonomic nervous mechanisms. Application of modulators of the microbiota-gut-brain axis, such as probiotics, helminthes and certain special diets, may be a promising strategy for the treatment of ASD. This review mainly discusses the salient observations of the disruptions of the microbiota-gut-brain axis in the pathogenesis of ASD and reveals its potential therapeutic role in autistic deficits.

Liao, H. Y., Tao, L., Zhao, J., Qin, J., Zeng, G. C., Cai, S. W., Li, Y., Zhang, J., and Chen, H. G. Clostridium butyricum in combination with specific immunotherapy converts antigen-specific B cells to regulatory B cells in asthmatic patients. Sci.Rep. 2016; 6:20481

ABSTRACT: The effect of antigen specific immunotherapy (SIT) on asthma is supposed to be improved. Published data indicate that administration of probiotics alleviates allergic diseases. B cells play important roles in the pathogenesis of allergic diseases. This study aims to modulate antigen specific B cell property by the administration of Clostridium butyrate (CB) in combination with SIT. The results showed that after a 3-month treatment, the total asthma clinical score and serum specific IgE were improved in the patients treated with SIT, which was further improved in those treated with both SIT and CB, but not in those treated with CB alone. Treatment with SIT and CB increased p300 and STAT3 activation, up regulated the IL-10 gene transcription and increased the frequency of peripheral antigen specific B cells. In conclusion, administration with SIT in combination with CB converts Der p 1 specific B cells to regulatory B cells in asthma patients allergic to Der p 1. The data suggest a potential therapeutic remedy in the treatment of allergic diseases.

Lim, S. M., Jeong, J. J., Woo, K. H., Han, M. J., and Kim, D. H. Lactobacillus sakei OK67 ameliorates high-fat diet-induced blood glucose intolerance and obesity in mice by inhibiting gut microbiota lipopolysaccharide production and inducing colon tight junction protein expression. Nutr.Res. 2016; 36(4):337-348.

ABSTRACT: A high-fat diet (HFD) induces obesity and the associated increases in blood glucose and inflammation through changes in gut microbiota, endotoxemia, and increased gut permeability. To counteract this, researchers have suggested that the use of probiotics that suppress production of proinflammatory lipopolysaccharide (LPS). Here, we tested whether Lactobacillus sakei OK67, which inhibits gut microbiota LPS production selected from among the lactic acid bacteria isolated from kimchi, exerted antihypoglycemic or anti-inflammatory effects in HFD-fed mice. Mice were randomly divided into 2 groups and fed an HFD or a low-fat diet for 4 weeks. These groups were further subdivided; 1 subgroup was treated with L sakei OK67 and fed the experimental diet for 4.5 weeks, whereas the other subgroup was fed the experimental diet alone. L sakei OK67 treatment lowered HFD-elevated LPS levels in blood and colonic fluid and

significantly decreased HFD-elevated fasting blood glucose levels and the area under the curve in an oral glucose tolerance test. L sakei OK67 treatment inhibited HFD-induced body and epididymal fat weight gains, suppressed HFD-induced tumor necrosis factor-alpha and interleukin-1beta expression and nuclear factor-kappaB activation in the colon, and significantly increased HFD-suppressed interleukin-10 and tight junction protein expression in the colon. Oral administration of L sakei OK67 significantly downregulated HFD-induced expression of peroxisome proliferator-activated receptor gamma, fatty acid synthase, and tumor necrosis factor-alpha in adipose tissue. In addition, L sakei OK67 treatment strongly inhibited nuclear factor-kappaB activation in LPS-stimulated peritoneal macrophages. We report that L sakei OK67 ameliorates HFD-induced hyperglycemia and obesity by reducing inflammation and increasing the expression of colon tight junction proteins in mice.

Linares, D. M., Ross, P., and Stanton, C. Beneficial Microbes: The pharmacy in the gut. Bioengineered. 1-2-2016; 7(1):11-20.

ABSTRACT: The scientific evidence supporting the gut microbiome in relation to health maintenance and links with various disease states afflicting humans, from metabolic to mental health, has grown dramatically in the last few years. Strategies addressing the positive modulation of microbiome functionality associated with these disorders offer huge potential to the food and pharmaceutical industries to innovate and provide therapeutic solutions to many of the health issues affecting modern society. Such strategies may involve the use of probiotics and prebiotics as nutritional adjunct therapies. Probiotics are generally recognized to be a good form of therapy to keep harmful, intestinal microorganisms in check, aid digestion and nutrient absorption, and contribute to immune function. Probiotics are reported to improve microbial balance in the intestinal tract and promote the return to a baseline microbial community following a perturbing event (dysbiosis) such as antibiotic therapy. Prebiotics are selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora, which confers benefits upon host well-being and health.

Liu, J., Liu, Y., Li, G., Shen, J., Tao, Z., Tian, Y., Chen, L., Li, C., and Lu, L. Dynamic Comparation on the Usage of Probiotics in Organic Wastewater Treatment under Aerobic Conditions in a Diurnal Environment. J.Air Waste Manag.Assoc. 3-2-2016;

ABSTRACT: This study aims at evaluating and comparing pollution removal in wastewater treatment via the use of probiotics alone or in combination under aerobic conditions in diurnal cycles. Herein, 650 mL of organic wastewater was stored in 1 L conical flasks and then randomly divided into three treatment groups, each experiment was repeated three times. Group A was supplemented with 2% (v/v) photosynthetic bacteria (Rhodopseudomonas palustris) alone; group B was supplemented with 2% (v/v) B. subtilis alone; group C was supplemented with 1% (v/v) photosynthetic bacteria (PSB) and 1% (v/v) B. subtilis. Results showed that the pH increases were in the order: Group A >== Group C >== Group B. The performance of the probiotics in terms of ammonia nitrogen and TN removal was in the order: Group A >== Group C >==Group B, while in terms of TOC and TC removal were: Group C >== Group B >== Group A. CONCLUSION: The effect of probiotics combination treatment on ammonia nitrogen and TN removal was better than that using B. subtilis alone, but worse than that using PSB alone. The effect of B. subtilis alone treatment on TOC and TC removal was better than that using PSB alone,but

the combination of PSB and B. subtilis showed greater benefits on TOC and TC removal. IMPLICATIONS: Photosynthetic bacteria and B. subtilis were used in this study to investigate the carbon and nitrogen metabolism via the use of different probiotics and then study further on comparing and achieving the best pollution removal performance in probiotics alone or in combination treatment. To make observations realistic, the experiments were conducted under aerobic conditions in a diurnal cycle environment.

Liu, Z., Liu, W., Ran, C., Hu, J., and Zhou, Z. Abrupt suspension of probiotics administration may increase host pathogen susceptibility by inducing gut dysbiosis. Sci.Rep. 2016; 6:23214

ABSTRACT: In this study, we investigated the risk associated with suspension of probiotics administration in tilapia, an animal model that may mimic immune-compromised conditions in humans. Tilapias were fed for 14 days using a probiotics-supplemented diet, followed by a three-day suspension of probiotics treatment and a subsequent challenge by Aeromonas hydrophila. Unexpectedly, the suspension of a probiotic strain Lactobacillus plantarum JCM1149 significantly triggered susceptibility of the host to A. hydrophila. We further observed that suspension of JCM1149 resulted in host gut microbiota dysbiosis and the subsequent disorder in the intestinal metabolites (bile acids, amino acids, and glucose) and damage in the intestinal epithelium, giving rise to a condition similar to antibiotics-induced gut dysbiosis, which collectively impaired tilapia's gut health and resistance to pathogenic challenges. Additionally, we determined that JCM1149 adhered relatively poorly to tilapia intestinal mucosa and was rapidly released from the gastrointestinal tract (GIT) after suspension, with the rapid loss of probiotic strain probably being the direct cause of gut dysbiosis. Finally, three other probiotic Lactobacillus strains with low intestinal mucosa binding activity showed similar rapid loss phenotype following administration suspension, and induced higher host susceptibility to infection, indicating that the risk is a generic phenomenon in Lactobacillus.

Lopez, P., de, Paz B., Rodriguez-Carrio, J., Hevia, A., Sanchez, B., Margolles, A., and Suarez, A. Th17 responses and natural IgM antibodies are related to gut microbiota composition in systemic lupus erythematosus patients. Sci.Rep. 2016; 6:24072

ABSTRACT: Intestinal dysbiosis, characterized by a reduced Firmicutes/Bacteroidetes ratio, has been reported in systemic lupus erythematosus (SLE) patients. In this study, in vitro cultures revealed that microbiota isolated from SLE patient stool samples (SLE-M) promoted lymphocyte activation and Th17 differentiation from naive CD4(+) lymphocytes to a greater extent than healthy control-microbiota. Enrichment of SLE-M with Treg-inducing bacteria showed that a mixture of two Clostridia strains significantly reduced the Th17/Th1 balance, whereas Bifidobacterium bifidum supplementation prevented CD4(+) lymphocyte over-activation, thus supporting a possible therapeutic benefit of probiotics containing Treg-inducer strains in order to restore the Treg/Th17/Th1 imbalance present in SLE. In fact, ex vivo analyses of patient samples showed enlarged Th17 and Foxp3(+) IL-17(+) populations, suggesting a possible Treg-Th17 trans-differentiation. Moreover, analyses of fecal microbiota revealed a negative correlation between IL-17(+) populations and Firmicutes in healthy controls, whereas in SLE this phylum correlated directly with serum levels of IFNgamma, a Th1 cytokine slightly reduced in patients. Finally, the frequency of Synergistetes, positively correlated with the Firmicutes/Bacteroidetes ratio in healthy controls, tended to be reduced in patients when anti-dsDNA titers were increased and showed a strong negative correlation with IL-

6 serum levels and correlated positively with protective natural IgM antibodies against phosphorylcholine.

Lu, C., Sang, J., He, H., Wan, X., Lin, Y., Li, L., Li, Y., and Yu, C. Probiotic supplementation does not improve eradication rate of Helicobacter pylori infection compared to placebo based on standard therapy: a meta-analysis. Sci.Rep. 2016; 6:23522

ABSTRACT: This meta-analysis included eligible randomized controlled trials (RCTs) with the aim of determining whether probiotic supplementation can improve H. pylori eradication rates. PUBMED, EBSCO, Web of Science, and Ovid databases were searched. We included RCTs that investigated the effect of combining probiotics, with or without a placebo, with standard therapy. A total of 21 RCTs that reported standard therapy plus probiotics were included. Compared to the placebo group, the probiotics group was 1.21(OR 1.21, 95% CI: 0.86, 1.69) and 1.28 (OR 1.28, 95% CI: 0.88, 1.86) times more likely to achieve eradication of H. pylori infection in intent-to-treat (ITT) analysis and per protocol (PP) analysis, respectively. Probiotics with triple therapy plus a 14-day course of treatment did not improve the eradication of H. pylori infection (OR 1.44, 95% CI: 0.87, 2.39) compared to the placebo. Moreover, the placebo plus standard therapy did not improve eradication rates compared to standard therapy alone (P = 0.816). However, probiotics did improve the adverse effects of diarrhea and nausea. These pooled data suggest that the use of probiotics plus standard therapy does not improve the eradication rate of H. pylori infection compared to the placebo.

Lytvyn, L., Quach, K., Banfield, L., Johnston, B. C., and Mertz, D. Probiotics and synbiotics for the prevention of postoperative infections following abdominal surgery: a systematic review and meta-analysis of randomized controlled trials. J.Hosp.Infect. 2016; 92(2):130-139.

ABSTRACT: BACKGROUND: Postoperative infections, particularly surgical site infections (SSIs), cause significant morbidity and mortality. Probiotics or synbiotics are a potential prevention strategy. AIM: To evaluate the efficacy of probiotics/synbiotics for reducing postoperative infection risk following abdominal surgery. METHODS: We searched AMED, Central, CINAHL, Embase, Medline, and grey literature for randomized controlled trials of elective abdominal surgery patients administered probiotics or synbiotics compared to placebo or standard care. Primary outcome was SSIs. Secondary outcomes were adverse events, respiratory tract infections (RTIs), urinary tract infections (UTIs), combined infections, length of hospital stay, and mortality. Using random-effects meta-analyses, we estimated the relative risk (RR) or mean difference (MD) and 95% confidence interval (CI). Tests were performed for heterogeneity, subgroup and sensitivity analyses were conducted, and the overall evidence quality was graded. FINDINGS: We identified 20 trials (N = 1374 participants) reporting postoperative infections. Probiotics/synbiotics reduced SSIs (RR: 0.63; 95% CI: 0.41-0.98; N = 15 studies), UTIs (RR: 0.29; 95% CI: 0.15-0.57; N = 11), and combined infections (RR: 0.49; 95% CI: 0.35-0.70; N = 18). There was no difference between groups for adverse events (RR: 0.89; 95% CI: 0.61-1.30; N = 6), RTIs (RR: 0.60; 95% CI: 0.36-1.00; N = 14), length of stay (MD: -1.19; 95% CI: -2.94 to 0.56; N = 12), or mortality (RR: 1.20; 95% CI: 0.58-2.48; N = 15). CONCLUSION: Our review suggests that probiotics/synbiotics reduce SSIs and UTIs from abdominal surgeries compared to placebo or standard of care, without evidence of safety risk. Overall study quality was low, owing mostly to imprecision (few patients and events, or wide CIs); thus larger multi-centered trials are needed to further assess the certainty in this estimate.

Machado, M. V. and Cortez-Pinto, H. Diet, Microbiota, Obesity, and NAFLD: A Dangerous Quartet. Int.J.Mol.Sci. 2016; 17(4)

ABSTRACT: Recently, the importance of the gut-liver-adipose tissue axis has become evident. Nonalcoholic fatty liver disease (NAFLD) is the hepatic disease of a systemic metabolic disorder that radiates from energy-surplus induced adiposopathy. The gut microbiota has tremendous influences in our whole-body metabolism, and is crucial for our well-being and health. Microorganisms precede humans in more than 400 million years and our guest flora evolved with us in order to help us face aggressor microorganisms, to help us maximize the energy that can be extracted from nutrients, and to produce essential nutrients/vitamins that we are not equipped to produce. However, our gut microbiota can be disturbed, dysbiota, and become itself a source of stress and injury. Dysbiota may adversely impact metabolism and immune responses favoring obesity and obesity-related disorders such as insulin resistance/diabetes mellitus and NAFLD. In this review, we will summarize the latest evidence of the role of microbiota/dysbiota in diet-induced obesity and NAFLD, as well as the potential therapeutic role of targeting the microbiota in this set.

Mackos, A. R., Galley, J. D., Eubank, T. D., Easterling, R. S., Parry, N. M., Fox, J. G., Lyte, M., and Bailey, M. T. Social stress-enhanced severity of Citrobacter rodentium-induced colitis is CCL2-dependent and attenuated by probiotic Lactobacillus reuteri. Mucosal.Immunol. 2016; 9(2):515-526.

ABSTRACT: Psychological stressors are known to affect colonic diseases but the mechanisms by which this occurs, and whether probiotics can prevent stressor effects, are not understood. Because inflammatory monocytes that traffic into the colon can exacerbate colitis, we tested whether CCL2, a chemokine involved in monocyte recruitment, was necessary for stressor-induced exacerbation of infectious colitis. Mice were exposed to a social disruption stressor that entails repeated social defeat. During stressor exposure, mice were orally challenged with Citrobacter rodentium to induce a colonic inflammatory response. Exposure to the stressor during challenge resulted in significantly higher colonic pathogen levels, translocation to the spleen, increases in colonic macrophages, and increases in inflammatory cytokines and chemokines. The stressor-enhanced severity of C. rodentium-induced colitis was not evident in CCL2(-/-) mice, indicating the effects of the stressor are CCL2-dependent. In addition, we tested whether probiotic intervention could attenuate stressor-enhanced infectious colitis by reducing monocyte/macrophage accumulation. Treating mice with probiotic Lactobacillus reuteri reduced CCL2 mRNA levels in the colon and attenuated stressor-enhanced infectious colitis. These data demonstrate that probiotic L. reuteri can prevent the exacerbating effects of stressor exposure on pathogen-induced colitis, and suggest that one mechanism by which this occurs is through downregulation of the chemokine CCL2.

Madan, J. C. Neonatal Gastrointestinal and Respiratory Microbiome in Cystic Fibrosis: Potential Interactions and Implications for Systemic Health. Clin.Ther. 3-10-2016;

ABSTRACT: PURPOSE: The gastrointestinal microbiome plays a critical role in nutrition and metabolic and immune functions in infants and young children and has implications for lifelong health. Cystic fibrosis (CF) transmembrane conductance regulator (CFTR)

mutations in CF result in viscous mucous production, frequent exposure to antibiotics, and atypical colonization patterns, resulting in an evolving dysbiosis of the gastrointestinal and respiratory microsystems; dysbiosis in CF results in systemic inflammation, chronic infection, and dysregulation of immune function. Dysbiosis in both the respiratory system and gut contributes to undernutrition, growth failure, and long-term respiratory and systemic morbidity in infants and children with CF. Understanding the role that the gut and respiratory microbiome plays in health or disease progression in CF will afford opportunities to better identify interventions to affect clinical changes. METHODS: Summary was done of the pertinent literature in CF and the study of the microbiome and probiotics. FINDINGS: New studies have identified bacteria in the respiratory tract in CF that are typically members of the intestinal microbiota, and enteral exposures to breast milk and probiotics are associated with prolonged periods of respiratory stability in CF. IMPLICATIONS: Understanding the complex interactions between the CFTR mutations, microbial colonization, and mucosal and systemic immunity is of major importance to inform new treatment strategies (such as restoring a healthier microbiome with probiotics or dietary interventions) to improve nutritional status and immune competence and to decrease morbidity and mortality in CF.

Madjd, A., Taylor, M. A., Mousavi, N., Delavari, A., Malekzadeh, R., Macdonald, I. A., and Farshchi, H. R. Comparison of the effect of daily consumption of probiotic compared with low-fat conventional yogurt on weight loss in healthy obese women following an energy-restricted diet: a randomized controlled trial. Am.J.Clin.Nutr. 2016; 103(2):323-329.

ABSTRACT: BACKGROUND: Despite evidence for the beneficial effects of probiotics and low-fat dairy products, to our knowledge, no study has compared the beneficial effect on weight loss of consuming a probiotic yogurt (PY) compared with a standard low-fat yogurt (LF) during a hypoenergetic program. OBJECTIVE: We compared the effect of the PY with LF yogurt consumption on body weight and cardiometabolic risk factors in women during a weight-loss program. DESIGN: Overweight and obese women [body mass index (in kg/m(2)): 27-40; age: 18-50 y) who usually consumed standard LFs were asked to consume either PY or LF every day with their main meals for 12 wk while following a weight-loss program. RESULTS: A total of 89 participants were randomly assigned to one of the 2 intervention groups. Baseline variables were not significantly different between groups. A statistically significant reduction in anthropometric measurements and significant improvements in cardiometabolic risk characteristics were observed over the 12 wk in both groups. However, no significant differences in weight loss and anthropometric measurements were seen between groups after the intervention. Compared with the LF group, the PY group had a greater (mean +/- SD) decrease in total cholesterol (PY = -0.36 +/- 0.10 mmol/L, LF = -0.31 +/- 0.10 mmol/L; P = 0.024), low-density lipoprotein cholesterol (PY = -0.35 +/- 0.10 mmol/L, LF = -0.31 +/- 0.11 mmol/L; P = 0.018), homeostasis model assessment of insulin resistance (PY = -0.55 +/- 0.32, LF = -0.42 +/- 0.20; P = 0.002), 2-h postprandial glucose (PY = -0.61 +/- 0.24 mmol/L, LF = -0.44 +/- 0.19 mmol/L; P < 0.001), and fasting insulin concentration (PY = -1.76 +/- 1.01 mU/mL, LF = -1.32 +/- 0.62 mU/mL; P = 0.002), as secondary endpoints after the study. No significant differences were found for fasting plasma glucose, high-density lipoprotein cholesterol, or triglycerides within both groups after the 12 wk. CONCLUSION: Consumption of PY compared with LF with main meals showed no significant effects on weight loss. However, it may have positive effects on lipid profiles and insulin sensitivity during a weight-loss program. This trial was registered at http://www.irct.ir/ as IRCT201402177754N8.

Mahboubi, M. and Kazempour, N. The Effects of Inulin on Characteristics of Lactobacillus paracasei TD3 (IBRC-M 10784) as Probiotic Bacteria in vitro. Arch.Iran Med. 2016; 19(2):92-95.

ABSTRACT: INTRODUCTION: Probiotics, as live microbial food ingredients or functional foods, are often related to health promotion and diseases prevention. The growth of these beneficial probiotics was improved by non-digestible food ingredients that are defined as prebiotics. Inulin is one of the known prebiotics that improves the gastrointestinal health. AIM: The aim of this study was to evaluate the functional properties of Lactobacillus paracasei TD3 (IBRC-M 10784) and the effects of inulin as prebiotic on these properties in vitro. METHODS: The tolerance of L. paracasei TD3 for low pH and pepsin (stomach condition), bile salt and trypsin conditions (small intestine conditions), antibiotics and pathogenic bacteria were evaluated by different methods. RESULTS: Lactobacillus paracasei TD3 was obtained from TakGene Company. L. paracasei TD3 was resistant to acidic pH higher than 3.5 and was tolerant to pepsin condition lower than 0.723 microg/mL and to bile salts </= 0.4% w/v and tripsin condition equal to 72.32 u/g and lower. This strain was resistant to vancomycin, nalidixic acid, colistin and gentamicin while was sensitive to penicillin, streptomycin and tetracycline. L. paracasei TD3 showed antimicrobial activity against S. typhimurium, Sh. dysanteriae, Sh. flexneri and E. coli, respectively. Inulin promoted the growth of L. paracasei TD3 (log CFU) in different conditions and increased the antibacterial activity of L. paracasei TD3 against pathogenic bacteria without changing the tolerance of bacteria to pH and other conditions (P > 0.05). CONCLUSION: L. paracasei TD3 (IBRC-M 10784) in combination with inulin can be considered as valuable strain for further investigations in human clinical studies.

Majeed, M., Nagabhushanam, K., Natarajan, S., Sivakumar, A., Eshuis-de, Ruiter T., Booij-Veurink, J., de Vries, Y. P., and Ali, F. Evaluation of genetic and phenotypic consistency of Bacillus coagulans MTCC 5856: a commercial probiotic strain. World J.Microbiol.Biotechnol. 2016; 32(4):60

ABSTRACT: Commercial probiotics preparation containing Bacillus coagulans have been sold in the market for several decades. Due to its high intra-species genomic diversity, it is very likely that B. coagulans strain may alter in different ways over multiple years of production. Therefore, the present study focuses to evaluate the genetic consistency and probiotic potential of B. coagulans MTCC 5856. Phenotypic and genotypic techniques including biochemical profiling, 16S rRNA sequencing, GTG 5'', BOX PCR fingerprinting, and Multi-Locus-Sequence typing (MLST) were carried out to evaluate the identity and consistency of the B. coagulans MTCC 5856. Further, in vitro probiotic potential, safety and stability at ambient temperature conditions of B. coagulans MTCC 5856 were evaluated. All the samples were identified as B. coagulans by biochemical profiling and 16S rRNA sequencing. GTG 5'', BOX PCR fingerprints and MLST studies revealed that the same strain was present over 3 years of commercial production. B. coagulans MTCC 5856 showed resistance to gastric acid, bile salt and exhibited antimicrobial activity in in-vitro studies. Additionally, B. coagulans MTCC 5856 was found to be non-mutagenic, non-cytotoxic, negative for enterotoxin genes and stable at ambient temperature (25 +/- 2 degrees C) for 36 months. The data of the study verified that the same strain of B. coagulans MTCC 5856 was present in commercial preparation over multiple years of production.

Maldonado-Barragan, A., Caballero-Guerrero, B., Martin, V., Ruiz-Barba, J. L., and Rodriguez, J. M. Purification and genetic characterization of gassericin E, a novel co-culture inducible bacteriocin from Lactobacillus gasseri EV1461 isolated from the vagina of a healthy woman. BMC.Microbiol. 2016; 16(1):37

ABSTRACT: BACKGROUND: Lactobacillus gasseri is one of the dominant Lactobacillus species in the vaginal ecosystem. Some strains of this species have a high potential for being used as probiotics in order to maintain vaginal homeostasis, since they may confer colonization resistance against pathogens in the vagina by direct inhibition through production of antimicrobial compounds, as bacteriocins. In this work we have studied bacteriocin production of gassericin E (GasE), a novel bacteriocin produced by L. gasseri EV1461, a strain isolated from the vagina of a healthy woman, and whose production was shown to be promoted by the presence of certain specific bacteria in co-culture. Biochemical and genetic characterization of this novel bacteriocin are addressed. RESULTS: We found that the inhibitory spectrum of L. gasseri EV1461 was broad, being directed to species both related and non-related to the producing strain. Interestingly, L. gasseri EV1461 inhibited the grown of pathogens usually associated with bacterial vaginosis (BV). The antimicrobial activity was due to the production of a novel bacteriocin, gassericin E (GasE). Production of this bacteriocin in broth medium only was achieved at high cell densities. At low cell densities, bacteriocin production ceased and only was restored after the addition of a supernatant from a previous bacteriocin-producing EV1461 culture (autoinduction), or through co-cultivation with several other Gram-positive strains (inducing bacteria). DNA sequence of the GasE locus revealed the presence of two putative operons which could be involved in biosynthesis and immunity of this bacteriocin (gaeAXI), and in regulation, transport and processing (gaePKRTC). The gaePKR encodes a putative three-component regulatory system, involving an autoinducer peptide (GaeP), a histidine protein kinase (GaeK) and a response regulator (GaeR), while the gaeTC encodes for an ABC transporter (GaeT) and their accessory protein (GaeC), involved in transport and processing of the bacteriocin. The gaeAXI, encodes for the bacteriocin gassericin E (GasE), a putative peptide bacteriocin (GaeX), and their immunity protein (GaeI). CONCLUSIONS: The origin of the strain (vagina of healthy woman) and its ability to produce bacteriocins with inhibitory activity against vaginal pathogens may be an advantage for using L. gasseri EV1461 as a probiotic strain to fight and/or prevent bacterial infections as bacterial vaginosis (BV), since it could be better adapted to live and compete into the vaginal environment.

Malik, A., Sumayyah, S., Yeh, C. W., and Heng, N. C. Identification and sequence analysis of pWcMBF8-1, a bacteriocin-encoding plasmid from the lactic acid bacterium Weissella confusa. FEMS Microbiol.Lett. 3-13-2016;

ABSTRACT: Members of the Gram-positive lactic acid bacteria (LAB) are well-known for their beneficial properties as starter cultures and probiotics. Many LAB species produce ribosomally-synthesized proteinaceous antibiotics (bacteriocins). Weissella confusa MBF8-1 is a strain isolated from a fermented soybean product that not only produces useful exopolysaccharides but also exhibits bacteriocin activity, which we call weissellicin MBF. Here, we show that bacteriocin production by W. confusa MBF8-1 is specified by a large plasmid, pWcMBF8-1. Plasmid pWcMBF8-1 (GenBank accession number KR350502), which was identified from the W. confusa MBF8-1 draft genome sequence, is 17,643 bp in length with a G+C content of 34.8% and contains 25 open reading frames (ORFs). Six ORFs constitute the weissellicin MBF locus, encoding three putative double-glycine-motif

peptides (Bac1, Bac2, Bac3), an ABC transporter complex (BacTE) and a putative immunity protein (BacI). Two ORFs encode plasmid partitioning and mobilization proteins, suggesting that pWcMBF8-1 is transferable to other hosts. To the best of our knowledge, plasmid pWcMBF8-1 not only represents the first large Weissella plasmid to be sequenced but also the first to be associated with bacteriocin production in W. confusa.

Mandal, H., Jariwala, R., and Bagchi, T. Isolation and characterization of lactobacilli from human faeces and indigenous fermented foods for their potential application as probiotics. Can.J.Microbiol. 2016; 62(4):349-359.

ABSTRACT: This study was conducted to select Lactobacillus strains from various sources on the basis of their probiotic attributes, such as acid and bile tolerance, binding to intestinal cells, and antimicrobial activity. Twelve isolates were obtained from human and food sources and were evaluated against standard probiotic Lactobacillus rhamnosus GG (LGG). Isolates were also studied for their antibiotic susceptibility. Isolate Lactobacillus fermentum GPI-6 showed the best survival profile at 0.3% and 1% bile salt, as compared with LGG. Isolates Lactobacillus plantarum GRI-2 and Lactobacillus salivarius GPI-4 showed no reduction in survival rate at pH 2.5. As expected, isolates showed strain-specific differences when comparing various attributes. Isolates GPI-4, GPI-7, and FA-5 showed better adhesion to HT-29, while isolate GPI-4 adhered better to Caco-2 cells than did LGG. However, when studying their ability to compete with Escherichia coli O26:H11, isolates GPI-6 and GPI-7 significantly inhibited E. coli adhesion to both HT-29 and Caco-2 cells compared with LGG. In conclusion, isolates GPI-4, GPI-7, and FA-5 showed excellent binding ability and antagonistic activity and better tolerance to acidic pH (pH 2.5) and to different bile salt concentrations in comparison with LGG, and hence, they could be considered as potential probiotic candidates.

Mariman, R., Reefman, E., Tielen, F., Persoon-Deen, C., van de, Mark K., Worms, N., Koning, F., and Nagelkerken, L. Lactobacillus plantarum NCIMB8826 ameliorates inflammation of colon and skin in human APOC1 transgenic mice. Benef.Microbes. 3-11-2016; 7(2):215-225.

