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Monitoring immune modulation in the general population

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Optimal immune function is crucial to human health, and nutrition is one of the major factors modulating immune function. Whole diets, individual nutrients and food components such as phytochemicals, prebiotics and probiotics have been shown to affect different aspects of immune function. Substantiated solutions to help optimise immune function are much sought after by consumers and industry alike. To provide further guidance on the assessment and interpretation of immune modulation due to nutrition, ILSI Europe commissioned a group of experts to prepare a guidance document. First, the criteria to evaluate usefulness of immune markers were defined. Over 75 markers were scored within the context of three distinct immune system functions: defence against pathogens, avoidance of allergy, and control of low-grade (metabolic) inflammation. The most useful markers were subsequently classified depending on whether they signify clinical relevance and/or involvement of immune function. Next, five theoretical scenarios were drafted describing potential changes in marker values compared to a relevant reference range. Finally, all elements were combined, providing a framework to aid the design and interpretation of studies assessing effects of nutrition on immune function. This step-wise approach offers a clear rationale for selecting markers for future trials and provides a framework for the interpretation of outcomes. A similar step-wise approach may also be useful to rationalise the selection and interpretation of markers for other physiological processes critical to the maintenance of health and well-being.

Gut microbiota manipulation and weight modification

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Obesity is caused by alterations in the balance between food intake and energy expenditure. The gut microbiota plays an important part in the harvesting, storage, and expenditure of energy obtained from the diet and several studies have correlated imbalances in the composition of the human gut microbiota with obesity. Both antibiotics and probiotics can modify the gut microbiota and are widely used as growth promoters in agriculture. Related probiotic strains can differ significantly in genotype and phenotype. Human beings have used probiotics for many years and as in animals, specific probiotics species are associated with weight gain or loss. The results of food research are at greater risk of bias and are easier to manipulate than are those for antibiotic research. The effect of a probiotic species on the digestive flora depends on the strain and is largely determined by bacteriocin production. The ability of probiotics to inhibit pathogens is supported by their use to prevent and treat acute antibiotic-associated and travellers' diarrhoea. Probiotics can cause disease in humans and the mortality among patients with acute pancreatitis treated with probiotics was about twice as high as that of patients who received placebo. The careful selection of probiotics is essential because these agents may have various biological effects, many with implications for the human gut microbiota and potential weight modification.

Suggestive comprehensive regulatory guidelines for safe and judicious use of probiotics

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Probiotics have always been a unique microbial category due to established evidences of their applications in wellness of human beings. Currently, regulatory bodies across the globe consider probiotics under several categories depending upon their intended use as biologics, pharmaceuticals, food and nutritional supplements. In order to clear the ambiguity related to regulatory specifications, assurance of quality and premarketing safety assessment, drafting of comprehensive guidelines with global acceptance is need of the hour. For the present study, literature has been surveyed in context to study current regulatory guidelines on probiotics in different countries and hence recommendations have been suggested. In contrast to problematic issues, novel probiotic based products consisting of new species and novel strains are constantly being reported and hence a common regulatory framework is required which will certainly solve considerable confusions and challenges ahead. Major issues related to efficacy and safety can be resolved by following appropriate identification, evaluation, manufacturing and approval process, which will ensure clinical results of probiotic drugs. Understanding of the term probiotics is becoming a necessity to impose good regulatory decisions, to fully realise and utilise the exciting potential of probiotic products as foods and as drugs. To conclude, selection and design of probiotics remains an important challenge for the scientific community in concern with their safety factors for the regulatory bodies, manufacturers and consumers about the claims associated with probiotics, which needs to be addressed for the successful marketing and judicious usage of probiotics as functional foods and as well as pharmaceutical formulations.

Gut microbiota and glucagon like peptide-1: is there a role for probiotics?

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Alteration of gut microbiota affects expression of the hormone glucagon like peptide-1 (GLP-1) from enteroendocrine L-cells. GLP-1 is involved in nutrient mediated insulin release, appetite and gastrointestinal physiology. However, the mechanism controlling its expression is not known. Furthermore, it is unknown whether small intestinal or colonic L-cells functions similarly. To investigate whether the gut microbiota modulates GLP-1 production, we rederived a reporter mouse strain expressing YFP under the proglucagon promotor as germ free. GLP-1 expressing L cells from Ileum and colon of germ free and conventionally raised mouse were sorted using flow cytometry and were subjected to microarray analysis. We found that gut microbiota affects L cell biology in different intestinal compartments with differential regulation of transmembrane receptor expression and cellular signalling in colon and ileum. Finally, we investigated potential of engineered *Lactococcus lactis* expressing mouse GLP-1 to promote glucose stimulated insulin secretion in primary mouse islets. Our results demonstrated that genetically modified microbe derived GLP-1 stimulated insulin release under *ex vivo* conditions in mouse islets.

An update on the clinical application of probiotics for selected paediatric conditions

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The use of probiotic supplements has become more common for both inpatient and outpatient paediatric conditions. This presentation will review recent data regarding the use of probiotic interventions for diarrhoeal disease, colic, the potential prevention of eczema and the potential prevention of necrotising enterocolitis. The interpretation of the current literature is difficult due to the heterogeneity of probiotic interventions and regimens, the differences in the clinical populations and the presence of multiple confounding factors. There are varying levels of evidence to support the use of probiotic interventions for specific paediatric conditions. We will discuss potential applications and limitations of these data to paediatric clinical practice.

The microbiome-gut-brain axis: from bowel to behaviour

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There is a growing appreciation of the relationship between gut microbiota, and the host in maintaining homeostasis in health and predisposing to disease. Bacterial colonisation of the gut plays a major role in postnatal development and maturation of key systems that have the capacity to influence central nervous system (CNS) programming and signalling, including the immune and endocrine systems. Individually, these systems have been implicated in the neuropathology of many CNS disorders and collectively they form an important bidirectional pathway of communication between the microbiota and the brain in health and disease. Over the past 5 years substantial advances have been made in linking alterations in microbiota to brain development and even behaviour and the concept of a microbiota-gut brain axis has emerged. Animal models have been essential in moving forward this frontier research area. In order to assess such a role we use studies involving, germ free mice and early-life microbiota manipulations and finally probiotic administration in adulthood. We assess neurochemical, molecular and behavioural effects following these manipulations. Our data show that the gut microbiota is essential for normal stress, antidepressant and anxiety responses. Moreover, microbiota is essential for both social cognition and visceral pain. Finally, there are critical time-windows early in life when the effects of microbiota on brain and behaviour appear to be more potent. Our data also demonstrates that these effects may be mediated via the vagus nerve, spinal cord, or neuroendocrine systems. Such data offer the enticing proposition that specific modulation of the enteric microbiota by dietary means may be a useful 'psychobiotic'-based strategy for both stress-related and neurodevelopmental disorders ranging from depression to autism.

