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for NUTRITION & HUMAN and MICROBIOTA HEALTH

2ND

SCIENCE & BUSINESS SYMPOSIUM



Abstracts

Effects of Resveratrol in a Mouse Model of Alcohol Addiction

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Alcohol addiction elicits oxidative imbalance and it is well known that polyphenols as resveratrol may have antioxidant properties. We investigated whether or not resveratrol could confer a protective potential against alcohol-induced oxidative stress.

We administered (per os) for two months 10 mg/kg of resveratrol in alcoholic adult male mice. Resveratrol metabolites as resveratrol sulfate, dihydroresveratrol glucuronide, and dihydroresveratrol sulfate but not resveratrol *per se* were found in the serum of mice administered with resveratrol. Oxidative stress was evaluated by FORT (free oxygen radical test) and FORD (free oxygen radical defense) tests. Alcoholic mice showed a high oxidative status compared to non-alcoholic mice (higher FORT and lower FORD) but resveratrol supplementation partially counteracted the alcohol pro-oxidant effects, as evidenced by FORT. We have also shown that in the liver brain derived neurotrophic factor (BDNF) elevation caused by alcohol intoxication may be reduced by resveratrol supplementation. In conclusion, a better understanding of the antioxidant protection provided by polyphenols might be of primary interest for drug discovery and dietary- based prevention of the damage associated with chronic alcohol abuse.

Putative Probiotic Lactic Acid Bacteria Isolated from Greek Protected Designation of Origin Cheeses Using Traditional and Metagenomic Microbiological Analysis

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Objective: The microbiological characteristics of indigenous Protection Designation of Origin (P.D.O.) Greek cheeses were determined by plate counting and metagenomic analysis. Three different samples of each of six cheese types were examined: Anevato, Batzos, Feta, Galotyri, Kalathaki Limnou and Kopanisti.

Methods: Amplicon metabarcoding libraries were constructed based on the V3- V4 hypervariable regions of the 16S rRNA gene. Putative lactic acid bacteria isolated by plate counting were sequenced (16SrRNA) and examined for their ability to ferment fructooligosaccharides (FOS) and galactooligosaccharides (GOS).

Results: Highest overall microbial populations by plate counting were recorded in Feta and Galotyri and the lowest in Kopanisti. Feta cheese had the highest number of lactic acid bacteria, whereas Kopanisti cheese had the least. The highest levels of coliforms, enterococci, presumptive *Listeria* spp. and presumptive *St. aureus* were all recorded in Batzos cheese, while Feta and Kopanisti contained the lowest numbers of these bacteria. In total, 8,869 unique bacterial taxa were detected, mainly belonging to Streptococcaceae and Lactobacillaceae families. *Lactococcus* and *Lactobacillus* were the most abundant genera. The most diverse bacterial profile was detected in Kopanisti and Batzos and the lowest in Feta and Kalathaki Limnou. Presumptive lactic acid bacteria isolated from the cheeses were identified to the level of genus and most likely species. Of the 33 isolates examined, 25 were *Lactobacillus* sp., six were *Enterococcus* sp., one *Pediococcus* sp. and one *Leuconostoc* sp. All but four isolates grew well on GOS (as well as on glucose), while 2/3 grew well on FOS.

Production of Fruit Based Drinks as Carriers of Probiotic Bacteria *Lactobacillus Rhamnosus* SP

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Objective: In recent years, there has been a growing interest in the development of non-dairy probiotic products due to vegetarianism, cholesterol contents of milk products, milk allergy and other factors. For example, fruits might be ideal carriers for probiotics because of their beneficial components like minerals, vitamins, dietary fibers, and antioxidants. In this study, it was aimed to produce a novel healthy fermented beverage based on sour cherry, apricot and peach juices as a probiotic bacteria carrier.

Methods: Sour cherry, apricot and peach juices were pasteurized at 65°C for 30 minutes after adjusting the fruit concentration to 35%, 40% and 50% (w/v), respectively and adding sugar of 12% (w/v). The pasteurized juices were inoculated with probiotic *Lactobacillus rhamnosus* 99, which was precultured at 37°C overnight in MRS broth, at a targeted level of 10⁹-10¹⁰cfu/mL, and then they were fermented at 37°C for 48 hours. During the fermentation, survival of *L. rhamnosus* 99, pH changes and sensory properties were evaluated at 24h intervals.

Results: Increases in the numbers of probiotic *L. rhamnosus* 99 were more than 1 log, and it was statistically significant ($P < 0.05$) in all of the juices fermented. There was no significant ($P > 0.05$) changes in pH value of the sour cherry, while pH values of the apricot and peach juices were significantly ($P < 0.05$) lower than those of the unfermented counterparts. The findings indicated that all the juice matrices and degree of acidity could have the potential for the growth and activity of the lactic acid bacteria. According to sensory evaluation, the fermented fruit juices had an almost same overall acceptability compared to the non-fermented counterparts, except peach juice, due to intense consistency. Moreover, 24 hours fermented fruit nectars were more acceptable in terms of the taste than that of the unfermented and 48 hours fermented samples. Especially sugar content of the fermented nectars was found to be more balanced by the panelists compared to the control samples.

Conclusions: In conclusion, it was observed that the fermented sour cherry, apricot and peach juices had sufficient number of probiotic bacteria to provide health benefits for their use and thus, they could be a promising alternative food matrix for the probiotic carrier in place of dairy products. On the other hand, further studies should be conducted to determine the survival and stability of the probiotic bacteria during the extended storage periods.

Gut Microbiota Composition and Immunity of Ageing Mice Supplemented with Cow Milk Containing a Different Casein Profile

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Objective: Ageing is characterised by nutritional deficiencies and functional alterations of the digestive and immune system, accompanied by altered gut microbiota composition. Cow milk containing A2 beta-casein was reported to be better tolerated in subjects with unspecific milk intolerance, in comparison to conventional milk containing A1 beta-casein. The aim of this study was to evaluate whether supplementation with milk containing A2 beta-casein could improve gut microbiota composition and immunity in an animal model of ageing mice.

Methods: Twenty-four, 20 months old Balb-c mice were fed three nutritionally balanced, isocaloric and isoproteic diets: standard (Control) or supplemented with cow milk containing either A1/A2 (Standard milk) or A2/A2 beta-casein (Test milk). At day 30, fecal samples were collected for Next Generation Sequencing (NGS) and HPLC for microbial composition and SCFA analysis, respectively. Lymphocyte subpopulations were evaluated by flow cytometry in jejunum.

Results: Beta diversity analysis highlighted specific, significant variations of faecal microbiota composition in Test milk-supplemented mice with respect to the other groups. Comparison of taxonomical assignments among groups identified Lachnospiraceae as characterizing family within the two milk-supplemented mice. Moreover, Test milk-supplemented group showed a higher content of faecal SCFA (in particular, butyrate). Immunological analysis revealed higher percentage of T-helper and B lymphocytes, paralleled by decreased numbers of T-cytotoxic lymphocytes, in mice supplemented with Test milk.

Conclusions: Taken together, results suggest a positive role of milk containing A2 beta-casein when consumed by aged mice as supplementation, both in terms of increased differentiation of T-helper and B lymphocytes and of higher concentration of beneficial microbiota-derived fermentation products.

Vitamin D Receptor Contributes to the Health Benefits of Probiotic Consumption

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Objective: Inflammatory bowel disease affect millions worldwide. Probiotics are known to improve inflammatory conditions by modulating gut microbiota, however, the exact mechanisms involved are not well-understood. Meanwhile, vitamin D receptor (VDR), besides mediation of vitamin D functions, is involved in cell differentiation, growth, anti-inflammatory actions, and a key factor for shaping gut microbiome. This work aimed to evaluate the impact of probiotic fermented milk (FM) on the inflammatory responses and expression of VDR *in vivo*.

Methods: Probiotic FM was produced with the co-culture *Streptococcus thermophilus* TH-4 and *Lactobacillus paracasei* subsp. *paracasei* F19. Animal experiments were performed with wild-type (WT) and VDR knockout (VDRKO) C57BL/7 mice in a dextran sulfate sodium (DSS) colitis model (5% in drinking water 24 h after first gavage). PBS or FM were gavaged (100 microliter) daily for 7 days.

Results: Probiotic FM showed an anti-inflammatory effect only for WT mice, worsening the inflammation in VDRKO mice. After DSS treatment, IL-6 level was significantly lower in the WT-FM+DSS group when compared with WT-PBS+DSS ($P < 0.05$). In contrast, for VDRKO mice, the IL-6 levels were dramatically high in VDRKO-FM+DSS group when compared with VDRKO-PBS+DSS ($P < 0.05$). Moreover, at mRNA level, FM increased the VDR relative expression in colon cells when compared with control groups.

Conclusions: The probiotic FM produced with the co-culture *Streptococcus thermophilus* TH-4 and *Lactobacillus paracasei* subsp. *paracasei* F19 presented a promising anti-inflammatory potential against DSS induced colitis in mice, but VDR expression is needed. Therefore, enhancing VDR levels may contribute to potential health benefits driven by probiotic consumption.

Fortunella Margarita: The Citrus Fruit of the Moment? Investigation of Bioactive Compounds: Vitamins, Macroelements and Polyphenols

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Objective: Citrus fruits are preferred in the choice of consumers. Kinkan (*F. margarita*) is unconventional citrus that has been increasing consumer interest. The aim of the study is nutritionally analyzing *F. margarita* to determination and quantification of vitamins, bioactive compounds and minerals and to verify if the fruits can be as a good option like a functional food.

Methods: Vitamins C and E, carotenoids and flavonoids were analyzed by HPLC. Polyphenolic compounds and antioxidant capacity were determined by spectrophotometry. Minerals were determined by ICP-OES. The results of the Kinkan analysis were expressed using descriptive statistics (mean ± standard deviation) with four replicates.

Results: In peel and pulp of Kinkan were detected the presence of vitamin C (2.32 ± 44.24 mg/100 g). The most expressive component of vitamin E were α -tocopherol (569.00 ± 10.20 μ g/100 g) and β -tocotrienol (66.89 ± 39.93 μ g/100 g). The polyphenol content was (98.55 ± 1.93 mg GAE/100 g). The majority flavonoids detected were apigenin (38.1573 ± 0.53 mg/100 g) and eriodictiol (36.8809 ± 0.38 mg/100 g). The antioxidant activity was (62.01%). In Kinkan fruit, the macroelements most expressive were K, Ca and Mg.

Conclusions: The Kinkan flavonoids are described as a significant promise as a skin cancer chemo preventive agent. The consumption of the fruits daily provides the organism can be contribute the necessary intake, which is economically feasible. Also, it is emphasized that the combination of high levels of phenolic compounds, antioxidant activity and potassium can be very important in diets of salt-dependent hypertensive patients.

Effect of Gluten Free Diet on Intestinal Microbiota in Pediatric Celiac Patients

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Objective: Aim of the present study is determination of time-dependent changes of microbiota according to gluten-free diet (GFD) exposure and explore a possible microbiota pattern specific to celiac disease (CD). To be able to develop recommendations for improving the effectiveness of the GFD by determining the relationship between changes in nutrient and food intake in celiac patients.

Methods: Eleven children between 4-12 years were enrolled at Gazi University. After biopsy-proven diagnosis, children were directed to dietitian to get GFD treatment. Before treatment, demographic data and history of antibiotic/probiotic usage were taken, body weight and height were measured. Each participant was followed during six-month under GFD treatment and ensured to get negative serologic markers specific to CD after six-month. Before and after the follow-up, fecal and blood samples were taken, dietary intakes were questioned by three consecutive days. Factor analysis will be performed to divide dietary patterns of children. The 16S rRNA sequencing and analysis of collected fecal samples will be done.

Results: The preliminary results of the present study will be available to presentation by the date of the congress.

Conclusions: It is expected that a possible microbiota pattern specific to pediatric CD will be identified. Results of the study can provide guidance for alternative diagnose or treatment methodologies (probiotic use, diet content, etc.). Time-dependent microbiota changes after gluten-free diet therapy, the trend of this change and the possible association of gluten-free diet with microbiota changes will enhance our knowledge with regard to increase the nutritional efficacy of GFD therapy.

Detection of New Probiotics in the Presence of Viral Gastroenteritis

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Objective: The purpose of this study was to investigate new probiotic lactobacilli in the presence viral gastroenteritis such as norovirus and rotavirus infection.

Methods: ELISA assay was used to identify norovirus and rotavirus antigen in fecal samples from 188 patients with gastroenteritis and from 40 without gastroenteritis. Lactobacilli were identified by selective media, gram reaction, colony morphology, classical identification (API) and molecular (16sRNA) tests. Acid resistance (pH 1.5-8.2), bile tolerance (0.01-0.4%), antimicrobial activity against *E.coli* ATCC 11229, exopolysaccharide (EPS) production, taurocholic and glycocholic acid deconjugation and cholesterol removal of the isolated were identified.

Results: Lactobacilli were identified in 31 of the 92 fecal samples were found positive for rotavirus antigen (33.69%) and in 46 of the 107 fecal samples were found positive for norovirus antigen (42.9%). The EPS production ranges were 32.24-148.14 mg/L. The cholesterol elimination rates ranged between 6.21-41.16%. Furthermore, a positive and strong correlation was determined between EPS production and the presence of cholesterol ($r=0.882$, $P<0.001$). The deconjugation rates for the sodium glycocholate group was higher than the sodium taurocholate group. Norovirus (+) strains had higher EPS production, deconjugation and cholesterol removal compared to in the rotavirus (+), norovirus (-), rotavirus (-) and without gastroenteritis. Significant differences were observed among groups in all parameters ($P<0.05$).

Conclusions: Given the increased number of norovirus and rotavirus cases in Turkey and worldwide, it is very crucial to add new probiotic bacteria in the diets of children with viral gastroenteritis to improve the staminal functions.

Anti-campylobacter Effects of Probiotics In Vitro and In Vivo

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Objective: To investigate the effects of probiotics on growth of campylobacteria in nutrient agar medium and on campylobacter persistence in the intestine of animals.

Methods: The antagonistic activity of probiotic cultures *Enterococcus faecium* L3 (Laminolact) and *Lactobacillus acidophilus* (Acipol) against 5 strains of *Campylobacter coli* and *C.jejuni* taken the bacterial cultures collection of Pediatric Research and Clinical Center for Infectious Diseases was studied using the double-layer agar method with different doses of probiotics 5-8 lg CFU/mL.

The experiments in vivo were performed on white outbreed mice, which were injected with *C. jejuni* taken in a dose of 10 lg CFU per animal (i.p.). One day before and for 5 days after infection the animals got probiotics Laminolact (group E) or Acipol (group L) in dose 7 lg CFU /mouse for 5 days. Animals of the control group (C) did not get probiotic.

Results: Probiotic enterococci and lactobacilli in minimal concentrations 5 and 6 lg CFU/mL, correspondently, inhibited growth of all campylobacter strains. After infection, dyspepsia symptoms were not noted. But the weight in the control group gained worse than in groups with probiotic therapy. On the 14th day of observation, *Campylobacter jejuni* were found in 50.0%, 33.1% and 62.5% in E, L and C groups, respectively.

Conclusions: Campylobacter spp. are sensitive to the probiotic lactobacilli, enterococci and their metabolites when they grow in the culture medium. Various efficacy of probiotics for the treatment of campylobacter infection has been proven, which should be considered when prescribing therapy.

Microbiota Changes in Children With Viral Gastroenteritis

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Background: Microbiota changes in viral gastroenteritis (VG) is of considerable importance. There is some data showing that viral infection can lead to prolonged dysbiosis and intestinal symptoms retention. This study aimed to possible establish microbiota changes and immune response due to rotavirus infection that can lead to formation of functional gastrointestinal disorders in children.

Methods: The study included 143 children aged 1-7 years with rotavirus (n = 60), norovirus (n = 55) and rota-norovirus gastroenteritis approved with PCR in feces. The study group included 34 boys (51.1%) and 26 girls (48.9%), the average age of which was 2.9 ± 0.9 years. Immune status (definition of the main subpopulations of leukocytes, evaluation of interferon status, determination of the concentration of serum immunoglobulins) was carried twice in the 3rd-5th and 18th-21st days of the disease. Evaluation of intestinal microbiocenosis was carried in the same periods as immune response with both PCR-RT and cultural methods. The convalescents of RI were observed outpatiently in Saint-Petersburg Pasteur Research Institute for 24 months (with clinical examinations 1 time in every 3 mo) with the purpose of revealing FGD in accordance with Rome IV criteria.

Results: FGD were diagnosed in 14 (23.3%) convalescents of RI. Changes in microbiological and immunological parameters were found. Microbiota changes in RI were characterized with a significant increase in the level of opportunistic *Bacteroides fragilis*, decrease in the number of symbiotic *Lactobacillus* spp., as well as the obligate microorganisms *Bacteroides thetaiotaomicron*, *Faecalibacterium prausnitzii*, which are difficult to cultivate on ordinary nutrient media. This parameters has also shown high level of statistical differences between children with and without FGD. In the period of convalescence, the ratio of *Bacteroides fragilis*/*Faecalibacterium prausnitzii*, which reflects the degree of severity of anaerobic imbalance, was highly informative. These parameter was significantly increased in convalescents who had formed FGD ($P=0.01$). Study of immune status, has shown that in acute period of RI more frequently children had virus-induced IFN production suppression ($P=0.03$), and CD8 lymphocytes level decrease ($P=0.05$). Children with FGD after RI had an increase in the level of CD25 + lymphocytes ($P=0.05$), and relatively lower levels of serum IgA ($P=0.04$).

Conclusions: An increased risk of FGD formation is observed in children after RI if decrease in the number of symbiotic *Lactobacillus* spp., *Bacteroides thetaiotaomicron*, *Faecalibacterium prausnitzii*, suppression of virus-induced IFN production, decrease in CD8 lymphocytes in acute period of VG, an increase of CD25 lymphocytes during the convalescence period of RI.

NOD2 Promotes Host Defense and Recovery from Candida Tropicalis Infection During Experimental Colitis

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Objective: Increasing evidence supports the importance of mycobio-microbiome interactions in IBD. NOD2 recognizes fungally-derived chitin; our goal was to determine the potential role of NOD2 in fungally-infected colitic mice.

Methods: NOD2-deficient (*Nod2*^{-/-}) and wild-type (WT) mice were infected with *Candida tropicalis* (*Ct*), a fungal species prevalently found in IBD patients, and subjected to 5d DSS to induce colitis, followed by 1-wk recovery; disease activity was monitored throughout experiment. Colons and mesenteric lymph nodes (MLNs) were harvested to assess histologic evidence of colitis and quantify group 3 innate lymphoid cells (ILC3s) and ILC3-derived IL-17 by FACS. Colonic *Il17* and *Il22* were measured by qPCR and fecal samples collected to measure fungal load.

Results: *Ct* did not exacerbate colitis in DSS-treated WT mice; however, *Ct*-infected *Nod2*^{-/-} mice had a higher fungal burden and delayed recovery versus *Ct*-infected WT controls, suggesting a protective role of NOD2 in colitic mice with *Ct* infection. *Ct*-infected *Nod2*^{-/-} mice also expressed a dramatic reduction in colonic IL-22 and IL-17, cytokines important in maintaining epithelial barrier integrity, and IL-17+ILC3s, known to clear opportunistic fungal infections, were significantly decreased in colons/MLNs of *Ct*-infected, DSS-colitic *Nod2*^{-/-} versus WT mice, indicating that delayed fungal clearance and recovery of *Nod2*^{-/-} mice may be due

to their inability to mount appropriate, protective type-3 immune responses.

Conclusions: Specific opportunistic fungal infections impede gut mucosal healing, and NOD2 promotes recovery of colitis and resolution of inflammation, possibly via an IL-17-mediated mechanism.

Healthy Protection of Bergamot is Linked to the Modulation of Microbiota

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Objective: This study aimed to evaluate a new food grade delivery system of bergamot, Bergamot Phytosome®, and its effects on human microbiota correlated to cardiovascular health, obesity and gastrointestinal disorders.

Methods: A simulated gastric and duodenal human digestion of Bergamot Phytosome (Vazguard™) was performed *ex-vivo* before adding it to the batch culture system. Fecal samples were obtained from 3 healthy women (45–53 y). Individual fecal slurries (1% w/v) were inoculated to the batch-culture systems containing basal nutrient media and the digested Bergamot Phytosome (1000 mg/L) or a control solution. After incubation at 37°C in anaerobic condition DNA was extracted and a 16S Metagenomic Sequencing Analysis was performed.

Results: In this experimental model the modulation of microbiome diversity was observed after *ex-vivo* Bergamot Phytosome treatment. 25 different phyla were identified, of which 4 major phyla were modulated: *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*. A decreased ratio of *Firmicutes/Bacteroidetes* and increased of *Proteobacteria* were observed indicating an intestinal microbial and cardiovascular health positive modulation.

Regarding genus levels, 418 different genera were identified. 8 major genera (62%) were modulated: *Escherichia*, *Serratia*, *Bacteroides*, *Prevotella*, *Enterococcus*, *Bifidobacterium*, *Blautia* and *Faecalibacterium*.

Conclusions: For the first time, the modulation of microbiome was associated to Bergamot Phytosome supporting its clinical efficacy in CVD e obesity.

Effects of B-Fructans Fiber on Bowel Function: A Systematic Review and Meta-analysis

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Objective: The aim of this systematic review and meta-analysis was to assess the effects of beta-fructans on bowel function in humans.

Methods: A comprehensive literature search using PubMed and EUPMC was performed on stool parameters (frequency, consistency and weight) followed by a meta-analysis. A meta-regression was also conducted, including the impact of degree of polymerization (DP), dose, duration, age and body mass index (BMI).

Results: The search resulted into 2343 hits that were screened on relevance. Forty-seven publications were analyzed in detail which resulted into 31 cases on stool frequency, 6 cases on stool consistency, 12 cases on stool wet weight and 6 cases on stool dry weight. All beta-fructans contributed to increase in stool frequency ($+0.28 \pm 0.06$ defecations per day). This was mostly explained by short-chain fructans (DP <10) and not by long-chain fructans (DP ≥ 10). The meta-regression describes a relationship between the frequency of bowel movements and the BMI.

In addition, stool consistency, as reported by the Bristol Stool Scale, was positively impacted by beta-fructans. The effect was more outspoken with short-chain beta-fructans. No effect was observed on stool dry weight while stool wet weight was increased when using beta-fructans, with most prominent effect due to beta-fructans with DP <10.

Conclusions: Regular bowel movements are an important factor affecting the quality of life and they could be achieved by consuming more dietary fiber. When the intake of dietary fiber is insufficient, the consumption of foods containing short-chain beta-fructans can contribute to an improved bowel function.

Compliance to Probiotic Therapy in Irritable Bowel Syndrome in Clinical Practice: A Real-life Study

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Introduction: Probiotics have been evaluated in multiple clinical trials on irritable bowel syndrome (IBS) showing efficacy on different IBS-related symptoms. Among them, the multistrain probiotic VSL#3 (manufactured by Nutrilinea Srl and distributed by Ferring SPA) has been the object in clinical trials evaluating its administration for 4 to 8 weeks. However, whereas in clinical trials patients are closely monitored for compliance, in real-life setting long term compliance could be low. Furthermore, in many countries, probiotics are fully paid by patients and the cost of therapy could further limit the compliance to probiotic therapy.

Aims and Methods: This is a single-center, observational, prospective study to evaluate the compliance to prescription of probiotic therapy in real life and to identify factors able to influence adherence to therapy. Patients diagnosed with IBS according to Rome IV criteria and receiving a clinical prescription of VSL#3 for their IBS symptoms were evaluated for eligibility. Patients providing informed consent received a diary at the start of therapy to evaluate safety and effect of treatment for two months. After two months a final visit (at clinic or by telephone) was made to assess compliance and eventual reasons for discontinuation.

Results: Fifty patients (mean age $41 \pm SD 14.4$ years, 26% males) have been enrolled and 49 completed the planned follow up. IBS subtypes are distributed as following: 44% diarrhea, 42% constipation and mixed in the remaining cases. Eighty-six percent of patients received a 4-week prescription of one sachet per day. Sixty percent of patients resulted adherent in the FAS population. Among the 20 patients with reduced compliance, 5 assumed less than 50%, 12 assumed 50% and 2 assumed more than 50 but less than 80% of prescribed doses. Principal reasons of not adherence among the 20 patients are the price of the product (40%), mild adverse events (AEs) (30%) and poor appreciation of flavour (15%). Furthermore, one patient (5%) forgot to take the treatment, one (5%) stopped the treatment for inefficacy and, for the patient who was lost at follow up (5%), the reason was not available. About AEs, 20% of patients experienced at least one (only one patient two AEs). All AEs were mild, and they were: bloating (6/10 patients), constipation (2/10 patients) and flatulence (3/10 patients). The AEs were considered related to treatment in 9/10 cases and they were a reason for discontinuation in 6/10 cases. All AEs completely resolved without sequelae. No serious adverse events have been reported. Sixty-two percent of patients who assumed the therapy reported overall satisfactory benefit on their IBS symptoms with the prescribed therapy.

Conclusions: According to our results, despite a good safety profile, 60% of patients assumed all the prescribed probiotic therapy in real life setting with reported overall satisfactory benefit. The main reasons for lack of compliance were price of the product, mild AEs (mainly bloating) and low palatability.

Probiotics in Metabolic Syndrome

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Objective: The aim of this study was to investigate the importance of intestinal microbiota and probiotics in patients with metabolic syndrome.

Methods: Lactobacilli and Bifidobacteria were identified by selective media, gram re-action, colony morphology, biochemical identification by API and 16sRNA tests from feces of 42 patients diagnosed with metabolic syndrome and 20 healthy individuals. Acid resistance (pH 1.5-3.5), bile tolerance (0.01 and 0.2%), antimicrobial activity against *E.coli* ATCC 11229 and *P. aeruginosa* ATCC 27853, exopolysaccharide (EPS) production, bile deconjugation and cholesterol assimilation of the all isolated strains were identified.

Results: Eight Lactobacilli and 2 Bifidobacteria strains were isolated from 42 feces samples with metabolic syndrome. 16 Lactobacilli and

8 Bifidobacteria strains were isolated from 20 feces samples of healthy individuals. The low pH resistance and bile tolerance of strains were determined. EPS production ranges were 31.10-88.52 mg/L. The cholesterol assimilation rates ranged between 8.66-52.06%. A positive and strong correlation was determined between EPS production and cholesterol assimilation ($r=0.674$, $P<0.005$). The glycocholate rates was higher than the taurocholate in all strains. *Lactobacillus* spp. had higher EPS production, deconjugation and cholesterol assimilation compared to in the *Bifidobacterium* spp.