ABSTRACT: Genetic predisposition and environmental factors, including the gut microbiota, have been suggested as major factors in the development and progression of atopic dermatitis. Hyperlipidemic human APOC1(+/+) transgenic mice display many features of human atopic dermatitis, such as scaling, lichenification, excoriations, and pruritus, along with a disturbed skin barrier function. Cytokine analysis of serum shows an increase of various pro-inflammatory cytokines, including interleukin (IL)-12p40, IL-6, and IL-1alpha, but lower levels of interferon-gamma. These mice also display aspects of colitis evident from macroscopic and histological abnormalities. Genome-wide transcriptome analysis of the intestine shows up-regulation of several genes associated with mast cells and eosinophils and this observation was confirmed by demonstrating increased numbers of IgE(+) and FcRepsilon(+) mast cells in the colon and in the skin. Oral treatment with Lactobacillus plantarum NCIMB8826 resulted in decreased numbers of mast cells in the colon. Moreover, this L. plantarum strain ameliorated skin pathology, evident from improved skin barrier integrity, absence of skin thickening, and less excoriations. These results suggest that modulation of intestinal immune homeostasis contributes to the suppression of atopic dermatitis.

Marom, T., Marchisio, P., Tamir, S. O., Torretta, S., Gavriel, H., and Esposito, S. Complementary and Alternative Medicine Treatment Options for Otitis Media: A Systematic Review. Medicine (Baltimore). 2016; 95(6):e2695

ABSTRACT: Otitis media (OM) has numerous presentations in children. Together with conventional medical therapies aimed to prevent and/or treat OM, a rising number of complementary and alternative medicine (CAM) treatment options can be offered. Since OM is common in children, parents may ask healthcare professionals about possible CAM therapies. Many physicians feel that their knowledge is limited regarding these therapies, and that they desire some information. Therefore, we conducted a literature review of CAM therapies for OM, taking into account that many of these treatments, their validity and efficacy and have not been scientifically demonstrated.We performed a search in MEDLINE (accessed via PubMed) using the following terms: "CAM" in conjunction with "OM" and "children. Retrieved publications regarding treatment of OM in children which included these terms included randomized controlled trials, prospective/retrospective studies, and case studies.The following CAM options for OM treatment in children were considered: acupuncture, homeopathy, herbal medicine/phytotherapy, osteopathy, chiropractic, xylitol, ear candling, vitamin D supplement, and systemic and topical probiotics. We reviewed each treatment and described the level of scientific evidence of the relevant publications.The therapeutic approaches commonly associated with CAM are usually conservative, and do not include drugs or surgery. Currently, CAM is not considered by physicians a potential treatment of OM, as there is limited supporting evidence. Further studies are warranted in order to evaluate the potential value of CAM therapies for OM.

Martin-Cabezas, R., Davideau, J. L., Tenenbaum, H., and Huck, O. Clinical efficacy of probiotic as an adjunctive therapy to non-surgical periodontal treatment of chronic periodontitis: A systematic review and meta-analysis. J.Clin.Periodontol. 3-11-2016;

ABSTRACT: FOCUSED QUESTION: What is the clinical influence of probiotics as an adjunctive therapy of scaling and root planing (SRP) when compared with SRP alone or in combination with placebo in the treatment of chronic periodontitis (CP). METHODS: Electronic databases were searched up to July 2015. Randomized controlled trials (RCTs) comparing SRP + probiotic versus SRP were included. PPD reduction and CAL gain were selected as primary outcome variables. RESULTS: Independent screening resulted in 4 eligible publications for the systematic review and 3 were included in the meta-analysis. Meta-analysis showed a statistically significant CAL gain (-0.42 mm, p=0.002) and bleeding on probing (BOP) reduction (-14.66, p=0.003) for SRP + probiotic treatment versus SRP at short-term. Only a tendency (p=0.06) has been observed in terms of overall PPD reduction, whereas results were significant when stratified for moderate (-0.18, p=0.001) and deep pockets (-0.67, p<0.001). CONCLUSION: Within the limitations of this study, the findings of this meta-analysis seem to support the adjunctive use of L.reuteri to SRP in CP treatment at short-term, especially in deep pockets. Heterogeneity and limited available data may reduce the impact of these conclusions. Future long-term RCTs evaluating the clinical efficacy of adjunctive probiotics to SRP are needed. This article is protected by copyright. All rights reserved.

Matelski, L. and Van de, Water J. Risk factors in autism: Thinking outside the brain. J.Autoimmun. 2016; 67:1-7.

ABSTRACT: Autism spectrum disorders (ASD) are complex neurodevelopmental conditions that have been rising markedly in prevalence for the past 30 years, now thought to affect 1 in 68 in the United States. This has prompted the search for possible explanations, and has even resulted in some controversy regarding the "true" prevalence of autism. ASD are influenced by a variety of genetic, environmental, and possibly immunological factors that act during critical periods to alter key developmental processes. This can affect multiple systems and manifests as the social and behavioral deficits that define these disorders. The interaction of environmental exposures in the context of an individual's genetic susceptibilities manifests differently in each case, leading to heterogeneous phenotypes and varied comorbid symptoms within the disorder. This has also made it very difficult to elucidate underlying genes and exposure profiles, but progress is being made in this area. Some pharmaceutical drugs, toxicants, and metabolic and nutritional factors have been identified in epidemiological studies as increasing autism risk, especially during the prenatal period. Immunologic risk factors, including maternal infection during pregnancy, autoantibodies to fetal brain proteins, and familial autoimmune disease, have consistently been observed across multiple studies, as have immune abnormalities in individuals with ASD. Mechanistic research using animal models and patient-derived stem cells will help researchers to understand the complex etiology of these neurodevelopmental disorders, which will lead to more effective therapies and preventative strategies. Proposed therapies that need more investigation include special diets, probiotics, immune modulation, oxytocin, and personalized pharmacogenomic targets. The ongoing search for biomarkers and better treatments will result in earlier identification of ASD and provide much needed help and relief for afflicted families.

Mazidi, M., Rezaie, P., Kengne, A. P., Mobarhan, M. G., and Ferns, G. A. Gut microbiome and metabolic syndrome. Diabetes Metab Syndr. 2-11-2016;

ABSTRACT: The gut microbiome contributes approximately 2kg of the whole body weight, and recent studies suggest that gut microbiota has a profound effect on human metabolism, potentially contributing to several features of the metabolic syndrome. Metabolic syndrome is defined by a clustering of metabolic disorders that include central adiposity with visceral fat accumulation, dyslipidemia, insulin resistance, dysglycemia and non-optimal blood pressure levels. Metabolic syndrome is associated with an increased risk of cardiovascular diseases and type 2 diabetes. It is estimated that around 20-25 percent of the world's adult population has metabolic syndrome. In this manuscript, we have reviewed the existing data linking gut microbiome with metabolic syndrome. Existing evidence from studies both in animals and humans support a link between gut microbiome and various components of metabolic syndrome. Possible pathways include involvement with energy homeostasis and metabolic processes, modulation of inflammatory signaling pathways, interferences with the immune system, and interference with the renin-angiotensin system. Modification of gut microbiota via prebiotics, probiotics or other dietary interventions has provided evidence to support a possible beneficial effect of interventions targeting gut microbiota modulation to treat components or complications of metabolic syndrome.

McFarland, L. V., Ozen, M., Dinleyici, E. C., and Goh, S. Comparison of pediatric and adult antibiotic-associated diarrhea and Clostridium difficile infections. World J.Gastroenterol. 3-21-2016; 22(11):3078-3104.

ABSTRACT: Antibiotic-associated diarrhea (AAD) and Clostridum difficile infections (CDI) have been well studied for adult cases, but not as well in the pediatric population. Whether the disease process or response to treatments differs between pediatric and adult patients is an important clinical concern when following global guidelines based largely on adult patients. A systematic review of the literature using databases PubMed (June 3, 1978-2015) was conducted to compare AAD and CDI in pediatric and adult populations and determine significant differences and similarities that might impact clinical decisions. In general, pediatric AAD and CDI have a more rapid onset of symptoms, a shorter duration of disease and fewer CDI complications (required surgeries and extended hospitalizations) than in adults. Children experience more community-associated CDI and are associated with smaller outbreaks than adult cases of CDI. The ribotype NAP1/027/BI is more common in adults than children. Children and adults share some similar risk factors, but adults have more complex risk factor profiles associated with more co-morbidities, types of disruptive factors and a wider range of exposures to C. difficile in the healthcare environment. The treatment of pediatric and adult AAD is similar (discontinuing or switching the inciting antibiotic), but other treatment strategies for AAD have not been established. Pediatric CDI responds better to metronidazole, while adult CDI responds better to vancomycin. Recurrent CDI is not commonly reported for children. Prevention for both pediatric and adult AAD and CDI relies upon integrated infection control programs, antibiotic stewardship and may include the use of adjunctive probiotics. Clinical presentation of pediatric AAD and CDI are different than adult AAD and CDI symptoms. These differences should be taken into account when rating severity of disease and prescribing antibiotics.

McFarland, L. V. Therapies on the horizon for Clostridium difficile infections. Expert.Opin.Investig.Drugs. 3-21-2016; 1-15.

ABSTRACT: INTRODUCTION: Clostridium difficile infections are a leading cause of healthcare facility outbreaks of gastrointestinal illness that may have serious complications and a high rate of recurrent disease. Despite the availability of standard antibiotic treatments, data from national surveillance programs indicate that the incidence of this disease continues to increase, placing a heavy burden on healthcare systems. New emerging strategies are being tested to replace or augment these standard antibiotics. Areas covered: Thirty-two current investigational agents focusing on different strategies for both prevention and treatment of C. difficile infections are reviewed. Data was gathered from a literature search of public databases for published trials from 1999-November 13, 2015 and from the author's compendium of knowledge. Agents reviewed included 13 antibiotics, two antibiotic inactivators, seven bacteria or yeasts acting to enhance the normal microbiome, seven immunizing agents and three toxin binders. Of the 32 investigational treatments reviewed, 8 (25%) showed significant efficacy in phase II or III clinical trials and are actively being developed as new therapies for C. difficile infections. Expert opinion: A number of potential treatments have floundered during their development process, while others have shown promising results. The strongest efficacy has been in the areas of newer antibiotics, probiotics, monoclonal antibodies and vaccines. By targeting the pathogenic pathway of C. difficile infections, multiple strategies for prevention and treatment have been developed.

McKenney, K. M. and Alfonzo, J. D. From Prebiotics to Probiotics: The Evolution and Functions of tRNA Modifications. Life (Basel). 2016; 6(1)

ABSTRACT: All nucleic acids in cells are subject to post-transcriptional chemical modifications. These are catalyzed by a myriad of enzymes with exquisite specificity and that utilize an often-exotic array of chemical substrates. In no molecule are modifications more prevalent than in transfer RNAs. In the present document, we will attempt to take a chemical rollercoaster ride from prebiotic times to the present, with nucleoside modifications as key players and tRNA as the centerpiece that drove the evolution of biological systems to where we are today. These ideas will be put forth while touching on several examples of tRNA modification enzymes and their modus operandi in cells. In passing, we submit that the choice of tRNA is not a whimsical one but rather highlights its critical function as an essential invention for the evolution of protein enzymes.

Mello, A. M., Paroni, G., Daragjati, J., and Pilotto, A. Gastrointestinal Microbiota and Their Contribution to Healthy Aging. Dig.Dis. 2016; 34(3):194-201.

ABSTRACT: Studies on populations at different ages have shown that after birth, the gastrointestinal (GI) microbiota composition keeps evolving, and this seems to occur especially in old age. Significant changes in GI microbiota composition in older subjects have been reported in relation to diet, drug use and the settings where the older subjects are living, that is, in community nursing homes or in a hospital. Moreover, changes in microbiota composition in the old age have been related to immunosenescence and inflammatory processes that are pathophysiological mechanisms involved in the pathways of frailty. Frailty is an age-related condition of increased vulnerability to stresses due to the impairment in multiple inter-related physiologic systems that are associated with an increased risk of adverse outcomes, such as falls, delirium, institutionalization, hospitalization and death. Preliminary data suggest that changes in microbiota composition may contribute to the variations in the biological, clinical, functional and psycho-social domains that occur in the frail older subjects. Multidimensional evaluation tools based on a Comprehensive Geriatric Assessment (CGA) have demonstrated to be useful in identifying and measuring the severity of frailty in older subjects. Thus, a CGA approach should be used more widely in clinical practice to evaluate the multidimensional effects potentially related to GI microbiota composition of the older subjects. Probiotics have been shown to be effective in restoring the microbiota changes of older subjects, promoting different aspects of health in elderly people as improving immune function and reducing inflammation. Whether modulation of GI microbiota composition, with multi-targeted interventions, could have an effect on the prevention of frailty remains to be further investigated in the perspective of improving the health status of frail 'high risk' older individuals.

Messora, M. R., Pereira, L. J., Foureaux, R., Oliveira, L. F., Sordi, C. G., Alves, A. J., Napimoga, M. H., Nagata, M. J., Ervolino, E., and Furlaneto, F. A. Favourable effects of Bacillus subtilis and Bacillus licheniformis on experimental periodontitis in rats. Arch.Oral Biol. 2016; 66:108-119.

ABSTRACT: OBJECTIVES: The purposes of this study were to evaluate, in rats: i) the effects of Bacillus species on the development of experimental periodontitis (EP) via microtomographic, immunological and hematological assays (Experiment 1-E1); ii) the effects of Bacillus species as adjuncts to scaling and root planing (SRP) for the treatment of EP via histomorphometric and immunohistochemical analyses (Experiment 2-E2). METHODS: In E1, 24 rats were divided into groups C1 (control), PROB1, EP1 and EP-PROB1.

In groups with EP, the mandibular first molar of each animal received a ligature for 14days. In groups PROB1, animals received Bacillus species for 44days, starting 30days before EP induction in Group EP-PROB1. In E2, 24 rats were assigned to groups C2 (control), PROB2, EP-SRP2 and EP-SRP-PROB2. In groups with SRP, EP was induced as described in E1. The ligatures were removed after 14days and SRP was performed. In groups PROB2, animals received Bacillus species for 15days, starting after SRP in Group EP-SRP-PROB2. RESULTS: In E1, Group EP1 presented bone loss (BL) and eosinophil numbers greater than Group EP-PROB1 (P<0.05). In Group EP-PROB1, the receptor activator of nuclear factor-kB ligand (RANKL)/osteoprotegerin (OPG) ratio was similar to that of groups without EP. In E2, Group EP-SRP-PROB2 presented fewer TRAP-positive osteoclasts, lower immunolabeling pattern for a proinflammatory cytokine and decreased BL and attachment loss than Group EP-SRP2 (P<0.05). CONCLUSIONS: Bacillus species supplementation provided a protective effect against BL and enhanced the effects of SRP in the treatment of EP in rats.

Michael, D. R., Moss, J. W., Calvente, D. L., Garaiova, I., Plummer, S. F., and Ramji, D. P. Lactobacillus plantarum CUL66 can impact cholesterol homeostasis in Caco-2 enterocytes. Benef.Microbes. 2-3-2016; 1-10.

ABSTRACT: Hypercholesterolemia drives the development of cardiovascular disease, the leading cause of mortality in western society. Supplementation with probiotics that interfere with cholesterol metabolism may provide a contribution to disease prevention. Lactobacillus plantarum CUL66 (NCIMB 30280) has been assessed in vitro for its ability to impact cholesterol absorption. L. plantarum CUL66 tested positive for bile salt hydrolase activity and the ability to assimilate cholesterol from culture media. RT-qPCR analysis showed that the bacterium significantly decreased the expression of Niemann-Pick C1-like 1 and ATP-binding cassette transporter-1 in polarised Caco-2 cells after 6 h exposure. Conversely, the expression of ATP-binding cassette sub-family G member (ABCG)-5 and ABCG-8, and 3-hydroxy-3-methylglutaryl-CoA reductase were significantly increased. Using a radiolabelled assay, we also observed significant reductions in the uptake and basolateral efflux of cholesterol by Caco-2 cells exposed to L. plantarum CUL66. This in vitro study identified L. plantarum CUL66 as a cholesterol lowering bacteria by highlighting its ability to beneficially regulate multiple in vitro events associated with intestinal cholesterol metabolism and provides evidence of efficacy for its inclusion in future in vivo studies.

Miller, H., Ferris, R., and Phelps, B. R. The effect of probiotics on CD4 counts among people living with HIV: a systematic review. Benef.Microbes. 3-25-2016; 1-8.

ABSTRACT: Probiotics are defined by the WHO as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'. Ongoing research has shown probiotics provide benefits to humans, including protection and restoration of the gastrointestinal and other mucosal tracts. As human immunodeficiency virus (HIV) activates gut-associated lymphoid tissue (GALT), several studies have investigated the effect of probiotics on CD4 cell count and related outcomes among those living with HIV. These studies are summarised here. Manuscripts were identified using the search terms 'probiotics', 'synbiotics', 'HIV', and 'CD4', and were reviewed for relevance and inclusion of CD4 count as an immunologic endpoint. Bibliographies of relevant manuscripts were also reviewed for additional studies matching inclusion and

exclusion criteria. The search yielded 91 results; 13 included relevant outcomes. Seven of these studies produced beneficial CD4 outcomes, while the remaining 6 reported on insignificant beneficial or negative CD4 outcomes. The studies summarised here collectively suggest that daily consumption of probiotics over a prolonged period of time may improve CD4 count in people living with HIV.

Min, Y. N., Yang, H. L., Xu, Y. X., and Gao, Y. P. Effects of dietary supplementation of synbiotics on growth performance, intestinal morphology, sIgA content and antioxidant capacities of broilers. J.Anim Physiol Anim Nutr.(Berl). 3-28-2016;

ABSTRACT: Today, several strategies are being used to decrease the serious effects of antibiotics abuse on broilers industry and public health, among which synbiotics are one of the most promising antibiotic alternative. This study was undertaken to assess the effects of synbiotics, which composed of probiotics (Bacillus subtilis) and prebiotics (xylooligosaccharide and mannanoligosaccharide), on growth performance, intestinal morphology, sIgA content and antioxidant parameters of broilers. Four hundred and fifty one-day-old commercial Cobb48 broilers were assigned to five treatments consisting of six replicates of 15 birds each pen. Five dietary treatments include basal diets (control), basal diets plus antibiotics (4 mg/kg Xanthomycin), basal diets plus 1 g of probiotics B. subtilis product/kg of diets (4 x 108 cfu/kg), basal diets plus 150 mg/kg xylooligosaccharide (35%) and 1 g/kg mannanoligosaccharide (75%), and basal diets plus synbiotics (1 g of probiotics B. subtilis product/kg of diets (4 x 108 cfu/kg), 150 mg/kg xylooligosaccharide (35%) and 1 g/kg mannanoligosaccharide (75%). The results demonstrated that on 21 and 42 days, dietary supplementation of the synbiotics significantly increased daily weight gain ($p < 0.05$), feed efficiency ($p < 0.05$), the villus height and villus:crypt ratio in the duodenum, jejunum and ileum ($p < 0.05$), as well as intestinal mucosa sIgA content ($p < 0.05$), serum T-SOD activity ($p < 0.05$) and lysozyme content ($p < 0.05$), comparing with control group. In conclusion, synbiotics (B. subtilis and xylooligosaccharide and mannanoligosaccharide) is one of the safe and ideal dietary supplementations to increase broilers' growth performance by improving small intestinal morphology, sIgA content and antioxidant capabilities.

Mohkam, M., Nezafat, N., Berenjian, A., Mobasher, M. A., and Ghasemi, Y. Identification of Bacillus Probiotics Isolated from Soil Rhizosphere Using 16S rRNA, recA, rpoB Gene Sequencing and RAPD-PCR. Probiotics.Antimicrob.Proteins. 2016; 8(1):8-18.

ABSTRACT: Some Bacillus species, especially Bacillus subtilis and Bacillus pumilus groups, have highly similar 16S rRNA gene sequences, which are hard to identify based on 16S rDNA sequence analysis. To conquer this drawback, rpoB, recA sequence analysis along with randomly amplified polymorphic (RAPD) fingerprinting was examined as an alternative method for differentiating Bacillus species. The 16S rRNA, rpoB and recA genes were amplified via a polymerase chain reaction using their specific primers. The resulted PCR amplicons were sequenced, and phylogenetic analysis was employed by MEGA 6 software. Identification based on 16S rRNA gene sequencing was underpinned by rpoB and recA gene sequencing as well as RAPD-PCR technique. Subsequently, concatenation and phylogenetic analysis showed that extent of diversity and similarity were better obtained by rpoB and recA primers, which are also reinforced by RAPD-PCR methods. However, in one case, these approaches failed to identify one isolate, which in combination with the phenotypical method offsets this issue. Overall, RAPD fingerprinting, rpoB and recA along with concatenated genes sequence analysis discriminated closely

related Bacillus species, which highlights the significance of the multigenic method in more precisely distinguishing Bacillus strains. This research emphasizes the benefit of RAPD fingerprinting, rpoB and recA sequence analysis superior to 16S rRNA gene sequence analysis for suitable and effective identification of Bacillus species as recommended for probiotic products.

Mokoena, M. P., Mutanda, T., and Olaniran, A. O. Perspectives on the probiotic potential of lactic acid bacteria from African traditional fermented foods and beverages. Food Nutr.Res. 2016; 60:29630

ABSTRACT: Diverse African traditional fermented foods and beverages, produced using different types of fermentation, have been used since antiquity because of their numerous nutritional values. Lactic acid bacteria (LAB) isolated from these products have emerged as a welcome source of antimicrobials and therapeutics, and are accepted as probiotics. Probiotics are defined as live microbial food supplements which beneficially affect the host by improving the intestinal microbial balance. Currently, popular probiotics are derived from fermented milk products. However, with the growing number of consumers with lactose intolerance that are affected by dietary cholesterol from milk products, there is a growing global interest in probiotics from other food sources. The focus of this review is to provide an overview of recent developments on the applications of probiotic LAB globally, and to specifically highlight the suitability of African fermented foods and beverages as a viable source of novel probiotics.

Morales, A., Carvajal, P., Silva, N., Hernandez, M., Godoy, C., Rodriguez, G., Cabello, R., Garcia-Sesnich, J., Hoare, A., Diaz, P. I., and Gamonal, J. Clinical Effects of Lactobacillus Rhamnosus in Non-Surgical Treatment of Chronic Periodontitis: A Randomized Placebo-Controlled Trial With 1-Year Follow-up. J.Periodontol. 3-4-2016; 1-12.

ABSTRACT: BACKGROUND: Probiotics are living microorganisms that provide beneficial effects for the host when administered in proper quantities. The aim of this double- blind placebo- controlled parallel- arm randomized clinical trial was to evaluate the clinical effects of a Lactobacillus rhamnosus SP1-containing probiotic sachet as an adjunct to non-surgical therapy. MATERIAL AND METHODS: Twenty-eight systemically healthy volunteer with chronic periodontitis were recruited and monitored clinically at baseline and 3, 6, 9 and 12 months after therapy. Clinical parameters measured included plaque accumulation, bleeding on probing, pocket probing depths (PPD) and clinical attachment loss. Patients received non-surgical therapy including scaling and root planing (SRP) and were randomly assigned to a test (SRP + probiotic, n = 14) or control (SRP + placebo, n = 14) group. The intake, once a day for 3 months, of a L. rhamnosus SP1 probiotic sachet commenced after the last session of SRP. RESULTS: Both test and control groups showed improvements in clinical parameters at all time points evaluated. The test group, however, showed greater reductions in PPD than the control. Also, at initial visits and after 1-year follow-up, the test group showed a statistically significant reduction in number of participants with PPD >/= 6mm, indicating a reduced need for surgery, in contrast to the placebo group. CONCLUSIONS: The results of this trial indicate that oral administration of L. rhamnosus SP1 resulted in similar clinical improvements compared to SRP alone.

Moratalla, A., Gomez-Hurtado, I., Moya-Perez, A., Zapater, P., Peiro, G., Gonzalez-Navajas, J. M., Gomez Del Pulgar, E. M., Such, J., Sanz, Y., and Frances, R. Bifidobacterium pseudocatenulatum CECT7765 promotes a TLR2-dependent anti-inflammatory response in intestinal lymphocytes from mice with cirrhosis. Eur.J.Nutr. 2016; 55(1):197-206.

ABSTRACT: BACKGROUND: Intestinal homeostasis plays an important role in bacteria-derived complications in cirrhosis. Intestinal lymphocytes are responsible for immune effector functions and can be modulated by certain probiotics. We evaluate the interaction between Bifidobacterium pseudocatenulatum CECT7765 and intestinal lymphocytes in mice with cirrhosis. ANIMALS AND METHODS: Cirrhosis was induced by intragastrical administration of carbon tetrachloride in Balb/C mice. One week prior to laparotomy, animals received B. pseudocatenulatum CECT7765 (10(7), 10(9) or 10(10) cfu/daily) or placebo. Chemokine receptor and cytokine expression were evaluated in intestinal lymphocytes. Gut permeability was studied by FITC-LPS recovery in vivo. Luminal antigens, inflammation and functional markers were evaluated in liver samples. RESULTS: Bifidobacterium pseudocatenulatum CECT7765 decreased the expression of pro-inflammatory chemokine receptors CCR6, CCR9, CXCR3 and CXCR6 in intestinal lymphocytes from cirrhotic mice in a concentration-dependent manner. The bifidobacterial strain induced a shift towards an anti-inflammatory cytokine profile in this cell subset. B. pseudocatenulatum CECT7765-induced inflammatory modulation was TLR2-mediated, as in vitro TLR2 blockade inhibited the reduction of TNF-alpha and its receptors and the increase of IL-10 and IL-10 receptor secretion. The recovery rate of administered fluorescence-labelled endotoxin was significantly and dose-dependently lowered with the bifidobacterial strain. The reduced intestinal permeability was associated with a decreased burden of bacterial antigens in the liver of mice treated with B. pseudocatenulatum CECT7765. Liver function and inflammation were improved with the use of the bifidobacterial strain at the highest dose tested (10(10) cfu). CONCLUSION: Bifidobacterium pseudocatenulatum CECT7765 improves gut homeostasis and prevents gut-derived complications in experimental chronic liver disease.

Nagpal, R., Kumar, M., Yadav, A. K., Hemalatha, R., Yadav, H., Marotta, F., and Yamashiro, Y. Gut microbiota in health and disease: an overview focused on metabolic inflammation. Benef.Microbes. 3-11-2016; 7(2):181-194.

ABSTRACT: In concern to the continuously rising global prevalence of obesity, diabetes and associated diseases, novel preventive and therapeutic approaches are urgently required. However, to explore and develop such innovative strategies, a meticulous comprehension of the biological basis of these diseases is extremely important. Past decade has witnessed an enormous amount of research investigation and advancement in the field of obesity, diabetes and metabolic syndrome, with the gut microbiota receiving a special focus in the triangle of nutrition, health and diseases. In particular, the role of gut microbiota in health and diseases has been one of the most vigorous and intriguing field of recent research; however, much still remains to be elucidated about its precise role in host metabolism and immune functions and its implication in the onset, progression as well as in the amelioration of metabolic ailments. Recent investigations have suggested a significant contribution of the gut microbiota in the regulation and impairment of energy homeostasis, thereby causing metabolic disorders, such as metabolic endotoxemia, insulin resistance and type 2 diabetes. Numerous inflammatory biomarkers have been found to be associated with obesity, diabetes and risk of other associated adverse outcomes, thereby suggesting that a persistent low-grade inflammatory response is a potential risk factor. In this milieu, this

review intends to discuss potential evidences supporting the disturbance of the gut microbiota balance and the intestinal barrier permeability as a potential triggering factor for systemic inflammation in the onset and progression of obesity, type 2 diabetes and metabolic syndrome.

Namkin, K., Zardast, M., and Basirinejad, F. Saccharomyces Boulardii in Helicobacter Pylori Eradication in Children: A Randomized Trial From Iran. Iran J.Pediatr. 2016; 26(1):e3768

ABSTRACT: BACKGROUND: Helicobacter pylori infects around 50% of the human population and is asymptomatic in 70% of the cases. H. pylori eradication in childhood will not only result in peptic symptoms relief, but will also prevent late-term complications such as cancer. Today, probiotics are being increasingly studied in the treatment of gastrointestinal infections as an alternative or complement to antibiotics. OBJECTIVES: In this study we aimed to assess the effect of S. boulardii supplementation on H. pylori eradication among children in our region. PATIENTS AND METHODS: In this randomized double-blind placebo-controlled clinical trial 28 asymptomatic primary school children with a positive H. pylori stool antigen (HpSA) exam were randomly allocated into the study group, receiving Saccharomyces boulardii, and the control group receiving placebo capsules matched by shape and size, for one month. The children were followed up weekly and were reinvestigated four to eight weeks after accomplished treatment by HpSA testing. The significance level was set at $P < 0.05$. RESULTS: 24 children completed the study. The mean HpSA reduced from 0.40 +/- 0.32 to 0.21 +/- 0.27 in the study group, indicating a significant difference ($P = 0.005$). However, such difference was not observed in the control group ($P = 0.89$). Moreover, the HpSA titer showed a 0.019 +/- 0.19 decrease in the study group whereas the same value was 0.0048 +/- 0.12 for the controls, again stating a significant difference ($P = 0.01$). CONCLUSIONS: Saccharomyces boulardii has a positive effect on reducing the colonization of H. pylori in the human gastrointestinal system but is not capable of its eradication when used as single therapy.

Nishida, S., Ono, Y., and Sekimizu, K. Lactic acid bacteria activating innate immunity improve survival in bacterial infection model of silkworm. Drug Discov.Ther. 2016; 10(1):49-56.