Newly characterised probiotic lactobacilli for naturally protected and functional foods

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Since the food safety and functionality become priority of the modern society, there is a rising interest in study lactic acid bacteria (LAB) from different habitats. With this aim a collection of LAB from traditional Bulgarian dairy products (yoghurt, cheeses, 'katak') was established. 25 lactobacilli were *in vitro* evaluated as candidate probiotics. The strains which possess transit tolerance in the gut conditions, growth ability in the presence of prebiotics and antimicrobial activity were evaluated as immunomodulators. They were identified as species *Lactobacillus plantarum*, *Lactobacillus brevis* and *Lactobacillus bulgaricus*, by classical API tests and molecular methods – species-specific and RAPD-PCR, and 16S rDNA sequencing. *in vitro* and *in situ* a broad spectrum of activity against food deteriorative and toxigenic micromycetes from the genera *Penicillium, Fusarium, Aspergillus, Trichoderma* and bacterial pathogens *Salmonella enteritidis*, *Escherichia coli* and *Enterobacter* was established and 4 promising strains were selected. They were successfully applied as bio-protective adjuncts to industrial starters for Bulgarian yoghurt. Different effects from partial to fully suppression of fungal growth were observed in experimental samples of new fermented milks, supplemented with variants of bio-protective adjuncts added as live, thermally-treated or lyophilised LAB cultures. In addition, the lactobacilli demonstrate a high viability of ~10^6-10^7 cfu/ml during the production and the cold storage period (28 days) combined with positive effects on the sensory properties of the products. The obtained results seemed promising for further application of selected probiotic strains and for design of new safety and functional foods, able to fulfil different consumers' demands.

Effects of *Lactobacillus reuteri* on duration of diarrhoea in outpatient children with acute infectious diarrhoea

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Probiotics are considered by ESPGHAN as of possible benefit in the treatment of acute gastroenteritis. We enrolled children with acute infectious diarrhoea, and followed-up as outpatient. Exclusion criteria were use of antibiotics or probiotics 1 month before admission, severe malnutrition and chronic underlying disease including immunocompromised conditions. We enrolled 64 children as an outpatient group. Three children from the *Lactobacillus reuteri* group and 1 from the control group were excluded because of antibiotic prescription (post-randomisation): 29 in the probiotic and 31 in the control group. Median age in the *L. reuteri* group was 24 months and median age of the control group was 18 months; age distribution was similar between the probiotic and control group. Mean number of stool frequency during the 24 h prior to admission was 7.13±3.8 per day in the *L. reuteri* group and was 6.1±0.97 per day in control group (P>0.05). The duration of diarrhoea was significantly reduced in the *L. reuteri* group when compared to the control group (mean ± standard deviation) (60.4±24.5 h vs. 74.3±15.3 h, P<0.05). The effect (percentage diarrhoea-free children) of the *L. reuteri* started to be observed after 24 h of intervention and was best observed at 48 h of the probiotic intervention. At 48 h, 45% of the children receiving *L. reuteri* had watery diarrhoea while this was the case in 87% of children in the control group (P<0.01). At 72 h, 17% of children receiving *L. reuteri* had diarrhoea while 13% have still diarrhoea in control group (P>0.05). No adverse effects related to the probiotic use were noted. *L. reuteri* decreased the duration of acute diarrhoea in outpatients. Previously, similar findings were reported in in-patients.

Survival of probiotic bacteria in low sodium Minas cheeses added with arginine

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The survival of *Lactobacillus acidophilus* in low sodium Minas cheese with added arginine, a potential masking of metallic flavour, was investigated. Minas cheese was manufactured with starter and probiotic culture (*Lactococcus lactis* and *L. acidophilus*, 6 log cfu/g), packed in polyethylene bags and stored at 5 °C for 14 days. Along the salting step, four different treatments were performed: 100 NaCl, 75/25 NaCl/KCL, 50/50 NaCl/KCL and 50/50/1 NaCl/KCL/arginine (% w/w). Microbiological analyses were performed during 1, 7 and 14 days of refrigerated storage. Arginine did not prevented growth of the probiotic culture. *L. acidophilus*, counts ranged from 7.11 to 9.21 log cfu/g in cheese, while *L. lactis* counts were always above 8 log cfu/g. In probiotic cheese with added arginine, the probiotic counts ranged from 7.23 to 8.45 log cfu/g. Low sodium Minas cheese with added arginine was found to be a potential carrier of probiotic bacteria with long shelf life.

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Dissecting a probiotic: biochemical and immunological aspects

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Probiotics mediate their effects on host organisms through various potential mechanisms, and an individual probiotic bacterial strain most likely exerts it's *in vivo* effects through more than one of these mechanisms. *Bifidobacterium infantis* 35624 is a well-studied probiotic strain with proven clinical benefits. To better understand its mode of action, we have begun to characterise this strain and its components in various *in vitro* and *in vivo* models using biochemical and immunological techniques. The detailed characterisation of the molecules, derived from microbes, that induce immunological responses will support the development of novel pharmaceutical molecules for therapeutic purposes.

The colon mucus as the habitat for the commensal bacteria

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That commensal bacteria live in a symbiotic relation with the host has been understood for long time, but why we can handle that many bacteria in the colon without triggering an overt immune response has not been revealed. Our discovery of a two-layered mucus system where the inner mucus layer is devoid of bacteria has changed this. The inner colon mucus layer is formed by goblet cells synthesising highly glycosylated MUC2 mucin that upon release form enormous net-like structures. These are staggered on each other to form the inner mucus layer that act as a filter and inhibit bacterial penetration. This layer is converted to the outer mucus layer by host protease activities that cause the net-like structure to expand and by this opens for bacteria to penetrate. The outer mucus layer is thus the habitat for the commensal bacteria. The commensal bacteria attach to and use MUC2 mucin glycans as a food source and deliver short fatty acids back to the host. The glycan structures on the MUC2 mucin vary between species, but are surprisingly similar in the distal colon suggesting that these can act as attachment sites. By manipulating the glycans, it became obvious that shorter glycans are degraded faster and can allow bacteria to reach the epithelium. If epithelial cells are in contact with a massive amount of bacteria, this triggers an immune response and colitis. In fact, all mouse models that spontaneously developed colitis lacked an inner mucus layer or have defects in it that allowed bacteria to penetrate. Patients with ulcerative colitis also have a dysfunctional mucus layer. Recent studies on mice without bacteria and with different microbiota as determined by 16S rRNA sequencing suggest that the bacterial composition is very important for controlling the host and the properties of the inner mucus layer. Bacteria can thus affect the mucus phenotype with potential negative health effects.