Conclusions: In this study, probiotic flora in individuals with metabolic syndrome was found to be significantly reduced, probiotic specificity of bacteria obtained from healthy individuals was found to be better. Given the increased number of metabolic syndrome cases in worldwide, it is very crucial to add probiotic bacteria in the diets of patients with metabolic syndrome to improve the vital functions.

Obesity Management with Probiotics: Modulation of Gut Microbiota Composition and Body Weight Control

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Objectives: Apart from diet, hygiene and genetics, the gut microbiota play an important role in the emergence of obesity, through a dysbiosis status. We investigated whether gut microbiota of rats fed on Cafeteria-diet (CAF) changes compared to rats fed on Standard CHOW diet and if daily probiotics administration shows an effect on weight and gut microbiota.

Methods: Groups of Wistar-male rats were considered: CHOW-control, CAF, CAF plus probiotics (CAF+P). *Lactobacillus plantarum* IMC510 was administered for 84 days (10^8 cells/die). Weight and food consumption and microbiota composition, using Real-Time PCR, were examined.

Results: The obesity-induced Cafeteria-diet cause in rats an increase in *Firmicutes* and a decrease in *Bacteroidetes* levels, with a significantly higher body weight in CAF group compared to CHOW. After 84 days, CAF+P showed 12% of reduction in body weight compared to CAF. On the other hand, CAF caused a significant decrease in *Bacteroidetes-Prevotella-Porphyrionas* spp. related to the obesity status, while the probiotic treatment balanced this level and significantly increased *Lactobacillus* spp. and *Bifidobacterium* spp. in CAF+P.

Conclusions: Probiotics supplement can represent a valuable support in obesity management

A Randomized Controlled Trial on the Effects of a Synbiotic Compound on Markers of Inflammation in Obese Adolescents

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Objective: Growing body of evidence has proposed significant association between alterations in gut microbiota with obesity and related markers of inflammation. This trial aims to assess the effects of a synbiotic supplement on inflammatory markers in obese adolescents.

Methods: This randomized controlled trial was conducted among 60 participants aged 12 to 18 years. They were randomly assigned into two groups of equal number for receiving synbiotic or placebo for eight weeks. The synbiotic medication contained a combination of viable freeze-dried *Lactobacillus Casei*, *Lactobacillus Rhamnosus*, *Streptococcus Thermophilus*, *Bifidobacterium Breve*, *Lactobacillus Acidophilus*, *Bifidobacterium Longum*, *Lactobacillus Bulgaricus* of human origin with prebiotics (fructo oligosaccharides), as well as vitamins E, A, and C.

Results: Overall, 55 participants completed the study, i.e. 28 in the synbiotic group and 27 in the placebo group. At the end of the trial, higher decrease was documented in BMI Z-score, waist

circumference, and waist-to-hip ratio of the synbiotic group than in the placebo group. Moreover, the synbiotic group had significant decrease in mean levels of high-sensitive C-reactive protein and interleukin-6. These changes remained significant after adjustment for potential confounding factors including anthropometric indices.

Conclusion: The findings of this trial suggest that synbiotic supplementation might have beneficial effects on markers of inflammation in obese adolescents. This beneficial effect was independent of the change in anthropometric measures; future studies with longer follow up are necessary to document the clinical impacts of this finding.

Properties of Lactic Acid Bacteria Isolated from Fermented Cereal Foods

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In recent years, interest in fermented products is increased with the notice that the providing benefits in human health of consuming fermented products. Cereal-based fermented products are produced and consumed in different areas of the World, especially in Middle Asia, Middle East and Africa. The major fermented cereal-based products can be classified as rice-based fermented foods (e.g. idli, dosa and dhokla), wheat-based fermented foods (e.g. soy sauce, tarhana and kishk), corn-based fermented foods (e.g. ogi, pozol and kenkey), sorghum-based fermented foods (e.g. injera and kisra) and cereal-based fermented beverages (e.g. boza, sake, chichi and mahewu). It is indicated that lactic acid bacteria (LAB) commonly isolated from cereal-based fermented products belong to *Lactobacillus*, *Pediococcus* and *Leuconostoc* genus, while *Lactobacillus* species are usually taking over the predominant flora. *Lactobacillus plantarum* and *Lb. fermentum* are dominantly take part in most cereal-based fermentations among LAB. It is also reported that some other LAB such as *Lb. brevis*, *Lb. delbrueckii* ssp. *bulgaricus*, *Lb. rhamnosus*, *Lb. pentosus* and *Lb. paracasei* ssp. *paracasei*, *Lb. salivarius*, *Lactococcus lactis*, *Pediococcus pentosaceus*, *P. acidilactici*, *Leuconostoc mesenteroides* and *Weissella confusa* isolated from various cereal-based fermented products. Hence, it could be concluded that cereal-based fermented foods are good sources of probiotic bacteria.

Keywords: cereal, probiotic, lactic acid bacteria, fermentation

The High Polymerization Degree Influences the Inulin Bioaccessibility in Durum Wheat Spaghetti

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Objective: In order to evaluate how the polymerization degree (PD) could influence the permanence of inulin in small intestine, two inulins at high and low PD were used at 4% (w/w) concentration to enrich durum wheat spaghetti. They were characterized and inulin bioaccessibility assessed by an *in vitro* gastrointestinal digestion.

Methods: Two types of inulin: cardoon roots (CRI high PD) and chicory (CHI low PD) were used for the spaghetti production. The inulin analysis in cooked spaghetti was performed after acid hydrolysis (2 h, 70°C) and soluble carbohydrates analyzed (Dionex HPLC-PAD). Digestion performed according to D'Antuono et al 2016.

Results: Inulin content was similar (CRI, 8.1 and CHI, 8.2 mg/g FW) while after digestion its amount changed. In CRI spaghetti, bioaccessibility was lower (39%) than CHI spaghetti (99%), indicating that the drastic digestive condition hydrolyzed completely the inulin increasing the fructose release, while in CRI spaghetti the inulin was preserved in pasta.

Conclusions: These preliminary results show that the inulin bioaccessibility was influenced by its degree of polymerization, suggesting that the accumulation in pasta makes inulin directly available in the last intestinal tract as substrate for health promoting actions.

Effects of SYN BIO[®] Probiotic Formulation on Pathogens Isolated from Chronic Ulcerative Lesions: In Vitro Study

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Objective: The aim of this *in vitro* study was to isolate and classify pathogenic strains from difficult healing wounds in order to evaluate whether probiotics have a competitive effect against them. The efficacy of SYN BIO[®], probiotic formulation containing *Lactobacillus rhamnosus* IMC501[®] and *Lactobacillus paracasei* IMC502[®], was tested.

Methods: Pathogens were isolated from chronic ulcerative lesions and identified by morphological, biochemical and molecular techniques. SYN BIO[®] was investigated for antimicrobial activity, minimum inhibitory concentration (MIC), co-aggregation and adherence capacity to human keratinocytes and also tested in combination with some medical devices, using an *in vitro* model, to simulate a treatment of chronic ulcerative wound infection.

Results: SYN BIO[®] demonstrated an inhibitory action against all the pathogens and the percentage value of co-aggregation increased over time. MIC results indicated that a concentration of 2.34×10^7 CFU/dose of SYN BIO[®] was able to inhibit the pathogens growth. The adhesion percentage of probiotics to human keratinocytes was 43%, highlighting the possibility to create a protective environment preventing pathogens' biofilm formation.

Conclusions: This study highlights the opportunity to successfully use the probiotics as topical complement to conventional medication representing a new therapeutic approach in the treatment of chronic ulcerative lesions.

Skin Microbiota and Bacterial Biofilms in Patients With Atopic Dermatitis and in Healthy Human Hosts

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Introduction: Skin diseases such as atopic dermatitis (AD) depends on impaired immunological and microbial balance of microbiota. AD is a recurrent and chronic skin disease, which processes with colonization of the skin by microorganisms.

Aim: Comparative analysis of skin bacterial microbiota and its biofilms in patients with AD and in healthy human hosts. Investigations of morphophysiological properties and ultrastructural features of human skin samples with microorganisms and its biofilms by electron microscopy.

Materials and Methods: Skin samples of 107 patients with AD and of 80 control samples of skin were examined. The affiliation of species of bacteria in skin microbiota was carried out by standard methods. Human skin samples and bacterial biofilms were detected by electron microscope.

Results: An electron microscopy analysis revealed that bacteria colonized the upper layers of the epidermis. In healthy skin most part of cells was composed of physiologically active, dividing cells. The microbiota in AD formed biofilms includes complex of additional protective structures of a biopolymer matrix and a surface membrane-like membrane. *S. aureus*, isolated from patients with AD, are poly-resistant to many antibacterial drugs, including methicillin.

Conclusion: Electron microscopy shows that in healthy skin microbiota there is a suppression of the formation of biofilms. In the AD skin were found well-developed microbial communities – biofilms. To suppress the growth of biofilms in skin is necessary to destroy the complex of protective structures (surface film, intercellular matrix), that prevent the exposure of antibacterial drugs and immune protection factors.

Periodontal Diseases & Systemic Organs Interaction

Dr Piero Simeone. Active member of Italian Academy of Prosthetic Dentistry

Microbes appear and affect every aspect of human life. The human oral cavity contains a number of different habitats. Synergy and interaction of variable oral microorganisms help human body against invasion of undesirable stimulation outside. However, imbalance of microbial flora contributes to oral diseases and systemic diseases. Oral microbiomes play an important role in the human microbial community and human health. The use of recently developed molecular methods has greatly expanded our knowledge of the composition and function of the oral microbiome in health and disease.

Studies in oral microbiomes and their interactions with microbiomes in variable body sites and variable health condition are critical in our cognition of our body and how to make effect on human health improvement.

Oral Microbiota & Periodontal Diseases: Periodontal diseases frequently occur in human mouth, and can be divided into two categories, gingival diseases and periodontitis. Periodontal diseases cause destruction of periodontium (tooth-supporting tissues such as gingiva and alveolar bone) and constitute a potential risk factor for certain systemic diseases.

Oral cavity is a natural microbial culture medium, in which periodontal tissue has complex anatomy and organizational structure, physical and chemical properties, which indeed provides good conditions for growth of microorganisms.

Oral Microbiota & Gastrointestinal system diseases More and more gastrointestinal system diseases are proved to be associated with oral microbiomes. Inflammatory bowel disease (IBD) is one of the earliest to be found. Nowadays, there're more convincing evidences for correlations between liver cirrhosis, gastrointestinal cancers and oral microbiomes.

Oral Microbiota & Diabetes: Diabetes mellitus is characterized by hyperglycemia, inflammation and high oxidative stress, which can lead to systemic complications. There is a bidirectional relationship between periodontal disease and diabetes.

Microbiome plays a key role in homeostasis and affects several pathologic processes, including diabetes.

Diabetes is a risk factor for periodontitis and increases disease severity. In type I diabetics, an increase in the severity of periodontal diseases has been shown across most age ranges. Age itself has been shown to be a risk factor for periodontitis, and is likely to be a confounder. Type II diabetes has also been shown to be a risk factor for periodontal diseases. A study of association between diabetic status and periodontal conditions in 1,342 individuals showed increased risk for periodontitis

Interdisciplinary Therapy: The new classification of periodontal diseases and conditions also includes systemic diseases and conditions that affect the periodontal supporting tissues.

Such conditions are grouped as "Periodontitis as a Manifestation of Systemic Disease", and classification should be based on the primary systemic disease.

There are, however, common systemic diseases, such as uncontrolled diabetes mellitus, with variable effects that modify the course of periodontitis. These appear to be part of the multifactorial nature of complex diseases such as periodontitis and are included in the new clinical classification of periodontitis as a descriptor in the staging and grading process. Although common modifiers of periodontitis may substantially alter disease occurrence, severity, and response to treatment, current evidence does not support a unique pathophysiology in patients with diabetes and periodontitis.

Full-mouth scaling and root planing (FM-SRP) resulted in clinical and microbiological improvement 6 weeks post-treatment, but produced a moderate systemic acute-phase response including elevated inflammatory mediators 1 day post-treatment.

However this dental clinical approach should be accompanied by the reduction of systemic risk factors (smoke, nutrition, diabetes) and in cooperation with the interdisciplinary medical disciplines.

All periodontal patients must be included in a systematic recall programme to maintain the achieved outcomes.

Nanoemulsion of *Passiflora Edulis* as Dietary Preservative Proposal

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There is serious growing concern about the effects of the use of various chemical additives in food products. This situation has encouraged the research of new natural agents that are more efficient and healthier to complement the food preservation. *Passiflora edulis* has a variety of antioxidant compounds responsible for its bioactive properties, which justifies its use in this work to develop an oil-in-water nanoemulsion for natural preservative in foods. The main objective of this work was to study potential of *P. edulis* extract for food preservation and develop an oil-in-water nanoemulsion. The formulation was composed by Hydrogenated PEG-40 Castor oil; *P. edulis* extract at 0.5% (w/w); Span 60 and Sunflower oil and ultrapure water. Physicochemical characteristics as size, polydispersion index (PDI) and zeta potential were analysed in Zetasizer. Thermal stress and stability tests were conducted too. Contents of flavonoids compounds were determined, the phenolic compounds was determined by Folin-Ciocalteu method and antioxidant activity was analysed using DPPH method. The average size of nanoemulsion was 70 nm and mean zeta potential value was -22 mV. The formulation showed physicochemical stability after 2 months and when submitted to 70°C, with no statistically significant variation of size and zeta potential. Nanoemulsion was considered monodisperse since values of PDI were lower than 0.3. Analyses of extract indicates that total phenolic values were 51.25 (± 0.41) mg GAE/g, flavonoids content was 14.52 (± 0.25) mg/g and antioxidant activity was EC50 = 25.2 mg/mL. These results suggest the use of *P. edulis* nanoemulsion as a natural preservative in foods.

Keywords: DPPH, preservative, food

Corn (ZEA MAYS L). Extracts as Source of Active Compounds with Promising Effects in Reducing Trigeminal Pain Through Microbiota Modulation

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Objective: Corn (*Zea mays*) is a valuable source of polyphenols with potential healthy properties. The purpose of the study was: (1) a chemical characterization of different corn varieties, including yellow, pigmented and hybrid corns; (2) to assess the role of anthocyanins from pigmented corns in modulating trigeminal pain and the gut microbiota balance.

Methods: Spectrophotometric methods were used to characterize the total polyphenol and anthocyanin content in corn extracts and to measure their antioxidant activity. Trigeminal pain was stimulated by unilateral injection of Complete Freund's Adjuvant in male rats that had received for 10 days extracts from: (1) yellow corn, (2) anthocyanin-rich purple corn, or (3) water as controls. Microglia/macrophages activation was analyzed by immunohistochemistry. The bacterial taxonomic profile was studied from fecal samples by 16S rRNA profiling protocol.

Results: Corn varieties showed an interesting polyphenol profile, with a total content ranging between 3.61 and 11.12 mg/g dry weight (gallic acid equivalents); this content was well correlated with the antioxidant activity ($R^2 > 0.90$). If compared with yellow varieties, purple corn administration prevented orofacial allodynia and the trigeminal infiltration of macrophages. In addition, purple

corn modulated the gut microbiota composition toward an anti-inflammatory taxonomic profile.

Conclusions: Selected corn varieties could represent a dietetic source of polyphenols with anti-inflammatory and antioxidant activity able to prevent trigeminal pain through different mechanisms. Further studies are in progress to investigate the correlation between the polyphenol profile and the biological activity of different corn varieties, with particular attention to the possible involvement of the gut-brain axis.

Effects of the Dietary Intervention with Green Dwarf Banana Flour on the TNBS Relapse Model of Intestinal Inflammation

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Objective: Inflammatory bowel disease (IBD) includes Ulcerative colitis and Crohn's disease, two inflammatory processes in gastrointestinal tract. Current pharmacological treatments are associated with several side effects, particularly after long-term use. Different functional foods have been studied as complementary treatment for IBD in humans, including dietary intervention with prebiotics. In previous study we demonstrated diet-rich with green dwarf banana (*Musa sp* AAA) acted as intestinal anti-inflammatory product in acute phase of experimental intestinal inflammation, modulating oxidative stress and short-chain fatty acid production by intestinal microbiota. Based on that, we decided to study the effects of dietary intervention containing green dwarf banana flour in the relapse phase of TNBS (trinitrobenzenesulphonic acid) model of inflammation in order to simulate IBD human relapse.

Methods: Rats were divided in 3 groups: healthy (standard diet), TNBS-control (standard diet) and treated (green dwarf banana flour diet by 50 days before second induction and 2 days thereafter). Intestinal inflammation was induced twice: first in 36th day after onset of treatment and second, 14 days after the first induction using 0.25 mL of TNBS (40 mg/mL) solution in ethanol 50%. All animals were killed 48h after second induction and colons were analysed macroscopically and biochemically.

Results: Green dwarf banana flour was able to decrease damage macroscopic score and this effect was associated with a decrease in myeloperoxidase activity when compared with TNBS-control.

Conclusion: In conclusion, remission or activity did not change SCFAs profile in UC or CD patients.

The Use of Fermented Sour Soba Rich in Prebiotics, Probiotics and Postbiotics as Part of Sustainable Diet. Recent Finding Based on Randomized Controlled Trials (RCT)

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The popular use of probiotic foods is blooming in line with public awareness for healthy food consumption. Traditional fermented foods are rich sources of natural probiotic bacteria strains, prebiotics and postbiotics and are good candidates for microbiome-based therapy targeting the gut homeostasis. Sour sobya (SS) is rice based fermented porridge has acidic pH of 3.5 in which *Lactobacillus* and yeast exist in symbiotic relationship. In vitro, the bioactive peptides of SS strongly inhibited the growth of seven virulent microbes. The bioactivities of SS were assessed in series of randomized controlled trials (RCT) on healthy children, adolescents and adults. The SS was served daily in portions providing dosages of 4.4 ± 1 billion colonies forming units for a duration of 3 weeks. The stool, urine samples and blood samples were collected before and at the last day of each trial for subsequent microbiological, biochemical and immunological tests were completed and the data were subjected to statistical analysis.

Results: The results were in favor of SS intake compared to placebo or the control group. The intake of SS was associated with significant increase in total fecal *Lactobacilli* with concurrent

significant decreases in the count of total Enterobacteriaceae. Significant increases in the production of the fecal short chain fatty acids with lowering in the fecal pH. The urinary excretion of thio-barbituric acid, biomarker of oxidative stress was reduced significantly among the participants belonging to all age groups. The urinary excretion of total hydroxyproline index, biomarker of growth velocity increased dramatically in all children. The gut barrier function improved among adolescents. In adults, the levels of lipid profile parameters improved significantly as compared to the respective baseline levels. The mean plasma total homocystein; concentration was reduced significantly. The systemic immunoglobulin A concentrations increased significantly among the children and the adult groups.

Conclusion: Sour sobya [SS] is a potential microbiome-based therapeutic agent in clinical and molecular nutrition. Investigations aiming product development with prolonged shelf life stability is warranted.

Comparative Studies Concerning the Bioactivity of Peptides Obtained by Kefir-kombucha Fermentation of Bovine Colostrum

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Objective: The aim of this study was to perform a comparative evaluation of peptides bioactivity obtained from colostrum fermented with Kombucha, with Kefir grains or with a combination of these two microorganism consortia.

Methods: Colostrum fermentation: at 30°C, for 96 hours. Variants of fermentation tested: with artisanal Kefir grains (1), with Kombucha (2) and with a combination of these two consortia (3). Control: unfermented colostrum. Peptides of 3000 Da separation: by centrifugal filtration. Quantitative determination of released peptides: using bicinchoninic acid (BCA) assay kit. Peptides antioxidant activity: by the ABTS and DPPH assays. Capacity to inhibit the angiotensin-converting enzyme (ACE) activity: using hippuryl-L-histidyl-L-leucine as substrate. The in vitro cytomodulation: by MTS assay on NCTC fibroblasts and on colon adenocarcinoma HT29 cells.

Results: All peptide extracts presented antioxidant activity and a good ACE inhibition, suggesting antihypertensive potential. The ABTS, DPPH and ACE results were significantly higher (> 10%) in kefir - Kombucha fermented colostrum (3) compared with unfermented colostrum. The biocompatibility on normal fibroblasts showed a good viability (> 80%), at concentrations between 0.1 and 1 mg/mL. The highest tumoral HT-29 cell growth inhibition was determined by peptides obtained from colostrum fermented with both microorganism consortia.

Conclusions: All data demonstrated that colostrum fermented with both Kombucha and kefir consortia represents a valuable source of peptides with improved biological activities, compared to those obtained from unfermented (control) or fermented colostrum with a single microbial consortium. Specific bioactivity and functionality of isolated peptides make them suitable as ingredients in functional foods and nutraceuticals.

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Phytochemical and Nutritional Characterization of Psidium Guajava L. (GUAVA) Leaves Extract

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Introduction: *Psidium guajava* L. leaves have been used for treatment of many diseases by population. In many pharmacological studies, extraction of the bioactive components are performed in water, what poses some limitation for the stability of the components. Here, we sought to characterize an ethanolic extract of *P. guajava* leaves. Therefore, the purpose of this work is to analyze the phytochemical profile of *P. guajava* leaves extract aiming standardize this raw material for the develop phytoformulations.

Methods: *P. guajava* leaves extract were made out of whole leaves through the process of maceration in ethanol. Tannins were measured in the extract as an index of antinutritional factors. Phytochemical profile was evaluated using high-performance liquid chromatography (HPLC). Elements composition was determined in ICP-OES from samples chemically digested with a mixture do hydrogen peroxide (20% v/v) and nitric acid (80% v/v) for 24 hours at 150°C.

Results: Tannin content was 0.20 ± 0.007 mg/g (expressed as catechin). HPLC chromatogram revealed fifteen compounds identified by R_t . The three major compounds were rutin, quercetin and galocatechin detected at retention times 24.8; 29.5 and 30.6 minutes, respectively. From the measured elements by ICP-OES, the majors were: K (13,422.5 mg/g), P (1,333.6 mg/g) and S (2,298.2 mg/g).

Discussion: Results suggest that the extract is low in tannins and chromatographic analyzes indicated that the extract is rich in phenolic compounds and flavonoids. Micronutrient analysis revealed expected amounts do K, P and S in the extract and no exciting levels of Cd and Pb.

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Targeted Short-term Probiotic Intervention Effectively Restores the Function and Structure of Damaged Kidney in Gout as Detected by Ultrasonography

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Objective: Gout is extremely spread pathology involving kidney, liver and joints, is met more often in men. Gout has deep interplay with metabolic syndrome (MetS), including obesity and leads to chronic renal failure in every fourth patient. Ultrasound (US) can clearly determine symptoms of gouty nephropathy also at early stages, can be used as main method for screening and monitoring treatment. Modulating gut microbiome has great potential to improve metabolic health in gout.

The Aim was to study the efficacy of individualized probiotic intervention on signs of gout and MetS by monitoring sonographic diagnostic markers of gouty nephropathy.

Methods: We included twelve patients (age 32-68 y) with BMI > 30, waist circumference (WC) > 110, who met criteria of MetS with hyperuricemia (the levelsof uric acid over 400 μmol/L) and increased blood pressure. All patients underwent extensive general clinical, lab tests; multiparameter US of kidneys, joints, liver, measuring visceral fat (FV). The typical symptoms of gouty nephropathy in sonographic display were precisely documented. Patients were given probiotics (*B. animalis* VKB/*B. animalis* VKL strains at a dose 10⁸ CFU daily during 10 d); in cases of liver fibrosis - *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKB, *L. casei* IMV B-7280 (considered individually according to the knowledge on treatment mechanics obtained from in vivo in vitro studies and existing evidence).

Results: We registered ultrasound signs of symptoms of gouty nephropathy in all patients, namely detection of small hyperechoic inclusions in parenchyma (chalk-stone), increase in resistive index (RI) in segmental vessels over 0.7, thinning of parenchyma (less than 13 mm), fibrotic changes in parenchyma, hilly kidney margins, anechoic strips under the capsule. Weight, BMI, WC and VF decreased, liver structure, blood pressure improved, joints tophacae

decreased in size, normalized creatinine levels and uric acid levels (was 273 $\mu\text{mol/L}$) in all patient after focused probiotic administration. Most signs of gouty nephropathy improved, namely increasing in kidneys size and parenchyma, decreased IR under 0.7, decreased petrifications, small cystic lesions; fibrotic changes retained in parenchyma after short- term treatment. In 6 patients microsplenitis was detected, size improved to norm after treatment.

Conclusions: Short-term individualized probiotic therapy is effective to treat the signs of MetS and hyperuricemia and can successfully restore the function and structure of damaged kidney in gout.

Proteolytic Activity of *Enterococcus Faecalis* OB15, a Probiotic Strain Isolated from Rigouta Tunisian Dairy Product

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Objective: The study aimed to assess the technological properties such as acidifying ability, proteolytic and antibacterial activities of *Enterococcus faecalis* OB15, an interesting probiotic candidate previously isolated from Rigouta, a Tunisian dairy fermented product, before its potential use in food industry (Baccouri et al, 2019, Front Microbiol. doi: 10.3389/fmicb.2019.00881.).

Methods: The following parameters were studied:

- Fermentation activity by measuring the pH, the bacterial counts and the titratable acidity.
- Proteolytic activity in different systems (in UHT skim milk and in non- proliferative cell system) by electrophoresis (SDS-PAGE).
- Enzymatic profile using API-Zym galleries.
- Effect of temperature, pH and inhibitors on proteolytic activity.
- Antimicrobial activity against some pathogenic strains.
- Production of undesirable biogenic amines, i.e. histamine and tyramine.