ABSTRACT: Lactic acid bacteria (LAB) have been thought to be helpful for human heath in the gut as probiotics. It recently was noted that activity of LAB stimulating immune systems is important. Innate immune systems are conserved in mammals and insects. Silkworm has innate immunity in response to microbes. Microbe-associated molecular pattern (ex. peptidoglycan and beta-glucan) induces a muscle contraction of silkworm larva. In this study, we established an efficient method to isolate lactic acid bacteria derived from natural products. We selected a highly active LAB to activate the innate immunity in silkworm by using the silkworm muscle contraction assay, as well. The assay revealed that Lactococcus lactis 11/19-B1 was highly active on the stimulation of the innate immunity in silkworm. L. lactis 11/19-B1 solely fermented milk with casamino acid and glucose. This strain would be a starter strain to make yogurt. Compared to commercially available yogurt LAB, L. lactis 11/19-B1 has higher activity on silkworm contraction. Silkworm normally ingested an artificial diet mixed with L. lactis 11/19-B1 or a yogurt fermented with L. lactis 11/19-B1. Interestingly, silkworms that ingested the LAB showed tolerance against the pathogenicity of Pseudomonas aeruginosa. These data suggest that Lactococcus lactis 11/19-B1 would be expected to be useful for making yogurt and probiotics to activate innate immunity.

Nova, E., Perez de, Heredia F., Gomez-Martinez, S., and Marcos, A. The Role of Probiotics on the Microbiota: Effect on Obesity. Nutr.Clin.Pract. 2-11-2016;

ABSTRACT: The microbiota and the human host maintain a symbiotic association. Nowadays, metagenomic analyses are providing valuable knowledge on the diversity and functionality of the gut microbiota. However, with regard to the definition of a "healthy microbiota" and the characterization of the dysbiosis linked to obesity, there is still not a clear answer. Despite this fact, attempts have been made to counteract obesity through probiotic supplementation. A literature search of experimental studies relevant to the topic was performed in PubMed database with the keywords "probiotic" and "obesity" and restricted to those with "Lactobacillus" or "Bifidobacterium" in the title. So far, evidence of an antiobesity effect of different lactobacilli and bifidobacteria has been mainly obtained from animal models of dietary-induced obesity. Using these experimental models, a substantial number of studies have reported reductions in weight gain and, in particular, fat tissue mass at different locations following administration of bacteria, as compared with controls. Antiatherogenic and anti-inflammatory effects-including regulation of expression of lipogenic and lipolytic genes in the liver, reduction in liver steatosis, improvement of blood lipid profile and glucose tolerance, decreased endotoxemia, and regulation of inflammatory pathways-are also reported in many of them. The number of human studies focused on probiotic administration for obesity management is still very scarce, and it is too soon to judge their potential efficacy, especially when considering the fact that the actions of probiotics are always strain specific and the individual response varies according to intrinsic factors, the overall composition of diet, and their interactions.

Nowak, A., Czyzowska, A., Huben, K., Sojka, M., Kuberski, S., Otlewska, A., and Slizewska, K. Prebiotics and age, but not probiotics affect the transformation of 2-amino-3-methyl-3H-imidazo[4,5-f]quinoline (IQ) by fecal microbiota - An in vitro study. Anaerobe. 3-23-2016;

ABSTRACT: Heterocyclic aromatic amines (HAAs) are carcinogens which are formed in meat cooked using high-temperature methods. The human gastrointestinal (GI) microbiota plays a crucial role in maintaining health in humans of different ages, and especially in the elderly. However, the GI microbiota, whose metabolism and composition changes with age, may also be responsible for the activation of mutagenic substances reaching the colon with diet. Probiotics and prebiotics are promising in terms of reducing the destructive effects of HAAs. The aim of the study was to determine if fecal microbiota derived from the feces of 27 volunteers: infants (up to 18 months), adults (aged 23-39 years), the sub-elderly (aged 64-65 years), and the elderly (aged 76-87 years), and the presence of probiotics or prebiotics, affected the transformation of IQ (2-amino-3-methylimidazo[4,5-f]quinoline) to 7-OH-IQ (2-amino-3,6-dihydro-3-methyl-7H-imidazo[4,5-f]quinoline-7-one). The compounds were identified using LC-MSn, NMR, and FTIR. Their genotoxicity was compared in the comet assay. Individual strains capable of IQ transformation were also identified. 7-OH-IQ was detected in six persons (two children and four elderly individuals). The degree of IQ conversion ranged from 26% (4-month-old girl) to 94% (81-year-old woman) of the initial quantity. Four Enterococcus isolates: two Enterococcus faecium and two Enterococcus faecalis strains, as well as one Clostridium difficile strain (LOCK 1030, from the culture collection) converted IQ to 7-OH-IQ. The genotoxicity of samples containing 7-OH-IQ was even three times higher ($P < 0.05$) than those with IQ and was correlated with the degree of IQ conversion and 7-OH-IQ concentration.

Oh, B., Kim, J. W., and Kim, B. S. Changes in the Functional Potential of the Gut Microbiome Following Probiotic Supplementation during Helicobacter Pylori Treatment. Helicobacter. 3-18-2016;

ABSTRACT: BACKGROUND: Probiotic supplementation is utilized to alleviate the side effects associated with antibiotic therapy for Helicobacter pylori infection. Several studies have described the effects of administration of probiotics on the gut microbiota during antibiotic therapy. However, most of these studies have focused on specific bacteria, thereby providing limited information on the functional roles of the altered microbiota. Therefore, we examined the impact of probiotic supplementation on the structure and functional dynamics of the gut microbiota during H. pylori eradication, using whole-metagenomic sequence analysis. METHODS: Subjects were divided into two groups: the antibiotics group, which received only antibiotics, and the probiotics group, which received antibiotics with probiotic supplementation. The structural and functional profiles of gut microbiota was analyzed using metagenomic DNA extracted from the feces during treatment by Illumina MiSeq system. RESULTS: The overall alterations in microbiota, as revealed by whole metagenome sequencing, were similar with results from our previous 16S rRNA gene-based analysis. The proportional shift in functional gene families was greater in the antibiotics group than in the probiotics group. In particular, the proportion of genes related to selenocompound metabolism was reduced in the probiotics group, whereas genes associated with the metabolism of nucleotide sugars were increased. CONCLUSION: The functional alterations of gut microbiota may link to the reduction in intestinal irritation and maintenance of bacterial diversity observed following probiotic supplementation with antibiotic therapy. The potential beneficial roles of altered gut microbiota following probiotic supplementation are expected a reduction in side effects such as intestinal irritation and antibiotics resistance.

Ohshima, T., Kojima, Y., Seneviratne, C. J., and Maeda, N. Therapeutic Application of Synbiotics, a Fusion of Probiotics and Prebiotics, and Biogenics as a New Concept for Oral Candida Infections: A Mini Review. Front Microbiol. 2016; 7:10

ABSTRACT: Candida is a major human fungal pathogen causing infectious conditions predominantly in the elderly and immunocompromised hosts. Although Candida resides as a member of the oral indigenous microbiota in symbiosis, some circumstances may cause microbial imbalance leading to dysbiosis and resultant oral candidiasis. Therefore, oral microbial symbiosis that suppresses the overgrowth of Candida is important for a healthy oral ecosystem. In this regard, probiotics, prebiotics, and synbiotics can be considered a potential therapeutic and preventive strategy against oral candidiasis. Prebiotics have a direct effect on microbial growth as they stimulate the growth of beneficial bacteria and suppress the growth of pathogens. Probiotics render a local protective effect against pathogens and a systemic indirect effect on immunological amelioration. Synbiotics are fusion products of prebiotics and probiotics. This mini review discusses the potential use and associated limitations of probiotics, prebiotics, and synbiotics for the prevention and treatment of oral candidiasis. We will also introduce biogenics, a recent concept derived from the work on probiotics. Biogenics advocates the use of beneficial bioactive substances produced by probiotic bacteria, whose activities are independent from the viability of probiotic bacteria in human bodies.

Ojeda, P., Bobe, A., Dolan, K., Leone, V., and Martinez, K. Nutritional modulation of gut microbiota - the impact on metabolic disease pathophysiology. J.Nutr.Biochem. 2016; 28:191-200.

ABSTRACT: The obesity epidemic afflicts over one third of the United States population. With few therapies available to combat obesity, a greater understanding of the systemic causes of this and other metabolic disorders is needed to develop new, effective treatments. The mammalian intestinal microbiota contributes to metabolic processes in the host. This review summarizes the research demonstrating the interplay of diet, intestinal microbiota and host metabolism. We detail the effects of diet-induced modifications in microbial activity and resultant impact on (1) sensory perception of macronutrients and total energy intake; (2) nutrient absorption, transport and storage; (3) liver and biliary function; (4) immune-mediated signaling related to adipose inflammation; and (5) circadian rhythm. We also discuss therapeutic strategies aimed to modify host-microbe interactions, including prebiotics, probiotics and postbiotics, as well as fecal microbiota transplantation. Elucidating the role of gut microbes in shaping metabolic homeostasis or dysregulation provides greater insight into disease development and a promising avenue for improved treatment of metabolic dysfunction.

Ojha, K. S., Kerry, J. P., Alvarez, C., Walsh, D., and Tiwari, B. K. Effect of high intensity ultrasound on the fermentation profile of Lactobacillus sakei in a meat model system. Ultrason.Sonochem. 2016; 31:539-545.

ABSTRACT: The objective of this study was to investigate the efficacy of high intensity ultrasound on the fermentation profile of Lactobacillus sakei in a meat model system. Ultrasound power level (0-68.5W) and sonication time (0-9min) at 20 degrees C were assessed against the growth of L. sakei using a Microplate reader over a period of 24h. The L. sakei growth data showed a good fit with the Gompertz model (R(2)>0.90; SE<0.042). Second order polynomial models demonstrated the effect of ultrasonic power and sonication time on the specific growth rate (SGR, mu, h(-1)) and lag phase (lambda, h). A higher SGR and a shorter lag phase were observed at low power (2.99W for 5min) compared to control. Conversely, a decrease ($p<0.05$) in SGR with an increase in lag phase was observed with an increase in ultrasonic power level. Cell-free extracts obtained after 24h fermentation of ultrasound treated samples showed antimicrobial activity against Staphylococcus aureus, Listeria monocytogenes, Escherichia coli and Salmonella typhimurium at lower concentrations compared to control. No significant difference ($p<0.05$) among treatments was observed for lactic acid content after a 24h fermentation period. This study showed that both stimulation and retardation of L. sakei is possible, depending on the ultrasonic power and sonication time employed. Hence, fermentation process involving probiotics to develop functional food products can be tailored by selection of ultrasound processing parameters.

oki-Yoshida, A., Saito, S., Fukiya, S., Aoki, R., Takayama, Y., Suzuki, C., and Sonoyama, K. Lactobacillus rhamnosus GG increases Toll-like receptor 3 gene expression in murine small intestine ex vivo and in vivo. Benef.Microbes. 3-25-2016; 1-10.

ABSTRACT: Administration of Lactobacillus rhamnosus GG (LGG) has been reported to be therapeutically effective against acute secretory diarrhoea resulting from the structural and functional intestinal mucosal lesions induced by rotavirus infection; however, the underlying mechanisms remain to be completely elucidated. Because Toll-like receptor 3

(TLR3) plays a key role in the innate immune responses following the recognition of rotavirus, the present study examined whether LGG influences TLR3 gene expression in murine small intestine ex vivo and in vivo. We employed cultured intestinal organoids derived from small intestinal crypts as an ex vivo tissue model. LGG supplementation increased TLR3 mRNA levels in the intestinal organoids, as estimated by quantitative real-time polymerase chain reaction. Likewise, single and 7-day consecutive daily administrations of LGG increased TLR3 mRNA levels in the small intestine of C57BL/6N mice. The mRNA levels of other TLRs were not substantially altered both ex vivo and in vivo. In addition, LGG supplementation increased the mRNA levels of an antiviral type 1 interferon, interferon-alpha (IFN-alpha), and a neutrophil chemokine, CXCL1, upon stimulation with a synthetic TLR3 ligand, poly(I:C) in the intestinal organoids. LGG administration did not alter IFN-alpha and CXCL1 mRNA levels in the small intestine in vivo. Supplementation of other bacterial strains, Bifidobacterium bifidum and Lactobacillus paracasei, failed to increase TLR3 and poly(I:C)-stimulated CXCL1 mRNA levels ex vivo. We propose that upregulation of TLR3 gene expression may play a pivotal role in the therapeutic efficacy of LGG against rotavirus-associated diarrhoea. In addition, we demonstrated that intestinal organoids may be a promising ex vivo tissue model for investigating host-pathogen interactions and the antiviral action of probiotics in the intestinal epithelium.

Olson, J. K., Rager, T. M., Navarro, J. B., Mashburn-Warren, L., Goodman, S. D., and Besner, G. E. Harvesting the benefits of biofilms: A novel probiotic delivery system for the prevention of necrotizing enterocolitis. J.Pediatr.Surg. 3-2-2016;

ABSTRACT: BACKGROUND/PURPOSE: Probiotics reduce the incidence of necrotizing enterocolitis (NEC) albeit only when administered at high frequency (at least daily). We have developed a novel probiotic delivery system in which probiotics are grown as a biofilm on microspheres, allowing enhanced efficacy with only a single treatment. METHODS: Neonatal rats were subjected to experimental NEC. Pups received a single enteral dose of: (1) vehicle only, (2) unloaded microspheres, (3) MRS (broth)-loaded microspheres, (4) Lactobacillus reuteri, (5) L. reuteri grown on unloaded microspheres, or (6) L. reuteri grown on MRS-loaded microspheres. Intestinal injury was graded histologically and intestinal permeability determined by serum levels of enterally administered fluorescein isothiocyanate-labeled dextran. RESULTS: 69% of untreated pups developed NEC, whereas 32% of pups treated with L. reuteri grown as a biofilm on unloaded microspheres (p=0.009) and 33% of pups treated with L. reuteri grown as a biofilm on MRS-loaded microspheres (p=0.005) developed NEC. No other group had a significant reduction in NEC. Furthermore, pups treated with L. reuteri grown as a biofilm had significantly reduced intestinal permeability. CONCLUSIONS: A single dose of Lactobacillus biofilm grown on biocompatible microspheres significantly reduces NEC incidence and severity. This novel probiotic delivery system may be beneficial in the prevention of NEC in the future.

Orlando, A., Linsalata, M., and Russo, F. Antiproliferative effects on colon adenocarcinoma cells induced by co-administration of vitamin K1 and Lactobacillus rhamnosus GG. Int.J.Oncol. 3-29-2016;

ABSTRACT: Vitamin K (VK), an essential nutrient associated with the clotting cascade, has also been demonstrated to have anticancer properties in various cancer cells including colon cancer cells. Also probiotics have gained interest as potential anticancer agents.

Among them, Lactobacillus rhamnosus GG (L.GG) has been shown to inhibit cell proliferation and polyamine biosynthesis as well as to induce apoptosis in different human gastrointestinal cancer cells. Nevertheless, the exact mechanisms involved in these actions are not completely elucidated. Therefore, the aims of the present study were to evaluate in three differently graded human colon cancer cells (namely Caco-2, HT-29 and SW480) the effects of increasing VK1 concentrations, administered alone or in combination with viable L.GG, on the cell proliferation evaluated by MTT test, apoptosis investigated by Bax/Bcl-2 ratio and the percentage of the apoptotic cells, and the cell cycle evaluated by MUSE cell analyzer. Both VK1 and L.GG administered alone up to 72 h, caused inhibition of proliferation, induction of apoptosis and the cell cycle arrest in all the tested colon cancer cells. When VK1 and L.GG were co-administered, the addition of increasing VK1 concentrations potentiated the probiotic antiproliferative effect in a dose-dependent manner, being also related to the individual features of each cell line. The effect was more evident in Caco-2 and HT-29 cells compared to the less differentiated SW480. The enhanced antiproliferative efficacy due to co-administration of L.GG and VK1 could represent a suitable option in a functional food strategy for cancer growth inhibition and chemoprevention.

Orso, G., Mandato, C., Veropalumbo, C., Cecchi, N., Garzi, A., and Vajro, P. Pediatric parenteral nutrition-associated liver disease and cholestasis: Novel advances in pathomechanisms-based prevention and treatment. Dig.Liver Dis. 2016; 48(3):215-222.

ABSTRACT: Parenteral nutrition constitutes a life-saving therapeutic tool in patients unable to ingest/absorb oral or enteral delivered nutrients. Liver function tests abnormalities are a common therapy-related complication, thus configuring the so-called Parenteral Nutrition Associated Liver Disease (PNALD) or cholestasis (PNAC). Although the damage is frequently mild, and resolves after discontinuation of parenteral nutrition, in some cases it progresses into cirrhotic changes, especially in neonates and infants. We present a literature review focusing on the pathogenetic mechanisms-driven prevention and therapies for the cases where parenteral nutrition cannot be discontinued. Ursodeoxycholic acid has been proposed in patients with cholestatic hepatopathy, but its efficacy needs to be better established. Little evidence is available on efficacy of anti-oxidants, antibiotics, probiotics and anti TNFalpha. Lipid emulsions based on fish oil with a high content of long-chain polyunsaturated fatty acids omega-3 appear effective both in decreasing intrahepatic inflammation and in improving biliary flow. Most recent promising variations such as soybean/MCT/olive/fish oil emulsion [third generation lipid emulsion (SMOFlipid)] are under investigation. In conclusion, we remark the emergence of a number of novel pathomechanisms underlying the severe liver impairment damage (PNALD and PNAC) in patients treated with parenteral nutrition. Only few traditional and innovative therapeutic strategies have hitherto been shown promising.

Ortiz, A. M., Klase, Z. A., DiNapoli, S. R., Vujkovic-Cvijin, I., Carmack, K., Perkins, M. R., Calantone, N., Vinton, C. L., Riddick, N. E., Gallagher, J., Klatt, N. R., McCune, J. M., Estes, J. D., Paiardini, M., and Brenchley, J. M. IL-21 and probiotic therapy improve Th17 frequencies, microbial translocation, and microbiome in ARV-treated, SIV-infected macaques. Mucosal.Immunol. 2016; 9(2):458-467.

ABSTRACT: Increased mortality in antiretroviral (ARV)-treated, HIV-infected individuals has been attributed to persistent immune dysfunction, in part due to abnormalities at the gastrointestinal barrier. In particular, the poor reconstitution of gastrointestinal Th17 cells

correlates with residual translocation of dysbiotic, immunostimulatory microflora across a compromised intestinal epithelial barrier. We have previously demonstrated that oral probiotics promote increased intestinal CD4(+) T-cell reconstitution during ARV treatment in a non-human primate model of HIV infection; however, essential mucosal T-cell subsets, such as Th17 cells, had limited recovery. Here, we sought to promote Th17 cell recovery by administering interleukin (IL)-21 to a limited number of ARV-treated, probiotic-supplemented, Simian Immunodeficiency Virus (SIV)-infected pigtailed macaques. We demonstrate that probiotic and IL-21 supplementation of ARVs are associated with enhanced polyfunctional Th17 expansion and reduced markers of microbial translocation and dysbiosis as compared with infected controls receiving ARVs alone. Importantly, treatment resulted in fewer morbidities compared with controls, and was independent of increased immune activation or loss of viral suppression. We propose that combining ARVs with therapeutics aimed at restoring intestinal stasis may significantly improve disease prognosis of ARV-treated, HIV-infected individuals.

Patil, P. K., Muralidhar, M., Solanki, H. G., Patel, P. P., Patel, K., and Gopla, C. Effect of culture intensity and probiotics application on microbiological and environmental parameters in Litopenaeus vannamei culture ponds. J.Environ.Biol. 2016; 37(1):21-29.

ABSTRACT: The present study examined the complex interaction among stocking density and extent of probiotic use with production and environmental parameters in Litopenaeus vannamei culture ponds to suggest suitable management strategies. The study was conducted inL. vannamei culture ponds with stocking density of 35 nos sq m(-1) (Group I) and 56 nos sq m(-1) (Group II) and probiotic application @16.5 kg ha(-1) and 157 kg ha(-1), respectively. There was no significant difference noted between the two groups of ponds in respect to ammonia oxidizing bacteria (AOB) in sediment and nitrite oxidizing bacteria (NOB) in water samples, whereas significantly higher levels of AOB in water samples of high intensity culture ponds (Group II) and NOB in sediment samples of Group I were observed. The levels of sulphur oxidizing bacteria (SOB) and sulphur reducing bacteria (SRB) in Group I pond water and in Group II sediment were significantly higher than their corresponding levels in the other group. In both the groups, ammonia, nitrite and sulphide concentrations were below toxic limits prescribed for shrimp farming. Comparing the production parameters at harvest revealed that low intensity culture ponds (Group I) had higher growth rate, average body weight and significantly lower FCR and higher survival rate than high intensity culture ponds (Group II). The results indicated that application of microbial products in higher quantities did not benefit significantly, and there is a need to regulate quantum and schedule of biological product usage for economically sustainable shrimp culture.

Patole, S. K., Rao, S. C., Keil, A. D., Nathan, E. A., Doherty, D. A., and Simmer, K. N. Benefits of Bifidobacterium breve M-16V Supplementation in Preterm Neonates - A Retrospective Cohort Study. PLoS.One. 2016; 11(3):e0150775

ABSTRACT: BACKGROUND: Systematic reviews of randomised controlled trials report that probiotics reduce the risk of necrotising enterocolitis (NEC) in preterm neonates. AIM: To determine whether routine probiotic supplementation (RPS) to preterm neonates would reduce the incidence of NEC. METHODS: The incidence of NEC >/= Stage II and all-cause mortality was compared for an equal period of 24 months 'before' (Epoch 1) and 'after' (Epoch 2) RPS with Bifidobacterium breve M-16V in neonates <34 weeks. Multivariate logistic regression analysis was conducted to adjust for relevant confounders. RESULTS: A

total of 1755 neonates (Epoch I vs. II: 835 vs. 920) with comparable gestation and birth weights were admitted. There was a significant reduction in NEC >/= Stage II: 3% vs. 1%, adjusted odds ratio (aOR) = 0.43 (95%CI: 0.21-0.87); 'NEC >/= Stage II or all-cause mortality': 9% vs. 5%, aOR = 0.53 (95%CI: 0.32-0.88); but not all-cause mortality alone: 7% vs. 4%, aOR = 0.58 (95% CI: 0.31-1.06) in Epoch II. The benefits in neonates <28 weeks did not reach statistical significance: NEC >/= Stage II: 6% vs. 3%, aOR 0.51 (95%CI: 0.20-1.27), 'NEC >/= Stage II or all-cause mortality', 21% vs. 14%, aOR = 0.59 (95%CI: 0.29-1.18); all-cause mortality: 17% vs. 11%, aOR = 0.63 (95%CI: 0.28-1.41). There was no probiotic sepsis. CONCLUSION: RPS with Bifidobacterium breve M-16V was associated with decreased NEC>/= Stage II and 'NEC>/= Stage II or all-cause mortality' in neonates <34 weeks. Large sample size is required to assess the potential benefits of RPS in neonates <28 weeks.

Patterson, E., Ryan, P. M., Cryan, J. F., Dinan, T. G., Ross, R. P., Fitzgerald, G. F., and Stanton, C. Gut microbiota, obesity and diabetes. Postgrad.Med.J. 2-24-2016;

ABSTRACT: The central role of the intestinal microbiota in the progression and, equally, prevention of metabolic dysfunction is becoming abundantly apparent. The symbiotic relationship between intestinal microbiota and host ensures appropriate development of the metabolic system in humans. However, disturbances in composition and, in turn, functionality of the intestinal microbiota can disrupt gut barrier function, a trip switch for metabolic endotoxemia. This low-grade chronic inflammation, brought about by the influx of inflammatory bacterial fragments into circulation through a malfunctioning gut barrier, has considerable knock-on effects for host adiposity and insulin resistance. Conversely, recent evidence suggests that there are certain bacterial species that may interact with host metabolism through metabolite-mediated stimulation of enteric hormones and other systems outside of the gastrointestinal tract, such as the endocannabinoid system. When the abundance of these keystone species begins to decline, we see a collapse of the symbiosis, reflected in a deterioration of host metabolic health. This review will investigate the intricate axis between the microbiota and host metabolism, while also addressing the promising and novel field of probiotics as metabolic therapies.

Pradhan, B., Guha, D., Ray, P., Das, D., and Aich, P. Comparative Analysis of the Effects of Two Probiotic Bacterial Strains on Metabolism and Innate Immunity in the RAW 264.7 Murine Macrophage Cell Line. Probiotics.Antimicrob.Proteins. 4-1-2016;

ABSTRACT: Probiotic and potential probiotic bacterial strains are routinely prescribed and used as supplementary therapy for a variety infectious diseases, including enteric disorders among a wide range of individuals. While there are an increasing number of studies defining the possible mechanisms of probiotic activity, a great deal remains unknown regarding the diverse modes of action attributed to these therapeutic agents. More precise information is required to support the appropriate application of probiotics. To address this objective, we selected two probiotics strains, Lactobacillus acidophilus MTCC-10307 (LA) and Bacillus clausii MTCC-8326 (BC) that are frequently prescribed for the treatment of intestinal disorders and investigated their effects on the RAW 264.7 murine macrophage cell line. Our results reveal that LA and BC are potent activators of both metabolic activity and innate immune responses in these cells. We also observed that LA and BC possessed similar activity in preventing infection simulated in vitro in murine macrophages by Salmonella typhimurium serovar enterica.

Principi, N. and Esposito, S. Antibiotic administration and the development of obesity in children. Int.J.Antimicrob.Agents. 2016; 47(3):171-177.

ABSTRACT: Antibiotics are the most common prescription drugs administered at the paediatric age, however their administration can cause unwanted problems. Among these issues, antibiotic-induced gut microbiota dysbiosis has appeared as an emerging issue and has been associated with obesity. This problem is particularly relevant in children because they are frequently treated with antibiotics. Early development of obesity increases the risk of adult obesity, which is associated with the emergence of very severe clinical problems. Dysbiosis induced in the first periods of life can have the most relevant practical consequences because a decrease in the number of microbes and their substitution with other microbes dramatically modifies the development of the immune system as well as glucose and lipid metabolism. Unfortunately, not all of the mechanisms that could explain the relationship between gut microbiota modification and the development of obesity have been defined. Consequently, no definitive therapeutic approach has been elucidated. Probiotics and prebiotics could play a role in treating microbial dysbiosis because the addition of specific bacterial strains has been associated with normal weight and has been demonstrated to be useful in clinical conditions other than obesity that are caused by microbiota disruption. Considering that antibiotics are commonly prescribed and that obesity is increasing in paediatric patients, further studies specifically designed to evaluate how to disrupt the relationship between antibiotics and dysbiosis are urgently needed. Presently, paediatricians have to consider dysbiosis to be a new and serious reason for the judicious use of antibiotics in clinical practice.

Priyamvada, S., Anbazhagan, A. N., Kumar, A., Soni, V., Alrefai, W. A., Gill, R. K., Dudeja, P. K., and Saksena, S. Lactobacillus acidophilus Stimulates Intestinal P-glycoprotein Expression via a c-Fos/c-Jun Dependent Mechanism in Intestinal Epithelial Cells. Am.J.Physiol Gastrointest.Liver Physiol. 2-11-2016; ajpgi

ABSTRACT: Our previous studies showed that L. acidophilus culture supernatant (LA CS) increased P-glycoprotein (P-gp/MDR1) function, expression and promoter activity in Caco2 cells. Current studies were designed to elucidate the molecular mechanisms mediating the stimulatory effects of LA CS on Pgp promoter activity. Deletion analysis indicated that LA CS response element (s) is located between -172/+428 bp region and sequence analysis of this region revealed the presence of 3 potential binding sites for c-Fos or c-Jun, namely proximal AP1a, distal AP1b (-119/-78 bp) & AP1c (+175/+196 bp). LA CS (24h) showed an increase in the protein expression of both c-Fos and c-Jun by ~ 2 fold in Caco2 cells. EMSA showed that LA CS markedly increased the binding of Caco-2 nuclear proteins to AP1a & AP1b but not AP1c. DNA-protein complex was completely eliminated by c-Fos antibody, while c-Jun antibody partially eliminated the complex. ChIP analysis also showed that LA CS enhanced the association of c-Fos and c-Jun (by ~4 fold and 1.5 fold, respectively) with endogenous Pgp promoter in Caco2 cells (p-172/+1). Interestingly, over-expression of c-Fos or c-Jun activated Pgp promoter by ~2 fold each. This increase was further enhanced (~14 fold) when both c-Fos and c-Jun were simultaneously over-expressed, suggesting that the presence of one of these transcription factors potentiates the effect of the other. These studies for the first time provide evidence for the involvement of c-Fos/c-Jun in the stimulation of Pgp gene expression by L. acidophilus CS in the human intestine.

Przemska-Kosicka, A., Childs, C. E., Enani, S., Maidens, C., Dong, H., Dayel, I. B., Tuohy, K., Todd, S., Gosney, M. A., and Yaqoob, P. Effect of a synbiotic on the response to seasonal influenza vaccination is strongly influenced by degree of immunosenescence. Immun.Ageing. 2016; 13:6

ABSTRACT: BACKGROUND: Ageing increases risk of respiratory infections and impairs the response to influenza vaccination. Pre- and probiotics offer an opportunity to modulate anti-viral defenses and the response to vaccination via alteration of the gut microbiota. This study investigated the effect of a novel probiotic, Bifidobacterium longum bv. infantis CCUG 52,486, combined with a prebiotic, gluco-oligosaccharide (B. longum + GI-OS), on the response to seasonal influenza vaccination in young and older subjects in a double-blind, randomized controlled trial, taking into account the influence of immunosenescence markers at baseline. RESULTS: Vaccination resulted in a significant increase in total antibody titres, vaccine-specific IgA, IgM and IgG and seroprotection to all three subunits of the vaccine in both young and older subjects, and in general, the increases in young subjects were greater. There was little effect of the synbiotic, although it tended to reduce seroconversion to the Brisbane subunit of the vaccine and the vaccine-specific IgG response in older subjects. Immunological characterization revealed that older subjects randomized to the synbiotic had a significantly higher number of senescent (CD28(-)CD57(+)) helper T cells at baseline compared with those randomized to the placebo, and they also had significantly higher plasma levels of anti-CMV IgG and a greater tendency for CMV seropositivity. Moreover, higher numbers of CD28(-)CD57(+) helper T cells were associated with failure to seroconvert to Brisbane, strongly suggesting that the subjects randomized to the synbiotic were already at a significant disadvantage in terms of likely ability to respond to the vaccine compared with those randomized to the placebo. CONCLUSIONS: Ageing was associated with marked impairment of the antibody response to influenza vaccination in older subjects and the synbiotic failed to reverse this impairment. However, the older subjects randomized to the synbiotic were at a significant disadvantage due to a greater degree of immunosenscence at baseline compared with those randomized to the placebo. Thus, baseline differences in immunosenescence between the randomized groups are likely to have influenced the outcome of the intervention, highlighting the need for detailed immunological characterization of subjects prior to interventions. TRIAL REGISTRATION: Clinicaltrials.gov NCT01066377.