A double blind randomised trial showing probiotics to be ineffective in acute diarrhoea in Indonesian children

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The objective of this study was to investigate the efficacy of probiotics added to oral rehydration solution and zinc in the treatment of acute infectious diarrhoea in Indonesian children. A prospective randomised double blind placebo-controlled trial was performed to test the efficacy of a probiotic food supplement in 112 children in the Kenari subdistrict, central Jakarta., aged 6-36 months with acute infectious diarrhoea and moderate dehydration. The supplemented group was given standard therapy (oral rehydration solution and zinc) and the probiotic strains *Lactobacillus rhamnosus* R0011 1.9×10^9 cfu/day and *Lactobacillus acidophilus* R0052 0.1×10^9 cfu/day for 7 days, while the control group was given standard therapy and placebo. Median duration of diarrhoea was 68.5 h (range 13-165) in the supplemented and 61.5 h (range 21-166) in the control group (P=0.596). Median daily frequency of defecation until diarrhoea stopped was 5.0 in the supplemented versus 5.5 in the control group (P=0.795). In conclusion, this probiotic food supplement tested did not reduce the duration of acute infectious diarrhoea compared to oral rehydration and zinc.

American gut project

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Recent advances in the field of human microbiome research has sparked tremendous public interest in their own personal microbiomes, yet traditionally scoped and funded projects such as the NIH's Human Microbiome Project have made this difficult to achieve. An alternative model is crowdsourcing of samples, which we have previously used for the Earth Microbiome Project, and crowd-funding the research itself, in which participants donating above a certain level that supports the cost of the project are able to claim as a 'perk' sequencing of one or more samples of their choice. The American Gut Project, a collaboration between the Earth Microbiome Project and the Human Food Project, has used this crowd-funded, crowd-sourced model to become the largest crowd-funded science project that we know of to date, having raised over half a million dollars from over 6,000 participants. In this talk I describe how we initiated the project, the technologies that we are deploying (building on lessons from the Human Microbiome Project and the Earth Microbiome Project), and the results of the thousands of samples that have been released to the public to date, focusing on associations with factors such as age, diet, exercise, and disease. Of particular interest to probiotics researchers will be the availability of a vast public dataset describing what kinds of microbiomes are out there 'in the wild', and the new 'How much can you change your microbiome?' challenge that we are issuing to participants interested in seeing how plastic the microbiome is with different dietary and probiotic interventions.

Personalised probiotics by *ex vivo* enrichment of beneficial microorganisms from the host

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Currently, all probiotics available on the market have been obtained by selection and cultivation of a limited number of microorganisms from a relatively small number of sources. Evidence accumulates that the genetic makeup of the host is a crucial factor for facilitating the colonisation of beneficial bacteria in the human body. Therefore, treatment of a patient suffering from a recurring infection could be more effective with one or more of the patient's indigenous species. The microbial population isolated from the infected site of the body is enriched for bacteria, which are associated with a healthy microbiome. The *ex vivo*-enriched-sample can be used for therapeutic purposes. This innovative method is designated to TripleA, referring to the three consecutive steps involved, including Acquirement, Alteration and Administration. One of the medical cases where TripleA could be advantageous is the treatment of bacterial vaginosis (BV) – cultivation experiments indicate enrichment of the BV-associated microbiota for lactobacilli. It should be noted that TripleA is truly personalised, but the methodology used is generic for each medical application. TripleA can become widely accessible via the development of a device or a kit, which can easily be applied by physicians. In the future, the TripleA concept may become suitable for self-sampling, cultivation at home, and self-treatment.

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Gut microbiota and metabolomics

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The gut microbiota provides vitamin K production, energy harvesting from indigestible complex carbohydrates, stimulates cell growth and trains the immune system. There is still much to elucidate to understand fully these interactions. Proton nuclear magnetic resonance (^{1}H NMR) and mass spectrometry (MS) applied to biological extracts has the potential to unravel novel mechanistic information in relation to gut health. Faecal and tissue extracts from various studies with regard to a gut pathogen, obesity and bowel disease (BD) were profiled using ^{1}H NMR and MS metabolomics. Metabolomics indicated that infecting two mouse strains with *Citrobacter rodentium* led to different host responses. Lipidomics and metabolomics applied to an obese mouse model showed that dietary lipids were stored and dietary phenolics metabolised in a different manner and ^{1}H NMR applied to faecal extracts from BD patients and healthy controls showed difference in metabolic profiles. Gut microbiota seems to play a role in key metabolic functions in the host such as resistance to infection, harvesting and processing of dietary nutrients or balance in the bile acids pool. Metabolomics related to the gastro-intestinal tract highlights the important role of bacteria in maintaining a healthy gut.

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Public health impact of probiotics in common respiratory tract infections

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Two recent systematic reviews, demonstrated that probiotics are effective in reducing duration and frequency of common respiratory tract infections (CRTI). Antibiotic use also appeared to be decreased. These results have been used to estimate public health and budgetary consequences of probiotics consumption in France. A virtual age- and gender-standardised population was generated using a Markov model. CRTI risk factors were age, active/passive smoking, and living in a community setting. Incidence rates came from a national general practitioner (GP) network over the 2011-2012 flu season; the analysis was limited to GP patients. Economic perspectives were society, national health system and family. Data on resource utilisation came from the GP network. Outcomes included numbers of CRTI days and episodes, antibiotics courses, sick leave, medical and indirect costs. The influence of environmental risk factors like daily life in shared environments or active and passive smoking, were also investigated. Potential public health impact of probiotics appears to be substantial, whether taking into consideration the reduction of CRTI episodes' frequency or duration and shows positive consequences to all economic agents: it benefits society, the national health system and the family. Probiotics could be taken into consideration when searching for population-oriented strategies to limit the consequences of CRTI in primary health care and household settings during the winter season.

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Probiotic cheese attenuates exercise-induced immune suppression in Wistar rats

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Intense physical activity results in substantial stress and hence a significant risk for immune suppression in athletes. Consumption of probiotic cheese can potentially attenuate immune suppression induced by exhausting exercise in rats. A popular Brazilian fresh cheese (Minas Frescal cheese) containing *Lactobacillus acidophilus* LA14 and *Bifidobacterium longum* BL05 was fed for 2 weeks to adult Wistar rats, which then were brought to exhaustion on the treadmill. 2 h after exhaustion, the rats were killed and blood samples were collected for biochemical and haematological analysis. The exercise was efficient in reducing lymphocyte counts, irrespective of the type of ingested cheese, but the decrease in the probiotic cheese fed group was 22% compared with 48% in the animals fed regular cheese. Monocyte counts were unaltered in the rats fed probiotic cheese, when compared with a significant decrease in the rats fed the regular cheese. Most importantly, ingestion of the probiotic cheese resulted in a >100% increase in serum high-density lipoprotein cholesterol and a 50% decrease in triacylglycerols. We conclude that probiotic Minas Frescal cheese may be a useful alternative to enhance the immune system and could be used to prevent infections, particularly those related to the physical overexertion of athletes.