Results: *E. faecalis* OB15 was able to degrade casein fractions (α -casein, β -casein) to various extents, with optimal proteolysis for temperature in the range of 37-42°C and a neutral pH of 6.5. Proteolytic activity was highly inhibited in the presence of EDTA and slightly in the presence of PMSF. The strain displayed high activities of leucine, valine and cystine-aminopeptidase, α - and β -glucosidase, phosphatases and N-acetyl- β -glucosaminidase. Medium esterase lipase (C4 and C8), proteases (α -chymotrypsin and trypsin) and β -galactosidase activities were also found in *E. faecalis* OB15. Lipase, α -galactosidase and Naphthol AS-BI-phosphohydrolase activities were weak, and *E. faecalis* OB15 showed no β -glucuronidase activity. This bacterium did not harbor vancomycin resistance that could be transferred to other bacteria, was unable to produce histamine, and interestingly was found to be active against several food pathogens including *Staphylococcus aureus*, *Listeria innocua* and *Salmonella* sp.

Conclusions: These results suggest the safety of *E. faecalis* OB15 and its potential use in food industries for biopreservation or manufacture of novel hypoallergenic products.

Thermal or Membrane Processing for Infant Milk Formula-effect on Protein During Simulated Gastrointestinal Infant Digestion

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Objective: Infant Milk Formula (IMF) is designed as a breast milk substitute to satisfy the nutritional requirements of infants during the first months of life. We have recently produced a new IMF by

cascade membrane filtration (CMF) as an alternative to thermal processing, with equivalent safety but with enhanced bio accessibility, bioavailability and digestion of proteins. Herein we investigate the fate of milk proteins during *in vitro* gastro- intestinal digestion from pilot scale IMF produced by CMF versus standard thermal processing.

Methods: The IMF products were exposed to a static simulated gastrointestinal digestion (SGID) protocol to model the infant gut. Three different time points of SGID- G0, G60, and I60 were collected. Amino acid composition and SDS-PAGE analysis were performed. In addition, the effects of SGID samples on intestinal barrier health were assessed using Caco2 monolayers cell line.

Results: SDS-PAGE revealed different protein patterns with intact proteins notably visible in G60 of CMF compared to the thermal treatment. At I60 there were significant differences ($P < 0.05$) in the amino acid composition of CMF-IMF and thermally treated IMF compared to their GO time. The incubation of Caco-2 cells for 4 hours with IMF digested samples at a concentration of 200 $\mu\text{g/mL}$ revealed significant ($P < 0.05$) alterations in trans epithelial electrical resistance (TEER) between the two different treatments.

Conclusion: The type of processing treatment has an impact on the rate of protein digestion during *in vitro* infant digestion. Assessing the health benefits of IMF, produced by alternative processing such as CMF, is important for the production of next generation IMF products.

Stress Hormones Increase Biofilm Formation of *Enterococcus Faecalis*

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Objective: *Enterococcus faecalis* is a Gram positive, commensal bacterium of the human gut. This bacterium has controversial status due to its emerging role in nosocomial infections, while some strains with beneficial effects are used as probiotics and starter cultures in dairy industry. All these bacteria can be found on skin or in gut where they are continually exposed to various eukaryotic molecules. In this context, the aim of our work was to evaluate the effect of stress hormones on some *Enterococcus* strains.

Methods: Three *E. faecalis* strains were included in this study: *E. faecalis* MMH594 and *E. faecalis* V583, pathogenic strains of clinical origin, and *E. faecalis* OB15, a probiotic strain, previously isolated from tunisian Rigouta (Baccouri et al, 2019, Front Microbiol. doi: 10.3389/fmicb.2019.00881). These bacteria were exposed to epinephrine and norepinephrine (1-100 μM), and their capacity to form biofilm were evaluated by Confocal Laser Scanning Microscopy.

Results: Stress hormones (epinephrine and norepinephrine) were found to modulate the formation of biofilm (biomass, thickness) in *E. faecalis*. The major effect was observed for *E. faecalis* OB15 with significant increases of biomass (+25%) and thickness (+27%).

Conclusions: This study showed for the first time that stress hormones could increase biofilm formation in *E. faecalis*. Future experiments will aim to decipher the mechanisms involved and to identify an adrenergic putative sensor in *E. faecalis*. This may help to develop new strategies of antagonism to prevent the colonization by opportunistic pathogens.

Metabarcoding Analysis of Gut Microbiota of Healthy Individuals Reveals Impact of Probiotic Consumption

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Objective: Our recently published double-blind, placebo-controlled study showed probiotics intake exerted a positive effect on sleep quality and on the depressive mood state with a general improvement across time in different aspects of the profile of mood state, like sad mood, anger, and fatigue on healthy individuals. Beside results published in Martotta et al 2019, this work investigates the impact of probiotics on the human gut composition of the same cohort through a metabarcoding analysis.

Methods: 38 individuals assumed a daily-dose of probiotics (*Bifidobacterium*, *Lactobacillus fermentum*, *Lactobacillus rhamnosus* and *Lactobacillus plantarum* strains) or placebo for 6 weeks. 16S rRNA amplicon sequencing technique was used to sequence fecal samples, and the output sequences were analysed with bioinformatic and statistic methods to infer the most abundant taxonomic units, calculate alpha and beta diversities, and identify biomarkers.

Results: The probiotic cohort was characterized by *Akkermansia* and *Lactobacillus rhamnosus* after 3 weeks of treatment, shifting to a more diverse microbial composition comprehensive of *Romboutsia*, *Lactobacillus*, *Ruminococcus* and *Akkermansia* towards the end.

Amplicons related to *L. rhamnosus* and *B. longum* confirmed the presence of these two probiotic species throughout the assumption.

Conclusions: Probiotics may impact on the microbiota composition and functions. Further studies are on-going to understand how probiotics influence the human gut microbiota in relation to the psychological benefits.

Reference

Marotta A, Sarno E, Del Casale A, et al. Effects of probiotics on cognitive reactivity, mood, and sleep quality. *Front Psychiatry*. 2019;10:164.

Increased Incidence of Clostridium-like Species and Lower Diversity of Common Commensal Bacteria in Gut Microbiota of Children with Neurodevelopmental Disorders

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Objective: Clinical reports indicated increased incidence of gastrointestinal (GI) disturbances and dysbiosis in children with neurodevelopmental disorders (NDD) as well as acceleration of recovery after the dysbiosis treatment. The aim of this study was to evaluate gut microbiota diversity and to identify bacterial strains which incidences were different between patients' and control group. This is the first study evaluating gut microbiota diversity and composition in children with NDD.

Methods: Thirty-sixth children from Serbia with some of the NDD and 28 healthy children participated in the study. DGGE analyses of rDNA amplicons obtained with bacterial DNA as a template and three sets of primers (universal, *Lactobacillus* and *Bifidobacteria* specific primers) were performed. rDNA amplicons which incidences were statistically different between patient and control groups were selected for sequencing.

Results: According to range-weighted richness index it was observed that microbial diversity was lower in the patient group. Dice analyses revealed that DGGE profiles of autism, pervasive developmental disorder-non specified and mixed specific developmental disorder were similar. Increased incidence of potentially harmful bacteria, closely related to *Clostridium* species and lower incidence of several common commensal bacteria was detected in NDD group.

Conclusions: Our study reveals that the intestinal microbiota from children with NDDs differs from the microbiota of healthy children. Similarity of microbiota composition in patient groups with similar neurological and behavioral symptoms indicate that intestinal microbiota might have role in pathophysiology of NDD. Supplementation with several health promoting strains, could be safe adjuvant therapy in treatment of NDD accompanied with GI disturbances.

Effect of Lactobacillus Farciminis Supplementation in Preventing LPS-induced Hippocampal Neuroinflammation, in Mice

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Objective: The discovery of adult neurogenesis in human dentate gyrus of the hippocampus opens new challenges on the role of a virtuous lifestyle (i.e. diet) aimed at counteracting the decay of new neurons production in the aged brain. The use of probiotics could represent an intriguing approach for the prevention of specific diseases. We studied the potential neuroprotective effect of prolonged consumption of *Lactobacillus farciminis* (LF), in a mouse model of acute neuroinflammation.

Methods: C57B6/J male mice (Charles River Laboratories) were divided in the following groups: (1) controls (2) mice supplemented with LF (10⁹CFU/day, for 14 days in drinking water); (3) mice treated with LPS (1 mg/kg, ip); (4) mice supplemented with LF and injected the 14th day with LPS. Study of changes in adult neurogenesis was conducted by immunofluorescence technique, 24 hours after LPS injection.

Results: Preliminary data highlight how the prolonged consumption of LF before the injection of LPS, is able to prevent the decrease in the number and morphological complexity of neural progenitors (DCX marker), in the dentate gyrus. Moreover, consumption of LF significantly counteracts LPS-induced activated microglial shape in the hippocampal dentate gyrus of the same animals.

Conclusions: Prolonged use of LF is able to prevent decline in cognitive processes, by limiting the onset of neuroinflammation of the hippocampus, and by favoring the genesis of new neurons. The analysis of intestinal permeability and inflammation (under investigation) will give indications on the role of the gut-brain axis, in the neuroprotective effect observed in our experimental conditions.

Interactions and Cross-talk Between Microbiota and Humans: A Preliminary Study on the Molecular Responses of Enterococcus Faecium NCIMB10415 to Bioactive Compounds and Feedback Signals

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Objective: Billions of bacteria co-exist with human cells in the gut, creating a complex network: unbalances in this could hesitate in some pathological conditions. The so-called microbiota-gut-brain-axis is acquiring more importance nowadays, still, there is a lack of information regarding the host signals involved and the molecular reactions exerted by bacteria. In the present study, we have evaluated the responses of the probiotic *Enterococcus faecium* NCIMB 10415 to serotonin and norepinephrine, as these molecules are abundant in the gut and involved in some pathological patterns.

Methods: Firstly, growth kinetics were determined for 24 hours of growth in Chemical Defined Medium and SAPI medium supplemented or not with the molecules. Successively, modifications of the probiotic attitude were tested by evaluating the resistance to gastric and intestinal juice, while biofilm formation was assessed with confocal microscopy and with the crystal violet method. Adhesion on confluent Caco-2/TC7 and Transepithelial Electric Resistance of Caco-2/TC7 differentiated cells were then examined. All the experiments were conducted comparing treated and untreated bacteria.

Results: Some modifications of growth patterns were observed during the late stationary phase, and in general nearly all the measured probiotic parameters we measured have been enhanced by the contact with the two molecules.

Conclusions: As previously demonstrated for pathogenic bacteria, also this probiotic strain can respond to human bioactive molecules, supporting the idea of an 'interkingdom signalling'. Work is in progress to evaluate bacterial metabolic pathways modifications

exerted by these compounds (proteomic approach) and possible feed-back effects induced on Caco-2/TC7 (IL-8) and other types of cells present in the gut, as immune cells (dendritic cells) and enteroendocrine cells by treated *in-toto* bacteria and their supernatants.

Brush Border Enzyme Activity in Patients with the Syndrome of Bacterial Overgrowth in the Small Intestine

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Objective: To study the relationship between the activity of small intestine enzymes and duodenal microbiocenosis in patients with *Helicobacter pylori* (HP) - associated duodenitis.

Methods: 65 patients (14-25 y) with duodenitis were examined. HP and genes of pathogenicity of ureC, cagA+, cagC+, Sade+, cagH+, giardiasis, determination of enzyme activity in bioplates were studied.

Results: Isolated HP did not affect the activity of brush border enzymes. The frequency of the combination of HP and syndrome of bacterial overgrowth (BOS) of the small intestine had a negative correlation with the activity of lactase and aminopeptidase M. Frequency of the combination of HP and giardiasis had a negative correlation with the activity of lactase and glycyl-L-leucinedipeptidase. The combination of HP, BOS and giardiasis decreased the activity of all three studied enzymes: lactase, aminopeptidases M and glycyl-L-leucinedipeptidase. Giardiasis and the presence of pathogenic strains of HP, having all of the studied genes pathogenicity was accompanied by a decreased activity of lactase in duodenum. Revealed negative correlation between the activity of the lactase gene and the presence of cagC+ and the activity of glycyl-L-leucinedipeptidase and genes cagA+ and cagH+. It was found that the activity of lactase is negatively affected by Actinomyces, Staphylococcus species, Pseudomonas aeruginosa, Peptococcus species, Candida albicans, Enterococcus faecalis. A positive correlation was found between lactobacilli in the small intestine and the activity of aminopeptidase M.

Conclusions: Microbiocenosis of the duodenum having HP - associated duodenitis and HP genetic characteristics affect the activity of enzymes of the brush border.

Novel Insights into the Strain-level Variations and Metabolic Potential of the Unique Indian Gut Microbiome

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Objective: India is a diverse country with different populations, lifestyles, and dietary-habits. The Indian gut microbiome is not well-explored, and our recent study is the largest gut metagenomic study that revealed the microbial and functional composition of Indian gut microbiome. Further, a multi-population analysis has been performed to identify the unique Indian gut strains, their variations, and role of diet in shaping the gut microbiome.

Methods: Metagenomic reads from 776 samples of 10 population datasets including Indian and nine other populations were assembled. Gene prediction followed by functional annotation using KEGG and eggNOG databases were performed. Binning based on tetranucleotide frequency and contig abundance was performed and the bin quality ($\geq 90\%$ completeness and $\leq 10\%$ contamination) was assessed, and Indian-specific genome bins were identified.

Results: 7714 genome bins were identified from ten different populations, of which 1988 were India-specific. Interestingly, 88 out of the total 163 Prevotella bins belonged to India, which reaffirms the abundance (up to 80%) of Prevotella in Indian gut. The functional analysis of gut microbiome genes showed an enrichment of carbohydrate-metabolism and plant-degrading enzyme genes, which aligns well with the carbohydrate and plant-rich diet of the Indian population.

Conclusions: This is the first comprehensive study to identify the Indian-specific genomic bins and their unique functional composition, the results of which may find several translational applications in therapeutics, FMT, development of probiotics, etc. for Indian and other populations having similar dietary habits and lifestyle.

Plasticity of Child Microbiota in the Development of Obesity

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Objective: Early prevention of obesity is based on a clear understanding of the role that the intestinal microbiota plays in the development of this pathology. Diet is important in the evolution of microbiota, but the health effects are also important in disease management and interactions with associated pathologies. This study, therefore, is aimed at identifying biomarkers (microbiological and biochemical) that occurred when changing the microbiota pattern of a child at the limit of the diagnosis of obesity until the age of 10 years.

Methods: The *in vitro* study carried out simulations of the microbiota at 6 months, 3 years, and 10 years of age (<http://www.gissystems.ro>). The evolution of the microbiota pattern was determined by qPCR. A metabolomic study was carried out that was aimed at the synthesis of essential organic acids as the microbiota developed a stable pattern.

Results: The results thus obtained revealed an increase in the synthesis of short-chain fatty acids, in contrast with the presence of lactic acid. If, apparently, increase in age was associated with equilibration of the microbial pattern, then after 3 years, there was a progressive development of *Firmicutes*, a balance in favourable strains, and a decrease in strains of the genus *Bacteroides*. These data were correlated with a progressive increase in appetite, especially for sweets.

Conclusions: Identifying the key point in the development of dysbiosis was a biomarker capable of representing an effective pre-clinical diagnosis to reduce the risk of further development of degenerative pathologies. From the data obtained, the influencing ratio of the present species was identified, which can be a pre-clinical result in the development of innovative strategies to reduce obesity in children.

The Analysis of Fecal Microbiota and Insulin Production in Diabetic Rats After Oral Administration of Probiotic *Lactobacillus Paraplantarum* BGCG11

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Objective: Our previous studies with *Lactobacillus paraplantarum* BGCG11 probiotic-treatment of diabetic rats showed decreased hyperglycemia and ameliorating effect on diabetes-associated damage of liver and kidneys. Hence, the aim of this study was to reveal the effects of BGCG11 probiotic on gut microbiota composition and monitoring the insulin production in pancreatic islets in diabetic rats.

Methods: Experiments were performed on albino Wistar rats divided into four groups: ND – non-diabetic control, D – streptozotocin (STZ) induced diabetes; P/D/P – BGCG11 pretreatment; D/P – BGCG11 treatment. The rats were orally administered with BGCG11, one week before (P/D/P) and after the STZ injection, for four weeks (P/D/P and D/P). Total DNA was isolated from all fecal samples and rDNA amplicons were analyzed by DGGE and 16S

rDNA genes sequencing. For immunohistochemical analysis, slides were stained with anti-insulin antibody and secondary antibody coupled with horseradish peroxidase.

Results: The results revealed the higher diversity of gut microbiota in D/P group comparing to D group, as well as the higher prevalence of *Flintibacter butyricus* (the major butyric producer), *Acetatifactor muris* (present in obese mouse) and *Eisenbergiella massiliensis* (found in obese woman), while the lipolytic bacterium *Aestuariuspira insulae* was more prevalent in diabetic rats. In both, P/D/P and D/P group, increased number of positive immunoreactions of β -cells for anti-insulin antibodies was displayed in compare to D group with islet atrophy.

Conclusions: The results of this study suggest that the positive effect of BGCG11 on STZ-induced diabetes in rats could be annotated to its protective role on the integrity of fecal microbiota.

Extended-spectrum-beta-lactamases in Escherichia Coli of Intestinal Microbiota of Piglets

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Objective: Antimicrobial resistance is one of the major public health challenges of 21st century. This concern is particularly in agriculture because use of antibiotics in livestock for growth promotion, prevention and control of infectious diseases. Pig manure may be used as fertilizer and antibiotic resistant bacteria will be spread in soils, surface waters and humans. The aim was study antibiotic resistance in intestinal microbiota of piglets in order to assess the risk to animal and public health.

Methods: Faeces from five piglets of a pig-farming in central Portugal, a dense livestock region, were obtained in slaughter house. *Escherichia coli*, as bacteria biomarker in samples, were quantified and identified. Four colonies of *E. coli* of each sample were taken. Determination of susceptibility to amikacin (AMK), ciprofloxacin (CIP), cotrimoxazole (SXT), aztreonam (AZT), ceftazidime (CAZ), cefepime (FEP) and amoxicillin + clavulanic acid (AMC) and screening of extended-spectrum-beta-lactamases (ESBL) and metallo-beta-lactamases (MBL) were performed.

Conclusions: The high resistant *E. coli* isolates allows classifying them as multi-drug-resistant (MDR) isolates because they presented resistance to different antibiotic classes. Also, these isolates showed ESBL. The majority of antibiotic resistant genes acquired by human pathogens were originated from natural environment. Therefore, these findings are very concerning for public health.

Identification of Novel Bioactive Metabolites Produced During the Cocultivation of Probiotic Strains and Clostridium Difficile in In Vitro Setup

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Objective: *Clostridium difficile* is a major enteric pathogen that causes pseudomembranous colitis in humans, and the infection can be fatal if not treated promptly. The aim of this project is to investigate the impact of a mix of 4 strains (*Barnesiella intestinihominis*, *Pseudoflavonifractor capillosus*, *Blautia hansenii* and *Clostridium scindens*) and *Clostridium scindens* alone in terms of production of bioactive metabolites (e.g. antimicrobials) once exposed to *Clostridium difficile* under simulated *in vitro* colonic conditions, making use of the SHIME® technology platform.

Methods and Results: For this project, we adapted a QuadSHIME with a modified configuration containing 8 distal colon compartments. Using short chain fatty acid (SCFA) analysis as surrogate for community stability, we successfully established the gut microbiota from a healthy individual in the SHIME system, and a subsequent colonization of the probiotic strains. Using qPCR and colony

counts specific to *C. difficile*, we verified the successful colonization of *C. difficile* during the infection period and gradual decrease of *C. difficile* population during the treatment period. By employing multi-omics (metagenomics, metatranscriptomics and metabolomics) analysis on the *in vitro* cultures we identified different signaling molecules and their biosynthetic pathways. In addition, the integration of multi-omics data helped us to understand community dynamics of different individual bacterial populations in the *in vitro* conditions using SHIME®.

Conclusion: By successfully establishing novel infection and treatment model in SHIME® platform using *C. difficile* as a model pathogen, we established an experimental setup that can be used for discovering new and unidentified antimicrobials against different enteric pathogens.

Phenotypic and Genotypic Characterization of Lactic Acid Bacteria Strains Isolated From Newborn Feces

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Objective: Phenotypic and genotypic characterization of lactic acid bacteria strains isolated from newborn feces samples, in order to determine their probiotic potential.

Methods: In the present study, ten feces samples were collected from newborns of different ages (between 0-5 d), born both naturally and by caesarean. A total of eight isolated LAB strains were identified using MALDI-TOF Mass Spectrometry system, after cultivation on selective medium (MRS with CaCO₃ and M17). In order to establish their pathogenic potential, the isolated strains were screened for soluble virulence factors production like hemolysin, lipase, gelatinase and DN-ase (using cultivation methods on specific substratum) and for adherence pattern on the cellular substrate represented by HeLa cells (using Cravioto's adapted method). The study continued with phenotypic determination of antibiotic susceptibility spectrum using disc diffusion standard method (according with CLSI standard, 2019) and genetic identification of potential resistance markers for *Enterococcus* isolated strains using Simplex PCR for the *vanA* gene and multiplex PCR for the *vanB* and *vanC* genes.

Results: In the present study were isolated 6 strains of *Enterococcus* belonging to the species *E. faecium* (3 strains), *E. faecalis* (1 strain), *E. durans* (3 strains), and only one *Lactobacillus rhamnosus*. *E. faecalis* 2M17 was the only strain that expressed soluble virulence factors like beta-hemolysin and gelatinase. *Lactobacillus rhamnosus* 9MRS strain, isolated from a newborn delivered vaginally and exclusively breastfed, showed a strongly adherence capability demonstrated by an aggregative pattern and 100% adherence index and no soluble virulence factors. Regarding the antibiotic sensitivity spectrum, two strains of *E. faecium* phenotypically expressed Vancomycin resistance demonstrated by the molecular amplification of *vanA* gene, but not of *vanB* and *vanC* genes. The three strains of *Enterococcus durans* showed sensitivity to Vancomycin, Linezolid, Gentamycin, Ampicillin and Levofloxacin and resistance to Tetracycline.

Conclusions: After de correlation of the expressed soluble virulence factors, the adherence pattern and antibiotic sensitivity spectrum, *Lactobacillus rhamnosus* 9MRS strain, isolated from a newborn delivered vaginally and exclusively breastfed, showed good properties to be considered a potential probiotic strain.

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Impact of Nutrient Starvation on the Modulation of the Inflammatory Response by Lactobacillus Strains in Intestinal Epithelial Cells and Macrophages

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A chronic inflammation state is associated with aging and with the onset and progression of many human diseases, including inflammatory bowel diseases, obesity, cardiovascular diseases and cancer. Among strategies developed to dampen inflammation, the use of probiotic bacteria with anti-inflammatory properties, from *Lactobacillus* and *Bifidobacterium* genus, represents a safe and low-cost promising treatment. However, despite encouraging *in vitro* and *in vivo* results, probiotic bacteria remain poorly effective in the treatment of human inflammatory-related diseases. Emerging evidence suggests that the immune system function might be heavily influenced by the sensing of nutrient, reinforcing the idea that diet can influence the inflammatory response. This may be particularly true for the intestine that face dynamic changes to nutrient bioavailability.

The purpose of this study is to investigate whether nutrient deprivation of host cells may potentiate the anti-inflammatory effects of *Lactobacillus* probiotic strain. Inflammatory responses were induced in intestinal epithelial cells (HT-29) using *Salmonella* infection and in macrophages (RAW, THP1 and J774A1) using lipopolysaccharide (LPS) treatment, in the presence or absence of nutrients in the host cells media. Inflammatory responses were analyzed by qRT-PCR and ELISA to follow the expression and secretion of various pro- and anti-inflammatory cytokines.

Our *in vitro* data shows that nutrient starvation allows to potentiate the abilities of *Lactobacillus* strains to downregulate pro-inflammatory responses in intestinal epithelial cells and macrophages, suggesting that diet may influence positively the efficiency of probiotics.

Keywords: Nutrient starvation, Probiotic, Macrophages, Intestinal Epithelial cells, Inflammatory response, *Lactobacillus*, *Salmonella*

Age and Gender Profiles of Selected Gut Microbiota Among Egyptian Children and the Sibling-specific Variation

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Gut microbiome has a potential role in the human health particularly in early life. Currently, there are no available data on the profile of gut microbiota among Egyptian children. The present study aims to fulfill this gap and to explore the abundances of 7 bacterial taxa belonging to the 4 major phylas Actinobacteria (*Bifidobacterium*), Bacteroidetes (*Bacteroides fragilis*, *Prevotella*), Firmicutes (*Clostridium leptum*, *Faecali bacterium*, *Lactobacillus*) and Proteobacteria (*Enterobacteriaceae*) among children of both sexes aging 3 - 9 years from unrelated or related families.

Fecal samples were collected from the children and the DNA was isolated by the ZR fecal DNA isolation kit. Primer pair targeting 16S rRNA bacteria group or species were used to quantify the bacteria species followed by the quantitative real time PCR technique.

The Results showed that the average genome was highest for *Bifidobacterium* (\log_{10} 7.44 genome copies per g wet feces), which is in good agreement with the international literature, due to the frequent milk drinking among children belonging to this age group. The relative mean high genome copies of *Enterobacteriaceae* (\log_{10} 5.8 genome copies per g wet stool) is of concern, being a signature of pathogenic bacteria. The low genome counts of *Prevotella* is noteworthy. Slight gender dependent differences were observed in few bacteria species. The sibling-specific variation in the genome copies of the fecal microbiota within families and the discriminant ability was ascertained. The results serve as baseline for further metagenomic studies.

Use of a New Probiotic Formulation in Allergic Patients: A Pilot Study

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Objective: Immune responses are regulated by T helper (Th) cells and allergy is associated to a Th2 response. Therapeutic approaches shifting the immune response may be beneficial for individuals with allergic rhinitis (AR). Here we show the results of a pilot clinical trial (RCT), in which a new probiotic formulation has been selected and tested.

Methods: A double-blind cross-over RCT was designed with a daily dose of probiotic formulation versus placebo. During the trial, patients underwent allergological exams, and fecal, serum and blood sampling.