Qiao, L. X., Zhu, W. Y., Zhang, H. Y., and Wang, H. Effect of early administration of probiotics on gut microflora and feeding in pre-term infants: a randomized controlled trial. J.Matern.Fetal Neonatal Med. 3-29-2016; 1-4.

ABSTRACT: BACKGROUND: This study aimed to evaluate the effect of early probiotic administration on gut microflora and influence on feeding in pre-term infants. METHODS: A double-blind, randomized, controlled clinical study was conducted to assess the effect of probiotics [live, combined lactobacillus and bifidobacterium (LCB)] supplementation in pre-term infants. Sixty hospitalized pre-term babies were randomly assigned to two groups: a probiotics-supplemented group and the control group. The primary endpoint was measurement of lactobacillus and bifidobacterium in the gut. The secondary outcome was the rate of feeding intolerance. RESULTS: In the first weekend, the quantity of gut lactobacillus and bifidobacterium was significantly higher in the probiotics-supplemented group than in the control group [7.84 +/- 0.35 versus 6.39 +/- 0.53 (log

copy number/g wet fecal weight), p = 0.013; 8.52 +/- 0.23 versus 7.01 +/- 0.48, p = 0.024, respectively]. In the second weekend, the amount of gut lactobacillus and bifidobacterium in the probiotics-supplemented group remained significantly higher (8.62 +/- 0.28 versus 7.34 +/- 0.59, p = 0.036 and 9.45 +/- 0.64 versus 7.85 +/- 0.43, p = 0.007, respectively). Fewer patients in the probiotics-supplemented group developed a feeding intolerance (13.3% versus 46.7%, p = 0.013). CONCLUSIONS: Probiotic supplementation in the hospitalized pre-term infants in the first 2 weeks of life resulted in higher amounts of lactobacillus and bifidobacterium in the gut and a concomitant lower rate of feeding intolerance.

Rao, S. C., thalye-Jape, G. K., Deshpande, G. C., Simmer, K. N., and Patole, S. K. Probiotic Supplementation and Late-Onset Sepsis in Preterm Infants: A Meta-analysis. Pediatrics. 2016; 137(3):1-16.

ABSTRACT: CONTEXT: Late-onset sepsis (LOS) is a major cause of mortality and morbidity in preterm infants. Despite various preventive measures, its incidence continues to remain high, hence the urgent need for additional approaches. One such potential strategy is supplementation with probiotics. The updated Cochrane Review (2014) did not find benefits of probiotics in reducing the risk of LOS in preterm infants (19 studies, N = 5338). Currently there are >30 randomized controlled trials (RCTs) of probiotics in preterm infants that have reported on LOS. OBJECTIVES: To conduct a systematic review including all relevant RCTs. DATA SOURCES: PubMed, Embase, Cochrane Central Register of Controlled Trials, Cumulative Index of Nursing and Allied Health Literature, and E-abstracts from the Pediatric Academic Society meetings and other pediatric and neonatal conference proceedings were searched in June and August 2015. STUDY SELECTION: RCTs comparing probiotics versus placebo/no probiotic were included. DATA EXTRACTION: Relevant data were extracted independently by 3 reviewers. RESULTS: Pooled results from 37 RCTs (N = 9416) using fixed effects model meta analysis showed that probiotics significantly decreased the risk of LOS (675/4852 [13.9%] vs 744/4564 [16.3%]; relative risk, 0.86; 95% confidence interval, 0.78-0.94; P = .0007; I(2) = 35%; number needed to treat, 44). The results were significant even after excluding studies with high risk of bias. CONCLUSIONS: Probiotic supplementation reduces the risk of LOS in preterm infants.

Razmpoosh, E., Javadi, M., Ejtahed, H. S., and Mirmiran, P. Probiotics as beneficial agents in the management of diabetes mellitus: a systematic review. Diabetes Metab Res.Rev. 2016; 32(2):143-168.

ABSTRACT: Probiotics have been suggested to play an important role in the management of diabetes. We conducted a systematic review on the role of probiotics in modulating parameters related to diabetes in animal and human experiments. We searched Pubmed, Scopus and Cochrane central until June 2014, concerning the effects of probiotics on hyperglycemia, hyperinsulinemia and their anti-diabetic efficacies by modulating the activities of proinflammatory and antioxidant factors. Our initial search retrieved 1120 reports. After screening titles and abstracts, 72 full-text articles were reviewed for eligibility. Ultimately, 33 articles met our inclusion criteria consisting of five human and twenty eight animal reports. Lactobacillus strains were, in particular, used in all studies with or without other strains. We found that probiotics have beneficial effects on glycemic controls, as all human studies showed significant reductions in at least one of the primary outcome endpoints which were the levels of fasting plasma

glucose, postprandial blood glucose, glycated haemoglobin, insulin, insulin resistance and onset of diabetes; similarly, all the animal reports, except for two, documented significant changes in these parameters. Regarding secondary outcome measures, that is, lipid profiles, pro-inflammatory and anti-oxidant factors, only one human and one animal study failed to show any significant changes in any of these parameters. This systematic review generally demonstrated beneficial effects of the probiotic administration, especially Lactobacillus sub-strains, on the management of diabetes-related blood parameters, although, more evidence, especially from human trials, is needed to confirm these effects and also to conduct a meta-analysis. Copyright (c) 2015 John Wiley & Sons, Ltd.

Rebollar, E. A., Antwis, R. E., Becker, M. H., Belden, L. K., Bletz, M. C., Brucker, R. M., Harrison, X. A., Hughey, M. C., Kueneman, J. G., Loudon, A. H., McKenzie, V., Medina, D., Minbiole, K. P., Rollins-Smith, L. A., Walke, J. B., Weiss, S., Woodhams, D. C., and Harris, R. N. Using "Omics" and Integrated Multi-Omics Approaches to Guide Probiotic Selection to Mitigate Chytridiomycosis and Other Emerging Infectious Diseases. Front Microbiol. 2016; 7:68

ABSTRACT: Emerging infectious diseases in wildlife are responsible for massive population declines. In amphibians, chytridiomycosis caused by Batrachochytrium dendrobatidis, Bd, has severely affected many amphibian populations and species around the world. One promising management strategy is probiotic bioaugmentation of antifungal bacteria on amphibian skin. In vivo experimental trials using bioaugmentation strategies have had mixed results, and therefore a more informed strategy is needed to select successful probiotic candidates. Metagenomic, transcriptomic, and metabolomic methods, colloquially called "omics," are approaches that can better inform probiotic selection and optimize selection protocols. The integration of multiple omic data using bioinformatic and statistical tools and in silico models that link bacterial community structure with bacterial defensive function can allow the identification of species involved in pathogen inhibition. We recommend using 16S rRNA gene amplicon sequencing and methods such as indicator species analysis, the Kolmogorov-Smirnov Measure, and co-occurrence networks to identify bacteria that are associated with pathogen resistance in field surveys and experimental trials. In addition to 16S amplicon sequencing, we recommend approaches that give insight into symbiont function such as shotgun metagenomics, metatranscriptomics, or metabolomics to maximize the probability of finding effective probiotic candidates, which can then be isolated in culture and tested in persistence and clinical trials. An effective mitigation strategy to ameliorate chytridiomycosis and other emerging infectious diseases is necessary; the advancement of omic methods and the integration of multiple omic data provide a promising avenue toward conservation of imperiled species.

Reid, G., Kumar, H., Khan, A. I., Rautava, S., Tobin, J., and Salminen, S. The case in favour of probiotics before, during and after pregnancy: insights from the first 1,500 days. Benef.Microbes. 2-3-2016; 1-10.

ABSTRACT: Successful human reproduction requires microbial homeostasis in the female reproductive tract, and colonisation of the newborn with beneficial microbes. In order to prevent several complications associated with dysbiosis, the administration of probiotics is more often being considered. The objective of the enclosed review was to examine the rationale for probiotic utility before and during pregnancy and in the early phase of infant life. The conclusions emerged from a panel of researchers who met during the

International Scientific Association for Probiotics and Prebiotics (ISAPP) workshop held in Washington, DC, USA in 2015. The group concluded based upon the current literature, that a case can be made for the use of a specific sets of probiotic organisms during the first 1,500 days of life, with the goal of a healthy pregnancy to term, and a healthy start to life with lowered risk of infections and inflammatory events. The key to successfully translating these recommendations to practice is that products be made available and affordable to women in developed and developing countries.

Ribera Casado, J. M. [Intestinal microbiota and ageing: A new intervention route?]. Rev.Esp.Geriatr.Gerontol. 3-3-2016;

ABSTRACT: Intestinal microbiota (IM) has continued to be the subject in all types of studies and publications. More is known on its different components and functions, as well as the changes that take place in IM through the life cycle, and the role of the factors involved in these changes. The aim of this review is to update the relationship between IM and aging. The presentation in 4 sections: (i)main factors of the human ageing process, underlining those related with gut changes; (ii)conceptual meaning of words like microbiota and other related terms; (iii)to comment on the most current findings as regards the changes in IM that occur in the ageing process, whether arising from the physiology or from disease situations, or other factors (environment, diet, drugs, etc.), and the health-consequences of these changes, and (iv)possibilities of different active positive interventions, with emphasis on diet measures.

Ricci, G., Cipriani, F., Zuccotti, G., Meneghin, F., Aceti, A., Gori, D., and Corvaglia, L. Probiotics and prevention of eczema: have we enough data to draw conclusions? Allergy. 2016; 71(3):426-428.

Abstract Not Available.

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Ricci, G., Cipriani, F., Cuello-Garcia, C. A., Brozek, J. L., Fiocchi, A., Pawankar, R., Yepes-Nunes, J. J., Terraciano, L., Gandhi, S., Agarwal, A., Zhang, Y., and Schunemann, H. J. A clinical reading on "World Allergy Organization-McMaster University Guidelines for Allergic Disease Prevention (GLAD-P): Probiotics". World Allergy Organ J. 2016; 9:9.

Abstract Not Available.

Ripert, G., Racedo, S. M., Elie, A. M., Jacquot, C., Bressollier, P., and Urdaci, M. C. Secreted compounds of the probiotic Bacillus clausii O/C inhibit the cytotoxic effects induced by Clostridium difficile and Bacillus cereus toxins. Antimicrob.Agents Chemother. 3-21-2016;

ABSTRACT: Although the use of probiotics based on Bacillus strains to fight off intestinal pathogens and antibiotic-associated diarrhea is widespread, the mechanisms involved in producing their beneficial effects still remain unclear. Here, we studied the ability of the probiotic Bacillus clausii O/C secreted compounds to counteract the cytotoxic effects induced by toxins of two pathogens, Clostridium difficile and Bacillus cereus, by evaluating eukaryotic cell viability and expression of selected genes. Co-incubation of C. difficile and B. cereus toxic culture supernatants with the B. clausii supernatant completely prevented the damage induced by toxins in Vero and Caco-2 cells. The

hemolytic effect of B. cereus was also avoided by the probiotic supernatant. Moreover, in these cells, the expression of RhoB, encoding to a Rho GTPase target for C. difficile toxins, was normalized when C. difficile supernatant was pretreated using the B. clausii supernatant. All of the beneficial effects observed with the probiotic were abolished by the serine protease inhibitor PMSF. Suspecting the involvement of a secreted protease in this protective effect, a protease was purified from the B. clausii supernatant and identified as a serine protease (M-protease, Q99405). Experiments on Vero cells demonstrated the antitoxic activity of the purified protease against pathogen supernatants. This is the first report showing the capacity of a protease secreted by probiotic bacteria to inhibit the cytotoxic effects of toxinogenic C. difficile and B. cereus strains. This extracellular compound could be responsible, at least in part, for the protective effects observed for this human probiotic in antibiotic-associated diarrhea.

Rivkin, A. and Rybalov, S. Update on the Management of Diarrhea-Predominant Irritable Bowel Syndrome: Focus on Rifaximin and Eluxadoline. Pharmacotherapy. 2016; 36(3):300-316.

ABSTRACT: Diarrhea-predominant irritable bowel syndrome (IBS-D) is one of the most common diagnoses made by gastroenterologists. Current pharmacologic treatments for IBS-D include fiber supplements, antidiarrheal over-the-counter medications, probiotics, antispasmodics, antidepressants, and a 5-hydroxytryptophan 3 receptor antagonist. All of these options have limited efficacy in managing IBS-D. Rifaximin, a nonabsorbable antibiotic, has been evaluated in patients with IBS-D. In two randomized, double-blind, placebo-controlled phase III trials evaluating rifaximin 550 mg by mouth 3 times/day for 14 days, the primary efficacy end point was achieved by 9% more patients randomized to the rifaximin group compared with placebo (40.7% vs 31.7%, p<0.001, number needed to treat ~11). The primary efficacy end point was defined as the proportion of patients having adequate relief of global IBS symptoms for at least 2 of the 4 weeks during the primary follow-up period (weeks 3-6). In the phase III trial examining the efficacy and safety of repeated courses of rifaximin in patients who responded to the initial 2-week course, rifaximin given for up to two additional courses provided a statistically significant incremental benefit (33% vs 25%, p=0.02). Eluxadoline is a gut-targeting mu and kappa opioid receptor agonist and a delta opioid receptor antagonist. The dual mechanism of eluxadoline may explain the antidiarrheal and abdominal pain-modulating properties and lack of profound constipation. In two identically designed randomized, double-blind, placebo-controlled phase III studies, 10.3% more patients in an eluxadoline 100 mg by mouth twice/day group met the primary efficacy end point during the follow-up 1-12 week period compared with placebo (p<0.001). The primary efficacy end point was a composite response, defined as improvement in worst abdominal pain and stool consistency at the same time on most (50% or more) days during the follow-up period. This review evaluates evidence for the use of rifaximin and eluxadoline in patients with IBS-D. Rifaximin provides an additional modality for the management of IBS-D patients; it has mild to moderate efficacy similar to other currently available treatment options. Rifaximin is relatively safe, lacks significant drug-drug interactions, and can be used for up to two additional retreatment courses. This may make rifaximin a possible initial or second-line treatment option. Eluxadoline can also offer relief to patients with IBS-D. While effective, because of several limitations, including drug-drug interactions and drug disease contraindications, as well as current lack of clinical experience, it may be tried as a second- or third-line agent.

Rodenas, C. L., Lepage, M., Ngom-Bru, C., Fotiou, A., Papagaroufalis, K., and Berger, B. Effect of Formula Containing Lactobacillus Reuteri DSM 17938 on Fecal Microbiota of Infants Born by Cesarean-Section. J.Pediatr.Gastroenterol.Nutr. 3-28-2016;

ABSTRACT: OBJECTIVES: Microbiota modulation by probiotics in infants born by cesarean (C)-section is poorly understood. We aimed at assessing the response of C-section-delivered infant microbiota to a formula containing L. reuteri DSM 17938 and comparing it with that of vaginally-delivered infants. METHODS: Infants delivered by C-section (C) and vaginally (V) were randomized to receive either control formula (CCt, n = 10; VCt, n = 10) or the same formula containing L. reuteri (CLr, n = 11; VLr, n = 9) within 72 hours following birth. Stool samples were collected at 2 weeks and 4 months of age. Microbial DNA was extracted, amplified and pyrosequenced. RESULTS: The phylogenetic profiles of the CLr, VCt and VLr microbiota were not significantly different at any age, but diverged from that of CCt at 2 weeks. Compared with VCt, CCt displayed lower Bifidobacterium and higher Enterobacter, unclassified Enterobacteriaceae, Enterococcus, Clostridium and unclassified Clostridiaceae relative abundance at 2 weeks, as well as lower Collinsella and higher Enterococcus and Coprococcus abundance at 4 months. The level of most of these taxa was not significantly different between the CLr and the vaginal-delivery groups. Compared with VCt, the only difference observed in VLr microbiota was higher Lactobacillus at the 2 study ages and Coprococcus at 4 months. CONCLUSIONS: Our results show that a formula containing L. reuteri DSM 17938 does not essentially alter the microbiota in vaginally-born infants. However, in C-section delivered infants this strain seems to play the role of keystone species by modulating the early development of the microbiota towards the composition found after vaginal delivery.

Ruiz, L., Hidalgo, C., Blanco-Miguez, A., Lourenco, A., Sanchez, B., and Margolles, A. Tackling probiotic and gut microbiota functionality through proteomics. J.Proteomics. 3-18-2016;

ABSTRACT: Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Many strains exert their beneficial effects after transiently colonizing the human gut, where they interact with the rest of the intestinal microorganisms and with the host mucosa. Indeed the human gut harbours a huge number of microorganisms also known as gut microbiota. Imbalances in the relative abundances of the individual components of the gut microbiota may determine the health status of the host and alterations in specific groups have been related to different diseases and metabolic disorders. Proteomics provide a set of high-throughput methodologies for protein identification that are extremely useful for studying probiotic functionality and helping in the assessment of specific health-promoting activities, such as their immunomodulatory activity, the intestinal colonization processes, and the crosstalk mechanisms with the host. Furthermore, proteomics have been used to identify markers of technological performance and stress adaptation, which helps to predict traits such as behaviour into food matrices and ability to survive passage through the gastrointestinal tract. The aim of this review is to compile studies in which proteomics have been used to assess probiotic functionality and to identify molecular players supporting their mechanisms of action. SIGNIFICANCE: Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Molecular basis underlying the functional properties of probiotic bacteria responsible for the health promoting effects have been in the background for many years. Breakthrough of omics technologies in the probiotic and microbiota fields has had a very relevant impact in the elucidation of probiotic mechanisms and in the procedures to select these microorganisms, based on solid scientific evidence. It is unquestionable that,

in the near future, the evolution of proteomic techniques will play a pivotal role in the generation of knowledge about the functions of probiotics and gut commensals, still a pending issue in the field of intestinal microbiomics.

Ruiz, L., Moles, L., Gueimonde, M., and Rodriguez, J. M. Perinatal Microbiomes Influence on Preterm Birth and Preterms' Health: Influencing Factors and Modulation Strategies. J.Pediatr.Gastroenterol.Nutr. 3-17-2016;

ABSTRACT: Microbial communities inhabiting the human host play important roles in maintaining health status, including reproduction and early life programming which is particularly important in the context of preterm neonates' health. Preterm birth (PTB) is often the result of a microbial dysbiosis or infection. In addition, preterm neonates suffer from different levels of organ immaturity and an abnormal gut microbiota establishment, as compared to full term neonates. This exacerbates their developmental problems and can have negative consequences at systemic level. In addition, preterm babies are commonly exposed to delayed enteral feeding and hospital environments, which increases the risk of short- and long-term health problems. Some of these clinical conditions, such as necrotizing enterocolitis (NEC) or sepsis, may be life-threatening while others may translate into life-long conditions, including cognitive problems. Increasing scientific interest has focused on understanding developmental problems in preterm neonates related to abnormalities in the settlement of their microbial communities, with the final goal of selecting appropriate microbiome-targeted strategies (e.g. probiotics), to reduce preterm health risks and improve overall quality of life.This review aims to summarize current knowledge on microbiological factors influencing PTB initiation and gastrointestinal development, and on the health consequences to the preterm neonate. Scientific evidences on dietary strategies reducing PTB incidence and minimizing sequelae in this particularly sensitive human group subpopulation are also discussed.

Safari, R., Adel, M., Lazado, C. C., Caipang, C. M., and Dadar, M. Host-derived probiotics Enterococcus casseliflavus improves resistance against Streptococcus iniae infection in rainbow trout (Oncorhynchus mykiss) via immunomodulation. Fish.Shellfish.Immunol. 3-17-2016; 52:198-205.

ABSTRACT: The present study evaluated the benefits of dietary administration of host-derived candidate probiotics Enterococcus casseliflavus in juvenile rainbow trout Oncorhynchus mykiss. Experimental diets were prepared by incorporating the microorganisms in the basal feed at 3 inclusion levels (i.e. 107 CFU g-1 of feed [T1], 108 CFU g-1 of feed [T2], 109 CFU g-1 of feed [T3]). The probiotic feeds were administered for 8 weeks, with a group fed with the basal diet serving as control. The effects on growth performance, gut health, innate immunity and disease resistance were evaluated. Results showed that growth performance parameters were significantly improved in T2 and T3 groups. Activities of digestive enzymes such as trypsin and lipase were significantly higher in these two groups as well. Gut micro-ecology was influenced by probiotic feeding as shown by the significant increase in intestinal lactic acid bacteria and total viable aerobic counts in T2 and T3. Humoral immunity was impacted by dietary probiotics as total serum protein and albumin were significantly elevated in T3. The levels of serum IgM significantly increased in all probiotic fed groups at week 8; with the T3 group registering the highest increment. Respiratory burst activity of blood leukocytes were significantly improved in T2 and T3. Hematological profiling further revealed that neutrophil counts significantly increased in all probiotic fed groups. Challenge test

showed that probiotic feeding significantly improved host resistance to Streptococcus iniae infection, specifically in T2 and T3 where a considerable modulation of immune responses was observed. Taken together, this study demonstrated E. casseliflavus as a potential probiotics for rainbow trout with the capability of improving growth performance and enhancing disease resistance by immunomodulation.

Sah, B. N., Vasiljevic, T., McKechnie, S., and Donkor, O. N. Antibacterial and antiproliferative peptides in synbiotic yogurt-Release and stability during refrigerated storage. J.Dairy Sci. 3-16-2016;

ABSTRACT: The search for alternative therapeutics is on the rise due to the extensive increase in bacterial resistance to various conventional antibiotics and side effects of conventional cancer therapies. Bioactive peptides released from natural sources such as dairy foods by lactic acid bacteria have received attention as a potential source of biotherapeutic peptides. However, liberation of peptides in yogurt depends on proteolytic activities of the cultures used. Thus, this research was conducted to establish generation of inhibitory peptides in yogurt against pathogenic bacteria and cancer cells during storage at 4 degrees C for 28 d. Water-soluble crude peptide extracts were prepared by high-speed centrifugation of plain and probiotic yogurts supplemented with or without pineapple peel powder (PPP). The inhibition zones against Escherichia coli and Staphylococcus aureus by PPP-fortified probiotic yogurt at 28 d of storage were, respectively, 25.89 and 11.72 mm in diameter, significantly higher than that of nonsupplemented control yogurts. Antiproliferative activity against HT29 colon cancer cells was also significantly higher in probiotic yogurt with PPP than in nonsupplemented probiotic yogurt. Overall, crude water-soluble peptide extracts of the probiotic yogurt with PPP possessed stronger inhibitory activities against bacteria and cancer cells than controls, and these activities were maintained during storage. However, activities were lowered substantially during in vitro gastrointestinal digestion. These findings support the possibility of utilizing dairy-derived bioactive peptides in the development of a superior alternative to the current generation of antibacterial and anticancer agents, as well as a functional ingredient in foods, nutraceuticals, and pharmaceuticals.

Saiki, A., Ishida, Y., Segawa, S., Hirota, R., Nakamura, T., and Kuroda, A. A Lactobacillus mutant capable of accumulating long-chain polyphosphates that enhance intestinal barrier function. Biosci.Biotechnol.Biochem. 3-11-2016; 1-7.

ABSTRACT: Inorganic polyphosphate (polyP) was previously identified as a probiotic-derived substance that enhances intestinal barrier function. PolyP-accumulating bacteria are expected to have beneficial effects on the human gastrointestinal tract. In this study, we selected Lactobacillus paracasei JCM 1163 as a strain with the potential to accumulate polyP, because among the probiotic bacteria stored in our laboratory, it had the largest amount of polyP. The chain length of polyP accumulated in L. paracasei JCM 1163 was approximately 700 phosphate (Pi) residues. L. paracasei JCM 1163 accumulated polyP when Pi was added to Pi-starved cells. We further improved the ability of L. paracasei JCM 1163 to accumulate polyP by nitrosoguanidine mutagenesis. The mutant accumulated polyP at a level of 1500 nmol/mg protein-approximately 190 times that of the wild-type strain. PolyP extracted from the L. paracasei JCM 1163 significantly suppressed the oxidant-induced intestinal permeability in mouse small intestine. In conclusion, we have succeeded in breeding the polyP-accumulating Lactobacillus mutant that is expected to enhance intestinal barrier function.

Sakatani, A., Fujiya, M., Ueno, N., Kashima, S., Sasajima, J., Moriichi, K., Ikuta, K., Tanabe, H., and Kohgo, Y. Polyphosphate Derived from Lactobacillus brevis Inhibits Colon Cancer Progression Through Induction of Cell Apoptosis. Anticancer Res. 2016; 36(2):591-598.

ABSTRACT: Although probiotics are known to have antitumor activity, few bacteria-derived antitumor molecules have been identified. The present study explored an antitumor molecule derived from Lactobacillus brevis SBL8803 (L. brevis 8803) and the mechanisms that underlie its effects. Cell viability and apoptosis were assessed by a sulforhodamine B assay and terminal deoxynucleotidyl transferase dUTP staining, respectively. Phosphorylated extracellular signal-regulated kinase (ERK) and cleaved poly ADP-ribose polymerase (PARP) expression were detected by western blotting. The conditioned medium of L. brevis 8803 inhibited SW620 cells viability and the effect was reduced by the degradation of polyphosphate (poly P) in the conditioned medium. A xenograft model showed that poly P inhibited the growth of SW620 cells. Poly P induced the apoptosis of SW620 cells through activation of the ERK pathway. In contrast, in primary cultured cells derived from normal mouse, poly P did not affect cell viability. Probiotic-derived poly P is expected to be an antitumor drug with fewer adverse effects than conventional drugs.

Salvetti, E., Orru, L., Capozzi, V., Martina, A., Lamontanara, A., Keller, D., Cash, H., Felis, G. E., Cattivelli, L., Torriani, S., and Spano, G. Integrate genome-based assessment of safety for probiotic strains: Bacillus coagulans GBI-30, 6086 as a case study. Appl.Microbiol.Biotechnol. 3-8-2016;

ABSTRACT: Probiotics are microorganisms that confer beneficial effects on the host; nevertheless, before being allowed for human consumption, their safety must be verified with accurate protocols. In the genomic era, such procedures should take into account the genomic-based approaches. This study aims at assessing the safety traits of Bacillus coagulans GBI-30, 6086 integrating the most updated genomics-based procedures and conventional phenotypic assays. Special attention was paid to putative virulence factors (VF), antibiotic resistance (AR) genes and genes encoding enzymes responsible for harmful metabolites (i.e. biogenic amines, BAs). This probiotic strain was phenotypically resistant to streptomycin and kanamycin, although the genome analysis suggested that the AR-related genes were not easily transferrable to other bacteria, and no other genes with potential safety risks, such as those related to VF or BA production, were retrieved. Furthermore, no unstable elements that could potentially lead to genomic rearrangements were detected. Moreover, a workflow is proposed to allow the proper taxonomic identification of a microbial strain and the accurate evaluation of risk-related gene traits, combining whole genome sequencing analysis with updated bioinformatics tools and standard phenotypic assays. The workflow presented can be generalized as a guideline for the safety investigation of novel probiotic strains to help stakeholders (from scientists to manufacturers and consumers) to meet regulatory requirements and avoid misleading information.

Sanders, M. E., Shane, A. L., and Merenstein, D. J. Advancing probiotic research in humans in the United States: Challenges and strategies. Gut Microbes. 3-10-2016; 1-4.

ABSTRACT: This is a summary from a workshop convened as part of the 13th annual meeting of the International Scientific Association for Probiotics and Prebiotics. A group of 24 stakeholders, including clinical experts, researchers, federal government officials, funding agencies, lawyers and industry experts met to review the challenges of the current regulatory approach to human research on probiotics in the USA and to discuss ways to move research forward. There was agreement that some of the current regulatory requirements imposed on probiotic research in the United States hindered research progress and increased cost without improving study subject safety. Many situations were outlined by clinical investigators demonstrating the impact of regulatory delays on research progress. Additionally, research is compromised when study designs and outcomes require manipulation so as to invoke less burdensome regulatory requirements. These responses by investigators to regulatory requirements have placed United States' researchers at a disadvantage. The public ultimately suffer when research to clarify the role of these products on health is stalled. Workshop participants concurred that regulatory oversight should balance study subject vulnerability with documented safety for the intended use for the probiotic strain, and that human research on foods and supplements should not be be regulated as drug research. Challenges and potential improvement strategies are discussed.

Scaioli, E., Colecchia, A., Marasco, G., Schiumerini, R., and Festi, D. Pathophysiology and Therapeutic Strategies for Symptomatic Uncomplicated Diverticular Disease of the Colon. Dig.Dis.Sci. 2016; 61(3):673-683.