Probiotic yoghurt offers higher immune-protection than probiotic whey beverage

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Probiotics can be useful to improve the immune response in experimental challenges, like exhausting exercise, which causes immunosuppression. We have evaluated a probiotic yoghurt and a probiotic whey beverage using an exhausting physical-exercise protocol with rats. Wistar rats were given a daily 4 ml supplement of each type of conventional or probiotic yoghurt and whey beverage, manufactured with lactic culture of *Streptococcus thermophilus* TA040 and *Lactobacillus bulgaricus* LB340, and probiotic cultures of *Lactobacillus acidophilus* LA14 and *Bifidobacterium longum* BL05. The effects on the immune system were compared to those of pair-treated cohorts receiving the treatments for 14 days. Results demonstrated that the probiotic yoghurt outperformed the probiotic whey beverage in blood-cell indicators (neutrophils and lymphocytes), cytokines (tumour necrosis factor-α and interleukin-1β) and various standard health parameters. In conclusion, in this study, the treadmill exercise assay successfully produced immunosuppression in the rat and the combination of the nutrients and probiotic bacteria of the yoghurt reduced more effectively the adverse effects developed over the prolonged strenuous exercise than did a similar probiotic whey beverage.

Isolation and characterisation of natural probiotics targeted toward canines

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Due to the humanisation of pets, human health practices including probiotic consumption are transitioning into the companion animal market. Commercially-available canine probiotics are generally of human origin and are not optimal for companion animals since probiotic bacteria possess host-specific traits. As such, we are developing species-specific probiotic products for canines. Gram-positive bacteria were recovered from freshly-voided canine faeces and duplicate isolates were eliminated using RAPD PCR. Probiotic candidates are subjected to a series of tests including tolerance to low pH and bile, competitive exclusion assays, antimicrobial peptide production, ability to alter host immune function, and strain stability. Viable bacteria from canine faeces are present in smaller quantities and lower diversity than expected; however, we recovered several promising probiotic candidates. DNA sequencing determined these isolates are a range of lactic acid bacteria. We are developing a canine-specific probiotic product. Although the variety and number of lactic acid bacteria isolated from freshly-voided faeces is lower than expected, we recovered promising probiotic isolates. These strains will be subjected to the tests outlined above to determine their suitability for development into probiotic products. We aim to fill an unoccupied niche in the pet food and specialty markets where effective host-specific probiotic products are lacking.

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The study of growth performance of rainbow trout (*Oncorhynchus mykiss*) larvae fed fermented *Daphnia magna* meal with *Bacillus* probiotics

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One of the modern aspects of probiotic application in aquaculture is their use in raw material fermentation. The aim of this study was using the five *Bacillus* probiotics for fermenting of *Daphnia magna* meal based on an *in vitro* study. To this end 700 Rainbow trout (*Oncorhynchus mykiss*) larvae with mean initial body weight of 0.583±0.197 g were obtained and transferred to the laboratory and divided to 4 experimental treatments and control in a randomised study design. Daphnia were collected and washed and subsequently oven dried. The prepared daphnia meal was fermented for 15 days and then mixed with a commercial Rainbow trout feed at levels of 20 and 30%. Also the same levels of raw daphnia meal was added to the commercial diet and fed to the larvae for 60 days. The best performance was observed in treatments that contained fermented meal. In addition, the results related to the feeding of raw daphnia meal showed that this also might be efficient for growth and survival of rainbow trout larvae. The results also showed the high potential of fermented daphnia meal on improvement of blood factors of rearing larvae.

Bifidobacterium lactis Bi-07 in combination with galacto- or xylose-oligosaccharides: human intervention trials

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The aim of the study was to determine if the potential probiotic properties of *Bifidobacterium lactis* Bi-07. would be strengthened by either galacto-oligosaccharides (GOS) or xyloseoligosaccharides (XOS) in two separate human intervention trials. To test the influence of GOS or XOS, volunteers were randomised to receive either; one of the prebiotics (8 g/day), the probiotic (10^9 cfu/day), a pre- and probiotic combination or placebo in a cross over design. In only one of the studies, consumption of *B. lactis* Bi-07 lead to increased faecal *B. lactis* levels. Addition of the prebiotics did not influence this, although XOS increased total *Bifidobacterium* levels. *B. lactis* Bi-07 and XOS, independently modulated the measured immune markers. GOS failed to exhibit any measurable effect. While *B. lactis* Bi-07 and XOS did have independent effects, in particular on immune function and microbiota composition, no synergy was observed. It can be speculated that the prebiotics and the probiotic function in different parts of the intestine and therefore no synergy was observed. The concept of synbiotics maybe more challenging to accomplish than generally thought.

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Probiotic delivery: does the matrix matter?

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Probiotics have mainly been tested for their health benefits in the form of dairy products and capsules. However, also other matrices have been tested. Little is known about the potential influence the matrix has on efficacy. Literature searches were done on human intervention trials that used various matrices. Furthermore, manufacturers of probiotics (Valio, Lallemand and Chr. Hansen) were asked if they were aware of studies with their strains in various matrices looking at similar health outcomes. Studies were grouped by strain (combination) and 'health' outcome. Twenty-four studies on eight strains/combinations indicate that the matrix may influence faecal recovery in a strain dependent manner. Studies on microbiota composition suggest rather recovery of the consumed probiotic, than a shift in microbiota composition, regardless of the tested matrix. While biomarkers are often determined in intervention trials with different matrices, they have often not been measured in different populations. This is similar for clinical outcomes. Although many matrices have been investigated they often have been tested in different populations and for different end points, making it difficult to draw any firm conclusions. To answer the question on the influence of the food matrix on probiotic efficacy, dedicated studies should be performed in similar populations, on the same biomarkers and clinical endpoints.

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Bacterial identification from soy extract fermented by kefir grains

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Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Kefir grains are an irregular and gelatinous array composed of protein and polysaccharides containing bacteria and yeasts which are involved in the production of fermented probiotic beverages. The aim of this study has been to identify species of bacteria isolated from soy extract fermented by Kefir grains. The isolates were inoculated in MRS culture broth at 37 °C for 24 h for genomic DNA extraction. The extracted samples were subjected to electrophoresis in agarose gel at 1.0%. The 16S rRNA gene was amplified by PCR with oligonucleotide primers and 27F-1092R 16S rDNA gene amplification. Wizard® SV Gel and PCR Clean-Up System kits were used to purify the fragments. Then, the sequences were analysed in the program Package and submitted to Standen similarity search in genomic database. *Leuconostoc mesenteroides, Lactobacillus casei/paracasei* and *Lactobacillus diolivorans* species were identified and isolated. Such results are very promising and exciting for the development of new functional fermented beverages and raw materials to fermentation processes.