Results: Endolacoa® is a probiotic food supplement containing *L. plantarum* P17630 and *L. paracasei* 1688 -2 billions CFU/dose, Acticoa®, and vitamins D3 and B6. To test its effect on AR, we performed a double-blind cross-over RCT of Endolacoa® versus placebo. We enrolled 8 patients, with diagnosed dust mite allergy. These have been randomly allocated to an arm of the study (Endolacoa® or placebo) with a daily dose for 4 weeks with a wash-out period of 4-8 weeks before cross-over. There was a significant increase of regulatory T cells (CD4+/FoxP3+/CD25+/IL-10+) that inversely correlated with Th2-IL-4+ cells, in Endolacoa-treated patients versus placebo ($P < 0.05$). Moreover, we found a significant and positive correlation between Tregs, increase of lactobacilli in faecal samples and clinical scores ($P = 0.0072$ e $r = 0.8810$). DNA methylation analysis identified 3 dysregulated gene pathways upon probiotic administration.

Conclusions: Here, we obtained preliminary but promising results, suggesting that our new dietary supplemental could shift the immune system toward a less inflammatory response potentially beneficial to AR patients.

Typing of Surface Glycosilation of Microorganisms by Lectins with in House Elisa

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Objective: The goal of this work was detecting lectin-sugar interactions between 21 selected microorganisms and 7 plant lectins in a newly developed ELISA format, and the appraisal of the bacterium-lectin mutual interactions and characteristics.

Methods: Lectin molecules with previously defined specificities were WGA, SBA, LCA, RCA₁₂₀, MAA, UEA, all commercially available and recombinant ba- nana lectin¹. Lectins were biotinylated and biotinylation efficiency was assessed by dot blotting and titration against a mixture of microorganisms in ELISA to select appropriate dilutions. Subsequently analysis was performed with direct ELISA. 0.5 M monosaccharide mixture (glucose, man-nose and galactose) was used as inhibitor.

Results: All but one lectin binding was removed with 0.5 M monosaccharide solution, implying a specific lectin-sugar interaction. WGA could not be inhibited with this mixture. This lectin also showed lowest selectivity as it bound most of the bacteria tested. Of the tested microorganisms *L. helveticus* LAFTI and *L. acidophilus* Vivag showed highest binding to 4 of the 7 tested lectins.

Conclusions: The interaction of microorganisms with their host is dependent on the architecture of surface glycosilation. The simple methodology used is useful for studying the host colonisation capacity of microorganisms, as well as mutual relations between microorganisms such as displacement and competition.

Reduced Food Diversity in SIBO Patients

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Objective: Small intestinal bacterial overgrowth (SIBO) is a widespread disease characterized by a significant decrease in the quality of life. Antibiotic treatment with SIBO is not effective enough and the recurrence rate is high. Long-term dietary patterns can shift the composition of the microbiota. The aim of the study was to compare nutritional diversity in patients with SIBO H2 and in patients without excess hydrogen production.

Methods: Hydrogen-methane breath test with lactulose was performed in 630 patients, SIBO was diagnosed in 522 patients. Information on food intake was collected with 24 hours recall from all participants. According to food composition and portion all dishes in food diary were converted into constituent products and were sorted in the lists of unique values by group of products. The study compared the data of the analysis of nutritional diversity of patients with SIBO and those who did not have SIBO.

Results: A comparison of nutritional diversity in patients with SIBO revealed a lower species diversity in the groups of dairy products (2.70 ± 1.37 vs. 3.19 ± 1.34 , $P < 0.001$), vegetables (5.50 ± 2.22 vs. 6.29 ± 1.90 , $P < 0.001$), fruits (1.54 ± 1.38 vs. 1.99 ± 1.69 , $P = 0.018$). Diversity of grains, meats, fishes, fat products, nuts and legumes and sweets did not have significant differences.

Conclusions: According to the results of the study, significant differences in the nutritional diversity of patients with SIBO in relation to the consumption of dairy products, vegetables and fruits were established. The obtained data may be used to develop dietetic maintenance of SIBO therapy and relaps prevention.

Effect of Probiotics in Children with Infantile Eczema: A Double-blind Randomized Control Trial

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Objective: Probiotics are used in the treatment of several conditions: functional abdominal pain, infantile colic, celiac disease, irritable bowel syndrome, lactose intolerance, food allergy, Hp infection, asthma, atopic dermatitis and infantile eczema. To determine whether oral administration of the probiotic *Lactobacillus GG* under randomized, double-blinded, placebo-controlled conditions would improve symptoms of infantile eczema in children.

Patients and Methods: 41 children with infantile eczema were given *Lactobacillus GG* or placebo for 6 weeks and entered follow-up for 4 weeks. Children entered a randomized, double-blind, placebo-controlled trial.

Results: LGG, but not placebo, caused a significant reduction of both frequency ($P < 0.01$) and severity ($P < 0.01$) of eczema. These differences still were significant at the end of follow-up ($P < 0.02$ and $P < 0.001$, respectively).

Conclusions: *Lactobacillus GG* was superior to placebo in the treatment of eczema in children. The intestinal microbial flora may contribute to the pathogenesis of allergic diseases, LGG significantly reduces the frequency and severity of infantile eczema and maybe because improves the gut barrier function and reduce the inflammatory response.

Addition of Mucin to Growth Medium Stimulates Adhesivity and Therapeutical Potential of *Lactobacillus Reuteri* E

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Objective: Adhesion of probiotic bacteria to the mucus layer covering the mucosa of the gastrointestinal tract is necessary for its effective colonization and specific therapeutic effects. Enrichment of growth medium with mucin might stimulate bacterial adhesion, probably by increasing expression of surface structures responsible for bacteria-gut epithelia interactions. The aim of this study was to determine if pre-cultivation of potentially probiotic *Lactobacillus reuteri* E (LRE) with mucin stimulates its ability to adhere and promote mucin expression in HT-29 cells.

Methods: HT-29 cell line expressing MUC2 and MUC5AC was co-cultivated for 2 hours with LRE grown in MRS broth or MRS broth enriched with pig gastric mucin. The ability of LRE to adhere to HT-29 cells was evaluated by staining and plate counting. The relative expression of *muc* genes in HT-29 cells was measured by qPCR.

Results: Pre-cultivation of LRE with mucin enriched medium significantly increased the degree of LRE adhesion to HT-29. Co-cultivation of LRE with HT-29 cell resulted in significantly increased expression of MUC2 and MUC5AC compared to the control group (lactobacilli-free HT-29) and HT-29 co-cultivated with non-stimulated LRE.

Conclusions: These results suggest that pre-cultivation of lactobacilli with mucin may not only stimulate its adhesion abilities but also promote its health beneficial potential.

Prebiotics: From Bench to Bedside

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Prebiotics are a group of nutrition only degraded by gut microbiota. The aim of this study was to review different aspects of prebiotics including their definition, types, sources, mechanisms, and clinical applications. Prebiotics are defined as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health”. Fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and trans-galacto-oligosaccharides (TOS) are the most common prebiotics. Since FOS and GOS naturally exist in our foods in low quantities, scientists are trying to produce them on an industrial scale. The prebiotics degradation products are short chain fatty acids (including lactic acid, butyric acid, and propionic acid) that can diffuse to blood circulation. Consequently, they affect not only the gastrointestinal tracts and relevant diseases (such as irritable bowel syndrome and Crohn’s disease), but also other distant organs. In this regards, the beneficial effects of prebiotics have been demonstrated against malignancy (e.g., colorectal cancer), cardiovascular diseases (e.g., dyslipidemia), skin (e.g., atopic dermatitis) and mental disorders (e.g., autism). A daily dose of 2.5-10 g prebiotics is required to exert their beneficial functions on human health. Most products of prebiotics in the market have doses of 1.5-5 g per portion. Prebiotics within their therapeutic doses can cause mild to moderate side effects such as flatulence and osmotic diarrhea. Considering the suggested health benefits of prebiotics and their safety as well as their production and storage advantages compared to probiotics, they seem fascinating candidates for promoting human health condition as replacement or along with probiotics (synbiotics).

Keywords: prebiotics; gut microbiota; short-chain fatty acids; fructo-oligosaccharides; galacto-oligosaccharides; clinical applications

The Human Protective Basic Lectin Supersystem with Probiotic Properties

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Objective: We studied human carbohydrates-reacted lectin/lectin-like systems (LS): probiotic metabolites (> 27 kD, pI4-8), protein hormones (erythropoietins) and complement (C4B+C4A)-system. LS recognized glycoconjugates (GC). The aim is to describe the

human protective lectin supersystems (HPLSS) including contributor LS.

Methods: LS were separated using isoelectric focusing in polyacrylamide gel, electroblotting and chemiluminescent live visualization using GC-biotin (<http://www.lectinity.com>), antibodies, biotin-streptavidin system, peroxidase conjugates registered in *Bio-Chemi System* (UVP). Activities were studied by standard procedures.

Results: The following properties of HPLSS were proposed: *diversity as major LS (participation in biotope infrastructure functioning) and minor LS (involvement of signals in fine biotope regulation); *recognition according to: a) "One lectin—Ranged panel of GC images", b) "One GC—Group of lectins"; *capability to form new sites and components upon assembling; *capability to create receptor like structures; *capability to form cell- cytokine cascades reversible at early steps; *action as metabolomebiotics (Network-in-Network); *realization of lectin-coupled activities in subcytoagglutinating doses; *capability to be converted in associative and dissociative LS; *primary action in places of cell synthesis of lectins and secondary action due to mucosal axes and blood transportation; *support of reached healthy biotope balance; *action as auxillary, correcting, conservative, supervising; *support of differentiated and mature cells, their GC decors in prevention of system diseases and tumors; *potential for LS types extension and cofunctioning to non-lectin protective systems; *synergism to antimicrobials and chemotherapy agents; *antagonism to LS of pathogens.

Conclusions: The HPLSS and contributor LS are perspective for applications in prophylaxis, therapy and biotechnology.

Probiotic Screening of Ten Lactic Acid Bacteria from Traditional Algerians Dried Meat (KHLIAA)

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Objective: Ten LAB isolated from dried meat were assessed for resistance or sensitivity tests recommended to study probiotic properties.

Methods: Antimicrobial activity, acidity, growth Kinetics, quantification, heat sensitivities were assayed against *St. aureus* ATCC 25923, *C. perfringens* CECT 486 and *L. ivanovii* CECT 148. Assessment of proteolytic, lipolytic, amylolytic, gelatinase, and bile salts hydrolase activities, capacity to produce acetoin and exopolysaccharides, acidity conditions, bile salts, gastric and intestinal resistance was determined. Survival lactic acid bacteria was then calculated using single plate-serial dilution spotting. Cholesterol assimilation, hemolysis and antibiotic resistance was characterized. Statistical analysis was performed using origin pro v9.5.

Results: The neutralized supernatant of Lbm3, Lbm18 and Lbm50 showed 9mm inhibition, *C. perfringens* CECT 486 was the most sensitive. This LAB were found to decrease by 2-3log CFU/mL on gastrointestinal conditions and to assimilate cholesterol by 80%. The peak of antagonistic was obtained at the stationary phase where pH 3.50 was reached. The supernatant was sensitive with enzymes and heating. All LAB showed protein digesting but not for starch, lipids, gelatin, bile salt, also showed no hemolytic activity. Most of them showed sensitivity to antibiotics and minority exhibited négative EPS and acetoin.

Conclusions: Lbm3, Lbm18 and Lbm50 showed the highest potential probiotic score. This study should be accomplished by a molecular identification.

Keywords: meat, lactic acid bacteria, probiotic potential, screening

Bio-control of Vibrio Species in Cultured Milk by In Situ Bacteriocin Production from Lactic Acid Bacteria

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Objective: This study was undertaken to elucidate the antagonistic activity of bacteriocin-producing Lactic acid bacteria against *Vibrio* species.

Methods: Lactic acid bacteria (LAB) were isolated from milk products and identified phenotypically. They were initially screened for antagonistic activity against the *Vibrio* species by the agar well diffusion assay. Bacteriocins produced by the LAB were characterised with respect to enzymes, pH and temperatures. The effect of *in situ* bacteriocin production by LAB on the survival of *Vibrio* species was determined in *Nono* during the storage period of 72 hours (12-hour interval).

Results: Of the 112 strains of LAB tested for antagonistic activity against *Vibrio* species, only twelve were selected based on the bacteriocin production. They were characterised phenotypically and identified to be *Pediococcus damnosus*, *P. acidilactici*, *Lactobacillus brevis* and *L. plantarum*. The bacteriocins produced by the LAB were heat stable at 90°C for 20 minutes, active over a wide pH range, stable in the presence of catalase but lost their activity in the presence of proteolytic enzymes. Bacteriocins produced by LAB showed antagonistic activity against *Vibrio* species with zone of inhibition ranges from 12–20 mm. *Vibrio* species counts reduced significantly to different extents in all samples of *Nono* and undetectable within 48 to 60 hours of storage. On the contrary, *Vibrio* species survived for 72 hours of storage in the control experiment that lack bacteriocin producing LAB.

Conclusions: This work demonstrates that bacteriocin-producing starter in milk fermentation can be exploited in inhibiting *Vibrio* species that causes diseases in man.

Gut Diseases, Birth Characteristics, Nutrition and Physical Development: Case-study on Children Aged 0-3 Years, Admitted into a Pediatric Hospital from Timisoara, Romania

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Objective: Gut microbiota of children is created and changed depending on their primary experiences with environment: birth, nutrition, physical development and immunity. The aim of the performed study was to investigate gut diseases of children-patients aged 0-3 years, admitted into a pediatric hospital from Timisoara, Romania, during 2 years (2016-2017), their birth characteristics, nutrition and physical development.

Methods: An observational case-study, with a retrospective investigation of the primary evidences was performed on a sample of 88 children (52.3% males, 47.7% females, aged 0-3 y) hospitalized with gut diseases into a pediatric hospital from Timisoara, during 2 years (1.01.2016-31.12.2017). Types (localization and etiology) of gut diseases, birth characteristics, nutrition (natural/artificial and diversification age), anthropometric parameters (at birth and actual) were investigated. Statistical analysis was performed by the aid of a SPSS20 program.

Results: Types of gut diseases by etiology were cow milk proteins allergies-30.7% patients, viral diseases-12.5%, microbial diseases-7.95% and parasitic diseases-5.68% in children-patients. Gut diseases by localization were enterocolitis-21.59% patients, gastroenteritis-13.64% and vomiting acute syndrome-32.95% patients. Children admitted into the pediatric hospital for gut diseases were borne to term-68.7%, 1st-order child-57.6%, with cranial presentation-94.3%, through caesarian-56.76% or natural-43.25% birth, with APGAR = 9-56.1%, weight at birth 2500-4000 grams-85.23%, length at birth 45-55 cm-77.27%. They were natural (0-2 mo-37.5%) or artificial (Nan-34.09%, Aptamil-15.9%) nourished, with diversification age at 6 months-58.1%. Actual weight (5-15 Kg) and actual length (50-80 cm) indicated normal development.

Conclusions: Children hospitalized for gut diseases (allergic, infectious) present certain birth characteristics (caesarian, APGAR = 9,

1st-order child), nutrition (artificial-NAN/natural = 0-2 mo) and physical development (normal).

Antioxidant Activity of Lactic Acid Bacteria as Potential Probiotics in Chronic Obstructive Pulmonary Disease (COPD)

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Objective: Chronic obstructive pulmonary disease (COPD) is chronic inflammatory lung disorder featured by increase of oxidative stress caused by inflammatory, immune and structural cells producing reactive oxygen species (ROS). Currently available pharmacological treatments are not effective in suppression of chronic inflammation in COPD patients and development of novel approaches is needed. Since probiotics are known for their beneficial effects on human health, development of appropriate probiotic cultures with antioxidant and anti-inflammatory effects is likely to be effective in the treatment of COPD.

Methods: The antioxidant activity of 21 LAB was evaluated by scavenging of DPPH radical's cation and the ability to resist hydrogen peroxide. Safety evaluation of selected LAB was performed by MIC determination and analysis of hemolytic activity. The ability of adherence to the BEAS-2B pulmonary cells of selected LAB was performed in the adhesion assay. The level of cytotoxicity in the cell cultures was measured by LDH.

Results: All 21 LAB strains were resistant to 1 mM hydrogen peroxide while four lactobacilli were highly resistant to 2 mM hydrogen peroxide. One *Lactobacillus brevis* and *Streptococcus thermophilus* and six *Lb. plantarum* strains exhibited DPPH-free-radical scavenging activity. All of these eight strains have QPS status and showed a high level of adherence to BEAS-2B with range of 46.4-69.95%. After cytotoxicity assay four lactobacilli were selected for further research.

Conclusions: According to results lactobacilli with antioxidative activity and ability to adhere to human pulmonary cells are candidate for further research in order to reduce inflammation in COPD.

Esophageal Varices are Associated with Gut Dysbiosis in Cirrhosis

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Objective: Gut dysbiosis and esophageal varices are common in cirrhosis, but the relation between them has not been assessed. The presence of the relation can become the basis for innovate management of this disorder.

Methods: The study included 50 cirrhotics. Their stool microbiome was assessed using v3-v4 16S rRNA gene sequencing. Standard esophagogastroduodenoscopy was performed.

Results: Patients had age 48.9 ± 12.9 years and gender distribution 24 male/26 female. Cirrhosis was Child-Turcotte-Pugh class A in 19 patients, B in 19, and C in 12. Etiology was alcohol (18 persons), viral (17), autoimmune liver diseases (11), and unclear (4). Esophageal varices Grade I was in 16 patients, Grade II - in 14, Grade III - in 10. Ten patients had not esophageal varices. In gut microbiome in cirrhosis with large esophageal varices (Grade II-III) in comparison with cirrhosis without these (Grade I and no varices), the abundance of Bacilli and Streptococcaceae increased (5.5% [1.8-17.8%] vs. 0.7% [0.2-7.3%], $P=0.005$; and 4.6% [0.4-14.4%] vs. 0.3% [0.1-3.0%], $P=0.011$), but the abundance of Clostridia decreased (66.9% [53.2-83.7%] vs. 81.3% [70.5-85.4%], $P=0.049$), the

abundance of Proteobacteria and Bacteroidetes didn't significantly change (0.8% [0.2-3.1%] vs. 1.7% [0.2-4.0%], $P=0.541$; and 6.8% [4.5-13.2%] vs. 5.7% [1.8-7.8%], $P=0.241$).

Conclusions: Esophageal varices are associated with gut dysbiosis in cirrhosis. In large esophageal varices, gram-positive facultative anaerobes are increased in gut microbiome that may lead to bacterial translocation these potential pathogenic bacteria, provide systemic inflammation, conduct splanchnic vasodilatation, and contribute esophageal varices formation. The management focused on gut dysbiosis can be perspective in these cases.

Gut Microbiome Analysis in Biopsies from Slovak Colorectal Adenomas and Carcinomas Patients

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Objective: According to the growing evidence of the role of microbial community colonizing the human gastrointestinal tract in colorectal cancer etiology, we have decided to compare the composition of gut microbiota in colorectal biopsies.

Methods: The set of specimens were split into 3 groups. Group 1 for controls; healthy subjects who underwent routine colonoscopy; Group 2 for patients with colorectal adenomas and Group 3 for those with colorectal carcinomas. For the characterization of the gut microbiota we used massive parallel sequencing of PCR amplicons of the V4 region of bacterial 16S rRNA gene.

Results: The *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Fusobacteria* were the most dominant bacteria phyla in all samples. A total of 40 different classes and 147 families were identified, many of which had shared presence in all samples while others were exclusive to different sample. The results showed higher levels of *Bacteroides/Prevotella* and *Bacteroides/Porphyromonas*, especially *Porphyromonas asaccharolytica*, in patients with colorectal cancer. On the other hand there was a lower level of *Lachnospiraceae*, *Ruminococcaceae/Faecalibacterium* in cancerous tissue compared to the control and colorectal polyps and adenomas.

Conclusion: This is the first study comparing the gut microbiota of CRC patients, patients with adenomas and noncancer control subjects. The identification of genuine CRC drivers will aid in early diagnosis and pave the way for novel microbiota based risk assessment tools and screening strategies for CRC.

Hypolipidaemic Effects of OGI, A Traditional Adult and Weaning Cereal Gruel Fermented with Probiotic Lactic Acid Bacteria and Yeasts in Rats FED High Cholesterol Diet

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Objective: Determination of cholesterol reducing ability of ogi (cereal gruel) fermented with probiotic lactic acid bacteria and yeast in rats fed with high cholesterol diet.

Methods: Appropriate phenotypic characterization, sequencing of the D1/D2 region of 26S rDNA and 16S rDNA of were used to identify the microorganisms. Selection of the test isolates was based on gastrointestinal survival, adherence ability and safety assessment. Total lipid profile of serum samples was determined by colorimetric reactions using enzymatic kits.

Results: *Lactobacillus plantarum* L65 and *Debaryomyces hansenii* Y73 were selected as test isolates. Both showed 99% gastrointestinal survival, 72% and 58% hydrophobicity to n-hexadecane, high bile salt hydrolysis and cholesterol reduction of 42-56% respectively. The groups fed on high cholesterol diet supplemented with ogi fermented with the probiotic strains had significantly lower levels of serum total cholesterol, triacylglycerol and low-density lipoprotein-cholesterol (LDL-C), when compared with the group fed high cholesterol diet without supplementation.

Conclusions: The study further revealed the possibility of employing probiotic microorganisms as complementary biotherapeutic agents to reduce the risk of cardiovascular diseases in humans.

Keywords: *L. plantarum*, *Debaryomyces hansenii*, Fermented cereal gruel, Cholesterol, Probiotic

Detection of New Probiotics in the Presence of Viral Gastroenteritis

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Objective: The main objective of our study was to investigate new probiotic lactobacilli in the presence viral gastroenteritis such as norovirus and rotavirus infection.

Methods: *Lactobacillus* spp. identification was performed in stool samples of 188 children with gastroenteritis and 40 healthy children who underwent rotavirus and norovirus research by ELISA.

Lactobacilli were selected according to gram reaction, morphology, classical identification (API) and molecular (16S rRNA) tests. Acid resistance (pH 1.5-8.2), bile tolerance (0.01-0.4%), antimicrobial activity against *E.coli* ATCC 11229, exopolysaccharide (EPS) production, bile acid deconjugation and cholesterol removal of isolates were identified.

Results: Lactobacilli were identified in 31 of 92 rotavirus positive (33.69%) fecal samples and in 46 of 107 norovirus positive (42.9%) fecal samples. The EPS production ranges were 32.24-148.14 mg/L. Cholesterol removal rates ranged between 6.21-41.16%. And also, a positive strong correlation was found between EPS production and cholesterol ($r=0.882$, $P<0.001$). The sodium glycocholate deconjugation was higher than sodium taurocholate. EPS productions, deconjugation rates and cholesterol removals in Noravirus (+) strains had higher compared to in the rotavirus (+), norovirus (-), rotavirus (-) and without gastroenteritis. Significant differences were observed among groups in parameters ($P<0.05$).

Conclusions: Increasingly rising norovirus and rotavirus infections in worldwide, it is very crucial to supplement new probiotic bacteria in the diets of children with viral gastroenteritis for quicker regulation of vital functions.

Probiotic as A Potential Therapeutics in Prevention and Treatment of Acute Diarrhoea with Gastrointestinal Infections in Children; A Conventional Therapeutics Approach

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In medicine, “diarrhoea” means “a flow through”, also defined as “the passage of three or more loose or liquid stools per day, more frequently than is normal for the personality”. If left untreated, diarrhoea can lead to severe dehydration, which can result in hospitalisation or even death. It is usually the symptom of gastrointestinal infection, most cases of diarrhoea in children result from infection caused by a variety of viruses, bacteria or parasites, which disturb the normal fluid and nutrient assimilation of the intestines. As per UNICEF data: Monitoring the situation of Children and Women report June 2016, diarrhoeal diseases accounting for 9 percent of all deaths among the children under five years of age making them the second largely common cause of child deaths worldwide. *Shigella* species is the second most widespread bacterial agents causing diarrhoea after *Escherichia coli*. Shigellosis is endemic to many developing countries and also occurs in outbreak causing substantial morbidity and mortality. Most *Shigella* infections result sporadically, but huge *Shigella* epidemic have been traced to contaminated food and water. In local Gulbarga district and surrounding region of Gulbarga (Karnataka state), diarrhoea has been estimated to be responsible for approximately 11-13% of all childhood illness, with a population of about 5,32,031. Among the four species of *Shigella*, *Shigella dysenteriae* and *Shigella flexnerii* were more predominant one. At present, multi-drug resistance has

complicated the assortments of empirical agents used for treatment of shigellosis, particularly in children. The emergence of fluoroquinolone resistance in *Shigella* spp and their dissemination across the countries, so the practicing the prevention and develop into protocols with natural pattern consider to be essential. The present research work is efforts made to develop alternative to conventional drugs. Now, the application of *Lactobacillus* species as a probiotic to the prevention, management and became possible option is use to probiotic *Lactobacillus* species as a biotherapeutics against enteric infections. Our results and its importance suggest that the *Lactobacillus* treatment is potentially useful for treatment of *Shigella* infections, especially in children. Scientific proof of the *in-vivo* efficacy and safety of probiotic bacteria as biotherapeutics has validated its clinical use in real-life situations for human health benefits. Hence, we conclude that, our *Lactobacillus* species as a probiotic showed more efficient results/ reports. Also this study provides the support for the formulation of novel probiotic as a biotherapeutic agent or supplements that can play a role in the prevention of gastro intestinal infection especially infantile diarrhoea and other related enteric infection.

Keywords: *Shigella* infection, Diarrhoea, Intestinal infection, Probiotics, *Lactobacillus*, Antibiotic resistance and Fluoroquinolones.

Prevention of Recurrent Urinary Tract Infections: Efficacy of a Formulation Containing the Selected *Lactobacillus Paracasei* LC11, Cranberry and D-mannose

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Objective: *Lactobacillus paracasei* LC11, (Proge Farm, Italy) has been tested in vitro for antibiotic activity versus different bacteria including *E. Coli* and for adhesion to the intestinal mucosa.

Considering the promising results, LC11 has been included in the formulation of a probiotic food supplement (Lactoflorene® Cist) in combination with Cranberry extract and D-Mannose.

The high antagonist activity against *E.Coli*, the high resistance to g.i. conditions, the efficient adhesion to intestinal epithelial cells are key factors of success for LC11 in the prevention of uncomplicated recurrent UTI in synergistic combination with the other ingredients.

Methods: 45 premenopausal women aged 18–50 years with acute UTI and a history of recurrences have been enrolled. Patient received phosphomicin once a day for two consecutive days and randomly assigned to group 1 (receiving Lactoflorene®Cist once a day for 10 d/mo for 3 mo), group 2 (receiving Lactoflorene®Cist once a day for 90 d) and group 3 no treatment (control).