ABSTRACT: Colonic diverticulosis imposes a significant burden on industrialized societies. The current accepted causes of diverticula formation include low fiber content in the western diet with decreased intestinal content and size of the lumen, leading to the transmission of muscular contraction pressure to the wall of the colon, inducing the formation of diverticula usually at the weakest point of the wall where penetration of the blood vessels occurs. Approximately 20 % of the patients with colonic diverticulosis develop abdominal symptoms (i.e., abdominal pain and discomfort, bloating, constipation, and diarrhea), a condition which is defined as symptomatic uncomplicated diverticular disease (SUDD). The pathogenesis of SUDD symptoms remains uncertain and even less is known about how to adequately manage bowel symptoms. Recently, low-grade inflammation, altered intestinal microbiota, visceral hypersensitivity, and abnormal colonic motility have been identified as factors leading to symptom development, thus changing and improving the therapeutic approach. In this review, a comprehensive search of the literature regarding on SUDD pathogenetic hypotheses and pharmacological strategies was carried out. The pathogenesis of SUDD, although not completely clarified, seems to be related to an interaction between colonic microbiota alterations, and immune, enteric nerve, and muscular system dysfunction (Cuomo et al. in United Eur Gastroenterol J 2:413-442, 2014). Greater understanding of the inflammatory pathways and gut microbiota composition in subjects affected by SUDD has increased therapeutic options, including the use of gut-directed antibiotics, mesalazine, and probiotics (Bianchi et al. in Aliment Pharmacol Ther 33:902-910, 2011; Comparato et al. in Dig Dis Sci 52:2934-2941, 2007; Tursi et al. in Aliment Pharmacol Ther 38:741-751, 2013); however, more research is necessary to validate the safety, effectiveness, and cost-effectiveness of these interventions.

Schaeck, M., Duchateau, L., Van den, Broeck W., Van, Trappen S., De, Vos P., Coulombet, C., Boon, N., Haesebrouck, F., and Decostere, A. Vibrio lentus protects gnotobiotic sea bass

(Dicentrarchus labrax L.) larvae against challenge with Vibrio harveyi. Vet.Microbiol. 3-15-2016; 185:41-48.

ABSTRACT: Due to the mounting awareness of the risks associated with the use of antibiotics in aquaculture, treatment with probiotics has recently emerged as the preferred environmental-friendly prophylactic approach in marine larviculture. However, the presence of unknown and variable microbiota in fish larvae makes it impossible to disentangle the efficacy of treatment with probiotics. In this respect, the recent development of a germ-free culture model for European sea bass (Dicentrarchus labrax L.) larvae opened the door for more controlled studies on the use of probiotics. In the present study, 206 bacterial isolates, retrieved from sea bass larvae and adults, were screened in vitro for haemolytic activity, bile tolerance and antagonistic activity against six sea bass pathogens. Subsequently, the harmlessness and the protective effect of the putative probiotic candidates against the sea bass pathogen Vibrio harveyi were evaluated in vivo adopting the previously developed germ-free sea bass larval model. An equivalence trial clearly showed that no harmful effect on larval survival was elicited by all three selected probiotic candidates: Bacillus sp. LT3, Vibrio lentus and Vibrio proteolyticus. Survival of Vibrio harveyi challenged larvae treated with V. lentus was superior in comparison with the untreated challenged group, whereas this was not the case for the larvae supplemented with Bacillus sp. LT3 and V. proteolyticus. In this respect, our results unmistakably revealed the protective effect of V. lentus against vibriosis caused by V. harveyi in gnotobiotic sea bass larvae, rendering this study the first in its kind.

Schneiderhan, J., Master-Hunter, T., and Locke, A. Targeting gut flora to treat and prevent disease. J.Fam.Pract. 2016; 65(1):34-38.

ABSTRACT: There's evidence to suggest that probiotics, prebiotics, and a high-fiber diet can help mange various illnesses. Here's how to translate gut flora research into practice.

Schreck, Bird A., Gregory, P. J., Jalloh, M. A., Risoldi, Cochrane Z., and Hein, D. J. Probiotics for the Treatment of Infantile Colic: A Systematic Review. J.Pharm.Pract. 3-2-2016;

ABSTRACT: OBJECTIVE: To evaluate whether clinical data support the safety and efficacy of probiotics for the management of infantile colic. BACKGROUND: Probiotics have been suggested as a potential strategy for infantile colic, and the specific species that have been studied in healthy infants are considered to be safe. METHODOLOGY: A systematic review was conducted to identify randomized controlled trials (RCTs) evaluating the use of probiotic supplementation in infants with colic. RCTs with a primary end point assessing crying or fussing time were selected. A meta-analysis comparing "responders" to "nonresponders" in infants receiving probiotic versus control was conducted. The quality of trials selected was assessed. RESULTS: Five RCTs assessing 2 different strains of the probiotic Lactobacillus reuteri in mostly breastfed infants were identified. Analysis of response rates showed that infants receiving probiotics had a 2.3-fold greater chance of having a 50% or greater decrease in crying/fussing time compared to controls (P = .01). Probiotic supplementation was not associated with any adverse events. CONCLUSION: Supplementation with the probiotic L. reuteri in breastfed infants appears to be safe and effective for the management of infantile colic. Further research is needed to determine the role of probiotics in infants who are formula-fed.

Schulberg, J. and De, Cruz P. Characterisation and therapeutic manipulation of the gut microbiome in inflammatory bowel disease. Intern.Med.J. 2016; 46(3):266-273.

ABSTRACT: Inflammatory bowel diseases are thought to develop as a result of dysregulation of the relationship that exists between the gut microbiota, host genetics and the immune system. The advent of culture-independent techniques has revolutionised the ability to characterise the role of the gut microbiota in health and disease based on the microbiota's genetic make-up. Inflammatory bowel diseases are characterised by dysbiosis which is an imbalance between pro- and anti-inflammatory bacteria and a reduction in bacterial diversity. Emerging data suggest that it is not only the presence of the gut microbiota but the functional activity of the microbiota that appears to play an important role in health and disease. Current strategies to manipulate therapeutically the gut microbiota using dietary modification, prebiotics, probiotics, antibiotics and faecal microbiota transplantation aim to restore the balance to a state of normobiosis. However, the ability of such strategies to correct dysbiosis and thereby achieve therapeutic benefit is yet to be fully characterised.

Sheikhi, A., Shakerian, M., Giti, H., Baghaeifar, M., Jafarzadeh, A., Ghaed, V., Heibor, M. R., Baharifar, N., Dadafarin, Z., and Bashirpour, G. Probiotic Yogurt Culture Bifidobacterium Animalis Subsp. Lactis BB-12 and Lactobacillus Acidophilus LA-5 Modulate the Cytokine Secretion by Peripheral Blood Mononuclear Cells from Patients with Ulcerative Colitis. Drug Res.(Stuttg). 2-24-2016;

ABSTRACT: Background: There are some evidences for the immunomodulation disorders in the response to intestinal microbiota in inflammatory bowel disease. Yogurt is a fermented milk product made with a starter culture consisting of different probiotics which could be colonized in intestine. However, the role of probiotics in the aetiopathogenesis of ulcerative colitis (UC) has not been clarified. To determine how the immune system responds to these bacteria this study was planned. Methods: Bifidobacterium lactis BB-12 (B. lactis) and Lactobacillus acidophilus LA-5 (L. acidophilus) were cultivated on MRS broth. PBMCs of 36 UC patients were separated by Ficoll-Hypaque centrifugation and co-cultured with different concentrations of UV killed bacteria in RPMI-1 640 plus 10% FCS for 48/72 h. IL-10, TGF-beta, IFN-gamma and TNF-alpha were measured in supernatant of PBMCs by ELISA. Results: Both bacteria significantly augmented IL-10, TGF-beta, IFN-gamma and TNF-alpha compared to control ($p<0.001$). The secretion levels of IL-10 and TGF-beta by B. lactis- compared to L. acidophilus-stimulated PBMCs were significantly higher ($p<0.05$, $p<0.01$ respectively). The secretion levels of TNF-alpha and IFN-gamma by PBMCs after 72 h were significantly lower compared to 48 h stimulation by B. lactis ($p<0.001$, $p<0.035$ respectively). Conclusion: These data show that both probiotics may trigger the pro- and anti-inflammatory immune response of UC patients. It seems that IL-10/TGF-beta uprising by B. lactis could be the reason of TNF-alpha/IFN-gamma reduction. Therefore albeit B. lactis still stimulates the effector Th cells but because of more stimulatory effect on Tregs, it could be a good potential therapeutic candidate for further investigation.

Sheth, R. U., Cabral, V., Chen, S. P., and Wang, H. H. Manipulating Bacterial Communities by in situ Microbiome Engineering. Trends Genet. 2016; 32(4):189-200.

ABSTRACT: Microbial communities inhabit our entire planet and have a crucial role in biogeochemical processes, agriculture, biotechnology, and human health. Here, we argue that 'in situ microbiome engineering' represents a new paradigm of community-scale genetic and microbial engineering. We discuss contemporary applications of this approach to directly add, remove, or modify specific sets of functions and alter community-level properties in terrestrial, aquatic, and host-associated microbial communities. Specifically, we highlight emerging in situ genome engineering approaches as tractable techniques to manipulate microbial communities with high specificity and efficacy. Finally, we describe opportunities for technological innovation and ways to bridge existing knowledge gaps to accelerate the development of in situ approaches for microbiome manipulations.

Shibahara-Sone, H., Gomi, A., Iino, T., Kano, M., Nonaka, C., Watanabe, O., Miyazaki, K., and Ohkusa, T. Living cells of probiotic Bifidobacterium bifidum YIT 10347 detected on gastric mucosa in humans. Benef.Microbes. 3-1-2016; 1-8.

ABSTRACT: The probiotic strain Bifidobacterium bifidum YIT 10347 has been demonstrated to inhibit Helicobacter pylori activity, prevent injury to the gastric mucosa, and improve general gastric malaise symptoms in H. pylori positive patients. This study aimed to investigate the adhering activity and localisation of B. bifidum YIT 10347 to gastric cells and tissue in vitro, and in human in vivo to clarify the mechanism of its beneficial effects on the stomach. The in vitro study found the adhesion rate of B. bifidum YIT 10347 to human gastric epithelial cells was about 10 times higher than that of lactic acid bacteria and other bifidobacteria. In the human study, 5 H. pylori negative and 12 H. pylori positive subjects ingested milk fermented with B. bifidum YIT 10347. B. bifidum YIT 10347 cells were measured by RT-qPCR for in gastric biopsy samples. Living B. bifidum YIT 10347 cells were detected in the biopsy samples in H. pylori negative subjects (105 cells/g and 104 cells/g at 1 h and 2 h after ingestion, respectively) and H. pylori positive subjects (104 cells/g at 1 h after the ingestion). Moreover, immunostaining analysis of tissue sections found that B. bifidum YIT 10347 cells were located at the interstitial mucin layer of the stomach. These results suggest that cells of probiotic B. bifidum YIT 10347 adhered to the human gastric mucosa in a live state, and that the higher adhering activity of B. bifidum YIT 10347 to the gastric mucosa may be involved in its beneficial effects on the human stomach.

Shim, Y. H., Lee, S. J., and Lee, J. W. Antimicrobial activities of Lactobacillus Strains against Uropathogens. Pediatr.Int. 2-10-2016;

ABSTRACT: BACKGROUND: The use of lactobacillus probiotics has been proposed as an alternative to prophylactic antibiotics for preventing urinary tract infection (UTI) in the era of antibiotic resistance. In this study, antimicrobial activities of lactobacillus strains against uropathogens, were evaluated and compared with those of antibiotics. METHODS: To evaluate inhibitory activities of lactobacilli against uropathogens, six lactobacillus strains [L. gasseri, L. rhamnosus, L. acidophilus, L. plantarum, L. paracasei, L. acidophilus and four representative uropathogens of infantile UTI [Extended-spectrum beta-lactamase (ESBL)(-) E. coli, ESBL(+) E. coli, Proteus vulgaris, Enterococcus fecalis] were selected. In vitro inhibition of lactobacillus strains to each uropathogen was evaluated by MRS agar well diffusion assay and were compared with those of commercial antibiotic discs. RESULTS: Average inhibitory zones of each six lactobacillus strains against four uropathogens showed slightly different but consistent inhibition (diameter of inhibitory zone: 10.5 ~ 20.0

mm). These were quite different from those of antibiotic discs with a wider range of inhibition (diameter of inhibitory zone: <6.0 ~ 27.5 mm) depend on the resistant pattern of uropathogens. The inhibitory zones of six lactobacillus strains were between those of sensitive and resistant antibiotics (P<0.05). CONCLUSIONS: Different lactobacillus strains possessed similar moderate antimicrobial activities against uropathogens. Further research is needed to ascertain the most ideal strains for probiotic potential.

Singh, D. P., Khare, P., Zhu, J., Kondepudi, K. K., Singh, J., Baboota, R. K., Boparai, R. K., Khardori, R., Chopra, K., and Bishnoi, M. A novel cobiotic-based preventive approach against high-fat diet-induced adiposity, nonalcoholic fatty liver and gut derangement in mice. Int.J.Obes.(Lond). 2016; 40(3):487-496.

ABSTRACT: BACKGROUND: High-fat diets (HFDs) induce systemic inflammation, gut microbial derangements and disturb metabolic homeostasis, resulting in weight gain, insulin resistance and nonalcoholic fatty liver (NAFL). Numerous antioxidants and prebiotic/probiotics per se may prevent HFD-associated comorbidities, but there are no reports related to their combination. OBJECTIVE: In the present study, we aim to evaluate a cobiotic combination of lycopene (antioxidant) and isomalto-oligosaccharides (IMOs, a prebiotic) for prevention of HFD-induced alterations. DESIGN: Male Swiss albino mice were fed either normal pellet diet (NPD) or HFD and lycopene (5 and 10 mg kg(-1)), IMOs (0.5 and 1 g kg(-1)) or their combination for 12 weeks. Systemic adiposity, glucose tolerance, insulin sensitivity, feeding regulators in hypothalamus, hepatosteatosis and liver inflammation, cecal short chain fatty acids (SCFAs), serum inflammatory cytokines, gut morphology and alterations in selected gut microbes were studied. RESULTS: Lycopene, IMOs and their combination prevented weight gain, adiposity, improved adipose tissue fat mobilization and reduced insulin resistance. Hypothalamic orexigenic and anorectic genes have also been modulated by these treatments. Dietary interventions prevented NAFL-like symptoms and improved glucose homeostasis. Improvement in selected gut microbial abundance and SCFA concentration along with reduced systemic inflammation, metabolic endotoxemia and improved ileal and colonic health were observed in mice supplemented with lycopene, IMOs and their combination. Interestingly, cobiotic combination synergistically improved many of the HFD-induced alterations. CONCLUSION: The present work provide evidence that new approach based on cobiotic combination (antioxidant plus prebiotic) can be employed to develop novel class of functional foods for their application against HFD-associated pathological complications.

Soderborg, T. K., Borengasser, S. J., Barbour, L. A., and Friedman, J. E. Microbial transmission from mothers with obesity or diabetes to infants: an innovative opportunity to interrupt a vicious cycle. Diabetologia. 2-2-2016;

ABSTRACT: Maternal obesity and diabetes dramatically increase the long-term risk for obesity in the next generation, and pregnancy and lactation may be critical periods at which to aim primary prevention to break the obesity cycle. It is becoming increasingly clear that the gut microbiome in newborns and infants plays a significant role in gut health and therefore child development. Alteration of the early infant gut microbiome has been correlated with the development of childhood obesity and autoimmune conditions, including asthma, allergies and, more recently, type 1 diabetes. This is likely to be due to complex interactions between mode of delivery, antibiotic use, maternal diet, components of breastfeeding and a network of regulatory events involving both the

innate and adaptive immune systems within the infant host. Each of these factors are critical for informing microbiome development and can affect immune signalling, toxin release and metabolic signals, including short-chain fatty acids and bile acids, that regulate appetite, metabolism and inflammation. In several randomised controlled trials, probiotics have been administered with the aim of targeting the microbiome during pregnancy to improve maternal and infant health but the findings have often been confounded by mode of delivery, antibiotic use, ethnicity, infant sex, maternal health and length of exposure. Understanding how nutritional exposure, including breast milk, affects the assembly and development of both maternal and infant microbial communities may help to identify targeted interventions during pregnancy and in infants born to mothers with obesity or diabetes to slow the transmission of obesity risk to the next generation. The aim of this review is to discuss influences on infant microbiota colonisation and the mechanism(s) underlying how alterations due to maternal obesity and diabetes may lead to increased risk of childhood obesity.

Song, J. A., Kim, H. J., Hong, S. K., Lee, D. H., Lee, S. W., Song, C. S., Kim, K. T., Choi, I. S., Lee, J. B., and Park, S. Y. Oral intake of Lactobacillus rhamnosus M21 enhances the survival rate of mice lethally infected with influenza virus. J.Microbiol.Immunol.Infect. 2016; 49(1):16-23.

ABSTRACT: BACKGROUND: Influenza viruses cause acute respiratory disease. Because of the high genetic variability of viruses, effective vaccines and antiviral agents are limited. Considering the fact that the site of influenza virus entry is the mucosa of the upper respiratory tract, probiotics that can enhance mucosal immunity as well as systemic immunity could be an important source of treatment against influenza infection. METHODS: Mice were fed with Lactobacillus rhamnosus M21 or skim milk and were challenged with influenza virus. The resulting survival rate, lung inflammation, and changes in the cytokine and secretory immunoglobulin A (sIgA) levels were examined. RESULTS: Because of infection (influenza virus), all the mice in the control group and 60% of the mice in the L. rhamnosus M21 group died; however, the remaining 40% of the mice fed with L. rhamnosus M21 survived the infection. Pneumonia was severe in the control group but moderate in the group treated with L. rhamnosus M21. Although there were no significant changes in the proinflammatory cytokines in the lung lysates of mice collected from both groups, levels of interferon-gamma and interleukin-2, which are representative cytokines of type I helper T cells, were significantly increased in the L. rhamnosus M21-treated group. An increase in sIgA as well as the diminution of inflammatory cells in bronchoalveolar lavage fluid was also observed in the L. rhamnosus M21-treated group. CONCLUSION: These results demonstrate that orally administered L. rhamnosus M21 activates humoral as well as cellular immune responses, conferring increased resistance to the host against influenza virus infection.

Sprenger, N., Odenwald, H., Kukkonen, A. K., Kuitunen, M., Savilahti, E., and Kunz, C. FUT2-dependent breast milk oligosaccharides and allergy at 2 and 5 years of age in infants with high hereditary allergy risk. Eur.J.Nutr. 2-24-2016;

ABSTRACT: PURPOSE: Manifestation of allergic disease depends on genetic predisposition, diet and commensal microbiota. Genetic polymorphism of mothers determines their breast milk glycan composition. One major determinant is the fucosyltransferase 2 (FUT2, secretor gene) that was shown to be linked to commensal microbiota establishment. We studied whether FUT2-dependent breast milk oligosaccharides are associated with allergic disease in breast-fed infants later in life.

METHODS: We analyzed FUT2-dependent oligosaccharides in breast milk samples of mothers (n = 266) from the placebo group of a randomized placebo-controlled trial of prebiotics and probiotics as preventive against allergic disease in infants with high allergy risk (trial registry number: NCT00298337). Using logistic regression models, we studied associations between FUT2-dependent breast milk oligosaccharides and incidence of allergic disease at 2 and 5 years of age. RESULTS: At 2 years, but not at 5 years of age, we observed a presumed lower incidence ($p < 0.1$) for IgE-associated eczema manifestation in C-section-born infants who were fed breast milk containing FUT2-dependent oligosaccharides. By logistic regression, we observed a similar relation ($p < 0.1$) between presence of FUT2-dependent breast milk oligosaccharides and IgE-associated disease and IgE-associated eczema in C-section-born infants only. When testing with the levels of breast milk oligosaccharide 2'-fucosyllactose as proxy for FUT2 activity, we observed significant ($p < 0.05$) associations in the C-section-born infants with 'any allergic disease,' IgE-associated disease, eczema and IgE-associated eczema. CONCLUSION: The data indicate that infants born by C-section and having a high hereditary risk for allergies might have a lower risk to manifest IgE-associated eczema at 2 years, but not 5 years of age, when fed breast milk with FUT2-dependent milk oligosaccharides. Further studies with larger cohorts and especially randomized controlled intervention trials are required to build on these preliminary observations.

Srivastava, S., Saha, S., Kumari, M., and Mohd, S. Effect of Probiotic Curd on Salivary pH and Streptococcus mutans: A Double Blind Parallel Randomized Controlled Trial. J.Clin.Diagn.Res. 2016; 10(2):ZC13-ZC16.

ABSTRACT: BACKGROUND: Dairy products like curd seem to be the most natural way to ingest probiotics which can reduce Streptococcus mutans level and also increase salivary pH thereby reducing the dental caries risk. OBJECTIVES: To estimate the role of probiotic curd on salivary pH and Streptococcus mutans count, over a period of 7 days. MATERIALS AND METHODS: This double blind parallel randomized clinical trial was conducted at the institution with 60 caries free volunteers belonging to the age group of 20-25 years who were randomly allocated into two groups. Test Group consisted of 30 subjects who consumed 100ml of probiotic curd daily for seven days while an equal numbered Control Group were given 100ml of regular curd for seven days. Saliva samples were assessed at baseline, after (1/2) hour 1 hour and 7 days of intervention period using pH meter and Mitis Salivarius Bacitracin agar to estimate salivary pH and S. mutans count. Data was statistically analysed using Paired and Unpaired t-test. RESULTS: The study revealed a reduction in salivary pH after (1/2) hour and 1 hour in both the groups. However after 7 days, normal curd showed a statistically significant ($p< 0.05$) reduction in salivary pH while probiotic curd showed a statistically significant ($p< 0.05$) increase in salivary pH. Similarly with regard to S. mutans colony counts probiotic curd showed statistically significant reduction ($p< 0.05$) as compared to normal curd. CONCLUSION: Short-term consumption of probiotic curds showed marked salivary pH elevation and reduction of salivary S. mutans counts and thus can be exploited for the prevention of enamel demineralization as a long-term remedy keeping in mind its cost effectiveness.

Staudacher, H. M. and Whelan, K. Altered gastrointestinal microbiota in irritable bowel syndrome and its modification by diet: probiotics, prebiotics and the low FODMAP diet. Proc.Nutr.Soc. 2-24-2016; 1-13.

ABSTRACT: Irritable bowel syndrome (IBS) is a functional bowel disorder characterised by abdominal pain or discomfort with disordered defecation. This review describes the role of the gastrointestinal (GI) microbiota in the pathogenesis of IBS and how dietary strategies to manage symptoms impact on the microbial community. Evidence suggests a dysbiosis of the luminal and mucosal colonic microbiota in IBS, frequently characterised by a reduction in species of Bifidobacteria which has been associated with worse symptom profile. Probiotic supplementation trials suggest intentional modulation of the GI microbiota may be effective in treating IBS. A smaller number of prebiotic supplementation studies have also demonstrated effectiveness in IBS whilst increasing Bifidobacteria. In contrast, a novel method of managing IBS symptoms is the restriction of short-chain fermentable carbohydrates (low fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) diet). Studies consistently demonstrate clinical effectiveness of the low FODMAP diet in patients with IBS. However, one unintentional consequence of this dietary intervention is its impact on the microbiota. This leads to an interesting paradox; namely, increasing luminal Bifidobacteria through probiotic supplementation is associated with a reduction in IBS symptoms while in direct conflict to this, the low FODMAP diet has clinical efficacy but markedly reduces luminal Bifidobacteria concentration. Given the multifactorial aetiology of IBS, the heterogeneity of symptoms and the complex and diverse nature of the microbiome, it is probable that both interventions are effective in patient subgroups. However combination treatment has never been explored and as such, presents an exciting opportunity for optimising clinical management, whilst preventing potentially deleterious effects on the GI microbiota.

Steinert, A., Radulovic, K., and Niess, J. Gastro-intestinal tract: The leading role of mucosal immunity. Swiss.Med.Wkly. 2016; 146:w14293

ABSTRACT: An understanding of mucosal immunity is essential for the comprehension of intestinal diseases that are often caused by a complex interplay between host factors, environmental influences and the intestinal microbiota. Not only improvements in endoscopic techniques, but also advances in high throughput sequencing technologies, have expanded knowledge of how intestinal diseases develop. This review discusses how the host interacts with intestinal microbiota by the direct contact of host receptors with highly conserved structural motifs or molecules of microbes and also by microbe-derived metabolites (produced by the microbe during adaptation to the gut environment), such as short-chain fatty acids, vitamins, bile acids and amino acids. These metabolites are recognised by metabolite-sensing receptors expressed by immune cells to influence functions of macrophages, dendritic cells and T cells, such as migration, conversion and maintenance of regulatory T cells and regulation of proinflammatory cytokine production, which is essential for the maintenance of intestinal homeostasis and the development of intestinal diseases, such as inflammatory bowel diseases. First interventions in these complex interactions between microbe-derived metabolites and the host immune system for the treatment of gastrointestinal diseases, such as modification of the diet, treatment with antibiotics, application of probiotics and faecal microbiota transplantation, have been introduced into the clinic. Specific targeting of metabolite sensing receptors for the treatment of gastrointestinal diseases is in development. In future, precision medicine approaches that consider individual variability in genes, the microbiota, the environment and lifestyle will become increasingly important for the care of patients with gastrointestinal diseases.

Steves, C. J., Bird, S., Williams, F. M., and Spector, T. D. The Microbiome and Musculoskeletal Conditions of Aging: A Review of Evidence for Impact and Potential Therapeutics. J.Bone Miner.Res. 2016; 31(2):261-269.

ABSTRACT: Recently, we have begun to realize that the billions of microorganisms living in symbiosis with us have an influence on disease. Evidence is mounting that the alimentary tract microbiome, in particular, influences both host metabolic potential and its innate and adaptive immune system. Inflammatory states characterize many bone and joint diseases of aging. This prompts the hypothesis that the gut microbiome could alter the inflammatory state of the individual and directly influence the development of these common and burdensome clinical problems. Because the microbiome is easily modifiable, this could have major therapeutic impact. This perspective discusses evidence to date on the role of the microbiome and the highly prevalent age-related disorders of osteoporosis, osteoarthritis, gout, rheumatoid arthritis, sarcopenia, and frailty. It also reviews data on the effects of probiotics and prebiotic interventions in animal and human models. Despite suggestive findings, research to date is not conclusive, and we identify priorities for research to substantiate and translate findings. (c) 2015 American Society for Bone and Mineral Research.

Sun, J., Wang, F., Ling, Z., Yu, X., Chen, W., Li, H., Jin, J., Pang, M., Zhang, H., Yu, J., and Liu, J. Clostridium butyricum attenuates cerebral ischemia/reperfusion injury in diabetic mice via modulation of gut microbiota. Brain Res. 3-29-2016;

ABSTRACT: Diabetes is known to exacerbate cerebral ischemia/reperfusion (I/R) injury. Here, we investigated the effects of Clostridium butyricum on cerebral I/R injury in the diabetic mice subjected to 30min of bilateral common carotid arteries occlusion (BCCAO). The cognitive impairment, the blood glucose level, neuronal injury, apoptosis, and expressions of Akt, phospho-Akt (p-Akt), and caspase-3 level were assessed. Meanwhile, the changes of gut microbiota in composition and diversity in the colonic feces were evaluated. Our results showed that diabetic mice subjected to BCCAO exhibited worsened cognitive impairment, cell damage and apoptosis. These were all attenuated by C. butyricum. Moreover, C. butyricum reversed cerebral I/R induced decreases in p-Akt expression and increases in caspase-3 expression, leading to inhibiting neuronal apoptosis. C. butyricum partly restored cerebral I/R induced decreases of fecal microbiota diversity, changes of fecal microbiota composition. Together, these findings highlight the important role of bacteria in the bidirectional communication of the gut-brain axis and suggest that certain probiotics might prove to be useful therapeutic adjuncts in cerebral I/R injury with diabetes.

Sun, J. and Buys, N. J. Glucose- and glycaemic factor-lowering effects of probiotics on diabetes: a meta-analysis of randomised placebo-controlled trials. Br.J.Nutr. 2016; 115(7):1167-1177.

ABSTRACT: This meta-analysis examined the effect of probiotics on glucose and glycaemic factors in diabetes and its associated risk factors. All randomised-controlled trials published in English in multiple databases from January 2000 to June 2015 were systematically searched. Only studies that addressed glucose- and glycaemic-related factors as outcome variables were included. The main outcomes of interest in trials were mean changes in glucose, HbA1c, insulin and homoeostasis model assessment-estimated insulin resistance (HOMA-IR). Using the Physiotherapy Evidence Database

(PEDro) scale to assess the quality of studies, a total of eleven studies with 614 subjects were included. The pooled mean difference and effect size with a 95 % CI were extracted using a random-effect model. It was found that there are statistically significant pooled mean differences between the probiotics and the placebo-controlled groups on the reduction of glucose (-0.52 mmol/l, 95 % CI -0.92, -0.11 mmol/l; P=0.01) and HbA1c (-0.32 %, 95 % CI -0.57, -0.07 %; P=0.01). There was no statistically significant pooled mean difference between the probiotics and the placebo-controlled groups on the reduction of insulin (-0.48 microIU/ml, 95 % CI -1.34, 0.38 microIU/ml; P=0.27) and HOMA-IR (pooled effect of -0.44, 95 % CI -1.57, 0.70; P=0.45). Meta-regression analysis identified that probiotics had significant effects on reduction of glucose, HbA1c, insulin and HOMA-IR in participants with diabetes, but not in participants with other risk factors. The present meta-analysis suggested that probiotics may be used as an important dietary supplement in reducing the glucose metabolic factors associated with diabetes.