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Newly found origin of probiotics in the Balkans

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Lactic acid fermentation has been known on the Balkans for many centuries. Till now lactic acid microorganisms have been isolated from yoghurt, diary domestic animals, infants' faeces and plants. The aim of this study is to research another origin of lactic acid strains with beneficial effects. We isolated eight natural strains of *Lactobacillus bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus lactis* and *Streptococcus thermophilus* from spring water in Bulgaria. These strains are able to live and reproduce in flowing spring waters under changing weather conditions. The strains stayed alive during fermentation, freeze-drying sublimation, and during storage at 24 °C for 3 years. Rehydrated, they survived the passage through the whole gastrointestinal tract and were able to colonise the colon. The strains originating from water have very good bacteriostatic effect against pathogenic microorganisms and reduce post-operative complications. The probiotic diary food containing these strains and natural amino acids significantly improved protein resorption and overcomes hypo- and dysproteinemia during the post-operative period. This study shows that the newly strains *Lactobacillus bulgaricus* and other lactic acid microorganisms isolated from water were viable and had an beneficial effect on human health. The isolation of probiotic lactic acid strains from spring water brings up the question of revision of the generally accepted opinion about the origin of the lactic acid bacteria species.

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Effect of probiotic on growth of *Staphylococcus aureus* and *Listeria monocytogenes* in cheese

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In this study, the effects of *Lactobacillus rhamnosus* on growth of *Staphylococcus aureus* and *Listeria monocytogenes* in Minas frescal cheeses during storage was evaluated. Minas frescal cheese is a semi-fat, non-ripened and high humidity cheese, which has been involved in food-borne disease outbreaks in Brazil. The experimental design used a 2×2×2 randomised factorial arrangement with 8 treatments and 4 replicates per treatment. The experimental cheeses were manufactured in a local dairy plant. Fresh cheeses were removed from the moulds and immediately assigned to each treatment, for inoculation with *L. rhamnosus* (10^9 cfu/g) and/or *S. aureus* (10^7 cfu/g) and/or *L. monocytogenes* (10^5 cfu/g). The Minas frescal cheeses were individually vacuum packed in polyethylene bags and stored at 7ºC for 21 days. *L. rhamnosus* and *S. aureus* counts in cheeses remained stable during 21 days of storage at 7 °C. However, *L. rhamnosus* was able to inhibit the growth of *L. monocytogenes* in cheeses ($P<0.05$), which counts decreased nearly 2 log cycles during 21 days. It is concluded that *L. rhamnosus* has a potential application for control of *L. monocytogenes* in Minas frescal cheeses during its shelf life.

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Pharmaceutical like validation of a probiotic dietary supplement for kidney health

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The probiotic dietary supplement market is growing globally at 9.6%, with sales expected to reach 2.10 billion USD by 2015. Newer applications are growing from lowering cholesterol and boosting immunity, to anxiety, allergy and autism. However, there is none targeting kidney health. Renadyl™ was developed over a ten year period to reduce serum uremic toxins and impart a better quality of life to kidney failure patients. Extensive *in vitro* experiments and simulator of human intestinal microbial ecosystem (SHIME) studies led to a formulation consisting of *Staphylococcus thermophilus* (KB19), *Lactobacillus acidophilus* (KB27) and *Bifidobacterium longum* (KB31), which could reduce uremic toxins. Animal studies followed using 5/6th nephrectomised rats, mini-pigs, chronic kidney disease (CKD) cats and dogs. Subsequently, a randomised double blind placebo controlled crossover trial in CKD 3/4 patients was carried out using a dose of 90×10^9 cfu/day at 4 sites, and an open label dose escalation study at 90×10^9, 180×10^9 and 270×10^9 cfu/day. Another double blind placebo controlled crossover study with haemodialysis patient was recently completed with a dose of 180×10^9 cfu/day. Outcomes were compared by measuring various uremic toxins, inflammatory biomarkers, faecal analysis and quality of life. Improvement in quality of life, reduction in levels of blood urea nitrogen, creatinine, C-reactive protein and indoxyl glucuronide was seen. Consumer acceptance of probiotics as dietary supplements will depend on major human clinical product validation as described.

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Survey of customer satisfaction with Renadyl™, a probiotic supplement for kidney health

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Renadyl™, a probiotic dietary supplement for kidney failure patients, is sold online. A survey questionnaire with 37 questions was sent to 998 customers that have taken Renadyl to evaluate the efficacy, safety and satisfaction with Renadyl usage. Questions were based on quality of life, onset and stage of kidney disease, physical, social and emotional health, medications, side effects, kidney function after taking the probiotic supplement with respect to blood urea nitrogen (BUN) and creatinine, product satisfaction with respect to customer service, pricing and product quality. 15% (147) customers responded. Median age was 70, 76% were over 50 years old, 46% were between 60 and 79. 57% were in chronic kidney disease (CKD) stage III or IV, 17% in CKD stage V (end stage renal disease) and 12% on dialysis. 4% had CKD for less than a year, 24% had CKD for 1-3 years, 20% for 3-5 years and 27% for 5-10 years. 71% had onset of CKD after 50 years of age. 95% experienced no side effects. 73-74% agreed that (1) kidney function improved (reduction in BUN and creatinine levels), (2) mood, energy and physical fitness improved, and (3) severity of the disease reduced. This consumer survey predominantly confirms the acceptance of the probiotic dietary supplement by kidney failure patients for their kidney health.

Understanding the vaginal microbiota to improve women's health

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The vaginal microbiota along with intrinsic host factors plays a major role in maintaining vaginal health. We hypothesised that differences in species composition of vaginal bacterial communities in health may affect the level of community dynamics, hence the risk of developing bacterial vaginosis or acquiring communicable diseases. We have analysed the composition and structure of the resident bacterial community in 400 healthy women and evaluated the level of community dynamics in more than 50 women sampled daily for 10 weeks using pyrosequencing. We have developed novel quantitative measure of community stability that incorporates three aspects of stability: constancy, resilience and persistence over time. This index was applied to identify five major classes of temporal community dynamics. As expected menses are associated with decreased stability and increased transitional events, but these effects were mitigated by community composition. A few communities experienced a lack of major transitional events, and those were often associated with *Lactobacillus crispatus, Lactobacillus gasseri* dominance and to a lesser extent, with communities lacking significant number of *Lactobacillus* spp. Community classes dominated by *Lactobacillus iners* demonstrated either instability or a remarkable level of stability. We showed that specific *L. iners* genomes are associated with high or low community stability. We showed that the genomic content of *L. iners* might be a good predictor of susceptibility to sexually transmitted diseases such as chlamydia. This work forms the basis of the development of mathematical models of vaginal community dynamics and suggests that longitudinal evaluation of the vaginal microbiota could form the basis to personalised treatments and evaluation of the risks to adverse outcomes. The findings form a framework for the rationale selection of strains of *Lactobacillus* spp. that could be developed as more personalised probiotics to reduce the unnecessary use of broad-spectrum antibiotics prescribed by doctors and in an attempt to restore vaginal health.