The Role of a Low Protein Diet (LPD) With and Without Probiotics in Patients with Advanced Renal Failure

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Objective: Previous studies demonstrated that certain pre and/or probiotics can reduce the levels of p-cresol (PC), indoxyl-sulphate (IS) and lipopolysaccharides (LPS) in chronic kidney disease (CKD) patients, but they did not evaluate the association with a low protein diet (LPD).

The aim of our study was to evaluate the association of LPD and selected probiotics, namely 5×10^9 CFU of *Bifidobacterium longum* (mix DLBL) and 1×10^9 CFU of *Lactobacillus reuteri* LRE02 (DSM 23878), in reducing the levels of PC, IS and Lipoprotein-associated

Phospholipase A₂ (Lp-PLA₂) in patients with advanced renal failure.

Methods: This single-centre, double-blind, placebo-controlled, randomised study enrolled adult subjects aged 18-80 years with glomerular filtration rate (GFR) <25 mL/min. A nephrological evaluation and biochemical analysis were performed at baseline, after 2 (T2) and 5 months (T5). Randomization to receive either probiotics or placebo occurred after 2 months.

Results: 9 drop-outs were recorded. A significant reduction of uric acid (5.9 ± 1.4 vs. 6.4 ± 1.4 mg/dL, $P=0.07$), triglycerides (172 ± 71 vs. 239 ± 210 mg/dL, $P=0.08$), C-reactive protein (71.4 ± 18.8 vs. 60.9 ± 16.2 g/24 h, $P=0.0077$) and Lp-PLA₂ (170.2 ± 52 vs. 158.5 ± 51.9 nmol/mL/min, $P=0.04$) was recorded the recurrent cystitis episodes were significantly lower in both group 1 and group 2 (53% vs. 16%, $P<0.01$) compared to control. During the 6 months follow up there were no differences in recurrent cystitis episodes in group 1 and group 2 (66% vs. 69%, $P<0.02$).

Conclusions: Lactoflorene®Cist was effective in the prevention of recurrent cystitis episodes. after T5 or T2 compared with T0. Lp-PLA₂ and PC were reduced by the LPD but were not affected by probiotics, while IS metabolism was affected by probiotics.

Conclusions: LPD/probiotics is a safe therapy for patients with advanced renal failure since no side effects were reported and positive effects on several biochemical and nephrological parameters were demonstrated.

Correlation of Anorectal Motility and Sensitivity Characteristics with Small Intestinal Bacterial Overgrowth in Patients with Diarrhea-predominant Irritable Bowel Syndrome

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Introduction: Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder with gastrointestinal motility disturbances (leading to diarrhea or constipation) and visceral hypersensitivity (leading to abdominal pain and distention). Small intestine bacterial overgrowth (SIBO) is also characterized by abdominal pain, bloating and diarrhea. SIBO is often associated with diarrhea-predominant IBS (IBS-D). The association between SIBO and anorectal motility and sensitivity disturbances in these group of patients has not been sufficiently studied.

Aim of the Study: To assess prevalence of SIBO in IBS-D patients and to assess correlation between SIBO and anorectal motility and sensitivity abnormalities in IBS-D patients.

Materials and methods: 35 patients with IBS-D (according to the ROME IV criteria) were analysed by the hydrogen breath test with glucose using Gastro+Gastrolyzer (Bedfont, UK) to determine SIBO. All patients were also examined by high-resolution anorectal manometry (HRAM) using 20 channel water-perfused catheter with a polyethylene (Solar GI, MMS, the Netherlands).

Results: SIBO was found in 24 patients with IBS-D (68.5%). According to HRAM IBS-D patients with SIBO had decreased absolute anal squeeze pressure ($P=0.012$), maximum absolute anal squeeze pressure ($P=0.011$), threshold for intense urge to defecate ($P=0.022$) and maximum tolerable volume ($P=0.025$) comparing to non-SIBO IBS-D patients.

Conclusions: IBS-D patients with SIBO are predisposed to decreased parameters of anorectal motility (absolute anal squeeze pressure and maximum absolute anal squeeze pressure) and sensitivity (threshold for intense urge to defecate and maximum tolerable volume), responsible for severity of diarrhea-predominant IBS symptoms.

Comparison of Specific IGG and IGA Subclass Levels to Lactobacillus and Streptococcus in Young Healthy Adults

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Objective: This study aimed to assess reactivity of serum antibodies of different IgG and IgA subclasses taken from young healthy adults to selected lactobacilli and streptococci.

Methods: Serum samples were collected from 22 young healthy adults, and were used as a pool.

The methodology consisted of attaching whole bacterial cells to ELISA plate, followed by blocking, the addition of pooled serum sample, which was diluted in PBS 500× for IgG, IgG1 and IgG2, 250× times for IgG3 and IgG4, 100× for total IgA analysis and 50× for IgA1 and IgA2. Commercial secondary antibodies were all monoclonal, biotinylated. For statistical analysis bacteria were grouped as *Lactobacillus* (8 strains), *Streptococcus* (5).

Results: The level of the main serum antibacterial antibody, of the IgG2 subclass showed no difference between *Lactobacillus* and *Streptococcus*. Only IgG1, IgG4 and IgA1 levels were significantly different between the two genera. The level of total IgG reflected the levels of IgG2. The reactivity toward lactobacilli was uniform across the tested species, which was not the case of streptococci.

Conclusions: We conclude that streptococci, due to persistent mucosal infection induce the production of IgA1, IgG1 and IgG4, which differentiates this genus from *Lactobacillus* genus in terms of antibody production. Lactobacilli bind almost equal levels of total IgG and IgG2 as streptococci, which is indicative of their inherent immunostimulative action which should be taken into consideration in individuals with overactive immune system such as in certain types of autoimmune diseases.

Prospects of Probiotic Lectins as Functional Food Ingredients

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Objective: We studied probiotic lectins (PL) recognizing polymeric polyvalent mucin like synthetic glycoconjugates (GC, <http://www.lectinity.com>) and investigated their properties. The aim is evaluation of prospects of PL as functional food (FF) ingredients.

Methods: PL of probiotic Acilact and bacteria were isolated and characterized using electrophoresis in the plate of polyacrylamide gel. Electroblooded on membrane PL reacted with GC-biotin and the live chemiluminescence of the bound streptavidin-peroxidase was registered in *BioChemi System* (UVP). Activities of acidic and alkaline preparations of lactobacilli and bifidobacteria were studied using standard procedures.

Results: 1. PL – imitators of probiotics: realization of prolonged antimicrobial activities which are synergistic to antibiotics (prospects are all aspects of application of pro/synbiotics/ posbiotics in FF). 2. PL act as “network-in- network” (carriage and deposit of GC type drugs, metabiotics, prebiotics and postbiotics). 3. Indirect support from side of PL during cofunctioning to complement, cytokines, macrophages, leukocytes, other protective cells (against system diseases, pathogens and diseases associated with geographic incompatibility of individual probiotic consortia). 4. Advantages of PL compared to probiotics: advancing in action; relative resistance upon storage and conditions of stress; prolonged presence in watersoluble and fat-soluble states; simplicity for preparation of metabolite combinations to create and choose effective mixtures of directed needed action; universality of regimes for PL applications (together with chemo/radiotherapy, antibiotics). 5. Suppliers of cationic metal and organic forms, detergents-like GC (extended use of PL in FF in combinations with nutraceuticals, for their support, or in variants of their carriage).

Conclusions: The data indicate spectrum of application of PL and extended prospects of their using in different categories and forms of FF. The possible approach is to use PL as a scaffold for effectors of FF to support their actions.

Study of Two Complementary Methods for the Quantification of Peptidoglycan in Gram-positive Bacteria

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Objective: The peptidoglycan (PG), also known as murein or bacterial mucopeptide, is a polymer that represents an essential component of the cell wall of bacteria responsible for both shape determination and cellular integrity under osmotic stress.

Its main role is to maintain the structure of the cell, provide a basis of attachment for the outermost proteins and take part in the processes of cell growth and division, so that the inhibition of its synthesis leads to lysis and, therefore, to cell death.

The PG layer is formed by saccharide chains joined by peptide bridges. A quantitative understanding of the relationships between PG architecture, morphogenesis, immune system activation and pathogenesis can provide molecular-scale insights into the function of this polymer involved in cell wall synthesis and cell growth.

Methods: With this study, we set up two alternative and complementary methods for the isolation and quantification of peptidoglycan in Gram-positive bacterial strains.

Results: The first method involves the development of a multistep method for the digestion by chemical and enzymatic means of the cellular components, except for peptidoglycan, followed by their quantification by gravimetric analysis. The second method provides the development of a process for the digestion of the previously isolated peptidoglycan in its individual components, and subsequent quantification by ultra-performance liquid chromatography (UPLC), in order to confirm and qualify also the first method.

Conclusions: Both methods could represent an innovative tool to more precisely investigate the peptidoglycans detectable in the cell walls of Gram-positive bacteria, including the most important probiotic genera.

Intestinal Epithelial Barrier Regulation with Probiotics

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Objective: The intestinal epithelium is composed of tightly connected monolayer of cells that shape epithelial barrier. The interactions of commensals with the gut mucosa help to regulate the intestinal epithelial barrier. Despite the large number of studies, the detailed mechanisms, of how commensal bacteria affect signaling pathways in intestinal epithelial cells, remain poorly understood.

Methods: The starting point of our research was the study on the dextran sulfate sodium (DSS) induced mouse model of colitis in which we investigated the protective effect of human commensal bacteria in intestinal inflammation. Based on the global gene expression analysis on the mice colon samples we developed series of *in vitro* experiments for mechanistic studies of the signaling pathways, responsible for the protective effect of probiotic bacterial strains *Lactobacillus (L.) gasseri* K7, *L. fermentum* L930BB, *Bifidobacterium animalis* subsp. *animalis* IM386 and *L. plantarum* WCFS1.

Results: We found that probiotics contribute to the regeneration of the intestinal epithelium by triggering anti-apoptotic pathways and pathways that impact on the organization of tight junction proteins and actin cytoskeleton. This probiotic immunomodulatory signaling cascades are initiated via Toll-like receptor 2 (TLR2). In addition, we also proved that probiotic surface components are responsible for TLR2 signalization.

Conclusions: We have shown that some immunomodulatory molecular complexes can be common to a larger taxonomic group, since all the studied strains have triggered the same signaling cascades in intestinal epithelial cells. By defining the probiotic signaling pathways, that strengthen the epithelial barrier, we have contributed to better understanding of probiotic efficiency.

Anticarcinogenic Activity of B. Longum BAA-999 Microencapsulates and Lycopene Against Azoxymethane-Dextran Sulfate Sodium (AOM-DSS) Induced Colon Carcinogenesis in CD-1 Mice

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Objective: The aim of this work was evaluated the chemopreventive potential of *Bifidobacterium longum* (BF) microencapsulated and complemented with lycopene (LYC) on the expression of members of the IGF-1/IGF-1R system and its relationship with the clinicopathological features in an AOM-DSS- induced colorectal carcinogenesis model.

Methods: BF was microencapsulated by Spray Drying Technique and daily gavaged with LYC for 16 weeks to male CD-1 mice in an AOM-DSS model. BF viability, pH values, and beta-glucuronidase activity (Beta-GA) were monitored in different segments of the murine GIT and feces. Subsequently, morphological and histopathological examinations of the colon were determined. Finally, protein expressions of IGF system were analyzed by IHC.

Results: BF and BF+LYC-treated groups had significantly lower inflammation grade, mean tumor number and tumor (13-38%) and adenocarcinoma (13-14%) incidence compared to AOM+DSS-treated animals (80%); with a higher distribution at the distal colon (67-100%), whereas LYC treatment only protected against inflammation grade and incidence. pH and Beta-GA values were significantly higher in the caecum, colon and feces samples of AOM +DSS control group and normalized by BF and/or LYC. Similar to the histopathology analysis, only BF and BF+LYC treatments significantly reduced both positive rate and expression grade of IGF-1 and IGF-1R proteins and normalized IGF1BP3 expression, however, LYC co-administration did not further improve these values.

Conclusions: Based on intestinal parameters related to the specific colon carcinogenesis in an AOM-DSS-induced model, supplementation of LYC and microencapsulated BF resulted in a significantly chemopreventive potential through modulation of over-expression of IGF-1/IGF-1R system.

Prunus Mahaleb Fruit Fermentation for New Functional Foods and Beverages

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Objective: *Prunus mahaleb* (*P. mahaleb*) is a deciduous tree native to the Mediterranean region, resistant to dry climate and several pests and diseases. *P. mahaleb* fruits are small, highly pigmented drupes representing a new source of bioactive compounds, above all anthocyanins, however due to astringent and sour taste, they are considered not-edible. This study aimed to improve sensory characteristics of *P. mahaleb* fruits after fermentation with different *Saccharomyces cerevisiae* and *Lactobacillus plantarum* strains.

Methods: Aqueous suspension of intact *P. mahaleb* fruits were inoculated with four lactic acid bacterial strains and one yeast strains, as single or mixed starter formulation. Microbial growth kinetics were followed during 20 days of fermentation characterizing both fermented fruits and fermentation broth by HPLC analysis and their organoleptic properties.

Results: Results indicated that all strains were able to grow and ferment fruits and that all *L. plantarum* strains were able to survive to simulated gastric and pancreatic digestions. However, the starter

L. plantarum FG69 + *S. cerevisiae* L1180-7 had the best impact on sensory characteristics.

Conclusions: Since sourness of *P. mahaleb* fruits was strongly reduced by fermentation with a new starter co-culture made up with *L. plantarum* and *S. cerevisiae* strains, it is possible to conclude that a new promising functional and probiotic food and beverage was obtained by exploiting fruits from trees currently growing on marginal land for agricultural production.

Tribiotics Design from Bovine Colostrum by Artisanal and Selected Cultures with Multiple Metabolic Activities

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Objective: Kefir grains as an artisanal culture for the colostrum fermentation offer new approaches for the tribiotics' obtainment. The bovine colostrum functionality was improved through metabolic transformation with a consortium based of artisanal and commercial probiotics.

Methods: Firstly, 10% bovine colostrum (Axyar, Belgium) suspension was hydrolysed by the selected *Candida lipolytica* MIUG D67 strain (10^6 CFU/100 mL), by incubation at 30°C for 48 hours. Further, the lactic acid fermentation was performed, for 48 hours at 30°C, using 2.5% kefir grains and 0.1% of different commercial cultures (Chr. Hansen, Denmark), *Bifidobacterium* (BB-12®), *Lactobacillus acidophilus* (LA-5®), *Lactobacillus casei* (L. casei 431®), FreshQ®4, ABY-3 Probio-Tec® or CHN-11. The fermented products' acidity, antioxidant and antimicrobial activity were analysed. The bioactive peptides were separated by centrifugal units with a 10 kDa cut off, whereas their functional properties were evaluated in vitro.

Results: The obtained fermented products showed acidity values in the range of 112.5-168.7°Th, an antioxidant activity between of 2.10-4.47 mM TE/g and an antimicrobial activity against spoilage microorganisms in the range of 2.5-7.0 mm. The fermented product in which FreshQ®4 culture was used revealed the highest acidification potential (168.74°Th), the best antimicrobial activity (5 mm) and a moderate antioxidant activity (3.15 mM TE/g). The peptides separated from the fermented products revealed high ABTS radicals scavenging activity and ACE inhibitory capacity.

Conclusions: The colostrum improvement by bioconversion and fermentation with a multiple microbial consortium assured the obtainment of products that have pre-, pro- and post-biotic properties, with valuable applications in the nutraceuticals and cosmeceuticals' production.

Use of Autochthonous Probiotic Yeasts to Improve the Aroma Profile of Fermented Chili Pepper Sauces

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The aim of the study was to investigate the different aroma profile produced by inoculation of yeast strains with probiotic potential, *Hanseniaspora opuntiae*, *Pichia kudriavzevii* and *Wickerhamomyces anomalus*, on the fermentation of Guajillo chili pepper sauce.

Methods: Guajillo chili pepper (*Capsicum annuum* L.) sauce was inoculated with the probiotic yeasts and incubated at 30°C for 4 days. Yeasts growth was analyzed by plate counting in Rose Bengal Agar. Flavor compounds produced during and at the end of fermentation were evaluated using HS-SPME-GC-MS and gas chromatography-olfactometry (GCO) analysis.

Results: A total of 78 volatile compounds were identified during the fermentation. Among them 34 were aroma active compounds

detected by GCO. Propanoic acid (cheesy), 3-methylbutanoic acid (sharp, cheese), ethyl 2-methylbutanoate (fruity), and 6-methyl-5-hepten-2-one (strong, citrus) were identified as key aroma contributors produced by the inoculation of the yeasts. All aroma compounds detected were affected by fermentation time, while aldehydes and terpenes, were not affected by yeast inoculation.

Conclusions: The use of the different yeast in the production of Guajillo chili pepper sauce fermentation provided different aroma notes. *P. kudriavzevii* and *W. anomalus* produced a similar aroma based on ester compounds, alcohols and branched chains acids. However, *W. anomalus* inoculation increased the cheesy character due to its ability to produce propanoic acid. In contrast, inoculation of *H. opuntiae* produced a different aroma and green notes based on high production of aldehydes, ketones and acetic acid.

Artemisia Umbelliformis SUBSP. Eriantha Extract Inhibits Growth and Invasion of Hepatocarcinoma Cell Lines

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Objective: Hepatocellular carcinoma (HCC) is the most frequent primary malignant disease of the liver and the second leading cause of cancer related mortality in the world. Discovering new compounds able to improve patient survival, alone or in combination, represents a priority. *Artemisia annua* L., a plant acknowledged for its anti-malarial effects, also shows important anticancer activities towards several kinds of tumors, including hepatocarcinoma. The aim of this study was to verify the antitumoral potential of other plants of *Artemisia* genus, such as the Central Apennine subendemic species *A. umbelliformis* ssp. *eriantha* (Ten.) Vallès-Xirau & Oliva Brañas.

Methods: Two hepatocarcinoma cell lines, HepG2 and Huh7, and a differentiated hepatocyte line, HepaRG, were used. Following treatment with alcoholic extracts of the aerial parts of the plant, cell proliferation was evaluated by neutral red assay, cell cycle and apoptosis by flow cytometry, cell migration by wound healing assay, and expression of cell cycle and apoptosis molecules by Western blotting.

Results: Treatment reduced cell growth of both HepG2 and Huh7 cells, with no effect on the differentiated HepaRG cells. In addition, Huh7 cells also exhibited lower healing after scratch wound, increased apoptotic cell fraction and elevated p21, p27 and p53 expression.

Conclusions: *A. umbelliformis* ssp. *eriantha* extract interferes with key factors of hepatocyte proliferation and invasion and emerges as a promising adjuvant for prevention and/or treatment of hepatocellular carcinoma.

Effect of Inulin DP on Quality Characteristics of Functional Whole-meal Spaghetti of Durum Wheat Old Varieties

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Objective: The aim of this work was to evaluate the effects of the addition of inulin with different polymerization degrees (DP) on the quality and sensory properties of whole-meal spaghetti using old varieties of durum wheat.

Methods: Four whole-meal flour of durum wheat ("Russello", "Senatore Cappelli", "Margherito" and "Timilia") compared to a commercial control were used for the production of fortified spaghetti with chicory inulin (CHI-DP about 20 fructose units) and cardoon inulin (CRI-DP about 80 fructose units), at 2 and 4% of substitution (w/w). On fresh and dry pastas, color, sensory attributes, cooking quality, loss of inulin (HPAEC-PAD, Thermofisher) were performed.

Results: In dry cooked spaghetti ANOVA showed that the cultivar influenced mainly the firmness, elasticity, fibrous, odor, taste and overall quality score (OQS). The inulin DP influenced significantly all the studied traits except firmness and bulkiness, while its substitution influenced all the traits. Out of cultivar, all the scores resulted higher than the threshold of acceptability, the taste resulted more palatable using CRI at 4%. The cvs Russello and Senatore Cappelli, with 4% CRI, obtained the highest OQS, good swelling index and water absorption, and lower OCT. "Margherito" gave the highest inulin losses after cooking.

Conclusions: Russello and Senatore Cappelli with 4% of CRI gave very interesting results and with a normal ratio of 100 g of pasta/die, it is possible to cover the 100% of the RDA for fructans; this value is enhanced if we consider the nutraceutical effect of the whole-grains used.

Biochemical Properties, Antioxidant and Anti-inflammatory Activity of Mulberry Fruits from South Apulia (South Italy)

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Objective: Simple sugars, organic acids, total phenol and anthocyanin contents, antioxidant (AA) and anti-inflammatory (AI) activity were investigated for the first time in fruits of *Morus alba* (cv. Legittimo nero and cv. Nello) and *Morus nigra* grown in Salento (South Apulia, South Italy).

Methods: Juice was obtained from the mulberry fruits by centrifugation and used to determine the glucose, fructose, sucrose, malic and citric acid using an enzymatic spectrophotometric kit. Phenols were extracted by cold acetone 70% acidified with HCl. Total phenols and ortho-diphenols content were determined spectrophotometrically; anthocyanins were analyzed by HPLC/DAD/MS. AA was determined by DPPH, ABTS and FRAP test; AI was estimated by cyclooxygenase (COX) inhibitory assay.

Results: The results showed that the sugars amount ranged between 6.29 and 7.66 g/100 g fresh weight (FW) while the malic and citric acids content was low, about 0.1-1 g/100 g FW. Mulberries are a good source of phenols which are present in higher values in *M. nigra* and *M. alba* cv. *Legittimo nero* (about 485 and 424 mg Gallic Acid Equivalent (GAE)/100 g FW, respectively). The HPLC/DAD/MS analysis identified 5 main anthocyanin compounds present in different concentrations in each variety of mulberry: cyanidin 3-sophoroside, cyanidin 3-glucoside, cyanidin 3-rutinoside, pelargonidin 3-glucoside, pelargonidin 3-rutinoside. The highest concentration of anthocyanins was determined in *M. alba Legittimo nero* (289 mg/100 g FW) while the lowest content (about 25 mg/100 g FW) was measured in *M. alba* cv. *Nello*. *M. nigra* showed a good AA in comparison with the different *M. alba* genotypes with all the used methods; its AA was equal 33, 26 and 21 μ mol Trolox/g using DPPH, ABTS and FRAP test, respectively. All genotypes showed an AI which was also compared with two commercial anti-inflammatory drugs.

Conclusions: The data obtained support the high biological qualities of mulberry fruits and their diffusion in human nutrition as an important source dietary antioxidant and anti-inflammatory components with possible beneficial effects on consumer's health.

Release, Stability and Bioaccessibility of Bioactive Peptides During In Vitro Digestion

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Objective: In the scientific literature, which is focused on the research and detection of biological activity of different food components, recently there is a great interest for investigation of their fate in process of gastrointestinal digestion. Biologically active compounds, such as peptides, polyphenols, flavonoids, carotenoids, prebiotics, probiotics, etc., generally, are chemically and thermally unstable, sensitive to oxidation and other environmental conditions. Therefore, their release,

stability and availability during the passage through the digestive tract can be affected. As the *in vivo* tests are time and financially demanding, there is a growing interest for *in vitro* tests, which are useful tools for examining and understanding of the changes, interactions, bioavailability and bioaccessibility of different nutrients and biologically active compounds.

Methods: A two stage *in vitro* digestion model system (by pepsin and pancreatin) was used to simulate the process of human gastrointestinal digestion on plant protein hydrolysates. The biologically active potential (antioxidant, antidiabetic and antihypertension activity) of the digests was determined.

Results: The results showed significantly higher the antioxidant, anti-hyperglycemic and ACE inhibitory potency of the digests of all studied protein hydrolysates, which indicating that it comes from the peptides obtained by hydrolysis with digestive enzymes.

Conclusions: This research indicates that this protein ingredient could be also source of natural antioxidant and potent antihypertensive and anti-hyperglycemic peptides released in the digestive system, after normal consumption.

Fermentation of Kiwicha (*Amaranthus Caudatus*), a Pseudocereals Originary from PERU, by *Lactobacillus Rhamnosus*

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Objective: *Kiwicha* is a pseudocereals crop from the Peruvian Andes with high nutritional value. The aim of this study was to assess the fermentative capacity and viability of *Lactobacillus rhamnosus* strains on beverage based on *Kiwicha*.

Methods: *Kiwicha* (Centenario variety) was suspended (20 % w/v) in water and heat treated (54°C×30 min, 65°C×60 min, 72°C×30 min). Overnight *L. rhamnosus* cultures were inoculated (v/v) with the following set: 1% CRL1505, 1% ATCC 53013, 0.1% LC705, 1% CRL505/0.1% LC705, and 1% ATCC 53013/0.1% LC705. Samples were incubated (37 °C, aerobiosis) for 10h. Total titratable acidity, pH and cell counts were assessed after 6 and 10h of fermentation and after 6, 12 and 22 days of storage at 4°C.

Results: After 10 hours of fermentation, the sample inoculated with ATCC53013 displayed the lowest pH (3.74), whereas that with LC705 had the highest pH (4.30). Cell counts after fermentation were (log orders CFU/g): 8.7 (CRL1505), 8.9 (ATCC 53013), 8.5 (LC705), 8.7 (CRL505/LC705), and 9.0 (ATCC53013/LC705). After 22 days storage, pH ranged from 3.45 to 3.66. Viability lost was negligible, except for LC705 (1.6 log orders CFU/g of reduction in cell counts).

Conclusions: *Kiwicha* may be a suitable substrate to be fermented by probiotic bacteria in order to develop new functional drinks, and also offered satisfactory protective capacity to maintain adequate levels of viable bacteria during 22 days of refrigerated storage.

Screening of *Lactobacillus* SP. Isolated from Wheat and Sorghum Sourdoughs May Hydrolyze Gluten During the Fermentation of a Gluten-containing Medium

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Objective: Screening of gluten-proteolytic *Lactobacillus* sp. isolated from wheat and sorghum sourdoughs.