Suo, H., Zhao, X., Qian, Y., Sun, P., Zhu, K., Li, J., and Sun, B. Lactobacillus fermentum Suo Attenuates HCl/Ethanol Induced Gastric Injury in Mice through Its Antioxidant Effects. Nutrients. 2016; 8(3)

ABSTRACT: The purpose of the study was to determine the inhibitory effects of Lactobacillus fermentum Suo (LF-Suo) on HCl/ethanol induced gastric injury in ICR (Institute for Cancer Research) mice and explain the mechanism of these effects through the molecular biology activities of LF-Suo. The studied mice were divided into four groups: healthy, injured, LF-Suo-L and LF-Suo-H group. After the LF-Suo intragastric administration, the gastric injury area was reduced compared to the injured group. The serum MOT (motilin), SP (substance P), ET (endothelin) levels of LF-Suo treated mice were lower, and SS (somatostatin), VIP (vasoactive intestinal peptide) levels were higher than the injured group mice. The cytokine IL-6 (interleukin 6), IL-12 (interleukin 12), TNF-alpha (tumor necrosis factor-alpha) and IFN-gamma (interferon-gamma) serum levels were decreased after the LF-Suo treatment. The gastric tissues SOD (superoxide dismutase), GSH-Px (glutathione peroxidase), NO (nitric oxide) and activities of LF-Suo treated mice were increased and MDA (malondialdehyde) activity was decreased compared to the injured group mice. By the RT-PCR assay, LF-Suo raised the occludin, EGF (epidermal growth factor), EGFR (epidermal growth factor receptor), VEGF (vascular endothelial growth factor), Fit-1 (fms-like tyrosine kinase-1), IkappaB-alpha (inhibitor kappaB-alpha), nNOS (neuronal nitric oxide synthase), eNOS (endothelial nitric oxide synthase), Mn-SOD, Cu/Zn-SOD, CAT (catalase) mRNA or protein expressions and reduced the COX-2, NF-kappaB (nuclear factor kappaB), and iNOS (inducible nitric oxide synthase) expressions in gastric tissues compared to the gastric injured group mice. A high concentration (1.0 x 10(8) CFU/kg b.w.) of LF-Suo treatment showed stronger anti-gastric injury effects compared to a low concentration of (0.5 x 10(8) CFU/kg b.w.) of LF-Suo treatment. LF-Suo also showed strong survival in pH 3.0 man-made gastric juice and hydrophobic properties. These results indicate that LF-Suo has potential use as probiotics for its gastric injury treatment effects.

Szajewska, H., Konarska, Z., and Kolodziej, M. Probiotic Bacterial and Fungal Strains: Claims with Evidence. Dig.Dis. 2016; 34(3):251-259.

ABSTRACT: BACKGROUND: Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit for the host. This review summarizes current (i.e. published in the last 5 years) key evidence on probiotic efficacy and its safety in

adults and children. To identify relevant data, searches of MEDLINE and the Cochrane Library databases were performed in August 2015 to locate randomized controlled trials (RCTs) or their meta-analyses. The focus was on commonly used, well-specified, bacterial and yeast probiotics for the treatment of gastrointestinal disorders. The MEDLINE database was also searched for evidence-based clinical practice guidelines, developed by scientific societies in the same timeframe. Data on safety of probiotics were obtained from a document developed by the US Agency for Healthcare Research and Quality. KEY MESSAGES: A number of relevant RCTs and meta-analyses are available. Saccharomyces boulardii is the most studied yeast probiotic, and Lactobacillus rhamnosus GG is the most studied bacterial probiotic. For both, the best documented fact is their efficacy for the treatment of acute gastroenteritis, especially in children, and for the prevention of antibiotic-associated diarrhea, both in adults and children. There is some evidence to support the use of probiotics to prevent or treat other diseases, such as necrotizing enterocolitis, infantile colic, Helicobacter pylori infection, and irritable bowel syndrome, but further studies are needed to identify which strain(s) is/are the most effective. Data on safety, particularly long-term safety, are limited. The risk of side effects is greater in people who have severe underlying health conditions. CONCLUSIONS: The evidence on bacterial and yeast probiotics has considerably expanded during recent years. Accumulated data allow one to make informed decisions about the effectiveness of probiotics and about how to reduce the use of those without proven efficacy.

Szajewska, H., Canani, R. B., Guarino, A., Hojsak, I., Indrio, F., Kolacek, S., Orel, R., Shamir, R., Vandenplas, Y., van Goudoever, J. B., and Weizman, Z. Probiotics for the Prevention of Antibiotic-Associated Diarrhea in Children. J.Pediatr.Gastroenterol.Nutr. 2016; 62(3):495-506.

ABSTRACT: This article provides recommendations, developed by the Working Group (WG) on Probiotics of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition, for the use of probiotics for the prevention of antibiotic-associated diarrhea (AAD) in children based on a systematic review of previously completed systematic reviews and of randomized controlled trials published subsequently to these reviews. The use of probiotics for the treatment of AAD is not covered. The recommendations were formulated only if at least 2 randomized controlled trials that used a given probiotic (with strain specification) were available. The quality of evidence (QoE) was assessed using the Grading of Recommendations Assessment, Development, and Evaluation guidelines. If the use of probiotics for preventing AAD is considered because of the existence of risk factors such as class of antibiotic(s), duration of antibiotic treatment, age, need for hospitalization, comorbidities, or previous episodes of AAD diarrhea, the WG recommends using Lactobacillus rhamnosus GG (moderate QoE, strong recommendation) or Saccharomyces boulardii (moderate QoE, strong recommendation). If the use of probiotics for preventing Clostridium difficile-associated diarrhea is considered, the WG suggests using S boulardii (low QoE, conditional recommendation). Other strains or combinations of strains have been tested, but sufficient evidence is still lacking.

Szajewska, H. What are the indications for using probiotics in children? Arch.Dis.Child. 2016; 101(4):398-403.

ABSTRACT: The health benefits of probiotics have been the subject of extensive research. Still, some questions are being repeatedly asked: should one use or not use probiotics? If

yes, how and when should probiotics be used? The purpose of this review is to summarise current evidence on specific probiotics' efficacy and safety to help healthcare professionals make evidence-based decisions on the indications for using specific probiotic strains or combinations in children. To identify relevant data, searches of MEDLINE and the Cochrane Library databases were performed in July 2015 to locate randomised controlled trials or their meta-analyses published in the last five years. The MEDLINE database also was searched for evidence-based clinical practice guidelines, developed by scientific societies. Considering that probiotics have strain-specific effects, the main focus was on data on individual probiotic strains, not on probiotics in general.

Takada, M., Nishida, K., Kataoka-Kato, A., Gondo, Y., Ishikawa, H., Suda, K., Kawai, M., Hoshi, R., Watanabe, O., Igarashi, T., Kuwano, Y., Miyazaki, K., and Rokutan, K. Probiotic Lactobacillus casei strain Shirota relieves stress-associated symptoms by modulating the gut-brain interaction in human and animal models. Neurogastroenterol.Motil. 2-20-2016;

ABSTRACT: BACKGROUND: This study aimed to examine the effects of Lactobacillus casei strain Shirota (LcS) on gut-brain interactions under stressful conditions. METHODS: Three double-blind, placebo-controlled trials were conducted to examine the effects of LcS on psychological and physiological stress responses in healthy medical students under academic examination stress. Subjects received LcS-fermented milk or placebo daily for 8 weeks prior to taking a national standardized examination. Subjective anxiety scores, salivary cortisol levels, and the presence of physical symptoms during the intervention were pooled and analyzed. In the animal study, rats were given feed with or without LcS for 2 weeks, then submitted to water avoidance stress (WAS). Plasma corticosterone concentration and the expression of cFos and corticotropin releasing factor (CRF) in the paraventricular nucleus (PVN) were measured immediately after WAS. In an electrophysiological study, gastric vagal afferent nerve activity was monitored after intragastric administration of LcS to urethane-anesthetized rats. KEY RESULTS: Academic stress-induced increases in salivary cortisol levels and the incidence rate of physical symptoms were significantly suppressed in the LcS group compared with the placebo group. In rats pretreated with LcS, WAS-induced increases in plasma corticosterone were significantly suppressed, and the number of CRF-expressing cells in the PVN was reduced. Intragastric administration of LcS stimulated gastric vagal afferent activity in a dose-dependent manner. CONCLUSIONS & INFERENCES: These findings suggest that LcS may prevent hypersecretion of cortisol and physical symptoms under stressful conditions, possibly through vagal afferent signaling to the brain and reduced stress reactivity in the PVN.

Talebi Bezmin, Abadi A. Vaccine against Helicobacter pylori: Inevitable approach. World J.Gastroenterol. 3-21-2016; 22(11):3150-3157.

ABSTRACT: Over three decades have passed since the discovery of Helicobacter pylori (H. pylori), and yet many questions about its treatment remain unanswered. For example, there is no certainty regarding continued use of current antibiotic therapy against H. pylori. The bad news is that even combined regimens are also unable to eradicate bacterial colonization. The worst problem with H. pylori chemotherapy is that even if we identify the most successful regimen, it cannot eliminate the risk of re-infection. This problem is further complicated by the fact that clinicians have no information as to whether probiotics are useful or not. Moreover, to date, we have no large scale produced vaccine effective against H. pylori. Due to the relatively rapid and abundant

dissemination of guidelines globally reported concerning management of gastric cancer prevention and therapeutic regimens, clinicians may choose a vaccine as better effective weapon against H. pylori. Therefore, a radical shift in adopted strategies is needed to guide ultimate decisions regarding H. pylori management. In light of failures in vaccine projects, we should identify better vaccine design targeting conserved/essential genes. The unique character and persistence of H. pylori pose obstacles to making an effective vaccine. Preferably, in developing countries, the best reasonable and logical approach is to recommend prophylactic H. pylori vaccine among children as an obligatory national program to limit primary colonization. Trying to produce a therapeutic vaccine would be postponed until later. In reality, we should not forget to prescribe narrow spectrum antibiotics. In the current review, I draw a route to define the best adopted strategy against this rogue bacterium.

Tan, L. T., Chan, K. G., Lee, L. H., and Goh, B. H. Streptomyces Bacteria as Potential Probiotics in Aquaculture. Front Microbiol. 2016; 7:79

ABSTRACT: In response to the increased seafood demand from the ever-going human population, aquaculture has become the fastest growing animal food-producing sector. However, the indiscriminate use of antibiotics as a biological control agents for fish pathogens has led to the emergence of antibiotic resistance bacteria. Probiotics are defined as living microbial supplement that exert beneficial effects on hosts as well as improvement of environmental parameters. Probiotics have been proven to be effective in improving the growth, survival and health status of the aquatic livestock. This review aims to highlight the genus Streptomyces can be a good candidate for probiotics in aquaculture. Studies showed that the feed supplemented with Streptomyces could protect fish and shrimp from pathogens as well as increase the growth of the aquatic organisms. Furthermore, the limitations of Streptomyces as probiotics in aquaculture is also highlighted and solutions are discussed to these limitations.

Tanida, M., Takada, M., Kato-Kataoka, A., Kawai, M., Miyazaki, K., and Shibamoto, T. Intragastric injection of Lactobacillus casei strain Shirota suppressed spleen sympathetic activation by central corticotrophin-releasing factor or peripheral 2-deoxy-d-glucose in anesthetized rats. Neurosci.Lett. 3-10-2016; 619:114-120.

ABSTRACT: Intragastric (IG) administration of probiotic strain Lactobacillus casei Shirota (LcS) decreases the sympathetic nerve outflow of anesthetized rats in a tissue-specific manner. In the present study, we examined the effects of IG administration of LcS on sympathetic activation induced by an intracerebroventricular (ICV) injection of corticotrophin-releasing factor (CRF) and an intravenous (IV) injection of 2-deoxy-d-glucose (2DG) or interleukin (IL)-1beta in urethane-anesthetized rats. The IG administration of LcS differently affected the stimulatory responses of sympathetic nerve outflow to CRF. LcS suppressed the increase in splenic sympathetic nerve activity (Spleen-SNA), induced by central CRF, in a dose-dependent manner; however, it did not alter adrenal sympathetic nervous activity (ASNA). In contrast, LcS did not affect spleen-SNA and ASNA following an IV injection of IL-1beta. On the other hand, IG administration of LcS suppressed the activation of ASNA following an IV injection of 2DG. These findings suggest that the suppression of central CRF-induced sympathetic activation by LcS is tissue-specific. Moreover, it can suppress the 2DG-induced sympathetic activation. Furthermore, we found that stomach-specific vagotomy attenuates the suppressive effect of LcS on CRF-mediated spleen-SNA activation. Thus, the present study suggests

that LcS administered to the stomach may act on the afferent vagal nerve and send afferent signals to the brain to regulate efferent SNA induced by sympathetic stimulators.

Tapiovaara, L., Lehtoranta, L., Poussa, T., Makivuokko, H., Korpela, R., and Pitkaranta, A. Absence of adverse events in healthy individuals using probiotics - analysis of six randomised studies by one study group. Benef.Microbes. 3-11-2016; 7(2):161-169.

ABSTRACT: Consumption of live bacteria as probiotic supplements is increasing. There is, however, a lack of information on the safety of ingested probiotics. The main objective of this study was to investigate the adverse events (AEs) of specific probiotics (Lactobacillus rhamnosus GG (LGG) alone or LGG in combination with L. rhamnosus Lc705, Propionibacterium freudenreichii JS, Bifidobacterium lactis BB12, or Bifidobacterium breve 99) studied in six of our study groups' clinical trials, by analysing individual participant data. A secondary objective was to study AEs associated with the consumed probiotic species and mixtures in three specific categories; 'gastrointestinal disorders', 'respiratory, thoracic and mediastinal disorders' and 'infections and infestations'. Six randomised, double-blind, placebo-controlled clinical studies by our study group were included in this AE analysis (study population n=1,909). All AE data were classified according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) v4.0. From the 26 CTCAE System Organ Classes, we identified AEs in 20 classes among 1,909 subjects. Probiotic ingestion did not result in statistically significant differences in AEs in different groups, when compared to placebo. A subgroup analysis of gastrointestinal, respiratory, thoracic and mediastinal disorders, infections and infestations, found no differences between the intervention groups or for different probiotic combinations (risk ratio (RR) = 0.97, 95% confidence interval (CI): 0.93-1.02, P=0.30; RR=0.99, 95% CI: 0.97-1.01, P=0.35; RR=0.99, 95% CI: 0.93-1.06, P=0.62, respectively). As a conclusion, ingestion of probiotic supplementations containing LGG alone, or LGG in combination with L. rhamnosus Lc705, P. freudenreichii JS, B. breve 99, or B. lactis BB12 did not seem to cause AEs in young and elderly subjects in this analysis.

Tapiovaara, L., Kumpu, M., Makivuokko, H., Waris, M., Korpela, R., Pitkaranta, A., and Winther, B. Human rhinovirus in experimental infection after peroral Lactobacillus rhamnosus GG consumption, a pilot study. Int.Forum Allergy Rhinol. 3-17-2016;

ABSTRACT: BACKGROUND: Data has emerged on possible beneficial effects of probiotics in respiratory tract viral infections, but it is unclear if the promising positive effects evidenced are due to a reduced viral load during infections. The aims of this work were to investigate the effect of peroral probiotic Lactobacillus rhamnosus GG (American Type Culture Collection [ATCC], Accession No. 53103) consumption on human rhinovirus (HRV) load in nasopharyngeal lavage samples in experimental HRV infection, and to correlate viral load to clinical symptoms. METHODS: Intranasal HRV A39 inoculation was performed on 59 adults, who had consumed juice enriched with live or heat-inactivated L. rhamnosus GG or control juice for 3 weeks prior to inoculation in a randomized, controlled, pilot trial setting. Nasopharyngeal lavage samples and symptom data were analyzed on day 0 before inoculation, and on days 2 and 5. Samples were subjected to quantitative HRV detection by polymerase chain reaction (PCR). RESULTS: Before inoculation 9 of 59 (15%) samples presented with another HRV strain than the studied A39. There was a tendency toward the lowest HRV loads in the L. rhamnosus GG groups and the highest in placebo group (log10 copies/mL, 95% confidence interval [CI], 6.20 [5.18 to 7.40] in live, 6.30 [4.91 to 7.08] in inactivated L. rhamnosus GG, and 7.25 [5.81 to

7.52] in placebo group, p = 0.57 in day 2) in the wild-type excluded population. The HRV load positively correlated with the symptom scores on days 2 and 5 (correlation coefficient 0.61 [p < 0.001] and 0.28 [p = 0.034], respectively). CONCLUSION: Results did not show statistical differences in viral loads in subjects using L. rhamnosus GG when compared to placebo. HRV load positively correlated with the total symptom scores.

Upadrasta, A. and Madempudi, R. S. Probiotics and blood pressure: current insights. Integr.Blood Press Control. 2016; 9:33-42.

ABSTRACT: Gut microbiota play a significant role in host metabolic processes, and recent metagenomic surveys have revealed that they are involved in host immune modulation and influence host development and physiology (organ development). Initially, probiotics are identified as potential therapeutics to treat gastrointestinal disorders and to revitalize the disturbed gut ecosystem. Currently, studies are exploring the potential for expanded uses of probiotics for improving the health conditions in metabolic disorders that increase the risk of developing cardiovascular diseases such as hypertension. Further investigations are required to evaluate targeted and effective use of the wide variety of probiotic strains in various metabolic disorders to improve the overall health status of the host. This review addresses the causes of hypertension and the hypotensive effect of probiotics, with a focus on their mechanistic action.

Urbaniak, C. and Reid, G. The potential influence of the microbiota and probiotics on women during long spaceflights. Womens Health (Lond Engl.). 2016; 12(2):193-198.

ABSTRACT: Humans have been exploring space for almost 55 years but space travel comes with many psychological and physiological changes that astronauts have to adapt to, both during and post flight missions. Now, with the reality of such missions lasting years, maintaining proper health of the flight crew is a high priority. While conditions such as nausea, bone loss, renal calculi and depression have been recognized, and approaches to medical and surgical care in space considered, the influence of the microbiota could be of added significance in maintaining astronaut health. While probiotics have long been part of the Russian cosmonaut diet, their use for specific health concerns of women has not been assessed. In this article, we explore the ways in which the microbiome may influence the health of female astronauts during long space flights, and present a rationale for the use of probiotics.

Urbanska, M., Gieruszczak-Bialek, D., and Szajewska, H. Systematic review with meta-analysis: Lactobacillus reuteri DSM 17938 for diarrhoeal diseases in children. Aliment.Pharmacol.Ther. 3-16-2016;

ABSTRACT: BACKGROUND: Not all probiotics are equal. AIM: To investigate the efficacy of Lactobacillus reuteri DSM 17938 (L. reuteri) in the management of various types of diarrhoeal diseases in children. METHODS: Medline, Embase, the Cochrane Library, trial registries and reference lists of included studies were searched in January 2016, with no language restriction, for randomised controlled trials (RCTs). RESULTS: Eight RCTs (n = 1229) met the inclusion criteria. In treatment trials, L. reuteri administration reduced the duration of diarrhoea (three RCTs, n = 256, mean difference, MD -24.82 h, 95% CI -38.8 to -10.8) and increased the cure rate on day 1 and day 2. However, heterogeneity and wide confidence intervals call for caution in interpreting results. In preventive trials carried out

in hospitalised children, based on the findings from two RCTs (n = 290), there was no significant reduction in the risk of nosocomial diarrhoea, rotavirus diarrhoea or diarrhoea of any origin with L. reuteri administration. Based on one RCT (n = 97), there was no effect of L. reuteri on the risk of antibiotic-associated diarrhoea. However, the evidence is limited because the overall frequency of diarrhoea was surprisingly low. In preventive studies carried out in apparently healthy children, L. reuteri reduced diarrhoeal outcomes in one RCT; the evidence from another trial was less convincing. CONCLUSIONS: In therapeutic settings, L. reuteri administration reduces the duration of diarrhoea and increases the chance of cure. In preventive settings, L. reuteri has the potential to reduce the risk of community-acquired diarrhoea in otherwise healthy children.

Van Beek, A. A., Hoogerland, J. A., Belzer, C., De, Vos P., De Vos, W. M., Savelkoul, H. F., and Leenen, P. J. Interaction of mouse splenocytes and macrophages with bacterial strains in vitro: the effect of age in the immune response. Benef.Microbes. 3-11-2016; 7(2):275-287.

ABSTRACT: Probiotics influence the immune system, both at the local and systemic level. Recent findings suggest the relation between microbiota and the immune system alters with age. Our objective was to address direct effects of six bacterial strains on immune cells from young and aged mice: Lactobacillus plantarum WCFS1, Lactobacillus casei BL23, Lactococcus lactis MG1363, Bifidobacterium breve ATCC15700, Bifidobacterium infantis ATCC15697, and Akkermansia muciniphila ATCC BAA-835. We used splenocytes and naive or interferon-gamma-stimulated bone marrow-derived macrophages (BMDM) as responder populations. All tested bacterial strains induced phenotypic and cytokine responses in splenocytes and BMDM. Based on magnitude of the cellular inflammatory response and cytokine profiles, two subgroups of bacteria were identified, i.e. L. plantarum and L. casei versus B. breve, B. infantis, and A. muciniphila. The latter group of bacteria induced high levels of cytokines produced under inflammatory conditions, including tumour necrosis factor (TNF), interleukin (IL)-6 and IL-10. Responses to L. lactis showed features of both subgroups. In addition, we compared responses by splenocytes and BMDM derived from young mice to those of aged mice, and found that splenocytes and BMDM derived from aged mice had an increased IL-10 production and dysregulated IL-6 and TNF production compared to young immune cells. Overall, our study shows differential inflammatory responses to distinct bacterial strains, and profound age-dependent effects. These findings, moreover, support the view that immune environment importantly influences bacterial immune effects.

Vanholder, R. C., Eloot, S., and Glorieux, G. L. Future Avenues to Decrease Uremic Toxin Concentration. Am.J.Kidney Dis. 2016; 67(4):664-676.

ABSTRACT: In this article, we review approaches for decreasing uremic solute concentrations in chronic kidney disease and in particular, in end-stage renal disease (ESRD). The rationale to do so is the straightforward relation between concentration and biological (toxic) effect for most toxins. The first section is devoted to extracorporeal strategies (kidney replacement therapy). In the context of high-flux hemodialysis and hemodiafiltration, we discuss increasing dialyzer blood and dialysate flows, frequent and/or extended dialysis, adsorption, bioartificial kidney, and changing physical conditions within the dialyzer (especially for protein-bound toxins). The next section focuses on the intestinal generation of uremic toxins, which in return is stimulated by uremic conditions. Therapeutic options are probiotics, prebiotics, synbiotics, and

intestinal sorbents. Current data are conflicting, and these issues need further study before useful therapeutic concepts are developed. The following section is devoted to preservation of (residual) kidney function. Although many therapeutic options may overlap with therapies provided before ESRD, we focus on specific aspects of ESRD treatment, such as the risks of too-strict blood pressure and glycemic regulation and hemodynamic changes during dialysis. Finally, some recommendations are given on how research might be organized with regard to uremic toxins and their effects, removal, and impact on outcomes of uremic patients.

Vastano, V., Pagano, A., Fusco, A., Merola, G., Sacco, M., and Donnarumma, G. The Lactobacillus plantarum Eno A1 Enolase Is Involved in Immunostimulation of Caco-2 Cells and in Biofilm Development. Adv.Exp.Med.Biol. 2016; 897:33-44.

ABSTRACT: The role of probiotics in prevention and treatment of a variety of diseases is now well assessed. The presence of adhesive molecules on the cell surface of probiotics has been related to the ability to confer health benefit to the host. We have previously shown that the enolase EnoA1 of Lactobacillus plantarum, one of the most predominant species in the gut microbiota of healthy individuals, is cell surface-expressed and is involved in binding with human fibronectin and plasminogen. By means of comparative analysis between L. plantarum LM3 (wild type) and its isogenic LM3-CC1 (DeltaenoA1) mutant strain, here we show that EnoA1 affects the ability of this bacterium to modulate immune response as determined by analysis of expression of immune system molecules in Caco-2 cells. Indeed, we observed induction of TLR2 expression in cells exposed to L. plantarum LM3, while no induction was detectable in cells exposed to LM3-CC1. This difference was much less consistent when expression of TLR4 was determined in cells exposed to the two strains. Pro-inflammatory (IL-6) and anti-inflammatory cytokines (IL-10, TGF-beta), and the antimicrobial peptide HBD-2 were induced in Caco-2 cells exposed to L. plantarum LM3, while lower levels of induction were detected in cells exposed to LM3-CC1. We also analyzed the ability to develop biofilm of the two strains, and observed a decrease of about 65 % in the development of mature biofilm in LM3-CC1 compared to the wild type.

Vazquez-Ortiz, M. and Turner, P. J. Improving the safety of oral immunotherapy for food allergy. Pediatr.Allergy Immunol. 2016; 27(2):117-125.

ABSTRACT: Food allergy is a major public health problem in children, impacting upon the affected individual, their families and others charged with their care, for example educational establishments, and the food industry. In contrast to most other paediatric diseases, there is no established cure: current management is based upon dietary avoidance and the provision of rescue medication in the event of accidental reactions, which are common. This strategy has significant limitations and impacts adversely on health-related quality of life. In the last decade, research into disease-modifying treatments for food allergy has emerged, predominantly for peanut, egg and cow's milk. Most studies have used the oral route (oral immunotherapy, OIT), in which increasing amounts of allergen are given over weeks-months. OIT has proven effective to induce immune modulation and 'desensitization' - that is, an increase in the amount of food allergen that can be consumed, so long as regular (typically daily) doses are continued. However, its ability to induce permanent tolerance once ongoing exposure has stopped seems limited. Additionally, the short- and long-term safety of OIT is often poorly reported, raising concerns about its implementation in routine practice. Most patients experience

allergic reactions and, although generally mild, severe reactions have occurred. Long-term adherence is unclear, which rises concerns given the low rates of long-term tolerance induction. Current research focuses on improving current limitations, especially safety. Strategies include alternative routes (sublingual, epicutaneous), modified hypoallergenic products and adjuvants (anti-IgE, pre-/probiotics). Biomarkers of safe/successful OIT are also under investigation.

Vecchio, A. L., Dias, J. A., Berkley, J. A., Boey, C., Cohen, M. B., Cruchet, S., Liguoro, I., Lindo, E. S., Sandhu, B., Sherman, P., Shimizu, T., and Guarino, A. Comparison of Recommendations in Clinical Practice Guidelines for Acute Gastroenteritis in Children. J.Pediatr.Gastroenterol.Nutr. 1-29-2016;

ABSTRACT: OBJECTIVE: Acute gastroenteritis (AGE) is a major cause of child mortality and morbidity. This study aimed at systematically reviewing clinical practice guidelines (CPGs) on AGE in order to compare recommendations and provide the basis for developing single universal guidelines. METHODS: CPGs were identified by searching MEDLINE, Cochrane-Library, National Guideline Clearinghouse and web sites of relevant societies/organizations producing and/or endorsing CPGs. RESULTS: The definition of AGE varies among the 15 CPGs identified. The parameters most frequently recommended to assess dehydration are skin turgor and sunken eyes (11/15, 73.3%), general appearance (11/15,66.6%), capillary refill time and mucous membranes appearance (9/15, 60%). Oral Rehydration Solution is universally recognized as first-line treatment. The majority of CPGs recommend hypo-osmolar (Na45-60 mmol/L, 11/15, 66.6 %) or low-osmolality (Na75 mmol/L, 9/15, 60%) solutions. In children who fail oral rehydration, most CPGs suggest intravenous rehydration and nasogastric tube insertion for fluid administration is preferred according by 5/15 CPGs (33.3%). Changes in diet and withdrawal of food are discouraged by all CPGs and early refeeding is strongly recommended in 13/15 (86.7%). Zinc is recommended as an adjunct to ORS by 10/15 (66.6%) CPGs, most of them from Low-Income Countries. Probiotics are considered by 9/15 (60%) CPGs, 5 from High-Income Countries. Antiemetics are not recommended in 9/15 (60%) CPGs. Routine use of antibiotics is discouraged. CONCLUSION: Key recommendations for the management of AGE in children are similar in CPGs. Together with accurate review of evidence-base this may represent a starting point for developing universal recommendations for the management of children with AGE worldwide.

Videhult, F. K. and West, C. E. Nutrition, gut microbiota and child health outcomes. Curr.Opin.Clin.Nutr.Metab Care. 2016; 19(3):208-213.

ABSTRACT: PURPOSE OF REVIEW: Diet is one of the main drivers of the composition and function of the gut microbiota. The scope of this review is to summarize recent studies assessing the role of gut microbiota in clinical pediatric conditions and to review studies using nutritional approaches to favorably modify the gut microbiota to improve health outcomes in children. RECENT FINDINGS: New studies underscore that breastfeeding and infant diet impact the gut microbiome and metagenome. A comprehensive study using metagenomic shotgun sequencing, suggests that the cessation of breastfeeding rather than the introduction of solid foods, drives the functional maturation of the infant gut microbiome toward an adult-like state. There is further support for the view that a disturbed early gut microbiota is implicated in allergic and autoimmune diseases. New studies using prebiotics, probiotics, and synbiotics in various pediatric disorders have yielded promising results, yet the evidence for specific guidelines on their use is still low.

SUMMARY: Intestinal dysbiosis is associated with several pediatric disorders but a cause-effect relationship remains to be clearly demonstrated in most conditions. Future studies using new systems biology approaches are anticipated to provide further insight into the functional capacities of the gut microbiome and its establishment in childhood. This may then lay the ground for improved treatment and prevention strategies targeting the gut microbiota.

Vieira, A. T., Rocha, V. M., Tavares, L., Garcia, C. C., Teixeira, M. M., Oliveira, S. C., Cassali, G. D., Gamba, C., Martins, F. S., and Nicoli, J. R. Control of Klebsiella pneumoniae pulmonary infection and immunomodulation by oral treatment with the commensal probiotic Bifidobacterium longum 5(1A). Microbes.Infect. 2016; 18(3):180-189.

ABSTRACT: Klebsiella pneumoniae (Kp) a common cause of pneumonia leads to intense lung injury and mortality that are correlated with infective exacerbations. Probiotics are a class of microorganisms that have immunomodulatory effects to benefit health. We investigated whether the probiotic Bifidobacterium longum 5(1A) induces protection in mice against lung infection induced by Kp and the potential involved mechanisms. Kp infection induced secretion of pro-inflammatory cytokines, neutrophil recruitment, significant bacterial load in the lung and 50% lethality. However, treatment with live B. longum 5(1A) induced faster resolution of inflammation associated with an increased production of IL-10, decreased lung damage with significantly reduction of bacterial burden that contributed to rescue 100% of mice from death. We found that these effects could be attributed, at least in part, to activation of the Toll-like receptor (TLR) adapter protein Mal, since B. longum 5(1A) treatment in Mal-deficient infected mice did not show the protection observed in wild type infected mice. Thus, we propose that live B. longum 5(1A) activates TLR-signaling pathway that results in ROS production and protects the host against pneumonia-induced death by finely tuning the inflammatory response and contributing to faster return to lung homeostasis.