Novel targets for probiotics in the mucosal immune system

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Many of the health benefits of probiotics for the (human) host, are related to functioning of the immune system. Thus, probiotics have been demonstrated to prevent and combat mucosal infections of the gastrointestinal, respiratory, and genital tract. In allergic as well as in autoimmune diseases, probiotics can restore and maintain the balance between Th1 and Th2 mediated immune responses by induction of regulatory T cells (Tregs). The mechanisms by which probiotics would exert these effects are thought to involve interaction between probiotics and dendritic cells present in the gut. In humans, most studies on immunoregulation by probiotics are performed with mononuclear cells derived from peripheral blood and thus focus on the systemic immune system. For the mucosal immune system, the players as well as the rules of the game differ from that of the systemic immune system. The so-called innate lymphoid cells specifically home in mucosal tissue and fulfil unique functional roles, such as production of interleukin-17. The development of mucosal Tregs is regulated by short chain fatty acids, in particular butyrate, produced by probiotic bacteria. The butyrate leads to acetylation of the promotor region of FoxP3, the key-transcription factor for Tregs. This finding could turn out to be the missing link between probiotics and regulation of the mucosal immune system.

The role of microbes in stress resilience and psychiatric disorders

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The immune system requires input from three categories of organism designated the 'Old Friends': (1) the commensal microbiota transmitted from mothers and family members; (2) organisms from the environment that diversify commensal microbiota; and (3) the 'old' infections that persisted in evolving hunter-gatherer groups as chronic infections. All these organisms had to be tolerated and so co-evolved roles in regulating the immune system. By contrast the 'crowd infections' (such as childhood virus infections) evolved later when urbanisation led to large communities, and did not evolve immunoregulatory roles because they either killed the host or induced solid immunity: they could not persist in isolated hunter-gatherer groups. High-income countries, where there is reduced exposure to immunoregulatory Old Friends, are undergoing increases in chronic inflammatory disorders including allergies, autoimmunity and inflammatory bowel diseases. Depression, anxiety and reduced stress resilience are comorbid with these conditions, or can occur in individuals with persistently raised circulating levels of inflammatory mediators in the absence of clinically apparent peripheral inflammatory disease. Psychological stress drives additional exaggerated release of inflammatory mediators when immunoregulation is defective. Moreover, poorly regulated inflammation during pregnancy might contribute to brain developmental abnormalities that underlie some cases of autism spectrum disorders and schizophrenia. In summary, faulty immunoregulation and inflammation predispose to psychiatric disease. Modulating the microbiota might target these problems of modern urban life.

Effects of *Bifidobacterium animalis* and *Bifidobacterium lactis* on growth parameters and blood profile of rainbow trout larvae

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Aquaculture industry is becoming increasingly important for animal protein production. Probiotic supplements have a special relevance in the development of rearing technologies for fish larvae. There has been no studies on the effect of *Bifidobacterium animalis* and *Bifidobacterium lactis* on growth and haematological profiles in rainbow trout (*Oncorhynchus mykiss*) larvae. A completely randomised experimental design was applied with three different bacterial concentrations of 1×10^9, 2×10^9 and 3×10^9 cfu/100 g dry feed, with each three replicates. Fish were fed four times a day for 60 days. The larvae fed a 1×10^9 cfu/100 g supplemented feed were significantly different from the fish in the other treatments and the control, with the highest mean final weight (24.983±6.109) and body weight gain (24.482±6.109). No significant difference in haemoglobin concentration was found between the experimental groups and the control within 2 months. The highest red and white blood cells concentrations were observed in the fish-fed 2×10^9 and 3×10^9 cfu/100 g probiotic bacteria. The present study showed that *B. animalis* and *B. lactis* improved growth performance and affected the haematologichal profiles of *O. mykiss* larvae. Probiotics reduced the mortality rate in experimental treatments and significant differences were observed between treatments and control. In conclusion, *B. animalis* and *B. lactis* improved the growth performance of rainbow trout.

Screening of lactobacilli and bifidobacteria from Bangladeshi indigenous poultry for their potential use as probiotics

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Despite the existence of potential sources, no probiotic has been developed yet in Bangladesh. Therefore, this study was undertaken to evaluate the probiotic bacteria from local poultry. A total 300 isolates belonging to the genera *Lactobacillus* and *Bifidobacteria* were identified following physical, biochemical and molecular methods from 61 healthy indigenous birds. Based on the auto-aggregation test, 12 lactobacilli and 7 bifidobacteria isolates were selected for antibacterial activity, resistance to low pH, resistance to bile extract, ability to produce H_2O_2, cell surface hydrophobicity and adhesion activities on crop epithelial cells to evaluate their probiotic potential. Three lactobacilli and two bifidobacteria strains were found to have potential as probiotic strains, as they met the criteria required to be used as probiotics. These findings are highly inspiring as they showed the potential of local Bangladeshi isolates to be used as probiotics. If the proposed strains can be used in commercial poultry industry as probiotics, it is expected that they would be more beneficial and save the huge amount of money that Bangladesh spends every year to import of such materials from abroad.

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Effect of intestinal microbiota on lymphoma and longevity in *Atm* deficient mice

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Intestinal microbiota play a role in the nutrient metabolism, modulation of the immune system, obesity and intestinal inflammation. We tested *Atm* deficient mice for genotoxicity, genetic instability, DNA damage, inflammation markers, cancer latency and longevity and high throughput sequencing of the intestinal microbiota. Isogenic mice from different facilities showed a 4-fold difference in life expectancy, a 4.5-fold difference in genetic instability and DNA damage. The onset of lymphomas was significantly 2-fold different. We sequenced the microbiota of both facilities and found *Lactobacillus johnsonii* 456 as the dominant bacterial strain in the health beneficial microbiota. Just this bacterium by itself reduced genotoxicity, reduced inflammation and reduced levels of cytotoxic T cells in the liver and blood. We also found similar differences in *Trp53* deficient and even in wildtype mice. We have shown that the intestinal microbiota is responsible for differences in genetic instability, genotoxicity, DNA damage, inflammation, latency of lymphoma and longevity. The underlying mechanisms is probably due to inflammation promotion or suppression. The understanding of this effect may lead to a breakthrough in the understanding of the causes of carcinogenesis, which might lead to prevention of ataxia telangiectasia, a currently incurable progressive disease and possibly other cancer-prone DNA repair deficient diseases or even wildtype mice and people.