Methods: Gluten-containing medium (GCM) was used to investigate the proteolytic activity of 120 lactic acid bacteria (LAB) isolated from two wheat (*Triticum aestivum*) and one sorghum (*Sorghum bicolor*) artisanal sourdoughs. Initially, LAB were screened by gram-positive staining and catalase test. The isolates were cultivated twice in MRS broth (24 h, 30°C), washed two times with NaCl (0.85% w/v) solution, and inoculated in the gluten citrate agar (GCA) surface (24 h, 37°C) to stimulate the production of proteolytic enzymes. Thereafter, microorganisms were harvested from the GCA and washed twice with sterile saline solution (Na Cl 0.85 w/v) supplemented with calcium chloride 10mM. Each inoculum (10% v/v) was added to GCM, and samples fermented (24h, 37°C) under agitation. The proteolytic activity was determined by SDS-PAGE and non-inoculated GCM was used as negative control. Specific primers for *Lactobacillus* sp. were used to identify gluten-degrading isolates at genus level by RT-qPCR.

Results: From 120 isolates, 23 were able to hydrolyze gluten when fermented GCM were compared with control. Besides, the results from RT-qPCR showed that all 23 proteolytic isolates were *Lactobacillus* sp.

Conclusions: Fermentation of gluten by selected lactobacilli is a potential tool to reduce gluten content in fermented gluten-based foods. These results are a preliminary effort to select gluten-degrading LAB to develop new foods with low content or absence of gluten for individuals with gluten allergy or intolerance. Additionally, more tests are necessary to verify the potential application of these lactobacilli as probiotics.

Investigating Potential Chemoprotective Role of Pomegranate Juice Through Analyzing Its Reciprocal Interactions with the Gut Microbiota

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Objective: While plants and their constituents have traditionally been used for preventing diseases, including cancer, the emerging concept of combination chemoprevention by multiple agents or “whole foods” is becoming increasingly attractive. This work aims to examine the chemopreventive role of polyphenol-rich pomegranate on the rat gut microbiota, as a step towards investigating its ability to neutralize polycyclic hydrocarbons-induced carcinogenesis.

Methods: Six seven-week-old male Sprague Dawley rats were randomly allocated to a control group, only fed a standard AIN76 diet, and a pomegranate group, fed the standard diet + 2.5 mL/kg/d standardized pomegranate juice. Pooled fecal samples from each group were collected twice weekly for eight weeks. DNA from 12 samples was extracted, quantified, and subjected to 16S rRNA amplicon sequencing by Illumina MiSeq. Sequencing data were analyzed by MG-RAST and MOTHUR. Additionally, GC/MS Metabolomic profiling was applied to pomegranate juice before and after its *in vitro* exposure to selected bacterial strains representing the gut microbiota.

Results: In pomegranate-fed rats, phylum Bacteroidetes (particularly genus *Prevotella*) and genera *Faecalibacterium* and *Blautia* were substantially more abundant, while *Eubacterium* and *Enterococcus*, among other Firmicutes, were less abundant. Overall, the Firmicutes-to-Bacteroidetes ratio increased by age and was also significantly lower in the pomegranate-fed rats. A significant decrease in amino acids and sugars was observed in the microbiota-treated juice, while organic acids significantly increased.

Conclusions: This pilot work serves as a standardization step for a model to test the potential chemoprotective effect of pomegranate

(and other natural polyphenols) against the carcinogenic effect of selected polycyclic hydrocarbons.

Akkermansia Muciniphila Robustness Towards Different Temperatures, Atmospheres and Gastrointestinal Conditions

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Objectives: Currently, *Akkermansia muciniphila* has been proposed as a next generation probiotic. However, detailed information addressing its viability under stressful conditions is scarce. Understanding a strain's resilience to potential stressful conditions is crucial for probiotic products manufacture. Herein, we evaluate the *A. muciniphila* susceptibility when exposed to environmental stresses including temperature, atmosphere and gastrointestinal passage (GIT).

Methods: For oxygen and temperature tolerance assays, *A. muciniphila* culture was incubated at different temperatures (4°C/22°C/37°C and 44°C), and under two atmospheres (aerobic and anaerobic) during 72 hours. Each 12 hours, pH values and *A. muciniphila* cultivable cell numbers were determined. To simulate GIT passage, an *in vitro* digestion method (1) was used.

Results: Overall, *A. muciniphila* exhibited a high oxygen tolerance with great stability in culturability (± 8.0 Log CFU/mL) detected after 72 hours exposure at 4°C and 22°C, 24 hours at 37°C and 12 hours at 44°C, as well when subjected to simulated GIT (7.8 ± 0.3 Log CFU/mL).

Conclusions: This work is the first to evaluate the resistant *A. muciniphila* culturability when subject to environmental stresses, suggesting that no strict technological contingencies are required when manufacturing probiotic products containing this bacterium as well its possible storage at household conditions or handling at higher temperatures.

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Postbiotic Characterization in the Amensalistic Symbiosis and Correlation to the Resilience of Human Microbiota

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Objective: Lactic acid bacteria constitute a large group of Gram positive organisms belonging to the human microbiota. Among these the genus *Lactobacillus* is the most widely characterized.

Many studies in the literature showed that culture medium and abiotic factors influence the production of antimicrobial substances. Furthermore, recent studies highlight the potential of metabolomics to predict antimicrobial activity among different *Lactobacillus* species. The purpose of the study was focused on the production of postbiotics by two human origin strains of *L. fermentum*. Moreover, the evaluation of antimicrobial activity and the characterization of metabolites were performed.

Methods: In particular, the production of antimicrobial molecules by varying the culture medium for the greatest yield was achieved.

Subsequently, the antimicrobial activity of the cell free supernatans (CFSs) was evaluated by agar well diffusion assay. Furthermore, metabolic characterization was assessed by ¹H-NMR analysis.

Results: The best yield of antimicrobial substances production was achieved with MRS medium with glycerol as supplement. CFSs showed antimicrobial activity against all pathogen strains tested. A panel of metabolites with variations in concentration were revealed by ¹H-NMR, but considerable differences among inter-species were not showed. Nevertheless, significant variances comparing the metabolites found in the supernatants of strains grown in MRS with glycerol and the same without supplements were recorded.

Conclusions: Despite the full characterization of the molecules present in CFSs has not yet occurred, the presence of sugars, amino acids and organic acids, suggesting the possible presence of bacteriocins or biosurfactants which could be linked to the antimicrobial activity.

Antimicrobial Activity of Cell-free Supernatant of *Lactobacillus Plantarum* IMC 509 Against Common Food Spoilage Microbes

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Objectives: This study aimed to evaluate the *in vitro* anti-spoilage property of *Lactobacillus plantarum* IMC 509 cell-free supernatant as a potential natural food preservative.

Methods: *L. plantarum* IMC 509 (10¹¹CFU/g) lyophilized powder was cultured in MRS broth. To eliminate cells, centrifugation and 0.2 µm filter membranes were used. CTR-supernatant was without treatment; in OA-supernatant pepsin, catalase, proteinase K were added to test the organic acids effects. HO- supernatant was treated with proteinase K and with neutralized pH to monitor the H₂O₂ effects. In BLS-supernatant catalase was added and pH neutralized to check bacteriocin-like-substances. Several Gram positive and Gram negative bacteria strains and one yeast were selected for the well diffusion test. Mueller-Hinton agars were inoculated with tested microbes and loaded with four supernatants. Plates were incubated and inhibition zones were checked and measured.

Results: The pH values of CTR and OA were lower than HO and BLS. The diameters of inhibition zone demonstrated that all tested strains were sensitive to both CTR and OA samples, while not to the HO and BLS samples, with the exception of *Klebsiella pneumoniae* which was sensitive to all four supernatants.

Conclusions: The results showed the lower pH value, the higher inhibition effects toward tested strains, demonstrating the antimicrobial properties of *L. plantarum* IMC 509 supernatants. The organic acids produced by the strain were the major active antimicrobial substances. Further studies are needed to evaluate its ability to extend food shelf-life by directly applying on food matrices.

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Viability and Synergistic Antibacterial Effects of Indigenous Probiotic Bacteria and Herbal Extracts in Invitro Conditions

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In present research we investigated the antibacterial potential of local isolates of probiotic bacteria (*Lactobacillus casei*, *L. plantarum*,

L.reuteri, *Enterococcus faecium* and *Pediococcus acidilacti*) in the presence of herbal extracts [Chamomile (*Matricaria chamomilla*), Fennel (*Foeniculum vulgare*), Peppermint (*Mentha piperita*) and thyme (*thymus vulgaris*)]. *Escherichia coli*, *Salmonella typhimurium* and *Salmonella enteritidis* were used as indicator organisms. The antibacterial activity of the mentioned probiotic isolates and the herbal extracts against the selected pathogens were examined by agar well diffusion method. The synergistic antibacterial effect and the viability (survival percentage) of the probiotic bacteria in combination with different concentrations of the mentioned herbal extracts were determined after 24, 48 and 72 hours. The minimum inhibitory concentrations (MIC) of the herbal extracts on the indicator organism (10⁶ cfu/mL) was measured by microdilution assay. All experiments were performed in triplicate and results analyzed statistically. According to the results, all selected LAB strains and thyme extracts were able to inhibit the growth of the tested gram negative. However, *L.plantarum* and *E.faecium* showed maximum inhibitory actions towards *S.typhimurium*. Probiotic bacteria mixed with thyme and peppermint showed synergistic effects and their combined use resulted in enhanced antibacterial actions. The viability of the probiotic bacteria in the presence of diluted (above 1:10) thyme and peppermint extracts were above 70%. In conclusion, the antibacterial activity exerted by probiotic bacteria against the pathogens could be significantly enhanced when combined with herbal extracts, and hence might be considered as suitable alternative to antibiotics for use in man and animals in future.

Keywords: Probiotic, herbal extracts, pathogens, Antibacterial activity, Synergistic actions

Short Chain Fatty Acids Profile During Remission or Activity Periods in IBD Patients

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Objective: Short chain fatty acids (SCFAs) are metabolites from colonic bacterial degradation of dietary fibre. They are important in the colon because affect colonocyte morphology and function, and produce, after absorption, a lot of pharmacological effects. The main SCFAs with beneficial effects on the host are acetate, propionate and butyrate. In the last decades, it became apparent that SCFAs might play a key role in the prevention and treatment of chronic diseases, including metabolic syndrome, bowel disorders, and cancer. Crohn's disease (CD) and Ulcerative colitis (UC) are characterized by a chronic, relapsing intestinal inflammation likely due to an inappropriate immune response together with intestinal microbiota dysbiosis, probably leading to a change in SCFAs profile during activity or remission periods of the disease. So, the aim of this work was to compare the SCFAs profile in patients with UC or CD in activity or remission of the symptoms.

Methods: Faeces were donated by IBD patients and SCFAs were measured by chromatographic analysis coupled to mass spectrometry. Patients were divided in two groups: remission or activity. All results are expressed by mean ± SEM.

Results: We identified no significant difference between patients in remission (r) or activity (a) for both diseases. In UC patients were observed acetate: 0.137(r) ± 0.008 versus 0.135(a) ± 0.008 mg/mL; propionate: 0.236(r) ± 0.007 versus 0.232(a) ± 0.007 mg/mL; butyrate: 0.067(r) ± 0.003 versus 0.070(a) ± 0.005 mg/mL. In CD patients were observed acetate: 0.132(r) ± 0.008 versus 0.134(a) ± 0.01 mg/mL; propionate: 0.252(r) ± 0.01 versus 0.244(a) ± 0.008 mg/mL; butyrate: 0.068(r) ± 0.004 versus 0.071(a) ± 0.005 mg/mL.

Conclusions: In conclusion, remission or activity did not change SCFAs profile in UC or CD patients.

Characterization of Microvesicles of Strain *Lactobacillus Plantarum* BGAN8 and Internalization by HT29 Cells

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Objective: Bacteria secrete membrane microvesicles (MVs) as a mechanism for intercellular communication. We report the characterization of MVs from the strain *Lactobacillus plantarum* BGAN8. The putative interaction of the MVs from *Lb. plantarum* BGAN8 with the intestinal epithelial cells was studied.

Methods: MVs were isolated from 20 hours liquid culture. Purified MVs were visualized by cryo-TEM. The protein content of isolated MVs was measured by Pierce BCA Protein Assay Kit. Proteins from MVs were visualized by SDS PAGE. Internalization of rhodamine B-R18-labeled MVs by HT29 monolayers were measured over time. HT-29 cells were pre-incubated, for 1 hour at 37°C, with (i) the lipid raft disrupting agents filipin III or nystatin, and (ii) with the inhibitors of the clathrin-mediated endocytosis pathway chlorpromazine or dynasore before adding the rhodamine B-R18-labeled MVs. CSLM techniques were used to capture images of MVs internalization in HT29 cells.

Results: All MVs were single-layer, with a size-range of 20 nm to 100 nm. Results from fluorescence quantification showed that MVs are internalized by HT29 cells. Use of clathrin inhibitors significantly reduced the fluorescence intensity, whereas no changes in the fluorescence were detected with inhibitors filipin III and nystatin. CLSM technique confirmed the entrance of vesicles into the cell in the presence of nystatin, while there was no fluorescence was observed if dynasore was used.

Conclusions: Internalization of the MVs from *Lb. plantarum* BGAN8 is mediated by clathrin mediated endocytosis pathway.

Probiotic and Postbiotic Effects of *L. Rhamnosus* GG Against RV-induced Diarrhea

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Objectives: *L. rhamnosus* GG (LGG) is included in the ESPGHAN guidelines as active treatment for Rotavirus (RV) induced acute gastroenteritis. Aim of this study was to evaluate the mechanisms of efficacy in an *in-vitro* model of RV diarrhea.

Methods: Oxidative stress was measure by reactive oxygen species. Apoptosis was evaluated by DAPI nuclear staining. NFκβ immunofluorescence was used for inflammation mechanism evaluation. Living LGG microorganisms or LGG conditioned medium (LGGm) were used to stimulate Caco-2 cell monolayers.

Results: RV induced an increase of ROS intracellular levels that was significantly reduced in the presence of LGG or LGGm compared to initial value ($P < 0,05$). LGG significantly reduced the number of RV-induced apoptotic nuclei and NFκβ nuclear translocation both in its living form and in form of conditioned medium ($P < 0,05$). The efficacy of LGG versus LGGm resulted at the same levels since no significant difference was observed.

Conclusions: LGG protects intestinal epithelial cells by RV damage acting on different lines of defense. LGG counteracts oxidative stress, apoptosis and inflammation both in its living form and in form of conditioned medium suggesting that specific moieties are involved.

Conflict of interest: The study was supported by Dicofarm SpA.

Effect of Probiotic and Autoprobiotic Enterococci on Contractile Activity of Rats Intesinum After Correction of Dysbiosis

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Objective: The aim of this study was to investigate changes of a pattern contractile activity in isolated rectum of the rat after probiotic and autoprobiotic correction of experimental dysbiosis.

Methods: Studies were performed on male Wistar rats without impact (group C2) and after induction of dysbiosis by ampicillin and metronidazole. After that suspensions of probiotic strains *Enterococcus faecium* L3 (group P) and autoprobiotic enterococci (group A) were administered to rats. Animals with dysbiosis from control 1 group (C1) got only the phosphatic buffer. The changes in the amplitude and frequency of spontaneous muscle contractions (SMC) of the rats rectum were studied using Physiological Ugo Basile (Italy).

Results: The SMC of the colon wall in C1 group was significantly changed: amplitude was reduced (in 4 time) and the frequency was increased (in 6,5 times) compared with the C2 group The frequency of SMC also remained high after the administration of autoprobiotic and probiotic. However the amplitude increased until full recovery after exposure to probiotic and partial after autoprobiotic correction. The frequency increased in these groups (A and P), no more than 1.5 times compared to C2.

Conclusions: Restoration of the tonic motor function of the colon occurred more effectively after exposure of probiotic (L3) and autoprobiotic *Enterococcus faecium*. The obtained results can be used in the development of new medical technologies for treating irritable bowel syndrome and disfunction of gastrointestinal tract accompanied by other human diseases.

This work was supported by Russian Science Foundation under grant no. 16-15-10085.

Secretome Modulation of CACO2 Cell Line Induced by a Multi-strain Probiotic

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Background: Probiotics are defined as live, non-pathogenic bacteria that confer health benefits beyond their nutritional value [1,2]. Particularly VSL#3, a probiotic mix containing 4 strains of Lactobacilli (*L. paracasei*, *L. plantarum*, *L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus**), 3 strains of Bifidobacteria (*B. longum****, *B. infantis***, *B. breve*) and *Streptococcus thermophilus*, has demonstrated efficacy in the management of diseases characterized by increased intestinal permeability such as irritable bowel syndrome and ulcerative colitis.

*Recently reclassified as *L. helveticus*.

**Recently reclassified as *B. animalis* subsp. *lactis*.

Aim: The aim of the present study was to study secreted bioactive factors in order to evaluate the mechanisms of action of VSL#3 to enhance intestinal epithelia.

Methods: Two different lots of VSL#3 (Manufacturer: Nutrilinea Srl, Gallarate (VA) – Italy, lot #802097 and lot #802100) were used. Caco2 cell line were treated with a conditioning media (CM) prepared using 1g of probiotic formula grown in D-MEM cell culture medium (free of serum and antibiotics) at 37°C for 48 hours without shaking and in anaerobic conditions. The CM will be centrifuged at 4100 rpm for 10 minutes to separate the bacteria, and the resulting supernatant will be filtered through a 0.2 mm syringe filter to remove any insoluble particles and diluted 1:10 and 1:25.

Caco2 were treated with diluted CM at 24 and 48 hours. Media culture for each conditions has been collected and analyzed by a deeper proteomics approach.

Label free proteomics analysis of secretome was performed as described by Greco V et al (ref). Briefly differential protein expression was evaluated by shotgun proteomics analysis based on nLC-HDMS^E and carried out on Synapt G2-Si mass spectrometer (Waters Corporation). Protein identification and protein expression analysis were performed by Protein Linx Global Server (PLGS v. 3.0.3, Waters Corp).

Results: The analysis of supernatants from Caco2, treated with CM from multistrain probiotic, showed the presence of bacteria strain-specific proteins, in particular proteins of metabolism. Human proteins synthesized from CaCo2 cell line were also identified, such as caspase 1, IL8, Heat Shock Protein (HSP) 70, HSP 70b, HSP 90, HSP 105. The production were time- and dose- dependent. Particularly, in CM diluted 1:10, probiotic derived proteins have been shown to be more expressed at 24 hours. human caspase 1, IL8, HSP 70, HSP 70b, HSP 90, HSP 105 were also found upregulated in Caco2 treated for 24 hours with CM diluted 1:10. On contrary, the expression of bacterial proteins has been resulted lower in sample from Caco2 treated with CM diluted 1:25, and the expression of human proteins cited above has been shown to be increased after 48 hours.

Conclusions: This work is one of few proteomic studies where the secretome were analyzed, and in particular this is the first time where a probiotic secretome was explored. The study on probiotic secretome is useful to understand if the probiotic was well reconstituted, especially because we found strain specific proteins from all 8 strains. Analysis of secretome from Caco2 treated with CM helped us to understand the mechanism by probiotics can enhance intestinal barrier: by strengthen the autophagy process, an arm of innate immunity, by overexpression of caspase 1, IL8 and HSP 70, and by HSPs dependent modulation of inflammation by producing anti-inflammatory cytokines in chronic inflammatory disease.

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Autoprotobiotics in Treatment of IBS Patients

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Objective: Irritated bowel syndrome (IBS) is a severe gastroenterological pathology, based on impaired micro ecology. Auto-probiotics are genetically tested strains of indigenous microbiota grown on the artificial media.

Methods: A total of 40 patients with IBS and 10 volunteers participated in the study. Fecal samples from the study participants were analyzed and their indigenous bacteria were selected grown as probiotics and provided to the patients as milk fermented food for three weeks. Fecal samples were studied by RT-PCR and by means of metagenome study with the following bioinformatics analysis.

Results: Studies have shown significant differences in the composition of microbiota of patients with IBS relatively to the control group. Clinically, patients had a shortening of the interval between bowel movements (100%), a decrease in general weakness (75%), a decrease or disappearance of nausea after a meal (90%), a decrease in abdominal discomfort after a meal (85%). Microbiologically an increase of Proteobacteria including Klebsiella, Clostridium difficile and C. perfringens, characteristic of patients with IBS was replaced by an increase in the number of lactobacilli and Faecalibacterium prausnitzii.

Conclusions: In conclusion a personalized microbial therapy with autoprotobiotics demonstrated significant degree of improvement of gut micro ecology as well as condition of the patients. The work was supported by Russian Science Foundation grant 16-15-10085.

Elderly People Intestinal Microbiota as a Target for Probiotic Functional Food Intervention

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Objective: PROBIOSENIOR is a research project that aims to evaluate the effect of a probiotic functional food based-diet on the reduction of low-grade inflammation, by improving the intestinal barrier function and the composition of the intestinal microbiota, in healthy senior subjects. It is an *in vivo*, double-blind, randomized, placebo-controlled study. Preliminary results obtained from a small boarding home will be presented here.

Methods: Eleven subjects have been selected following the inclusion criteria and a baseline characterization of the physiological, nutritional and lifestyle parameters have been performed sampling questionnaires and blood, urine, faecal samples. To analyse the biodiversity of gut microbiota, DNA extraction and Real-Time PCR were used detecting and quantifying the bacterial levels, while gas-chromatography coupled to flame ionization detection was used for Short Chain Fatty Acids quantification.

Results: Questionnaires collected data about lifestyle, eating habits, pathological and psychological anamnesis of the recruited subjects. The microbial analysis showed the amount and variability of the bacterial groups quantified (*Lactobacillus* spp., *Enterobacteriaceae*, *Bacteroides-Prevotella- Porphyromonas* spp., *Bifidobacterium* spp., *Staphylococcus* spp., *Cl. coccoides-Eu. rectale* group, expressed in log CFU/g of faeces). SCFAs were also quantified for each senior subject.

Conclusions: These preliminary results showed a correlation between dietary/lifestyle habits of this small community of elderly people and their gut microbial status. This is only a first piece of the PROBIOSENIOR project that will monitor a greater senior population following the changes made by the probiotic dietary intervention.

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Individualized Short-term Probiotic Therapy of Metabolic Syndrome According to the Host's Phenotype

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Objective: Metabolic syndrome (MetS) requires detection of visceral obesity plus any two of the other four factors (hyperglycemia, dyslipidemia, cardiovascular disease, hypertension). Individualized modulating gut microbiome should effectively improve metabolic health.

The Aim: Was to study the efficacy of individualized short-term probiotic therapy on MetS.

Methods: We included twenty patients (age 37-65 y) with BMI > 30, waist circumference (WC) > 110; all underwent extensive clinical examination and were stratified according to patterns of MetS as follows: DMT2; NAFLD; hyperuricemia; atherosclerosis; early age blood; and 3 patients with normal/low BMI with detected increased visceral fat (VF) on ultrasound. Selected ten patients were given probiotic strains at a dose 10⁸ CFU daily during 10 days individually according to the knowledge obtained from *in vivo* and *in vitro* studies and existing evidence and pathophysiological aspects of potential clinical effects of probiotic strains against particular symptom; and ten patients remained as controls.

Results: Weight, BMI, WC and VF decreased, liver structure, lipidogram and glucose levels improved after probiotic administration in all patient after focused probiotic administration. The following strains demonstrated highest efficacy in the following conditions: mixture of L. casei IMV B-7280/B. animalis VKB/B. animalis VKL strains was effective in decreasing cholesterol levels; L. casei IMV B-7280 - in DMT2; Th1/Th2 imbalance; L. delbrueckii subsp.

bulgaricus IMV B-7281, B. animalis VKB, L. casei IMV B-7280, B. animalis VKL - in NAFLD; strains L. plantarum LM VK7 and V. animalis VKB - in cholestasis; L. plantarum - in hypertension; in hyperuricemia, gout - Bifidobacterium spp.; in visceral ischemia - Bifidobacterium spp., L. reuteri and L. plantarum; in underweight individuals with increased VF, hyperglycemia and hypercholesterolemia - strains of Bifidobacterium spp., L. plantarum.

Conclusions: Short-term probiotic therapy is effective to treat MetS and visceral obesity. The highest anti-obesity effect was observed in strains L. casei IMV B-7280 and L. delbrueckii subsp. bulgaricus IMV B-7281, strain-specific approach was most effective for particular signs of MetS, in underweight individuals Bifidobacterium spp., L. plantarum strains improved metabolic signs.

Effect of a Synbiotic Supplement on Cardiovascular Risk Factors in Overweight or Obese Patients with Polycystic Ovary Syndrome

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Objective: Polycystic ovary syndrome (PCOS) is a reproductive disorder, which is also closely related to obesity and cardiovascular risk factors including dyslipidemia and hypertension. Currently, probiotics and synbiotics are of high interest due to beneficial effects in various chronic and metabolic disorders. The aim of this study was to investigate the effect of a synbiotic supplement on lipid profile and blood pressure in overweight or obese women with PCOS.

Methods: In this randomized double-blind placebo-controlled clinical trial, 88 women, body mass index ≥ 25 kg/m² and age 19-37 years, were enrolled. Patients received either a synbiotic supplement (1000 mg capsule containing seven strains of probiotics and inulin) or placebo for 12 weeks. Serum lipids profile and blood pressure were assessed at baseline and following supplementation.

Results: There were no statistically significant differences in cardiovascular risk factors between the two groups at baseline. Following 12 weeks supplementation diastolic blood pressure, but not systolic blood pressure ($P=0.234$), decreased significantly in the intervention compared with the control group (73 ± 10 vs. 74 ± 8 mmHg, $P=0.025$). Additionally, a significant improvement was observed in LDL serum levels in the intervention than the control patients (92 ± 19 vs. 95 ± 20 mg/dl, $P=0.038$) at the end of the trial. No other statistical or clinical changes were observed in lipid profile.

Conclusions: Twelve weeks supplementation with a 1000 mg synbiotic supplement improved some cardiovascular risk factors in overweight or obese women with PCOS. Further studies are required to understand the clinical efficacy of synbiotics on cardiovascular disorders in PCOS.

Lactobacillus Reuteri E May Activate Brown Adipose Tissue by Enhancing PPAR Gamma Expression

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Objective: Some probiotic bacteria are known to improve gut microbiota composition and represent an interesting target in battling obesity. Brown adipose tissue (BAT) plays a significant role in energy homeostasis by taking up fatty acids and glucose to form heat. It has been proposed that activation of BAT could decrease

the risk of developing obesity. PPAR gamma, among other effects, is known to promote adipogenesis in BAT and induce BAT recruitment - leading to increase of tissue mass and improvement of glucose homeostasis.