Villarino, N. F., LeCleir, G. R., Denny, J. E., Dearth, S. P., Harding, C. L., Sloan, S. S., Gribble, J. L., Campagna, S. R., Wilhelm, S. W., and Schmidt, N. W. Composition of the gut microbiota modulates the severity of malaria. Proc.Natl.Acad.Sci.U.S.A. 2-23-2016; 113(8):2235-2240.

ABSTRACT: Plasmodium infections result in clinical presentations that range from asymptomatic to severe malaria, resulting in approximately 1 million deaths annually. Despite this toll on humanity, the factors that determine disease severity remain poorly understood. Here, we show that the gut microbiota of mice influences the pathogenesis of malaria. Genetically similar mice from different commercial vendors, which exhibited differences in their gut bacterial community, had significant differences in parasite burden and mortality after infection with multiple Plasmodium species. Germfree mice that received cecal content transplants from "resistant" or "susceptible" mice had low and high parasite burdens, respectively, demonstrating the gut microbiota shaped the severity of malaria. Among differences in the gut flora were increased abundances of Lactobacillus and Bifidobacterium in resistant mice. Susceptible mice treated with antibiotics followed by yogurt made from these bacterial genera displayed a decreased parasite burden. Consistent with differences in parasite burden, resistant mice exhibited an elevated humoral immune response compared with susceptible mice. Collectively, these results identify the composition of the gut microbiota as a previously unidentified risk factor for severe malaria and modulation of the gut microbiota (e.g., probiotics) as a potential treatment to decrease parasite burden.

Vongbhavit, K. and Underwood, M. A. Prevention of Necrotizing Enterocolitis Through Manipulation of the Intestinal Microbiota of the Premature Infant. Clin.Ther. 2-9-2016;

ABSTRACT: PURPOSE: In spite of four decades of research, necrotizing enterocolitis (NEC) remains the most common gastrointestinal complication in premature infants with high mortality and long-term morbidity. The composition of the intestinal microbiota of the premature infant differs dramatically from that of the healthy term infant and appears to be an important risk factor for NEC. METHODS: We review the evidence of an association between intestinal dysbiosis and NEC and summarize published English language clinical trials and cohort studies involving attempts to manipulate the intestinal microbiota in premature infants. FINDINGS: Promising NEC prevention strategies that alter the intestinal microbiota include probiotics, prebiotics, synbiotics, lacteroferrin, and human milk feeding. IMPLICATIONS: Shaping the intestinal microbiota of the premature infant through human milk feeding and dietary supplements decreases the risk of NEC. Further studies to identify the ideal microbial composition and the most effective combination of supplements are indicated.

Wang, B. G., Xu, H. B., Xu, F., Zeng, Z. L., and Wei, H. Efficacy of oral Bifidobacterium bifidum ATCC 29521 on microflora and antioxidant in mice. Can.J.Microbiol. 2016; 62(3):249-262.

ABSTRACT: This study aimed to examine whether Bifidobacterium bifidum ATCC 29521, a species of colonic microflora in humans, is involved in the intestinal tract of mice. This study was also conducted to determine the antioxidant activity of this species by evaluating different microbial populations and reactive oxygen species isolated from feces and intestinal contents for 28 days of oral administration. Microbial diversities were assessed through bacterial culture techniques, PCR-DGGE, and real-time PCR. This study showed that the intake of B. bifidum ATCC 29521 significantly ($p < 0.05$) improved the ecosystem of the intestinal tract of BALB/c mice by increasing the amount of probiotics (Lactobacillus intestinalis and Lactobacillus crispatus) and by reducing unwanted bacterial populations (Enterobacter, Escherichia coli). Antioxidative activities of incubated cell-free extracts were evaluated through various assays, including the scavenging ability of DPPH radical (64.5% and 67.54% ($p < 0.05$), respectively, at 21 days in nutrients and 28 days in MRS broth), superoxide anion, and hydroxyl radical (85% and 61.5% ($p < 0.05$), respectively, at intestinal contents in nutrients and 21 days in MRS broth). Total reducing power (231.5 mumol/L ($p < 0.05$), 14 days in MRS broth) and mRNA level of genes related to oxidative stress were also determined. Results indicated that B. bifidum ATCC 29521 elicits a beneficial effect on murine gut microbiota and antioxidant activities compared with the control samples. This species can be considered as a potential bioresource antioxidant to promote health. Bifidobacterium bifidum ATCC 29521 may also be used as a promising material in microbiological and food applications.

Wang, J., Sun, C., Liu, C., Yang, Y., and Lu, W. Comparison of vaginal microbial community structure in healthy and endometritis dairy cows by PCR-DGGE and real-time PCR. Anaerobe. 2016; 38:1-6.

ABSTRACT: The normal vaginal microflora provides protection against infections of the reproductive tract. Previous studies have focused on the isolation and screening of probiotic strains from the vagina of cows; however, the vaginal microflora of postpartum

cows is poorly characterized. The present study was conducted to evaluate and characterize the vaginal microflora of healthy postpartum cows in relation to postpartum cows with endometritis by using PCR followed by denaturing gradient gel electrophoresis (PCR-DGGE) and Real-time PCR. The study population comprised 5 healthy cows and 5 cows with endometritis. The results indicated that the vaginal bacterial microflora of healthy postpartum cows was dominated by Lactobacillus sakei subsp. and Weissella koreensis, while there were no dominant bacterial species in the vaginal microflora of postpartum cows with endometritis. Common microorganisms such as Bacteroides spp., Fusobacterium spp., Enterococcus spp., Prevotella spp., Clostridium perfringens strains, and Escherichia coli were detected in both groups of cows by Real-time PCR. The bacterial diversity in the vagina of cows with endometritis was significantly higher than that in healthy cows. The results indicated that the vaginal microflora of cows with endometritis was more diverse and lacked dominant bacterial species as compared to that of the healthy cows, suggesting that disruption of the normal vaginal microflora may contribute to the onset of endometritis. This microbial community analysis provided information that might be used to develop probiotics to treat endometritis in cows; however, further investigation is needed.

Wang, W., Cao, J., Yang, F., Wang, X., Zheng, S., Sharshov, K., and Li, L. High-throughput sequencing reveals the core gut microbiome of Bar-headed goose (Anser indicus) in different wintering areas in Tibet. Microbiologyopen. 2-4-2016;

ABSTRACT: Elucidating the spatial dynamic and core gut microbiome related to wild bar-headed goose is of crucial importance for probiotics development that may meet the demands of bar-headed goose artificial breeding industries and accelerate the domestication of this species. However, the core microbial communities in the wild bar-headed geese remain totally unknown. Here, for the first time, we present a comprehensive survey of bar-headed geese gut microbial communities by Illumina high-throughput sequencing technology using nine individuals from three distinct wintering locations in Tibet. A total of 236,676 sequences were analyzed, and 607 OTUs were identified. We show that the gut microbial communities of bar-headed geese have representatives of 14 phyla and are dominated by Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes. The additive abundance of these four most dominant phyla was above 96% across all the samples. At the genus level, the sequences represented 150 genera. A set of 19 genera were present in all samples and considered as core gut microbiome. The top seven most abundant core genera were distributed in that four dominant phyla. Among them, four genera (Lactococcus, Bacillus, Solibacillus, and Streptococcus) belonged to Firmicutes, while for other three phyla, each containing one genus, such as Proteobacteria (genus Pseudomonas), Actinobacteria (genus Arthrobacter), and Bacteroidetes (genus Bacteroides). This broad survey represents the most in-depth assessment, to date, of the gut microbes that associated with bar-headed geese. These data create a baseline for future bar-headed goose microbiology research, and make an original contribution to probiotics development for bar-headed goose artificial breeding industries.

Wang, X., Farnell, Y. Z., Peebles, E. D., Kiess, A. S., Wamsley, K. G., and Zhai, W. Effects of prebiotics, probiotics, and their combination on growth performance, small intestine morphology, and resident Lactobacillus of male broilers. Poult.Sci. 3-4-2016;

ABSTRACT: Effects of commercial antimicrobials and the individual and combinational use of commercial prebiotics and probiotics in feed from d zero to 41 on the growth performance, small intestine size, jejunal morphology, and ileal resident bacteria population of broiler chickens were determined. A total of 1,040 one-day-old male Ross x Ross 708 broilers were randomly distributed to 80 floor pens (5 treatments, 16 replications per treatment, 13 chicks per pen). Five dietary treatments were employed: 1) a corn soybean-meal basal diet (served as a negative control diet, NC); 2) a basal diet supplemented with a commercial prebiotic product (Pre); 3) a basal diet supplemented with a probiotic product containing Bacillus subtilis spores (Pro); 4) a basal diet supplemented with both prebiotic and probiotic products (Pre + Pro); and 5) a basal diet supplemented with commercial antimicrobials (served as a positive control diet, PC). At d 14, Pre diets improved the relative level of Lactobacillus in ileal mucosa as compared to NC, Pro, or PC diets (P = 0.045) without improving broiler BW. Broilers fed PC diets exhibited the highest BW gain from d 15 to 27, the lowest duodenum, jejunum, and ileum relative weights as percentage of BW at d 27, and the highest breast weight at d 42 (P = 0.026, 0.035, 0.002, 0.025, and 0.035, respectively). Broilers fed Pro or Pre + Pro diets exhibited higher BW gain from d 28 to 41 (P = 0.005) and higher overall BW gain from d zero to 41 (P = 0.039) than those fed other diets. Dietary treatments did not affect jejunal morphology or ileal resident Escherichia coli level at any age. From our results, including spores of Bacillus subtilis in feed may stimulate growth at a later age and may facilitate broilers in reaching their target weight sooner. Therefore, probiotics are recommended as potential alternatives to antimicrobials in chicken diets, especially in grower and finisher feed.

Wang, Z., Wang, L., Chen, Z., Ma, X., Yang, X., Zhang, J., and Jiang, Z. In vitro evaluation of swine-derived Lactobacillus reuteri: probiotic properties and effects on intestinal porcine epithelial cells challenged with enterotoxigenic Escherichia coli K88. J.Microbiol.Biotechnol. 2-24-2016;

ABSTRACT: Probiotics are considered the best effective alternatives to antibiotics. The aim of this study was to characterize the probiotic potential of lactobacilli for use in swine farming by using in vitro evaluation methods. A total of 106 lactic acid bacterial isolates, originating from porcine feces, were first screened for the capacity to survive stresses considered important for putative probiotic strains. Sixteen isolates showed notable acid and bile resistance, antibacterial activity and adherence to intestinal porcine epithelial cells (IPEC-1). One isolate, LR1, identified as Lactobacillus reuteri, was selected for extensive study of its probiotic and functional properties in IPEC-1 cell models. L. reuteri LR1 exhibited good adhesion to IPEC-1 cells and could inhibit the adhesion of enterotoxigenic Escherichia coli (ETEC) to IPEC-1 cells. L. reuteri LR1 could also modulate transcript and protein expression of cytokines involved in inflammation in IPEC-1 cells; the lactobacillus inhibited the ETEC-induced expression of proinflammatory transcripts (IL-6 and TNF-alpha) and protein (IL-6), and increased the level of anti-inflammatory cytokine (IL-10). Measurement of the permeation of FD-4 showed that L. reuteri LR1 could maintain barrier integrity in monolayer IPEC-1 cells exposed to ETEC. Immunolocalization experiments showed L. reuteri LR1 could also prevent ETEC-induced tight junction ZO-1 disruption. Together, these results indicate that L. reuteri LR1 exhibits desirable probiotic properties and could be a potential probiotic for use in swine production.

West, C. E. Probiotics for allergy prevention. Benef.Microbes. 3-11-2016; 7(2):171-179.

ABSTRACT: Probiotics, given either as a supplement or in infant foods, have been evaluated in randomised controlled trials for allergy prevention. Here, the aim is to give an overview of the results from these primary prevention studies and to discuss current strategies. In most studies, single strains or a mixture of strains of lactic acid bacteria and bifidobacteria have been used - prenatally, postnatally or perinatally. Several meta-analyses have reported a moderate benefit of probiotics for eczema prevention, and the most consistent effect has been observed with a combined perinatal intervention in infants at high risk of allergic disease due to familial predisposition. In a recent meta-analysis, the use of multi-strain probiotics appeared to be most effective for eczema prevention. No preventive effect has been shown for other allergic manifestations. As long-term follow-up data on later onset allergic conditions (asthma and allergic rhinitis) are available only from a few of the initiated studies, reports from ongoing follow-up studies that are adequately powered to examine long-term outcomes are anticipated to provide more insight. Arguably, the differences in many aspects of study design and the use of different probiotic strains and combinations have made direct comparison difficult. To date, expert bodies do not generally recommend probiotics for allergy prevention, although the World Allergy Organization (WAO) in their recently developed guidelines suggests considering using probiotics in pregnant women, during breastfeeding and/or to the infant if at high risk of developing allergic disease (based on heredity). However, in concordance with other expert bodies, the WAO guideline panel stressed the low level of evidence and the need for adequately powered randomised controlled trials and a more standardised approach before clinical recommendations on specific strains, dosages and timing can be given.

Wideman, R. F., Jr. Bacterial chondronecrosis with osteomyelitis and lameness in broilers: a review. Poult.Sci. 2016; 95(2):325-344.

ABSTRACT: This review focuses on a specific cause of lameness known as bacterial chondronecrosis with osteomyelitis (BCO:) in broilers. Rapid increases in body weight impose excessive torque and shear stress on structurally immature epiphyseal and physeal cartilage, primarily in the proximal femora, proximal tibiae, and flexible thoracic vertebrae. Excessive mechanical stress creates osteochondrotic clefts among the chondrocytes of susceptible growth plates. These wound sites are colonized by hematogenously distributed opportunistic bacteria, culminating in the gross abscesses and necrotic voids that are pathognomonic for terminal BCO. Lameness attributable to characteristic BCO lesions can be reproduced by rearing broilers on wire flooring to create persistent footing instability and physiological stress, without the need to inoculate the birds with pathogenic bacteria that presumably are present but quiescent within the bird's microbial communities or in the environment. Experiments using the wire-flooring model revealed innate differences in the susceptibility of broiler lines to BCO, and demonstrated that BCO incidences can be reduced by prophylactically providing probiotics in the feed, by prophylactically adding 25-hydroxy vitamin D3 to the drinking water, or by therapeutically adding the antibiotic enrofloxacin to the drinking water. Hatchery and chick quality issues clearly influence the susceptibility of broilers to BCO. When broilers remain in a sitting posture for prolonged periods, the major arteries supplying their legs may be compressed. These episodes of inadequate blood flow may prevent chondrocyte maturation and trigger focal necrosis, thereby making the epiphyseal and physeal cartilage highly susceptible to osteochondrosis and BCO. Much remains to be revealed regarding the pathogenesis of BCO. Further revelations will be facilitated by the availability of the now-validated wire-flooring models that consistently trigger high incidences of BCO in experimental flocks.

Williams, B., Landay, A., and Presti, R. M. Microbiome alterations in HIV infection a review. Cell Microbiol. 3-6-2016;

ABSTRACT: Recent developments in molecular techniques have allowed researchers to identify previously uncultured organisms, which has propelled a vast expansion of our knowledge regarding our commensal microbiota. Interest in the microbiome specific to HIV grew from earlier findings suggesting that bacterial translocation from the intestines is the cause of persistent immune activation despite effective viral suppression with antiretroviral therapy (ART). Studies of SIV infected primates have demonstrated that Proteobacteria preferentially translocate and that mucosal immunity can be restored with probiotics. Pathogenic SIV infection results in a massive expansion of the virome, whereas non-pathogenic SIV infection does not. Human HIV infected cohorts have been shown to have microbiota distinctive from that of HIV negative controls and efforts to restore the intestinal microbiome via probiotics have often had positive results on host markers. The microbiota of the genital tract may play a significant role in acquisition and transmission of HIV. Modification of commensal microbial communities likely represents an important therapeutic adjunct to treatment of HIV. Here we review the literature regarding human microbiome in HIV infection.

Woloszynek, S., Pastor, S., Mell, J. C., Nandi, N., Sokhansanj, B., and Rosen, G. L. Engineering Human Microbiota: Influencing Cellular and Community Dynamics for Therapeutic Applications. Int.Rev.Cell Mol.Biol. 2016; 324:67-124.

ABSTRACT: The complex relationship between microbiota, human physiology, and environmental perturbations has become a major research focus, particularly with the arrival of culture-free and high-throughput approaches for studying the microbiome. Early enthusiasm has come from results that are largely correlative, but the correlative phase of microbiome research has assisted in defining the key questions of how these microbiota interact with their host. An emerging repertoire for engineering the microbiome places current research on a more experimentally grounded footing. We present a detailed look at the interplay between microbiota and host and how these interactions can be exploited. A particular emphasis is placed on unstable microbial communities, or dysbiosis, and strategies to reestablish stability in these microbial ecosystems. These include manipulation of intermicrobial communication, development of designer probiotics, fecal microbiota transplantation, and synthetic biology.

Woodman, Z. Can one size fit all? Approach to bacterial vaginosis in sub-Saharan Africa. Ann.Clin.Microbiol.Antimicrob. 2016; 15(1):16

ABSTRACT: Bacterial vaginosis (BV) is the most common vaginal disorder affecting women of reproductive age and is associated with increased risk of sexually transmitted infections such as human immunodeficiency syndrome (HIV-1). Sub-Saharan Africa has the highest BV and HIV-1 burden and yet very few studies have focused on understanding the aetiology of BV and its association with HIV in this region. It has been suggested that we need to accurately diagnose and treat BV to lower the risk of HIV infection globally. However, effective diagnosis requires knowledge of what constitutes a "healthy" cervicovaginal microbiome and current studies indicate that Lactobacillus crispatus might not be the only commensal protective against BV: healthy women from

different countries and ethnicities harbour alternative commensals. Microbiotas associated with BV have also shown global variation, further complicating effective diagnosis via culture-based assays as some species are difficult to grow. Antibiotics and probiotics have been suggested to be key in controlling BV infection, but the efficacy of this treatment might rely on reconstituting endogenous commensals while targeting a specific species of BV-associated bacteria (BVAB). Alternatively, therapy could inhibit essential BV bacterial growth factors e.g. sialidases or provide anti-microbial compounds e.g. lactic acid associated with a healthy cervicovaginal microbiome. But without global investigation into the mechanism of BV pathogenesis and its association with HIV, selection of such compounds could be limited to Caucasian women from certain regions. To confirm this suggestion and guide future therapy we require standardised diagnostic assays and research methodologies. This review will focus on research papers that describe the global variation of BV aetiology and how this influences the identification of determinants of BV pathogenesis and potential probiotic and antimicrobial therapy.

Wu, B. B., Yang, Y., Xu, X., and Wang, W. P. Effects of Bifidobacterium supplementation on intestinal microbiota composition and the immune response in healthy infants. World J.Pediatr. 2016; 12(2):177-182.

ABSTRACT: BACKGROUND: Intestinal microbiotas are thought to be the most important source of maturational stimuli to the development of the immune system. However, few studies have focused on the development of T helper (Th) 1 immune response and antibody response to vaccinations in healthy infants, especially in a large cohort. Through this randomized, double-blind control trial, we investigated the effects of Bifidobacterium longum BB536 (BB536) supplementation on intestinal microbiota composition and the immune response in term infants. METHODS: In total, 300 healthy newborns were recruited, randomized and fed formula either supplemented with BB536 or with no supplementation. Stool samples were analyzed at months 2, 4 and 11. The representative cytokine for Th1 [interferon-gamma (IFN-gamma)] and Th2 [interleukin-4 (IL-4)] secretion cells were measured using enzyme-linked immunospot assay at 4 and 7 months of age. The antibody response to vaccines was measured at months 7 and 11. RESULTS: A total of 264 infants completed the study. The amount of bifidobacteria and the bifidobacteria/ Enterobacteriaceae ratio (B/E) were significantly higher in the BB536 supplementation group at months 2 and 4. The number of IFN-gamma secretion cells and the ratio of IFN-gamma/IL-4 secretion cells were increased in the BB536 supplementation group at 7 months. Moreover, the higher value of B/E in the early stages seems to be related to the increased Th1 response. No difference was observed between groups in the antibody response after vaccination. CONCLUSION: BB536 has positive effects on establishing a healthy intestinal microbiota early in life, and it also plays an important role in improving the Th1 immune response.

Xia, L. P. and Jiang, Y. [Effect of probiotics in prevention and treatment of allergic diseases in children]. Zhongguo Dang.Dai Er.Ke.Za Zhi. 2016; 18(2):189-194.

ABSTRACT: The increasing incidence rate of allergic diseases has attracted global attention, and these diseases greatly threaten children′s health. The common pathogenesis of allergic diseases is the specific IgE- or cell-mediated immune response to common inhalant or food allergens. Epidemiological investigation, analysis of fecal flora, and clinical studies all suggest that the development and progression of allergic diseases

are closely related to the early disturbance of intestinal flora. Probiotics can regulate intestinal immune response, increase the barrier function of epithelial cells, inhibit the adhesion and colonization of pathogenic bacteria, and thus restore or reconstruct normal intestinal flora. With the increasing understanding of allergic diseases, the effect of probiotics in the prevention and treatment of such diseases will be taken more and more seriously.

Xu, L., Wang, Y., Wang, Y., Fu, J., Sun, M., Mao, Z., and Vandenplas, Y. A double-blinded randomized trial on growth and feeding tolerance with Saccharomyces boulardii CNCM I-745 in formula-fed preterm infants. J.Pediatr.(Rio J.). 3-3-2016;

ABSTRACT: OBJECTIVE: The use of probiotics is increasingly popular in preterm neonates, as they may prevent necrotizing enterocolitis sepsis and improve growth and feeding tolerance. There is only limited literature on Saccharomyces boulardii CNCM I-745 (S. boulardii) in preterm infants. METHOD: A prospective, randomized, case-controlled trial with the probiotic S. boulardii (50mg/kg twice daily) was conducted in newborns with a gestational age of 30-37 weeks and a birth weight between 1500 and 2500g. RESULTS: 125 neonates were enrolled; 63 in the treatment and 62 in the control group. Weight gain (16.14+/-1.96 vs. 10.73+/-1.77g/kg/day, p<0.05) and formula intake at maximal enteral feeding (128.4+/-6.7 vs. 112.3+/-7.2mL/kg/day, p<0.05) were significantly higher in the intervention group. Once enteral feeding was started, the time needed to reach full enteral feeding was significantly shorter in the probiotic group (0.4+/-0.1 vs. 1.7+/-0.5 days, p<0.05). There was no significant difference in sepsis. Necrotizing enterocolitis did not occur. No adverse effects related to S. boulardii were observed. CONCLUSION: Prophylactic supplementation of S. boulardii at a dose of 50mg/kg twice a day improved weight gain, improved feeding tolerance, and had no adverse effects in preterm infants >30 weeks old.

Yang, H., Zhao, X., Tang, S., Huang, H., Zhao, X., Ning, Z., Fu, X., and Zhang, C. Probiotics reduce psychological stress in patients before laryngeal cancer surgery. Asia Pac.J.Clin.Oncol. 2016; 12(1):e92-e96.

ABSTRACT: AIM: Laryngeal cancer is a common malignancy; surgery is the preferred treatment. Psychosocial stress is one of the negative impacts on patient recovery. This study aimed to elucidate the effect of probiotics on ameliorating anxiety, and on serum corticotropin-releasing factor (CRF) in laryngeal cancer patients before surgery. METHODS: A total 30 patients with laryngeal cancer and 20 healthy volunteers were recruited. During the 2 weeks before surgery, 20 patients were randomly allocated to receive probiotics or placebo twice a day. Heart rate was recorded daily. The degree of anxiety was assessed by the Hamilton Anxiety Scale (HAMA). RESULTS: Serum CRF levels in laryngeal cancer patients increased significantly in approaching surgery. After ingestion of probiotics, serum levels of CRF and heart rate did not increase before surgery. In addition, taking probiotics relieved the degree of anxiety of the patients from HAMA 19.8 to 10.2. CONCLUSION: Probiotics can ameliorate the clinical anxiety and biochemical features of stress in patients scheduled for laryngectomy.

Yang, X., Li, X. Y., Si, D., Yang, Z. B., He, Z. Y., Zhang, N. C., Zhang, S. S., and Shi, Z. Q. [Effect of dexamethasone contamination in drinking water on intestinal flora in mice]. Nan.Fang Yi.Ke.Da.Xue.Xue.Bao. 2016; 36(2):238-243.

ABSTRACT: OBJECTIVE: To evaluate the effect of water pollution with dexamethasone on intestinal flora in mice. METHODS: Twenty Balb/c mice were randomly divided into control group and low-, moderate- and high-dose dexamethasone groups. The mice in dexamethasone groups were exposed to dexamethasone sodium phosphate in drinking water at doses of 0.035, 0.225, and 2.25 ng for 36 days. The changes in behaviors, fur condition, and feces of the mice were observed daily. All the mice were sacrificed at 36 days and the tissues in the ileocecal region was collected for denaturant gradient gel electrophoresis (DGGE) of 16S rDNA V6 variable regions of microbes and sequence analysis with BLAST. RESULTS: The mice in the 3 dexamethasone groups all showed aggressive behaviors. Cluster analysis of DGGE graph showed relatively stable floras in the ileocecal region in all the mice, but principal component analysis identified differences in the dominating flora among the groups. Diversity analysis of the flora revealed significantly increased amount and types of bacteria in the intestinal flora in all the 3 dexamethasone groups ($P<0.05$ or 0.01) compared with the control group. Sequence analysis of 16S rDNA V6 regions showed 15 common bacterial species and 2 differential species between the dexamethasone groups and the control group with changes in the type and proportion of the dominating bacterium in the dexamethasone groups. Lactobacillus colonization was detected in the control group but not in moderate- and high-dose dexamethasone groups, and Shigella species were found in the latter two groups. CONCLUSIONS: Water contamination with dexamethasone can affect the nervous system of mice, cause changes in the types and amounts of intestinal bacteria and the dominating bacteria, and inhibit the colonization of probiotics in the intestinal floras to increase the risk of invasion by intestinal pathogenic bacteria.

Yang, Z., Wu, Q., Liu, Y., and Fan, D. Effect of Perioperative Probiotics and Synbiotics on Postoperative Infections After Gastrointestinal Surgery: A Systematic Review With Meta-Analysis. JPEN J.Parenter.Enteral Nutr. 2-10-2016;

ABSTRACT: BACKGROUND: Bacterial infection following gastrointestinal surgery remains a common morbidity. The aim of this study was to estimate the effect of the perioperative use of probiotics and synbiotics on postoperative infections. MATERIALS AND METHODS: We searched PubMed, Embase, and the Cochrane Library to identify pertinent randomized controlled trials (RCTs). The primary outcome was postoperative infection rate. The secondary outcomes were length of hospital and intensive care unit (ICU) stay, length of antibiotic therapy, and mortality. The pooled outcomes were calculated using random effects models. RESULTS: Twenty-eight RCTs involving 2511 patients were included in this systematic review. The incidence of infectious complications was lower among patients who received probiotics/synbiotics than among the controls (odds ratio [OR] = 0.35; 95% confidence interval [CI], 0.24-0.50), particularly regarding respiratory (OR = 0.44; 95% CI, 0.28-0.68), urinary tract (OR = 0.30; 95% CI, 0.16-0.55), and wound infections (OR = 0.58; 95% CI, 0.42-0.80). The lengths of hospital stay (mean difference [MD] = -3.20; 95% CI, -4.87 to -1.54) and duration of antibiotic therapy (MD = -3.40; 95% CI, -4.67 to -2.13) were shorter for patients who received probiotics/synbiotics than for controls. There were no significant differences in mortality (OR = 1.19; 95% CI, 0.52-2.74) or length of ICU stay (MD = -0.46; 95% CI, -1.07 to 0.14) between the compared groups. CONCLUSION: Probiotics and synbiotics may prevent postoperative infections in patients undergoing gastrointestinal surgery. However, the results need to be interpreted with caution due to the risk of bias in the included studies and the potential publication bias.

Yee, C. S. and Rachid, R. The Heterogeneity of Oral Immunotherapy Clinical Trials: Implications and Future Directions. Curr.Allergy Asthma Rep. 2016; 16(4):25

ABSTRACT: Food allergy is a potentially life-threatening disease which affects up to 8 % of children and 2-3 % of adults. Increasing food allergy prevalence poses a major public health concern. Induction of desensitization to food allergens through oral immunotherapy (OIT) is an expanding area of study encompassing peanut, egg, milk, and other food allergens. OIT consists of administering incremental doses of food allergen to food-allergic patients, to induce a state of desensitization. Safety, tolerability, and efficacy all remain ongoing concerns. Clinical trials for oral immunotherapy have encompassed many variations, including differences in dosage sizes and frequency, duration of build-up, type of allergen used, patient characteristics, and adjuvant therapies. Consequently, studies have also shown variation in rates of adverse effects, and successful desensitization. Here, we provide an overview of the key studies and discuss the implications of this heterogeneity. While desensitization is successful in the majority of patients, only a minority appear to develop sustained unresponsiveness even after years of therapy. Much larger and longitudinal studies using more homogenous protocols are needed in order to evaluate the clinical applicability of OIT, its long-term effectiveness, and effect on quality of life. The role of adjunctive therapies, including omalizumab and probiotics, requires further evaluation.