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Probiotics reduce inflammatory gene expression induced by a high fat diet

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In a recent study, we obtained some evidence that the probiotic supplement Lactibiane Tolerance® (LT) restores epithelial barrier disruptions induced by lipopolysaccharides. Here, we tested the beneficial effect of this probiotic mix on weight gain and glucose metabolism using a high-fat diet model in mice. Wild type C57/BL6J mice were fed with either normal or high fat diets. Some mice received LT probiotics. Animal weight, body fat percentage, and homeostasis model assessment for insulin resistance (HOMA IR) were evaluated. Inflammatory gene expression was examined in adipose and gut tissues using microfluidic tools. The administration of LT reduced the increase of body weight and insulin-resistance due to the high fat diet consumption. Gene expression analysis showed that LT inhibited the up-regulation of pro-inflammatory gene and leukotriene pathway in the adipose tissue. LT supplementation also increased mRNA levels of genes involved in the resolution and immunosuppression of inflammation in the gut tissue. LT significantly prevented body weight gain and insulin resistance. It modulated the expression of key genes involved in inflammation, its resolution and immunosuppression. These results suggest that improvement in the inflammatory state of the gut and adipose tissue might be a possible mechanism underlying the anti-obesity effect of LT.

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Gaps in current knowledge on probiotic supplementation, diet and gut-microbiota in old age

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Information on the influence of probiotic supplementation and diet on gut-microbiota in old age is limited and data is often conflicting. Our objective was to review intervention-studies on the impact of probiotic supplementation on gastrointestinal outcomes and identify gaps in current knowledge regarding individualised treatment strategies. The literature review used studies in PUBMED years 2000-2012 using probiotics as intervention evaluating gastrointestinal outcomes. Twelve studies that focused on increasing *Bifidbacterium* counts were included. Except for one, using *Bifidobacterium longum,* all showed increased *Bifidbacterium* counts following 14-180 days of intervention. Six studies showed an increase in defaecation, one a reduction in diarrhoea, and one a reduction of *Clostridium difficile* associated diarrhoea. Another study examined the impact of probiotics on gastroenteritis morbidity and indicated a shorter duration of fever. Most of these studies had small samples and short follow-up; some were poorly designed. We identified needs for more information on dosages, strain combinations in supplements and interactions with foods. The gastrointestinal system serves as the entry port of food and connecting the external and internal environments. Exploring relationships between diet, microbiota composition and health may enhance our ability to intervene using individually tailored supplementation. We are in the process of collecting diet and gut microbiota data on rehabilitation patients.

In vitro assessment of functional features of lactic acid bacteria isolated from horse faeces

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In equine production, probiotics are used to improve zootechnical performance and prevent intestinal infections. In this study, lactic acid bacteria were isolated from equine faeces to investigate their functional features *in vitro*. The isolates were identified by amplified ribosomal DNA restriction analysis of 16S-23S rRNA genes. The species found were *Weissella confusa* (64%), *Lactobacillus sanfranciscensis* (9%), *Enterococcus casselifavus* (9%), *Lactobacillus reuteri* (6%), *Lactobacillus crispatus* (6%) and *Lactobacillus equi* (6%). Most strains were resistant to acidic pH with slight to moderate growth inhibition by bile salts and about 40% had highly to moderate hydrophobic cellular surfaces. Several isolates exhibited strong antagonistic activity against the bacterial pathogens tested. All isolates exhibited the same antimicrobial susceptibility pattern, indicating an absence of horizontal transfer. Four strains exhibited better functional properties: low inhibition to gastric acids, low or moderate inhibition by bile salts, a moderate or high hydrophobicity and antagonism against several pathogens. These four strains deserve investigation as potential probiotics or mucosal delivery vectors for therapeutic proteins and DNA vaccines in horses.

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The influence of gut microflora on drug metabolism

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The aim of our study is to emphasise the role of gut microflora in drug metabolism since it has long been underestimated. We have analysed the original and review articles, published from 2000-2013, using the keywords 'gut microflora' and 'drug metabolism'. Gut microflora are capable of carrying out a wide spectrum of metabolic activities. Drugs which may be exposed to gut microflora are those that are orally ingested, but suffer from low solubility, low permeability or both. Gut microflora is mainly involved in reductive and hydrolytic reactions producing low molecular weight by-products. Additionally, the intestinal bacteria express various enzymes involved in processing of glutathione conjugates of xenobiotics excreted in the bile, reactions of decarboxylation, dehydroxylation, dealkylation, dehalogenation, deamination, etc. Drug transformation that occurs in gut microflora may be associated with increased toxicity, altered efficacy, the inactivation of drugs, increased production of toxic metabolites, or adverse drug interaction. In conclusion, the presence of bacterial community in the intestine and its metabolic activity may be linked with inter individual and intra individual differences in drug metabolism. Thus, it is necessary to gain more insight into reactions that may occur by enzymatic activity of gut microflora and into consequences that may be caused on that way.

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Probiotics in Mediterranean types of applications

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The Mediterranean basin is a vast storehouse of ancient culture, art and knowledge, including culinary tradition. The food culture has been formed upon geographical, climate, historic, social and religious elements. These traditional fermented foods are characterised by their high nutritional value and particular sensorial properties. These are due to the quality of the raw materials used, which, in turn, is determined by the climate conditions and thus the flora and fauna diversity. The same parameters have gradually shaped the microbial diversity of the traditional fermented foods. The members of this indigenous microbiota play a crucial role in the development of the nutritional and sensorial profile of these foods. The present paper describes snapshots of probiotic and protective cultures that have been isolated from traditional, naturally fermented foods and more specifically from Greek Kasseri cheese. *Lactobacillus fermentum* ACA-DC 179 displays probiotic potential *in vitro* and protects against trinitrobenzene sulfonic acid-induced colitis and *Salmonella* infection in murine models. Feed supplementation with *Lactobacillus plantarum* PCA236 modulates gut microbiota and milk fatty acid composition in dairy goats. Finally, *Streptococcus macedonicus* ACA-DC 198 produces the lantibiotic macedocin when grown in milk acting as a protective starter in cheese making.