Methods: 4-week old Wistar rats were fed sweetened cola drink *ad libitum* for 3 months; *Lactobacillus reuteri* E suspension was daily administered *per os* for last 30 days (CC+LRE). Rats drinking tap water (CON) or sweetened cola drink (CC) for three months *ad libitum* were used as control groups. Collected samples were stored at -80°C until processing for qPCR.

Results: Comparison between control groups and treated group did not show any difference in body weight, however we observed a significant increase of BAT tissue mass in group which was administered LRE. On molecular level we found a significant increase of PPAR gamma expression in this tissue. No changes in blood glucose or triglyceride levels were observed.

Conclusions: From the presented data we can conclude that LRE could have a positive effect on BAT activation through increasing PPAR gamma expression in this tissue. As we did not observe impact on weight gain or glucose and triglycerides blood levels, more research needs to be conducted to clarify the exact mechanism.

Paediatric Functional Gastrointestinal Disorders Treatment with Bifidobacterium ES 1 Longum: A Crossover, Double-Blind, Controlled Trial

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Functional gastrointestinal disorder (FGIDs) are one of the most common health complaints in children. It can interfere with school, sports, and regular daily activities. Aetiology is multi-factorial; the role of the "brain-gut axis" was underlined and innovative studies have shown the alteration and disruption of gut microbial composition and its contribution to the pro-inflammatory pathway. Bifidobacterium longum ES1 has been evaluated to induce cytokine production in order to revert the pro-inflammatory cytokine profile counteract the production of TNF- α and IFN- γ and to regulate IL-10 production and Th1/Th2 balance. The aim of our study was to compare the multisystem symptoms, quality of life, and functioning reducing dietary intake of fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) with/without Bifidobacterium longum ES1 supplementation in children with FGIDs. A crossover double-blind formula-controlled trial was carried out on 43 patient (4-15 y). The Pediatric Quality of Life Inventory (PedsQL™) and Gastrointestinal Symptom Rating Scale (GSRS) were filled out before inclusion in the study and during the follow up. Among 43 patients included, 21 (49%) patient were only suggested to low FODMAPs dietary intake and 22 (51%) had Bifidobacterium longum ES1 supplementation too. Of the patients enrolled in the study and treated with ES1 a full response was achieved in 19/21 (90,47%). Only low FODMAPs intake was usefulness only on 5/22 patient (22,7%). We underlined that low FODMAPs dietary intake with Bifidobacterium longum ES1 supplementation in children with FGIDs resulted efficacy and safety; we aim to continue our study to obtain potential statistically significant findings.

Antibiotic Resistance of Lactobacillus in Children

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Objective: To assess the prevalence of antibiotic resistance among indigenous and probiotic strains of lactobacilli in children.

Methods: Identified ranges minimum inhibitory concentrations (MIC) 50 and 90 to 16 antibiotics from 147 strains of *Lactobacillus* isolated from the intestine and 27 strains isolated from probiotics, synbiotics and Symbiotic A.

Results: MIC Benzylpenicillin more than 2 µg/mL is determined for 84.4% of strains of *Lactobacillus* from the intestine and 77.8% of probiotic *Lactobacillus*. Ampicillin MIC – 8 µg/mL is set at 33.3% of intestine, 22.2% – of probiotic *Lactobacillus*. MIC Cefazolin more than 2 µg/mL, Ceftriaxone more than 2 µg/mL was detected for most strains. MIC Tetracycline more than 2 µg/mL – in 97.3% of intestinal and 55.6% of probiotic strains. MIC Amikacin for more than 16 µg/mL – 91.8% of intestine, 66.7% of probiotic *Lactobacillus*. In the group of probiotic *Lactobacillus* strains, single isolates with a high level of resistance were found - MIC90 was more than 128 µg/mL. MIC Linco-mycin 8 µg/mL and more – for 93.2% of intestine, 70.8% of probiotic *Lactobacillus*. MIC for Oflox-acin and Ciprofloxacin 2 µg/mL or more for most strains.

Conclusions: A high level of resistance of *Lactobacillus* from the intestine was revealed. Resistance to Penicillin, Cefazolin, Ceftriaxone, Tetracycline, Amikacin, Ciprofloxacin set for 84% of the strains. 98% of *Lactobacillus* were drug resistant to 5 or more groups of drugs. Probiotic *Lactobacillus* resistance to Benzylpenicillin, Cefazolin, Ceftriaxone, Ofloxacin, Ciprofloxacin was established for 74% of strains. *Lactobacillus* from probiotic resistances to 5 or more groups of drugs – 81%.

Effects of a Probiotic Mixture on Moderate Atopic Dermatitis of Children: A Randomized Controlled Trial

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Objective: It is well documented that oral intake of some prebiotic/probiotic formulations might be effective in improving atopic dermatitis (AD) in the pediatric age group. This study was conducted to determine the effectiveness of a probiotic compound in reducing the AD symptoms.

Methods: This randomized, double blind, placebo-controlled trial was conducted among 50 Iranian children, aged 3-5 years, with moderate AD. They were randomly assigned to two groups (n=25 in each group) to receive prebiotic powder or placebo for 8 weeks. The prebiotic compound contained a mixture of *Bacillus subtilis* PXN 21, *Bifidobacterium infantis* PXN 27, and *Lactobacillus acidophilus* PXN 35 with maltodextrin as the carrier. The placebo contained only maltodextrin. The change in the severity scoring of atopic dermatitis (SCORAD) index was compared between groups.

Results: In total, 42 participants completed the trial (22 in the probiotic group and 20 in the placebo group). Comparison of data before and after the trial showed that the percent change in the SCORAD index was higher in the probiotic than in the placebo group (-65% vs. -27%, respectively, $P=0.02$).

Conclusions: The probiotic mixture used in the current trial was effective in reducing SCORAD index in children with moderate AD. Using various mixtures of probiotic strains in different populations is necessary to expand this finding.

In Vitro Study of the Potential for Inhibiting Haemolytic Pathogens by Selected Probiotic Strains: Safety Aspects and New Efficacy Horizons

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Objective: Haemolysis is the lysis of red blood cells (RBCs) and the subsequent release of their contents into surrounding fluid. There are three types of haemolysis, designated alpha, beta and gamma, that could be caused by different pathogenic microorganisms.

A vast body of scientific literature has demonstrated the ability of certain probiotic bacteria to antagonize Gram-positive and Gram-negative strains by secreting soluble molecules named bacteriocins. Anyway, insufficient data are currently available in relation to haemolytic bacteria. Our study focused on *Enterococcus faecalis* ATCC 19433 (γ -haemolysis), *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 8739 (α -haemolysis), *Staphylococcus aureus* ATCC 25923 (beta-haemolysis), and *Pseudomonas aeruginosa* ATCC 9027.

Methods: Six lactobacilli were selected for the study. Haemolytic bacteria were cultivated in Brain Heart Infusion (BHI) broth at 37°C for 18h. The agar spot assay was employed to quantify any possible inhibition. Broth cultures of pathogens at the proper density were poured on the plates previously spotted with probiotics. After incubation at 37°C in anaerobiosis for 48h, the diameters of inhibition zones around the spots were measured.

Results: *Lactobacillus plantarum* LP09 (DSM 25710) showed the strongest direct inhibition activity on all the haemolytic targets tested, with inhibition areas measuring on average 10 mm. *L. salivarius* LS01 (DSM 22775) and *L. plantarum* LP01 (LMG P-21021) were effective as well, with average inhibition diameters between 6 and 9 mm.

Conclusions: Selected probiotics could exert a focused protection effect against pathogenic bacteria responsible for RBCs lysis at various extent. Further investigations will be needed to investigate the underlying molecules responsible for inhibition.

Acid Tolerance of Lactic Acid Bacteria Isolated from Various Fermented Vegetables

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Objective: Probiotics should fulfill several criteria to be of benefit to human health. One of the most important criteria for probiotic microorganism is to be resistant to gastric acidity to survive until it reaches the intestine. Hence, the aim of the present study was to examine the acid tolerance of lactic acid bacteria (LAB) isolated from various fermented vegetable products.

Methods: Total eleven different samples [pickled turnips (A), mixed pickles (B, D, E), salt pickled red peppers (C), pickled tomatoes (F), sauerkraut (G, H), pickled beans (I), pickled okras (J) and pickled gappari (K)] produced at homes by traditional methods were collected around Izmir city in Turkey for the isolation of LAB strains. Among the samples, 114 LAB strains were isolated. In order to determine the acid tolerance of LAB isolates, MRS broth adjusted to pH 2, 3 and 4 were prepared. Each isolate was inoculated in MRS broths and the tubes were incubated at 30°C for 48 hours.

Results: The results showed that 3% of the isolates could grow at pH 2, 56% at pH 3 and 80% at pH 4. In general, significant proportion of the isolates (46%) can grow at low pH values, which shows that these isolates have possibility to show probiotic properties.

Conclusion: Hence, in the further studies, these isolates will be investigated in detail by subjecting their probiotic phenotypic data including bile tolerance, antibiotic susceptibility, cell surface hydrophobicity, aggregation, co-aggregation and antimicrobial activity.

Keywords: fermented vegetables, acid tolerance, probiotic, lactic acid bacteria

Modulation of Gut Microbiome Composition: Effects of Fruits and Vegetables

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Background/Objective: It has been well defined over the last decade that the diet influences the health of an individual, especially plant-based diet has been proposed to improve/maintain people's health.

But, it remains unclear until recently is the role of gut microbiome to this effect. So, in this study, we aimed to evaluate the beneficial effects of formulated fruits and vegetables supplementation (FVS) on the gut microbiome composition from healthy population.

Materials and Methods: This study is a randomized, double-blind, placebo-controlled study. This pilot study involves 30 healthy subjects aged 18-65 years, were randomly assigned to the treatment or placebo group, 15 subjects in each group and the duration of FVS supplementation/treatment was 6 weeks. Phenotypes, such as anthropometry, nutritional intake (by 24 h food recall method), biochemical parameters were recorded before and after treatment. The stool samples were collected before and after treatment. The gut microbial composition was evaluated by 16s rRNA sequencing on Illumina Miseq platform targeting V1-V3 hypervariable region. The short chain fatty acids (SCFA) were estimated using Agilent Gas Chromatography-MS. The anti-oxidant capacity was measured by Oxygen radical absorbance capacity (ORAC) method.

Results: The anti-oxidant level from plasma measured by ORAC was comparatively higher (+21%, $P=0.036$) in the FVS groups than the placebo group. The FVS group have shown a significant increase in the plasma folic acid level (+59.7%, $P=0.0001$), and vitamin B2 (VitB2; +25.6%, $P=0.04$) compared to placebo group. No significant differences were observed for vitamins A, E, K and serum potassium level. The 16s rRNA sequencing analysis have shown that FVS treatment greatly affects the bacterial lipid metabolism, gluconeogenesis and pentose pathways. In addition, gut microbiome composition positively correlated with butanoic, isobutanoic, ethanoic acids, and dietary intake of Lipids, Sugar, VitC and VitB in the FVS treatment group.

Conclusion: The formulated fruits and vegetables supplementation effectively modified gut microbial composition in healthy subjects.

Investigation of the Antagonistic Activity of Enterococci and Lactobacilli Against Multidrug-resistant Klebsiella Pneumoniae

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Objective: The aim of this study was to determine the antagonistic activity of probiotic strains against different strains of Klebsiella pneumoniae (KP) in vitro.

Methods: Antagonistic activity of probiotic Enterococcus faecium L-3, E.faecium L-X, E.faecium SF68, Lactobacillus plantarum 8PA3 and L.rhamnosus K32 strains was studied by double-layer agar method. Petri dishes contained MRS agar mixed with different dilutions (5-8 lgCFU/mL) of probiotic strains in the lower layer and Mueller-Hinton agar in the upper layer. The control plates were prepared without probiotic bacteria in the lower layer. Five KP strains (three of which were multidrug- and bacteriophage-resistant) in various dilutions were seeded onto solidified double-layer agar. Minimal concentrations (MC) of enterococci and lactobacilli strains required for inhibition of the KP growth were determined.

Results: Complete suppression of the KP growth was achieved by a presence in medium of E.faecium L-3 strain in a MC of 7.7 lgCFU/mL or L.plantarum 8PA3 strain in a MC of 8.2 lgCFU/mL. Growth inhibition of all KP strains was detected in presence of 5.8 lgCFU/mL of E.faecium L-3 strain; 6.24 lgCFU/mL of L.plantarum 8PA3 strain; 7.7 lgCFU/mL of L-X strain; 5.5 lgCFU/mL of SF68 strain and 7.9 lgCFU/mL of L.rhamnosus K32 strain.

Conclusions: The ability to suppress the growth of Klebsiella pneumoniae (including multidrug-resistant strains) allows considering the usage of probiotic and, in future prospects, autoprobiotic enterococci and lactobacilli for treatment of nosocomial infections and dysbiosis accompanied by KP overgrowth. This work was supported by Russian Science Foundation under grant no. 16-15-10085.

Probiotic Potential of Kombucha

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Kombucha tea, which has recently become popular among many traditional fermented foods, is a beverage of Manchurian origin, generally prepared from black tea with various bacteria and yeast involved in fermentation. Kombucha, a popular beverage in the World, stands out with its positive effects on health as well as its easy preparation and flavored taste. Acetic acid bacteria (AAB; *Komagataeibacter* spp., *Acetobacter* spp., *Gluconobacter* spp.), lactic acid bacteria (LAB; *Lactococcus* spp., *Lactobacillus* spp.) and yeasts (*Schizosaccharomyces pombe*, *Kloeckera apiculata*, *Saccharomyces cerevisiae*, *Saccharomyces ludwigii*, *Zygosaccharomyces bailii*, *Torulopsis delbrueckii*, *Brettanomyces bruxellensis*) involved in Kombucha fermentation. Kombucha is considered as a probiotic drink not only for the presence of the diversity of naturally occurring symbiotic microorganisms instead of single strain probiotics in Kombucha, but also providing short chain fatty acids (SCFAs) and other metabolites which improve immunity. At the end of the fermentation, Kombucha is rich in acetic, gluconic, glucuronic, tartaric, malic and less citric acids and limited amount of ethanol and CO. The resulting acids small intestine. The subjects continued their usual diet, except for the elimination of spirits, coffee in quantities greater than two daily cups, decrease the pH value of Kombucha tea and contribute to the formation of its characteristic sour taste. In the studies, it was determined that the mixed culture involved in the Kombucha fermentation has a strong symbiosis that inhibit the growth of pathogenic bacteria and has antiviral, antibacterial and antifungal effects. In this paper, the studies evaluating the probiotic potential of Kombucha were examined.

Keywords: Kombucha, probiotics, lactic acid bacteria, acetic acid bacteria, yeast

Dysbiosis in the Small Intestine: Towards an Optimal Therapy to Normalize the Intestinal Microbiota

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Background: Many studies evidenced the role of antibiotics in the modulation of Gut Microbiota (GM) and the reduction of gastrointestinal symptoms. In particular, Rifaximin showed its eubiotic function on GM with positive effects on human health. However, the characteristic of the GM always tends to return to its baseline characteristics.

Aim and Methods: To define, after the pharmacological correction of intestinal dysbiosis, the proper duration of the therapy, and the proper timing for the drug re-administration. We studied 6 male and 4 female subjects aged between 30 and 60, all complaining symptoms due to bacterial contamination of cold and carbonated drinks, and smoking. Metabolic pathologies were also excluded. The diagnosis of bacterial dysbiosis in the small intestine was confirmed through lactulose H2 breath test. Rifaximin was given to 5 subjects at the daily dose of 600 mg; 200 mg after each of the 3 meals, for 5 days per month. The other 5 subjects were treated with Rifaximin for two cycles of 5 days each every month. All subjects repeated the breath test after one month.

Results: (1) All subjects at the start of the test were found to have an intestinal "bacterial contamination": the first H2 peak in expired breath occurred within 40 minutes from the start of the test, reaching average levels of over 40/million, to then re-emerge in at least 2-3 average peaks of 60/million, remaining at high levels throughout the examination; (2) the test, repeated after a month, gave very different values in the two groups of cases studied: - in 3 subjects treated with only a cycle of 5 days a month of Rifaximin, the outcome of the breath test has substantially maintained its (pathological) characteristics. In the other 2 cases the first elevation of H2 occurred around 90 minutes and reached maximum levels around 35/million, and therefore the correction was very partial; - in

4 subjects treated with two cycles of 5 days each month the breath test indicated substantially normal results with elevation of H₂ after 100 minutes and never higher than 20/million; - only in one case there was an almost absent elevation of the H₂.

Conclusions: Overall, our study seems to suggest that Rifaximin may be able to correct intestinal dysbiosis, even in severe cases, and to maintain its outcome when administered every 15 days. However, we must consider these as preliminary results. Further investigation is needed on more cases, using higher doses of the drug and at different time of administration, also evaluating potential interferences and dosing CH4.

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Probiotic of Dehydrated Strawberry Puree Using Windows Refractive and Microencapsulation by Vibrating Technology

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Objective: The main purpose was to improve the resistance of probiotic cells using encapsulation process and introducing in a fruit matrix exposed to drying process.

Methods: *L. reuteri* DSM 17938 cells were microencapsulated by vibrating technology using alginate (2%) and alginate (2%)-chitosan (0.7%). Free and encapsulated cells were introduced in strawberry puree and dehydrated by Windows Refractive™ (50°C), the puree was located on the membrane of drying using a thin plastic template. The survival cells was evaluated to 50, 60 and 70 min of drying process, humidity and water activity were determined; after 70 min, some samples were exposed to gastrointestinal passage simulated and others were stored for 1 and 6 days in the conventional fridge to 9°C.

Results: Spherical capsules were observed with size particle 204.67 ± 22.64 μm and 156.00 ± 20.28 μm for alginate and alginate-chitosan respectively. Results of survival showed that during drying process of puree, cells encapsulated improved the resistance and kept more cells living, the most of assays were significantly ($P < 0.05$); the same situation was observed during the gastrointestinal passage and storage, cells protected with alginate-chitosan showed better results than cells protected only with alginate or unprotected. In the end, the humidity content in the puree dehydrated was between 16-21% and water activity 0.60-0.71.

Conclusions: The encapsulation process by vibrating technology provides greater protection to cells introduced in strawberry puree for to resist to drying process, also for gastric conditions and during storage, the low values in water activity are indicators that the product obtained can be stable and with low possibility of deterioration by microorganisms.

Physiological Aspects of Enterococcus Gallinarum CRL 1826, a Potential Probiotic for Ranaculture

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Objective: To evaluate the effect of gastrointestinal conditions on the maintenance of indigenous *Enterococcus gallinarum* CRL 1826 viability and probiotic properties.

Methods: The *E. gallinarum* strain was grown in MRS broth (pH 6.8, 37°C, 24 h). Cells were collected by centrifugation, washed and resuspended in PBS pH 7.4. 10⁹ CFU/mL of fresh cultures (FC), lyophilized (LC) and stored at 4°C for 1 year (SC), were subjected to simulated sequential gastrointestinal conditions to determine: cell viability (survival factor-SF), bacteriocin activity (Arbitrary Units-AU/mL) and surface properties (% hydrophobicity and auto-aggregation). Cells were added with 0.6% pepsin followed by a gradual descent of pH (7.4 to 2), 90 minutes. Then, 1% bile (pH 8) was added (10 min) and finally treated with 0.3% bile+0.1% pancreatin, 90 minutes.

Results: Bacterial viability diminished during the treatment with significant differences among FC, LC and SC; LC showing the highest values (SF=0.92). Under gastric conditions there were no variations in cell viability while it diminished during intestinal treatment, independently of the culture. Low bacteriocin activity (AU/mL) was detected in FC. In LC ~1300 were determined in gastric conditions, while bile treatment increased it up to ~2000 and then decreased (~800). In SC, it diminished during the gastric treatment, to then increase up to 1200 AU/mL. The surface properties did not change during treatments.

Conclusions: Considering that *E. gallinarum* CRL 1826 kept its probiotic properties during the sequential gastrointestinal digestion, it could prevent the gastrointestinal entrance of pathogens related to red-leg syndrome (a bacterial infectious disease) in bullfrog hatcheries.

Mycotoxin Removal Ability of Lactobacillus Acidophilus CIP 76.13 and L. Brevis CIP 102806T Isolated from Humans

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Objective: Mycotoxins are harmful secondary metabolites produced by fungi which contaminate a wide range of food and feed. Lactic acid bacteria (LAB) show a promising potential to degrade mycotoxins thus reducing their bioavailability in human and animal gastrointestinal systems. This study was aimed to investigate the ability of *Lactobacillus acidophilus* CIP 76.13 and *L. brevis* CIP 102806T, isolated from humans, in reducing AFB₁, OTA, ZEA, FB₁ and DON in order to be used as functional starter for feed and food industry.

Methods: Mycotoxin removal by viable and heat-inactivated cells was evaluated at 37°C for 24h in PBS containing 1 μg/mL of each mycotoxin. Mycotoxins were analysed by UHPLC-FLD/PDA methods. Technological and probiotic properties (proteolysis, lipolysis, and acidic tolerance at pH 3, 5, 7) were also studied. Antagonistic activity of each bacteria was evaluated by Burkholder agar diffusion assay against food borne pathogens.

Results: Viable cells of CIP 76.13 reduced ZEA, FB₁ and DON by 57 ± 2%, 36 ± 7% and 31 ± 3%, respectively, while CIP 102806T reduced ZEA by 100%. Percentage removal from heat inactivated cells were significantly lower (9 ± 4% for FB₁ and DON, and 28 ± 10% for ZEA, on average). Both strains survived at pH 3, 5 and 7 for 24 hours in PBS, and were lipolytic and proteolytic towards milk proteins. In addition, CIP 102806T showed antagonistic activity against *S. aureus* and *L. monocytogenes* strains.

Conclusions: These results reveal the potential use and broader application of *L. acidophilus* CIP 76.13 and *L. brevis* CIP 102806T for mycotoxin reduction in food and feed industry.

Different Probiotic Strains Improve Helicobacter Pylori Eradication and Gut Microbiota

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Objective: To investigate the effects of probiotics (P) in patients with *Helicobacter pylori* (Hp)-positive duodenal ulcer (DU).

Material and Methods: In this study 200 Hp - positive patients with DU were randomized into 4 groups, 50 patients in each. Hp was

confirmed histologically in samples obtained from the antrum and corpus of stomach. Intra-gastric and intraduodenal pH-metry by tool Gastroscan-5M, Investigation of gut microbiota, blood analyses also were performed. The following eradication regimens were recommended:

Group I: 10-days twice daily oral administration of PPIs in standard dose, amoxicillin 1000 mg, clarithromycin 500 mg, then 20-days twice daily PPIs plus twice daily oral administration of P, containing 3,025 billion alive lyophilized *Lactobacillus bulgaricus* DDS-14, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus* DDS-1 and *Bifidobacterium bifidum* during 1 month; Group II: PPIs and P twice daily and alkaline hydrocarbonate-chloride sodium mineral water (MW) 200 mL trice daily during 1 month; Group III: PPIs and P twice daily and chloride sodium MW 200 mL trice daily during 1 month; Group IV: P and PPIs once daily during 1 month. Therapeutic success was confirmed by a negative histological examination, performed in 4-12 weeks after therapy.

Results: Before treatment, all examined patients revealed intestinal dysbiosis. In Group I dyspeptic complaints (DC) disappeared in 74%, and decreased in 20%. Disappearance of DC was 78%, 76% and 74% in II, III and IV groups respectively. Decrease of DC was 20%, 22% and 24% in II, III and IV groups respectively. The eradication rates were 82%, 80%, 78% and 68% in I, II, III and IV groups, respectively. Healing of DU was noted in 84%, 86%, 84% and 78% of cases, in I, II, III and IV groups, respectively. Intra-gastric and intraduodenal pH increased in all groups, especially in II. In II, III and IV groups significantly decreased alanine transaminase, asparagines transaminase, blood bilirubin, alkaline phosphatase, cholesterol and triglycerides. After treatment gut microbiota became to normal ranges.

Conclusions: Adding P to standard triple therapy improves compliance and efficacy of Hp eradication. The combined use of PPIs, P and alkaline hydrocarbonate-chloride sodium MW improves efficacy of Hp eradication, reduces adverse effects with antibiotics, and gut microbiota.

This regimen may especially be helpful in patients with a history of gastrointestinal adverse effects with antibiotics, comorbid patients.

Prebiotic Effect of Onion on the Survival of Various Probiotics Inoculated in Koruk (Unripe Grape) Juice

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Objective: Unripe grape (*Vitis vinifera* L.) which is known as "Koruk" in Turkey, is a special fruit with high amounts of organic acids (mainly tartaric and malic acids) and phenolic compounds (gallic acid, caffeic acid, catechin, quercetin glycoside etc.). Acidic property and phenolic components of Koruk allow to show both antimicrobial and antioxidant effects. Koruk juice could be used as beverage, disinfectant for salad vegetables and marination liquids in meats. When it is used as marination liquid, it could be combined with onion juice, which is commonly used in marinate formulations. This kind of marination liquid could be enriched with probiotics to improve the product properties. Hence, in this study, the survival of *Lactobacillus acidophilus* LA5, *L. casei* 01, *L. rhamnosus* HN001 and *Bifidobacterium lactis* HN009 in Koruk juice and the prebiotic effect of onion juice on these probiotics were investigated.

Methods: Test cultures were inoculated (9 log CFU/mL) separately in prepared liquid including koruk and onion juices (1:1), where water was used instead of onion juice for control. The liquid was stored at 4°C and probiotics were counted after 2, 24, 48 hours storage.

Results: The results showed that all probiotics were survived in prepared liquids at higher than 7 log CFU/mL, except *L. acidophilus*, which survived at around 6 log CFU/mL in liquid excluding onion juice.

Conclusion: The highest numbers were found in the liquids prepared with onion juice, which confirmed the prebiotic effect of onion on probiotic cells.

Keywords: prebiotic, probiotic, *Vitis vinifera* L., koruk juice

Cashew and Grape Agroindustry Pomaces as Sources of Energy for Starter and Probiotic Strains

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Objective: Our objective was to evaluate the growth of probiotic and starter strains in a fibrous matrix of cashew and grape pomaces (CP and GP) as sources of energy.