Yoo, J. Y. and Kim, S. S. Probiotics and Prebiotics: Present Status and Future Perspectives on Metabolic Disorders. Nutrients. 2016; 8(3)

ABSTRACT: Metabolic disorders, including type 2 diabetes (T2DM) and cardiovascular disease (CVD), present an increasing public health concern and can significantly undermine an individual's quality of life. The relative risk of CVD, the primary cause of death in T2DM patients, is two to four times higher in people with T2DM compared with those who are non-diabetic. The prevalence of metabolic disorders has been associated with dynamic changes in dietary macronutrient intake and lifestyle changes over recent decades. Recently, the scientific community has considered alteration in gut microbiota composition to constitute one of the most probable factors in the development of metabolic disorders. The altered gut microbiota composition is strongly conducive to increased adiposity, beta-cell dysfunction, metabolic endotoxemia, systemic inflammation, and oxidative stress. Probiotics and prebiotics can ameliorate T2DM and CVD through improvement of gut microbiota, which in turn leads to insulin-signaling stimulation and cholesterol-lowering effects. We analyze the currently available data to ascertain further potential benefits and limitations of probiotics and prebiotics in the treatment of metabolic disorders, including T2DM, CVD, and other disease (obesity). The current paper explores the relevant contemporary scientific literature to assist in the derivation of a general perspective of this broad area.

Younan, S., Sakita, G. Z., Albuquerque, T. R., Keller, R., and Bremer-Neto, H. Chromium VI bioremediate by probiotics. J.Sci.Food Agric. 3-21-2016;

ABSTRACT: Chromium is a common mineral in the earth's crust and can be released into the environment from anthropogenic sources. Intake of hexavalent state, chromium (VI), through drinking water and food causes toxic effects, leading to serious diseases, and it is one commonly reported environmental problem. Microorganisms can mitigate or prevent the toxic effects caused by heavy metals in addition to having effective

resistance mechanisms to prevent cell damage and bind to this metal, sequestering them from the cell surface and removing them from the body. Species such as Lactobacillus spp, Streptococcus spp, Bacillus spp and Bifidobacterium spp present in the human mouth and gut and in fermented foods, have the ability to bind and detoxify some of these substances. This review address the primary topics related to chromium (VI) poisoning in animals and humans and the use of probiotics, as a way to mitigate or prevent the toxic effects caused by chromium (VI). Further advances in the genetic knowledge of such microorganism that may lead to discoveries that will clarify the most active microorganisms that act as bioprotectants in bodies exposed to chromium (VI) and are an affordable option for people and animals intoxicated by oral route.

Zacche, M. M. and Giarenis, I. Therapies in early development for the treatment of urinary tract inflammation. Expert.Opin.Investig.Drugs. 3-24-2016; 1-10.

ABSTRACT: INTRODUCTION: Urinary tract inflammation is a very common clinical condition. It is caused by several pathogens and antibiotic treatment is the mainstay of therapy. Increasing antimicrobial resistance and high recurrence rates represent a challenge. Consequently, there is an unmet need for new therapeutic options. Areas covered: The authors discuss the rationale of emerging management strategies and current experimentation. Furthermore, they focus on both acute and recurrent urinary tract infections (UTIs) and examine a range of therapeutics, including new antibiotics, vaccines, mannosides, hyaluronic acid, probiotics, immunomodulant agents and novel compounds derived from nanotechnology. Expert opinion: Basic science studies have elucidated the pathogenesis of UTIs and built up the ground for the development of new therapies. Evidence is mainly derived from animal studies on murine models of bacterial cystitis. However, clinical trials are scanty and cannot provide us with robust evidence. Hetereogeneity and virulence of uropathogens pose a threat that scientists and clinicians are struggling to overcome.

Zambori, C., Morvay, A. A., Sala, C., Licker, M., Gurban, C., Tanasie, G., and Tirziu, E. Antimicrobial effect of probiotics on bacterial species from dental plaque. J.Infect.Dev.Ctries. 2016; 10(3):214-221.

ABSTRACT: INTRODUCTION: The antimicrobial role of probiotic Lactobacillus casei subspecies casei DG (L. casei DG) and of the mix culture of probiotic Lactobacillus acidophilus LA-5 and Bifidobacterium BB-12 was tested on species of Staphylococcus, Streptococcus, Pasteurella, and Neisseria genera from supragingival sites from dogs with dental disease of different breed, age, sex, weight, and diet. The research was conducted on these four genera because of their importance in zoonotic infections after dog bites. METHODOLOGY: Species from Staphylococcus, Streptococcus, Pasteurella, and Neisseria genera were isolated and identified. To test the antimicrobial efficacy of L. casei DG and the mixed culture of probiotic L. acidophilus LA-5 and Bifidobacterium bifidum BB-12 on the pathogenic species, the agar overlay method was used. RESULTS: L. casei DG had a bactericidal effect on all analyzed species isolated from Staphylococcus, Streptococcus, Pasteurella, and Neisseria genera after 24 hours of incubation. The mixed probiotic culture made up of L. acidophilus LA-5 and Bifidobacterium BB-12 species had no bactericidal effect on the species of Staphylococcus and Streptococcus genera, which were resistant. However, it had a bacteriostatic effect on several species of Pasteurella and Neisseria genera. CONCLUSIONS: This work highlights the antimicrobial potential of probiotics in vitro,

demonstrating that the probiotic L. casei DG has a bactericidal effect on all analyzed species isolated from dental plaque and that the mix culture of probiotic L. acidophilus LA-5 and Bifidobacterium BB-12 has only a bacteriostatic effect.

Zarate, G., Palacios, J. M., Villena, J., and Zuniga-Hansen, M. E. Inhibition of enteropathogens adhesion to human enterocyte-like HT-29 cells by a dairy strain of Propionibacterium acidipropionici. Benef.Microbes. 3-1-2016; 1-12.

ABSTRACT: Adhesion to the host intestinal mucosa is considered relevant for orally delivered probiotics as it prolongs their persistence in the gut and their health promoting effects. Classical propionibacteria are microorganisms of interest due to their role as dairy starters as well as for their functions as probiotics. Propionibacterium acidipropionici Q4, is a dairy strain isolated from a Swiss-type cheese made in Argentina that displays probiotic potential. In the present work we assessed the ability of this strain to adhere to the human enterocyte-like HT-29 cell line and to counteract the adhesion of two common human enteropathogens, such as Escherichia coli C3 and Salmonella Enteritidis 90/390. The results were compared with those obtained with the well-known probiotic Lactobacillus rhamnosus GG. P. acidipropionici Q4 showed a high adhesion capacity, even higher than the reference strain L. rhamnosus GG (42.3+/-4.4% and 36.2+/-2.3%, respectively), whereas adhesion of enteropathogens was significantly lower (25.2+/-2.2% for E. coli and 21.0+/-3.4% for S. Enteritidis). Propionibacteria as well as lactobacilli were able to inhibit by exclusion and competition the adherence of E. coli C3 and S. Enteritidis 90/390 whereas only L. rhamnosus GG displaced S. Enteritidis from HT-29 intestinal cells. Inhibition of pathogens by propionibacteria was not exerted by antimicrobials or coaggregation but was mainly due to exclusion by cell surface components, such as proteins and carbohydrates. The relevance of cell surface proteins (CSP) for preventing pathogens infection was confirmed by their concentration dependent effect observed for both pathogens: 100 microg/ml of CSP inhibited E. coli attachment almost as untreated propionibacteria, whereas it partially inhibited the attachment of S. Enteritidis. Results suggest that P. acidipropionici Q4 could be considered for the development of propionibacteria containing functional foods helpful in counteracting enteropathogen infection.

Zeber-Lubecka, N., Kulecka, M., Ambrozkiewicz, F., Paziewska, A., Lechowicz, M., Konopka, E., Majewska, U., Borszewska-Kornacka, M., Mikula, M., Cukrowska, B., and Ostrowski, J. Effect of Saccharomyces boulardii and Mode of Delivery on the Early Development of the Gut Microbial Community in Preterm Infants. PLoS.One. 2016; 11(2):e0150306

ABSTRACT: BACKGROUND: Recent advances in culture-independent approaches have enabled insights into the diversity, complexity, and individual variability of gut microbial communities. OBJECTIVES: To examine the effect of oral administration of Saccharomyces (S.) boulardii and mode of delivery on the intestinal microbial community in preterm infants. STUDY DESIGN: Stool samples were collected from preterm newborns randomly divided into two groups: a probiotic-receiving group (n = 18) or a placebo group (n = 21). Samples were collected before probiotic intake (day 0), and after 2 and 6 weeks of supplementation. The composition of colonizing bacteria was assessed by 16S ribosomal RNA (rRNA) gene sequencing of fecal samples using the Ion 16S Metagenomics Kit and the Ion Torrent Personal Genome Machine platform. RESULTS: A total of 11932257 reads were generated, and were clustered into 459, 187, and 176 operational taxonomic units at 0 days, 2 weeks, and 6 weeks, respectively. Of the 17

identified phyla, Firmicutes Actinobacteria, Proteobacteria, and Bacteroidetes were universal. The microbial community differed at day 0 compared with at 2 weeks and 6 weeks. There was a tendency for increased bacterial diversity at 2 weeks and 6 weeks compared with day 0, and infants with a gestational age of 31 weeks or higher presented increased bacterial diversity prior to S. boulardii administration. Firmicutes and Proteobacteria remained stable during the observation period, whereas Actinobacteria and Bacteroidetes increased in abundance, the latter particularly more sharply in vaginally delivered infants. CONCLUSION: While the mode of delivery may influence the development of a microbial community, this study had not enough power to detect statistical differences between cohorts supplemented with probiotics, and in a consequence, to speculate on S. boulardii effect on gut microbiome composition in preterm newborns.

Zeng, J., Wang, C. T., Zhang, F. S., Qi, F., Wang, S. F., Ma, S., Wu, T. J., Tian, H., Tian, Z. T., Zhang, S. L., Qu, Y., Liu, L. Y., Li, Y. Z., Cui, S., Zhao, H. L., Du, Q. S., Ma, Z., Li, C. H., Li, Y., Si, M., Chu, Y. F., Meng, M., Ren, H. S., Zhang, J. C., Jiang, J. J., Ding, M., and Wang, Y. P. Effect of probiotics on the incidence of ventilator-associated pneumonia in critically ill patients: a randomized controlled multicenter trial. Intensive Care Med. 4-4-2016;

ABSTRACT: PURPOSE: To evaluate the potential preventive effect of probiotics on ventilator-associated pneumonia (VAP). METHODS: This was an open-label, randomized, controlled multicenter trial involving 235 critically ill adult patients who were expected to receive mechanical ventilation for >/=48 h. The patients were randomized to receive (1) a probiotics capsule containing live Bacillus subtilis and Enterococcus faecalis (Medilac-S) 0.5 g three times daily through a nasogastric feeding tube plus standard preventive strategies or (2) standard preventive strategies alone, for a maximum of 14 days. The development of VAP was evaluated daily, and throat swabs and gastric aspirate were cultured at baseline and once or twice weekly thereafter. RESULTS: The incidence of microbiologically confirmed VAP in the probiotics group was significantly lower than that in the control patients (36.4 vs. 50.4 %, respectively; P = 0.031). The mean time to develop VAP was significantly longer in the probiotics group than in the control group (10.4 vs. 7.5 days, respectively; P = 0.022). The proportion of patients with acquisition of gastric colonization of potentially pathogenic microorganisms (PPMOs) was lower in the probiotics group (24 %) than the control group (44 %) (P = 0.004). However, the proportion of patients with eradication PPMO colonization on both sites of the oropharynx and stomach were not significantly different between the two groups. The administration of probiotics did not result in any improvement in the incidence of clinically suspected VAP, antimicrobial consumption, duration of mechanical ventilation, mortality and length of hospital stay. CONCLUSION: Therapy with the probiotic bacteria B. Subtilis and E. faecalis are an effective and safe means for preventing VAP and the acquisition of PPMO colonization in the stomach.

Zeng, Z., Luo, J. Y., Zuo, F. L., Yu, R., Zhang, Y., Ma, H. Q., and Chen, S. W. Bifidobacteria possess inhibitory activity against dipeptidyl peptidase-IV. Lett.Appl.Microbiol. 2016; 62(3):250-255.

ABSTRACT: The incretin hormones are extremely rapidly metabolized by the ubiquitous enzyme dipeptidyl peptidase IV (DPP-IV). Therefore, DPP-IV inhibitors which can prolong the incretin effect are the newest and promising drugs for management of type 2 diabetes. In this study, we investigated whether Bifidobacteria colonizing the human gut

possess DPP-IV inhibitory activity. Cell-free intracellular extracts of 13 Bifidobacterium strains isolated from breast-fed infant faecal samples were prepared and screened for DPP-IV inhibitory activity, and two Bifidobacterium strains-Bif. longum BBMN68 and Bif. lactis Bb12-were used as reference strains. Most of the strains showed varying levels of DPP-IV inhibitory property (7-27%). Strains of Bifidobacterium adolescentis IF1-11 and Bifidobacterium bifidum IF3-211 showed the greatest DPP-IV inhibitory activity (27 and 25%) as well as good in vitro probiotic properties. This initial finding suggested that new beneficial function of Bifidobacteria is strain-dependent and the strains or their components may have the potential application for management of type 2 diabetes via inhibiting gastrointestinal DPP-IV activity. Further investigations into the isolation and identification of the bioactive components of Bifidobacteria are warranted. SIGNIFICANCE AND IMPACT OF THE STUDY: Our results show that Bifidobacteria isolated from breast-fed infants' faecal samples possess DPP-IV inhibitory activity. Strains of Bifidobacterium bifidum IF3-211 and Bifidobacterium adolescentis IF1-11, which showed excellent DPP-IV inhibitory properties as well as good in vitro probiotic properties, are expected to be beneficial for application as anti-diabetic probiotics.

Zhang, B., Wang, Y., Tan, Z., Li, Z., Jiao, Z., and Huang, Q. Screening of Probiotic Activities of Lactobacilli Strains Isolated from Traditional Tibetan Qula, a raw yak milk cheese. Asian-Australas.J.Anim Sci. 1-18-2016;

ABSTRACT: In this study, 69 lactobacilli isolated from Tibetan Qula, a raw yak milk cheese, were screened for their potential use as probiotics. The isolates were tested in terms of: their ability to survive at pH 2.0, pH 3.0, and in the presence of 0.3% bile salts; tolerance of simulated gastric and intestinal juices; antimicrobial activity; sensitivity against 11 specific antibiotics; and their cell surface hydrophobicity. The results show that out of the 69 strains, 29 strains (42%) had survival rates above 90% after 2 h of incubation at pH values of 2.0 or 3.0. Of these 29 strains, 21 strains showed a tolerance for 0.3% bile salt. Incubation of these 21 isolates in simulated gastrointestinal fluid for 3 h revealed survival rates above 90%; the survival rate for 20 of these isolates remained above 90% after 4 h of incubation in simulated intestinal fluid. The viable counts of bacteria after incubation in simulated gastric fluid for 3 h and simulated intestinal fluid for 4 h were both significantly different compared with the counts at 0 h ($P < 0.001$). Further screening performed on the above 20 isolates indicated that all 20 lactobacilli strains exhibited inhibitory activity against Micrococcus luteus ATCC 4698, Bacillus subtilis ATCC 6633, Listeria monocytogenes ATCC 19115, and Salmonella enterica ATCC 43971. Moreover, all of the strains were resistant to vancomycin and streptomycin. Of the 20 strains, three were resistant to all 11 elected antibiotics (ciprofloxacin, erythromycin, tetracycline, penicillin G, ampicillin, streptomycin, polymyxin B, vancomycin, chloramphenicol, rifampicin and gentamicin) in this study, and five were sensitive to more than half of the antibiotics. Additionally, the cell surface hydrophobicity of seven of the 20 lactobacilli strains was above 70%, including strains Lactobacillus casei 1133 (92%), Lactobacillus plantarum 1086-1 (82%), Lactobacillus casei 1089 (81%), Lactobacillus casei 1138 (79%), Lactobacillus buchneri 1059 (78%), Lactobacillus plantarum 1141 (75%), and Lactobacillus plantarum 1197 (71%). Together, these results suggest that these seven strains are good probiotic candidates, and that tolerance against bile acid, simulated gastric and intestinal juices, antimicrobial activity, antibiotic resistance, and cell surface hydrophobicity could be adopted for preliminary screening of potentially probiotic lactobacilli.

Zhang, F., Jiang, M., Wan, C., Chen, X., Chen, X., Tao, X., Shah, N. P., and Wei, H. Screening probiotic strains for safety: Evaluation of virulence and antimicrobial susceptibility of enterococci from healthy Chinese infants. J.Dairy Sci. 3-16-2016;

ABSTRACT: The aim of this study was to evaluate the safety of enterococci isolated from Chinese infants and screen out potential probiotic candidates. One hundred eight strains were isolated from feces of 34 healthy infants, and 38 strains of Enterococcus spp. were categorized as follows: E. faecalis (22), E. faecium (10), E. hirae (3), E. durans (2), and E. casseliflavus (1). Of these, 72.7% of E. faecalis came from infants delivered by cesarean and 62.5% of E. faecium from infants delivered vaginally. For safety evaluation of strains, we determined presence of virulence genes; production of hemolysin, gelatinase, and biofilm; and antimicrobial susceptibility of enterococci. Six out of 14 virulence genes were detected with a distribution of gelE (26.3%), cylA (39.4%), esp (15.8%), efaA (63.2%), asa1 (50.0%), and ace (50.0%). In phenotype analysis, 36.8% of the strains exhibited positive hemolytic activity and 17.5% were positive for production of gelatinase. Results of antimicrobial susceptibility showed that different percentages of the strains were resistant to ciprofloxacin (5.2%), vancomycin (7.8%), rifampicin (10.5%), erythromycin (52.6%), and gentamycin (52.6%); remarkably, none of the strains were resistant to ampicillin or chloramphenicol. In total, 10 strains, including 6 E. faecium, which are free of virulence determinants and sensitive to common antimicrobial agents (e.g., ampicillin and vancomycin), were further assessed for their probiotic properties. All strains survived well in simulated gastric fluid and intestinal tract, with maximum reductions of 0.600 and 0.887 log cfu/mL, respectively. Six strains of E. faecium could resist 0.3 to 1.0% bile salt, of which E. faecium WEFA23 presented the highest growth (75.06%) at 1.0% bile salt. All strains showed bile salt hydrolase activity on glycodeoxycholic acid, but only 3 of E. faecium showed activity on taurodeoxycholic acid. These results deliver useful information on the safety of enterococci in infants in China, and provide a protocol to screen probiotics for absence of virulence and antimicrobial susceptibility of enterococci.

Zhang, G. Q., Hu, H. J., Liu, C. Y., Shakya, S., and Li, Z. Y. Probiotics for Preventing Late-Onset Sepsis in Preterm Neonates: A PRISMA-Compliant Systematic Review and Meta-Analysis of Randomized Controlled Trials. Medicine (Baltimore). 2016; 95(8):e2581

ABSTRACT: The effect of probiotics on late-onset sepsis (LOS) in preterm neonates remains controversial. The authors systematically reviewed the literature to investigate whether enteral probiotic supplementation reduced the risk of LOS in preterm neonates in neonatal intensive care units.PubMed, Embase, and Cochrane Central Register of Controlled Trials were systematically searched for randomized controlled trials (RCTs) regarding the effect of probiotics in preterm neonates. The primary outcome was culture-proven bacterial and/or fungal sepsis. The Mantel-Haenszel method with random-effects model was used to calculate pooled relative risks (RRs) and 95% confidence intervals (CIs).Twenty-seven trials were included in our review, and 25 trials involving 6104 preterm neonates were statistically analyzed. Pooled analysis indicated that enteral probiotic supplementation significantly reduced the risk of any sepsis (25 RCTs; RR 0.83, 95% CI 0.73-0.94; I = 26%), bacterial sepsis (11 RCTs; RR 0.82, 95% CI 0.71-0.95; I = 0%), and fungal sepsis (6 RCTs; RR 0.57, 95% CI 0.41-0.78; I = 0%). This beneficial effect remains in very low birth weight infants (<1500 g) (19 RCTs; RR 0.86, 95% CI 0.75-0.97; I = 18%), but not in extremely low birth weight infants (<1000 g) (3 RCTs; RR 0.73, 95% CI 0.45-1.19; I = 53%). All the included trials reported no systemic infection caused by the supplemental probiotic organisms.Current evidence indicates that probiotic supplementation is safe, and effective in reducing the risk of LOS in preterm neonates in

neonatal intensive care units. Further studies are needed to address the optimal probiotic organism, dosing, timing, and duration. High-quality and adequately powered RCTs regarding the efficacy and safety of the use of probiotics in extremely low birth weight infants are still warranted.

Zhang, G. Q., Hu, H. J., Liu, C. Y., Zhang, Q., Shakya, S., and Li, Z. Y. Probiotics for Prevention of Atopy and Food Hypersensitivity in Early Childhood: A PRISMA-Compliant Systematic Review and Meta-Analysis of Randomized Controlled Trials. Medicine (Baltimore). 2016; 95(8):e2562

ABSTRACT: Most studies investigated probiotics on food hypersensitivity, not on oral food challenge confirmed food allergy in children. The authors systematically reviewed the literature to investigate whether probiotic supplementation prenatally and/or postnatally could reduce the risk of atopy and food hypersensitivity in young children.PubMed, Embase, the Cochrane Central Register of Controlled Trials, and 4 main Chinese literature databases (Wan Fang, VIP, China National Knowledge Infrastructure, and SinoMed) were searched for randomized controlled trials regarding the effect of probiotics on the prevention of allergy in children. The last search was conducted on July 11, 2015.Seventeen trials involving 2947 infants were included. The first follow-up studies were analyzed. Pooled analysis indicated that probiotics administered prenatally and postnatally could reduce the risk of atopy (relative risk [RR] 0.78; 95% confidence interval [CI] 0.66-0.92; I = 0%), especially when administered prenatally to pregnant mother and postnatally to child (RR 0.71; 95% CI 0.57-0.89; I = 0%), and the risk of food hypersensitivity (RR 0.77; 95% CI 0.61-0.98; I = 0%). When probiotics were administered either only prenatally or only postnatally, no effects of probiotics on atopy and food hypersensitivity were observed.Probiotics administered prenatally and postnatally appears to be a feasible way to prevent atopy and food hypersensitivity in young children. The long-term effects of probiotics, however, remain to be defined in the follow-up of existing trials. Still, studies on probiotics and confirmed food allergy, rather than surrogate measure of food hypersensitivity, are warranted.

Zhang, Q., Wu, Y., and Fei, X. Effect of probiotics on glucose metabolism in patients with type 2 diabetes mellitus: A meta-analysis of randomized controlled trials. Medicina (Kaunas.). 2016; 52(1):28-34.

ABSTRACT: OBJECTIVE: Our aim was to investigate the effects of probiotics on glucose metabolism in patients with type 2 diabetes mellitus using a meta-analysis of randomized, controlled trials. MATERIALS AND METHODS: Online databases Embase, Web of Science, and PubMed were searched until August 2014 to identify eligible articles. Finally, 7 trials were included. RESULTS: Probiotic consumption significantly changed fasting plasma glucose (FPG) by -15.92mg/dL (95% confidence interval [CI], -29.75 to -2.09) and glycosylated hemoglobin (HbA1c) by -0.54% (95% CI, -0.82 to -0.25) compared with control groups. Subgroup analysis was conducted to trials with non-yogurts control. Meta-analysis of trials with multiple species of probiotics found a significant reduction in FPG (weighted mean difference [WMD]: -35.41mg/dL, 95% CI: -51.98 to -18.89). The duration of intervention for >/=8 weeks resulted in a significant reduction in FPG (WMD: -20.34mg/dL, 95% CI: -35.92 to -4.76). Subgroup analysis of trials with species of probiotics did not result in a significant meta-analysis effect. Furthermore, the duration of intervention <8 weeks did not result in a significant reduction in FPG. The results also showed that probiotic therapy significantly decreased homeostasis model assessment of

insulin resistance (HOMA-IR) and insulin concentration (WMD: -1.08, 95% CI: -1.88 to -0.28; and WMD: -1.35mIU/L, 95% CI: -2.38 to -0.31, respectively). CONCLUSIONS: The present meta-analysis suggests that consuming probiotics may improve glucose metabolism by a modest degree, with a potentially greater effect when the duration of intervention is >/=8 weeks, or multiple species of probiotics are consumed.

Zhao, C., Lv, X., Fu, J., He, C., Hua, H., and Yan, Z. In vitro inhibitory activity of probiotic products against oral Candida species. J.Appl.Microbiol. 3-21-2016;

ABSTRACT: AIMS: To evaluate the inhibitory activity of probiotics against oral Candida species METHODS AND RESULTS: Four commercial probiotic products were screened. Bacillus subtilis R0179 was found to have a significant antifungal effect. Bacillus subtilis-Candida interactions were evaluated using disk diffusion tests, confocal laser scanning microscopy, scanning electron microscopy and interaction with engineered human oral mucosa tissue. Bacillus subtilis exhibited clear zones of inhibition for Candida albicans and Candida parapsilosis but not for Candida krusei. A remarkable reduction in the number of Candida cells and abundant Candida cell death were visualized with confocal laser scanning microscopy. Shrinkage and deformation of Candida cells was observed using scanning electron microscopy. Culture of Candida albicans on engineered human oral mucosa tissues resulted in the presence of a large number of yeast cells on the tissue surface and the development of large-scale tissue damage. However, comparatively fewer Candida cells were observed on Bacillus subtilis-treated tissues. We also use ultra performance liquid chromatography /time of flight mass spectrometry (UPLC/TOF MS) to explore the preliminary antifungal mechanism of Bacillus subtilis R0179 and to detect that whether it can secrete an antifungal agent, Iturin A. CONCLUSIONS: Bacillus subtilis R0179 exhibits a significant inhibitory effect on the growth of Candida species. SIGNIFICANCE AND IMPACT OF THE STUDY: Bacillus subtilis has the potential to be used in the prevention or treatment of oral candidiasis. This article is protected by copyright. All rights reserved.

Zhao, Y., Yuan, L., Wan, J., Sun, Z., Wang, Y., and Sun, H. Effects of potential probiotic Bacillus cereus EN25 on growth, immunity and disease resistance of juvenile sea cucumber Apostichopus japonicus. Fish.Shellfish.Immunol. 2016; 49:237-242.

ABSTRACT: This study was conducted to determine effects of potential probiotic Bacillus cereus EN25 (isolated from mud of sea cucumber culturing water bodies) on growth, immunity and disease resistance against Vibrio splendidus infection in juvenile sea cucumbers Apostichopus japonicus. Animals were respectively fed diets with B. cereus EN25 at 0 (control), 10(5), 10(7) and 10(9) CFU/g for 30 days. Results showed that dietary B. cereus EN25 had no significant effects on growth, total coelomocytes counts and acid phosphatase activity of A. japonicus ($P > 0.05$). Dietary EN25 at 10(7) CFU/g had significantly improved the phagocytosis, respiratory burst activity and total nitric oxide synthase activity of animals ($P < 0.05$). Compared to control, dietary EN25 at 10(5) or 10(7) CFU/g had no significant effects on superoxide dismutase activity of A. japonicus ($P > 0.05$), whereas dietary EN25 at 10(9) CFU/g had significantly decreased its activity ($P < 0.05$). The cumulative mortality after V. splendidus challenge decreased significantly in sea cucumbers fed with EN25 at 10(7) CFU/g ($P < 0.05$). The present study confirmed dietary B. cereus EN25 at 10(7) CFU/g could significantly improve immunity and disease resistance in juvenile A. japonicus.

Zheng, B., van, Bergenhenegouwen J., van de Kant, H. J., Folkerts, G., Garssen, J., Vos, A. P., Morgan, M. E., and Kraneveld, A. D. Specific probiotic dietary supplementation leads to different effects during remission and relapse in murine chronic colitis. Benef.Microbes. 3-11-2016; 7(2):205-213.

ABSTRACT: Although interest in using probiotics to prevent and treat intestinal diseases is increasing, the effects of specific probiotic strains still remain unclear. Here, we assess the therapeutic effects of two probiotic strains, Lactobacillus rhamnosus NutRes 1 and Bifidobacterium breve NutRes 204 on a dextran sodium sulphate (DSS)-induced chronic murine colitis model. The chronic colitis was induced by two DSS treatment cycles with a rest period of 10 days (the remission or resolution phase). The probiotic supplementation was started during the resolution phase, after the first DSS treatment cycle, and continued until the end of the experiment. In addition to clinical observations made during the experiment, cellular infiltration was measured along with mRNA expression of pro-inflammatory cytokines, T cell-associated cytokines, and Toll like receptors (TLR) in the inflamed colon after second DSS treatment cycle. L. rhamnosus, but not B. breve, rapidly and effectively improved the DSS-induced bloody diarrhoea during the resolution phase. However, a contradictory effect by both probiotic strains on the faecal condition was found after re-induction of colitis. The worsening of the faecal condition was accompanied by a reduced number of neutrophils and increased expression of interferon-gamma in the colons of DSS-treated mice. Furthermore, an increased expression of TLR2, TLR6 and pro-inflammatory markers including chemokine (C-C motif) ligand 2, interleukin (IL)-1beta, tumour necrosis factor alpha and IL-6 was found in DSS-treated mice with L. rhamnosus supplementation. These results indicate that therapeutic administration of specific probiotics might be beneficial during the resolution phase of colitis. However, caution should be taken as specific probiotic treatments reduce neutrophil influx, which may be the reason of exacerbation of chronic colitis.

Zhu, Y. and Wang, Y. Administering Probiotics in Duodenal-Jejunal Bypass Surgery Induces More Weight Loss in Sprague-Dawley Rats. Obes.Surg. 3-16-2016.

Abstract Not Available.

Zuccotti, G., Albani, E., and Meneghin, F. How Solid is the Scientific Evidence on Probiotics? J.Clin.Gastroenterol. 3-11-2016.

Abstract Not Available.

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