Prevention and treatment of diarrhoea with probiotics

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Probiotics have been largely studied to the prevention of acute infectious diarrhoea. Large, randomised controlled trials (RCT) provide evidence of a very modest effect (statistically significant, but of questionable clinical importance) of some probiotic strains (*Lactobacillus caseii* GG, *Lactobacillus reuteri, Bifidobacterium lactis*) on the prevention of community-acquired diarrhoea. The number needed to treat (NNT) to prevent one child from developing nosocomial Rota gastroenteritis is seven. However, the protective effect on prevention of diarrhoea becomes far less significant if the incidence of diarrhoea (episodes per patient-month) rather than the percentage of patients with diarrhoea are taken into account. The use of probiotics has been suggested in the treatment of acute gastroenteritis (AGE) in addition to early rehydration and avoidance of dietary restrictions. The pooled relative risk in a meta-analysis of 63 RCTs, which included 11,811 participants, indicated a statistically significant association of probiotic administration with reduction in antibiotic-associated diarrhoea (AAD) (relative risk = 0.58; 95% CI: 0.50 to 0.68). For every 7 patients that would develop diarrhoea while being treated with antibiotics, one fewer will develop AAD if also receiving probiotics. *Saccharomyces boulardii* (RR=0.43; 95% CI: 0.32-0.60) and *Lactobacillus rhamnosus* (RR=0.36; 95% CI 0.19-0.69) are the two best studied strains. In most studies, the probiotic is started together with antibiotic treatment. Probiotics have entered the mainstream of healthcare. The gastro-intestinal microbiota is fundamental for the development of the immune system. Although the main indications of the medical use of probiotics is still in the area of the prevention and treatment of gastro-intestinal related disorders, gradually more evidence is collected on extra-intestinal indications such as vaginitis, atopic dermatitis, respiratory tract infections. RCTs with the commercialised product in the claimed indications are mandatory before the use of a product can be recommended. Today, *L. caseii* GG and *S. boulardii* are the best studied strains. Although adverse effects have been reported, probiotics are considered as safe. Overuse and use of products that have not been validated constitute a major drawback.

Bacillus clausii and isomalto-oligosaccharides: investigation of a synbiotic potential

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Benefits of probiotic *Bacilli* are investigated for their resistance to stomach conditions under spore form. Prebiotics could promote establishment of probiotics in the intestine. This study investigated the prebiotic potential of isomalto-oligosaccharides (IMOS) on the probiotic strain *Bacillus clausii* O/C. IMOS are glucose oligomers from 3 to 30 units, with α-D-(1,6)-linkages. IMOS was characterised by ESI-MS and MALDI-MS. *B. clausii* O/C (Enterogermina®) growth kinetics were established in minimal medium with 1% glucose or IMOS. Proteomic analysis was done with 1D SDS-PAGE/MALDI-TOF-MS. Growth kinetics of spores and vegetative cells in medium with bile salts, containing glucose or IMOS, were evaluated. *B. clausii* growth rate was similar with IMOS or glucose as a carbon source. An α-glycosidase, which hydrolyses IMOS, is released in culture medium. Obtained dimers are accumulated in bacteria via a phosphoenolpyruvate-dependent maltose phosphotransferase system, and then used for glycolysis. Compared to glucose, IMOS as carbon source reduces the lag-time due to bile. This work highlights *B. clausii* enzymatic machinery modification to hydrolyse IMOS, improving resistance to bile. IMOS could promote germination of the strain in the intestine. Our study highlights the promising interest of a synbiotic combination of *B. clausii* O/C and IMOS.

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Probiotics/commensal bacteria, the gastrointestinal tract microbiome and musculoskeletal disease

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Osteoarthritis (OA) and rheumatoid arthritis (RA) comprise two of the most common forms of debilitating musculoskeletal disorders known. As such natural medicines are an attractive option for patients diagnosed with OA and RA. The gastrointestinal tract (GIT) and microbiota combined comprise one of the most metabolically and immunologically active organs. Microbial interactions in the GIT provide the necessary cues for the development of regulated pro- and anti-inflammatory signals that promotes immunological tolerance, metabolic regulation/stability and other factors which may then control local and extra-intestinal inflammation. The bacteria that colonise the GIT perform a number of functions that include (1) regulating the normal development and function of the mucosal barriers; (2) assisting the maturation of immunological tissues; (3) controlling nutrient uptake and metabolism; and (4) preventing the propagation of pathogenic microorganisms. Changes in the profile of resident GIT bacteria may reduce their beneficial functions and affect the regulation of GI immune and inflammatory responses. Numerous clinical studies have reported that probiotics have been reported to positively influence inflammatory processes. Several strains of *Lactobacilli* and *Bifidobacteria* among others (*Staphylococcus thermophilus*) indeed have been demonstrated to reduce GIT inflammatory conditions as well as at extra-intestinal sites (e.g. musculoskeletal joints) when administered orally. The mechanism may partly involve the rescue by probiotic bacteria of a dysbiotic GIT. We have posited that microbial cohorts (e.g. in the GIT) present bacterial communities with an overwhelming accuracy at identifying pathogens/pathogenic states than do current diagnostic methodologies; this then plausibly leading to future microbe directed site-specific therapeutics. Pharmacobiotics, using nutritional and functional food additives to regulate the gut microbiome is an exciting growth area of therapeutics, developing alongside an increased scientific understanding of gut-microbiome symbiosis in health and disease. This presentation will discuss the role of probiotics and commensal bacteria in musculoskeletal diseases such as OA and RA.

Early colonisation, probiotics and allergy

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Childhood asthma and allergies are the new epidemic of the 21st century. There is increasing evidence that the microbiome plays an important role for the development of these conditions. We have performed several cross-sectional and one birth cohort study among children raised on farms and in rural environments, but not on farms, respectively. Farm children living in environments with a high microbial burden have a significantly lower prevalence of asthma, hay fever and allergic sensitisation. Their innate immune response is already boosted by prenatal exposures of the pregnant mother. Postnatal exposures also boost regulatory T cells. The most relevant exposures for farm women and their children are animal sheds and the consumption of unprocessed cow's milk. Animal sheds are very rich in microbial exposures and this diversity has been related to the protection from childhood asthma. These findings indicate that the environmental microbiome plays a significant role in shaping a young child's immune responses and its risk to develop allergies. Understanding better the underlying microbial exposures and their role for the children's microbiome may eventually help develop novel preventive strategies.

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Gastrointestinal changes in autistic spectrum disorder children receiving probiotic Delpro®

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Children with Autism Spectrum Disorder (ASD) frequently exhibit gastrointestinal distress and may have imbalances of beneficial gut microbiota, which may lead to immunological and neurological conditions. Probiotics could be a solution for some of these problems. We investigated the use of the probiotic Delpro® for children with ASD. Delpro consists of five probiotic strains with 10×10^9 cfu of probiotic cells and 8 mg lysate powder of immunomodulator Del-Immune V®, derived from probiotic strain *Lactobacillus rhamnosus* DV. Participants were recruited by the Generation Rescue Foundation for Autism Spectrum Disorder. 33 ASD children participated in the survey. Respondents provided autism treatment evaluation checklist (ATEC) scores prior to and following initiation of the intervention. Pre- and post-treatment ATEC scores were analysed using a one-sided paired t test. All analyses were completed using Minitab 16. 48% of the respondents reported decreases in diarrhoea severity and 52% reported decreases in constipation severity. Overall, 88% reported a decrease in total ATEC score. Mean ATEC values decreased from 72.8 prior to treatment to 58.3 following treatment initiation. Participants also had significant improvements in all ATEC domains: speech/language/communication, sociability, sensory/cognitive awareness, and health/physical/behaviour. These data suggest that the probiotic/immunomodulator Delpro® may have significant benefit in the treatment of gastrointestinal distress among the ASD population.

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