Methods: Modified MRS broth (mMRS) with 1% of irradiated CP or GP were inoculated with 5 *Lactobacillus* strains (PCC, RC-14, 431 and F19), 3 *Bifidobacterium* strains (BB-02, BB-12 and BB-46); and 3 *Streptococcus thermophilus* starters (TH-4, TA-40, and ST-M6). Fermentation took place during 24 and 48 hours. Controls of pure mMRS broth and with 1% of specific carbohydrate were used. Results were expressed as mean variation in populations after fermentation (V24 h and V48 h) in log CFU/mL.

Results: V48h of ST-M6 in GP (4.3 ± 0.2) and CP (3.7 ± 0.1) were not significantly different ($P > 0.05$) and both higher than for the other starters. V24h of RC-14 in GP (4.3 ± 0.1) was higher ($P < 0.05$) than for all *Bifidobacterium* strains (in GP). V24h of BB-12 in CP (3.5 ± 0.2) was higher ($P < 0.05$) for BB-46 and PCC (in CP). Using PCA with 2 components (explaining 92.8% variability), similar patterns were observed for all the probiotic strains in CP, GP, and the carbohydrate control after 24h.; and for TA-40, TH-04, and the carbohydrate control (V24h) in GP.

Conclusions: *Streptococcus thermophilus* ST-M6 could be applied as starter together with the probiotic strains RC-14 in GP, and BB-12 in CP for the enrichment of new probiotic foods with sustainable dietary fibers.

Fermented Olive: Microflora and Probiotic Properties

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Olive, which is a member of the Oleaceae family (*Olea europaea* L.) maintains its significance for thousands of years. The most important producers are Spain, Greece, Italy, Turkey, Egypt, Algeria and Morocco. Natural fermentation is the most common production techniques of table olives. Microorganisms isolated from olive and brine mostly include *Enterobacteriaceae* spp., *Clostridium* spp., *Pseudomonas* spp., *Staphylococcus* spp., lactic acid bacteria, yeasts and rarely molds. During olive fermentation, lactic acid bacteria decrease pH value of the medium by producing lactic acid, which provides microbiological quality and safety of the product by preventing growth of spoilage and pathogen microorganisms. *Lactobacillus plantarum* and *Lb. pentosus* are among lactic acid bacteria that are dominant species in olive fermentation. Additionally, *Lb. brevis*, *Lb. casei*, *Lb. coryniformis*, *Lb. mali*, *Lb. paracasei*, *Lb. paraplantarum*, *Lb. rhamnosus*, *Lb. vaccinostercus*, *Leuconostoc mesenteroides*, *Leu. pseudomesenteroides*, *Lactococcus lactis*, *Enterococcus casseliflavus*, *E. italicus*, *Weissella cibaria*, *W. paramesenteroides* could take a part in the fermentation, depending on geographical location, variety of olive and production method. Although, olive fermentation is carried out by lactic acid bacteria, 4-6 log CFU/mL yeast population also exist in the media during whole process. Most often species isolated from different olive varieties include *Candida*, *Pichia*, *Saccharomyces* and rarely *Debaryomyces*, *Issatchenkia*, *Zygorulasporea* and *Wickerhamomyces*. Previous studies showed that microflora of fermented olive have probiotic properties. Hence, this product could be accepted as probiotic foods.

In Vitro Adherence and Anti-pathogens Effect of a Co-culture of Lactobacillus Plantarum and Lactobacillus Casei Cultivated in a Minimal Media Supplemented with Aloe Vera

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Objective: The study aimed to prove the *in vitro* adherence capacity and antimicrobial effect of a combined *Lactobacillus* spp. culture inoculum against two pathogenic bacterial and fungal strains.

Methods: A co-culture of *Lactobacillus* probiotic strains, cultivated in MRS and in an electrolytes solution, both enriched with 10% *Aloe vera* alcoholic lyophilized leaves extract was tested on HeLa-2 (human carcinoma cell line). The competitive adherence on HeLa-2 of *Lactobacillus* co-culture inoculum versus *S. aureus* and *C. albicans* pathogens, ATCC and clinical strains was also tested. The inhibitory capacity of the probiotic inoculum against the pathogens was calculated based on the pathogenic cells adhered to the cell line compared to the control.

Results: The adherence capacity was 70% (MRS inoculum) meaning $1 \cdot 10^8$ cfu/mL, respectively 75%, (electrolytes solution inoculum) meaning $1,13 \cdot 10^8$ cfu/mL. The inhibition capacity of *Lactobacillus* spp. against *S. aureus* ATCC reached a maximum value of 90%, respectively 80% for the clinical *S. aureus*. The optimum results against *C. albicans* were reached for the clinical strain, 80%.

Conclusions: The *L. casei* and *L. plantarum* lactic acid strains exhibited a certain adhesion degree to the HeLa-2 cell line, the optimal variant being the 1:1 combined inoculum grown in the electrolyte media supplemented with 10% *Aloe vera* alcohol extract. The products fermented with *L. plantarum* and *L. casei* in single cultures or as a combined inoculum (1:1) exhibited antimicrobial activity against some pathogenic microorganisms (*Staphylococcus aureus* and *Candida albicans*) with different potential depending on the culture medium. The supplementation of the media with *Aloe vera* increased the inhibitory capacity.

Clinical Trials on the Antagonistic Action of Probiotics Against Wound Pathogens

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Objective: Skin represents the first line of defense against environmental damage, including that caused by microorganisms. Skin damage can be the consequence of trauma, illnesses, surgery etc. The most effective wound management strategy is to prevent infections and promote healing. Probiotics can aid in skin healing by stimulating the production of immune cells and competitive exclusion of common pathogens of the skin. Our aim was to conduct a review of the recent literature on the efficacy of using probiotics against pathogens causing wound infections.

Methods: A literature search of clinical studies was conducted using the search terms: 'probiotic' AND 'wound infection' published after the year 2005. A comprehensive review and critique of the selected studies was carried out. A final yield of 20 articles was included.

Results: Topical application of probiotics was used in two studies on infected foot ulcers and burns. Three additional studies investigated the influence of oral probiotics on infections of burn injuries. The remaining 15 clinical studies used oral probiotics and were conducted on surgical patients with various surgical site wounds. The most commonly used probiotics were strains of *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*. Most studies showed a slight or significant lowering of surgical site infections, foot ulcer infection or burn infections in the probiotics group compared to the placebo group.

Conclusions: Exogenous and oral application of probiotics has demonstrated a reduction in wound infections and therefore the

potential use of probiotics for wound infections remains worthy of further studies.

Development and Evaluation of Whole Cell Probiotic Containing GEL for Topical Use

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Objective: Probiotics are emerging as a potential therapy. Clinical and experimental research indicates that topical probiotic application can favorably modulate skin flora and thus help manage disease. Present study describes a gelatin-based stable, patient compliant formulation containing whole cell probiotic (*Bacillus coagulans*) for diverse skin conditions.

Methods: Probiotic loaded emulgel was prepared using oil, surfactant, humectant and gelatin as base and characterized in terms of morphology, loading, water activity, viability on storage and germination on application. *In vivo* therapeutic efficacy in wound healing, dermal toxicity, translocation, stability and self preservative studies were also done.

Results: Microscopic images confirmed presence of probiotic spores enmeshed in honeycomb network. Self-preserving nature was confirmed by low water activity ($0.541 a_w$) and challenge test as per Indian Pharmacopoeia.

The probiotic count was maintained ($92 \pm 5 \times 10^6$) on storage for three months ($5^\circ\text{C} \pm 3^\circ\text{C}$ and $30^\circ\text{C} \pm 2^\circ\text{C}/65\% \text{RH} \pm 5\% \text{RH}$) confirming stability and cell viability. Spore germination started at 6 hours and only few non-germinated spores were left at 24 hours after application. The developed formulation was safe in terms of cytotoxicity, acute dermal irritation and permeation/translocation as $\text{NMT } 0.16 \pm .03\%$ of $8 \pm 4 \times 10^6$ cfu applied per day translocated to systemic circulation. Patient compliance in terms of application and removal was confirmed by texture and rheological studies. The *in vivo* therapeutic effect of developed formulation was confirmed in terms of wound healing and no statistically significant difference was found between marketed and probiotic-loaded formulations.

Conclusions: The developed formulation containing whole cell probiotic (*Bacillus coagulans*) can be considered as potential option for wound healing.

Development of a Rice-based Fermented Probiotic Beverage with Lactobacillus SPP. and Streptococcus Thermophilus

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Objective: Consumers, including elderly, vegans and lactose-intolerants demand for non-dairy functional foods towards a healthy lifestyle. Probiotics in cereal-based foods may improve their nutritional value by releasing nutrients during fermentation. Hence, the behavior of probiotic *Lactobacillus* spp. combined with yoghurt starter *Streptococcus thermophilus* in rice fermentation to develop a rice-based fermented beverage was studied.

Methods: Slurries of rice and water were prepared in two ratios (1:15 and 1:20 (w/w)) and inoculated with 3 different microbial consortia: nu-trish® *Lactobacillus casei* 01® with *Streptococcus thermophilus* (YoFlex® YF – L01 DA), *Lactobacillus rhamnosus* Rosell®.11 with *S.thermophilus* and nu-trish® *Lactobacillus acidophilus* 5® with *S. thermophilus* (Christian Hansen). Fermentation occurred at 39°C over 20 hours. Samples were collected every 2h and monitored for cell viability (CFU/mL), pH and viscosity. Glycemic index was determined.

Results: Consortium *L. casei/S. thermophilus* demonstrated the highest cell growth rate (2 log cycle) and pH decrease (6.6 to 4.5). Consortium *L. rhamnosus/S. thermophilus* presented a cell growth rate of 1 log cycle and a pH decrease of 6.6 to 4.9 whereas consortium *L. acidophilus/S. thermophilus* didn't reveal cell growth and exhibited the lowest pH variation (6.6 to 5.5). Slurries 1:15 revealed

the highest viscosity values. After fermentation both *L. casei*/S. *thermophilus* and *L. rhamnosus*/S. *thermophilus* consortia exhibited CFU/mL above the minimum probiotic threshold demonstrating to be good options for the elaboration of a low glycemic index rice-based symbiotic beverage.

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Keywords: probiotics, fermented beverage, non-dairy products, rice-based food

Storage Stability of Probiotic *Weissella Cibaria* N9 in Freeze-dried Form

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Objective: High storage stability of probiotics is one of the most important issues regarding product quality of dried probiotics and starter cultures. The viability of probiotics is influenced by various factors including presence or lack of oxygen, water activity and temperature. We aimed to examine the effect of different protective agents on the viability of *Weissella cibaria* N9 during drying and storage.

Methods: The optimum formulation of skim milk, lactose and sucrose as protective agents was determined by applying the Box-Behnken experimental design based on viability after freezing and freeze-drying. Storage stability of freeze-dried cells during storage for up to 180 days at different temperatures (4 and 25°C) was determined. Accelerated storage tests using temperatures of 50, 60 and 70°C were also used to develop a model system in order to predict the viability of freeze-dried probiotic cells.

Results: The optimal protective agent formulation was 5.65 (w/v) skim milk combined with 20 (w/v) lactose and 9.38 (w/v) sucrose, which provided high stability of cell (99 % <) after freezing and freeze-drying. The highest viability was observed at 4°C (9.11 log cfu/g) with an inactivation rate of $3.37 \times 10^{-3} \text{ day}^{-1}$, which indicate that viability of freeze-dried cells was more stable at refrigerated temperature than those stored at ambient temperature. The fastest cell reduction was observed at 70°C and protective agent effectively decreased thermal death.

Conclusions: Results indicated that this protective agent formulation was an effective for protecting the cell viability during freezing and storage, and accelerated storage testing was a useful technique with certain predictability in this study.

Keywords: *Weissella cibaria* N9, Prediction model, Probiotic, Protective agents

Whey Phage Ecology in Artisanal Traditional Italian “HARD” Cheese: Sustainability in Dairy Production

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Natural whey starters can be considered as a lactic acid bacteria culture, indispensable for the production of Italian traditional “hard” cheeses like Trentingrana. Daily maintained in the dairy activity, the main function of this culture is the acidification during the curd formation, having important effects such as whey drainage from the cocked curd and growth inhibition of photogenic and undesirable bacteria. Loss in whey starter fitness is often associated in poor acidification, mainly due to phage infection, that can strongly affect the cheese production and its final quality. Phage contamination in the dairy environment can not be completely eradicated, and there is fundamentally a co-evolution process that drives an ecological balance between phage and its microbial host, especially in whey starters

environments. Therefore knowledge on phages community represent an important aspect to reduce lost in cheese production and to maintain quality and excellence in cheese making.

To study the phage ecology in Trentingrana production chain, we have collected 400 samples in six Trentingrana dairies distributed on the autonomous province of Trento, Italy. Almost 1800 lactic acid bacteria have been isolated and more than 150 phages retrieved. Characterization of phage biotypes as well as phages genome sequencing is ongoing. The gaining knowledge together with the isolation of performing lactic acid bacteria resistant to phage infection, will be important to avoid milk and cheese spoilage, potentially leading to a more sustainable cheese production.

Lactobacillus Rhamnosus GR-1- the Best Studied Vaginal Probiotic Strain

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Objective: The genome sequences of various gastrointestinal and dairy probiotic *Lactobacillus* strains have been published, however, the vaginal strains are lagging behind. In this study, the complete genome sequence of the vaginal probiotic *L. rhamnosus* GR-1 was determined and compared to other *L. rhamnosus* strains at genomic and phenotypic level. The strain LGR-1 was originally isolated from a female urethra and was assessed with *L. rhamnosus* GG from a feces sample, and *L. rhamnosus* LC705 from a dairy product.

Methods: First the genome of LGR-1 was determined, followed by calculating the pan-genome of all publicly available *L. rhamnosus* strains and constructing a high-resolution phylogenetic tree. The phenotypic differences between LGR-1, LGG, and LC705 were evaluated by performing various experiments, such as carbohydrate utilization, adhesion to epithelial cells, detection of pili and EPS molecules, and different stress survival assays.

Results: A key difference is the absence in GR-1 and LC705 of the *spaCBA* locus required for pili-mediated intestinal epithelial adhesion. The LGR-1 genome contains a unique cluster for EPS production, which is postulated to synthesize glucose-rich, rhamnose-lacking exopolysaccharide molecules that are different from the galactose-rich EPS of LGG. Compared to LGG, LGR-1 was also genetically predicted and experimentally shown to better metabolize lactose and maltose and to better withstand oxidative stress, which is of relevance in the vagina.

Conclusions: Ultimately, this study could thus provide a molecular framework for the selection of the optimal probiotic strain for each targeted niche and condition.

Safety Assessment of a Microbial Food Culture for Specific Usage in the Food Chain: *Weissella Confusa*

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Introduction and Scope: The primary safety assessment for a new probiotic strain is based, in the European Union, on the history of safe use of the species it belongs. A strain specific safety assessment is also done through the Qualified Presumption of Safety (EFSA-BIOHAZARD Panel). The switch between the history of safe use of a species to the safety of a strain, however, can be complicated when the species does not belong to the most recent version of the “QPS list”. Some strains of *Weissella confusa* (former taxonomy *Lactobacillus confusus*) have been recently proposed as potentially probiotic. The species has been demonstrated for its safe use in fermented food, while it has also been recently reported to cause some clinical adverse effects. We propose here to perform the safety demonstration of this species following the recommendation of the QPS assessment and IDF-EFFCA inventory of microbial food cultures, by comparing “food” and “clinical” isolates.

Materials and Methods: A bibliographical review of opportunistic case reports of *W. confusa* and *L. confusus* was done to determine

the intrinsic and extrinsic factors of disease. A collection of food and clinical strains was done by collecting isolates from public and private collection in Europe. 22 food and 17 clinical isolates were compared for their genome sequence (WGS) an antibiotic resistance profile using the recommendation from EFSA-FEEDAP and Antibiotic used in hospital care setting.

Results. Discussion and Conclusion: Comparison of ATB profiles does not show differences between the different strains, food and clinical. WGS and sequence analysis is currently performed to analyse if any genomic rearrangement would explain the opportunistic pathogenicity of clinical isolates. If no phenotypic and genomic differences can be done between the food and clinical isolates, using the commonly accepted criteria, we propose to see the underlying disease factors of the host as well as the antibiotic use (First line, second line). Notably, Vancomycin is a recent antibiotic for usage in clinical settings, while heterofermentative Lactobacilli are commonly resistant to this drug. We therefore suggest that the recent episodes of Lactobacillaemia in the literature might be caused by the new drug usage, rather than a pathogenic potential of the different reported species.

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Protective Action of *B.Clausii* Probiotic Strains and Their Metabolites in an In Vitro Model of Rotavirus Infection

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Objective: The *B.clausii* probiotic, which includes 4 strains of *Bacillus*, has been reported to exert beneficial clinical effects, notably in the treatment of diarrhea and in the prevention of infectious diseases. We aimed to investigate the protective activities elicited by a mixture of 4 *B.clausii* probiotic strains, and its supernatant, on human enterocytes in a model of *Rotavirus* infection, the main etiological agent of acute gastroenteritis in pediatric age.

Methods: Caco-2 cells were pre-treated with *B.clausii* probiotic strains or with its filtered supernatant at 37°, CO₂ 5%, for 12 hours, and then RV were added to cells cultures. We evaluate the effects on epithelial integrity (Transepithelial Electric Resistance, TEER), on human enterocyte monolayer permeability (MUC5AC and tight junction expression), on reactive oxygen species (ROS) production, on analysis of pro-inflammatory cytokines (IL-8 and IFN-β) production and on toll-like receptor 3 (TRL3) pathway in a Caco-2 cells infected with RV with or without *B.clausii* strains and bacterial supernatant.

Results: *B.clausii* probiotic strains and its supernatant significantly prevented damage at epithelial barrier, ROS production, a decrease of MUC5AC, ZO-1 and occludin expression and IL-8 and IFN-β release in RV infected Caco-2 cells, showing a potential protective effect in RV infections. Moreover, a down-regulation of pro-inflammatory TLR3 pathway genes expression was observed in RV-infected Caco-2 cells treated with *B.clausii* probiotic strains and its supernatant.

Conclusions: *B.clausii* probiotic strains and its supernatant protect Caco-2 cells from RV through a positive modulation of epithelial integrity and monolayer permeability, a reduction of ROS production and pro-inflammatory cellular status. This *in vitro* study could be explain the molecular and cellular mechanisms of probiotic effects in the prevention of RV infections.

Lycoprogen: Tomato as a Functional Food

Piantelli M*, Fogliano V†, Iacobelli S‡, Natali PG*,†. *Center of Excellence on Aging and Translational Medicine (CeSI-Met), "G. d'Annunzio" University, Chieti, Italy; †Wageningen University, Department of Agrotechnology & Food Science, The Netherlands; ‡Janus Pharma S.r.l. Diet and nutrition have a substantial impact on human health. While the reductionist approach relying on micronutrients supplementation has limited value, dietary regimens containing given "functional foods" (FF) may be advantageous. In this context, the Mediterranean Diet (MD) still holds a leadership because of high content in fibers, vegetable and fruits. Among the latter, tomatoes and olives are relevant FF, as they are the source of complexes of micronutrients endowed with a variety of biological activities, which also concur to efficient scavenging of free radicals. As for any dietary approach, the questions remain regarding optimal bioavailability and compliance. With the aim of enhancing the functional food properties of whole tomato, we designed an original solvent-free processing method (US Patent No.0228484A1) resulting into a tomato powder (trade mark name "Lycoprogen": Italian Health Ministry, code 68843) which following addition of olive vegetation water has a defined chemical composition (100 g containing 250 mg of carotenoid, 120 mg of flavonoids, 8 mg ketosamines). Lycoprogen consumption increases the serum antioxidant activity in man and mice. Using the TRAMP model of prostate cancer, the tomato powder significantly decreased mortality and circulating inflammatory/angiogenic cytokines, as IL-6 and VEGF. In a phase II prospective, randomized double-blinded, placebo-controlled study involving 17 patients with benign prostatic hyperplasia, we found that those treated with Lycoprogen (one sachet daily for 60 d) had a significantly lower urinary tract symptoms, as assessed by the IPS score (7.44+1.11 SE) than placebo-receiving patients (5.75+1.01 SE, $P=0.0002$). A trend toward a reduction of total PSA levels was observed in Lycoprogen treated subjects (9.346 ng/mL+1.839 SE vs. 7.906+0.928 SE $P=0.100$). Finally, a significant reduction of basal PSA levels was seen in all but 5 obese or over-weight patients with basal PSA levels >10 ng/mL (18.520 ng/mL+2.747 SE vs. 10.323 ng/mL+2.073 SE, $P=0.009$). Lycoprogen is a functional food acting as antioxidant and a biological response modifier, thus representing a valuable dietary component to contrast chronic degenerative diseases amenable to be attenuated by the MD.

Probiotics, Prebiotics and Nutraceuticals in: Pediatrics the Therapeutic Efficacy of *Bifidobacterium Animalis* SUBSP. Lactis BB-12® in Infant Colic: A Randomized, Double Blind, Placebo-controlled Trial

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Objective: The pathogenesis of infant colic (IC) is poorly defined, but alterations in gut microbiota seem to be involved, supporting the potential therapeutic role of probiotics. We aimed to assess the rate of infants with a reduction of $\geq 50\%$ of mean daily crying duration after 28 days of intervention with the probiotic *Bifidobacterium animalis* subsp. *lactis* BB-12® (BB-12). Secondary outcomes were daily number of crying episodes, sleeping time, number of bowel movements and stool consistency.

Methods: RCT on otherwise healthy exclusively breastfed infants with infant colic (IC) randomly allocated to BB-12 (1×10⁹ CFU/d) or placebo. Infants assumed orally 6 drops/day of the assigned product for 28 days. Stool samples were collected to assess gut microbiota structure and butyrate, beta defensin 2 (HBD-2), cathelicidin (LL-37), secretory IgA (sIgA), calprotectin (CLP) levels.

Results: 80 infants were randomized, 40/group. The rate of infants with reduction of $\geq 50\%$ of mean daily crying duration was higher

in infants treated with BB-12 compared to placebo (80.0% vs. 32.5%, $P < 0.05$). The mean number of crying episodes decreased in both groups, but with a higher effect in BB-12 group (-4.7 ± 3.4 versus -2.3 ± 2.2 , $P < 0.05$). Mean daily stool frequency decreased in both groups but the effect was significantly higher in the BB-12 group; stool consistency was similar between the 2 groups. A increase in *Bifidobacterium* abundance, butyrate production, and HBD-2, LL-37, sIgA levels associated with a decrease in CLP fecal level was observed in BB-12 group. Increase in *Bifidobacterium* levels correlated significantly with the reduction of crying time.

Conclusions: Supplementation with BB-12 is effective in managing IC. The effect could derive from immune and non-immune mechanisms associated with a modulation of gut microbiota structure and function.

Precision Nutrition Tolerogenic Effect Elicited by Protein Fraction Derived from Different Hypoallergenic Formulas in PBMCs from Children with Cow Milk Allergy

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Objective: Different hypoallergenic formulas are available for the dietary treatment of cow's milk allergy (CMA). Safety and nutritional profile of these formulas have been well evaluated, but the potential tolerogenic activity elicited by their protein fraction is still largely undefined. We aimed to comparatively evaluate the tolerogenic effect elicited by protein fraction derived from different hypoallergenic formulas available for the dietary treatment of CMA.

Methods: Four hypoallergenic formulas were compared: extensively whey formula (EHWF), extensively hydrolyzed casein formula (EHCF), hydrolyzed rice formula (RHF), amino acid based formula (AAF). Formulas were reconstituted in water according to manufacturer's instructions, and subjected to *in vitro* infant gut simulated digestion using a sequential gastric and duodenal static model. Resulting protein fractions were purified using C18 reversed phase pre-packed cartridges (Sep-Pak, Waters, Milford, MA, USA), recovered in 70% acetonitrile/0.1% trifluoroacetic acid and finally vacuum-dried. Tolerogenic effects were evaluated in peripheral blood mononuclear cells (PBMCs) from 6 patients, with challenge-proven IgE-mediated CMA (age range 1-5 y, all Caucasians), stimulated with different doses of digested protein fractions (from 0.25 to 250 $\mu\text{g/mL}$) or b-lactoglobulin (BLG; 100 $\mu\text{g/mL}$) or bovine

serum albumin (BSA; 100 $\mu\text{g/mL}$) as positive and negative control respectively. The production of Th2 (IL-4, IL-5, IL-13) and Th1 (IL-10, IFN- γ) cytokines were assessed by ELISA. Modulatory action was also evaluated on immune (IL-33) and non-immune tolerogenic factors (mucin 5AC, tight-junction proteins ZO-1 and occludin) in human enterocytes (Caco-2 cells) by ELISA and Real Time PCR, respectively.

Results: Th2 cytokines were unaffected by the exposure to protein fraction from all study formulas, whereas only protein fraction from EHCF was able to positively modulate IL-10, IL-33, mucin 5AC, ZO-1 and occludin expression. All protein fraction from study formulas were able to increase INF- γ expression in PBMCs.

Conclusion: The results suggest a different regulatory action on immune and non-immune tolerogenic mechanisms elicited by protein fraction from different hypoallergenic formulas.

Microbiome Analysis at Strain-level: Study Design for the Pre-clinical and Clinical Development of Nutritional Supplements and Interventions

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Development of Nutritional Supplements and Interventions. According to the World Health Organization (WHO), more than 1.4 billion adults suffer from obesity or being overweight. In the United States, today nearly 60% of the population is overweight with a growing number of affected individuals being under the age of 11. In recent years, Next-Generation Sequencing has revolutionized microbiological sciences by revealing that virtually all environments, including the human body, are teeming with diverse microbial communities. Research has shown that the human microbiota contributes biological functions that are essential to our wellbeing. Conversely, disrupting the healthy homeostasis of host and microbiome can lead to dysbiosis and has been implicated with many diseases and pathologies. With mounting evidence linking the intestinal microbiome to metabolic disorders and obesity, research into novel nutritional and pharmacological interventions is carried out in the attempt to halt the obesity epidemic. In this seminar, we present an overview over the different phases involved in the development of probiotics and live biotherapeutic products (LBPs), including target discovery, pre-clinical and clinical research, production and quality assurance. We introduce common challenges and survey solutions specific to all phases of microbiome R&D using real-world examples.