MODULATION OF GUT MICROBIOTA AFTER SUPPLEMENTATION WITH CITRUS FRUIT EXTRACT USING THE TIM-2 IN VITRO MODEL OF THE HUMAN COLON

(1) S. Ahles, (2) M. Maurer Sost, (2) J. Verhoeven, (2) S. Verbruggen, (3) Y. Stevens, (2) K. Venema

(1) Department of Nutrition and Movement Sciences, Maastricht University, Maastricht, Netherlands
(2) Campus Venlo, Centre for Healthy Eating and Food Innovation, Maastricht University, Venlo, Netherlands
(3) Department of Internal Medicine, Maastricht University, Maastricht, Netherlands

OBJECTIVE: increasing intake of polyphenols could be a useful strategy to support and enhance gut health. Citrus flavonoids such as hesperidin and naringin have previously shown beneficial effects on barrier function and intestinal inflammation. The aim of this study was to assess effects of a natural citrus fruit extract (CFE) rich in hesperidin on gut microbiota composition and activity using the *in vitro* TIM-2 model of the human colon.
METHODS: the TIM-2 units were inoculated with human fecal samples and supplemented with 250 mg CFE, 350 mg CFE, or control for 72 hours. Samples were collected at baseline, after 24 h, 48 h, and 72 h. Gut microbiota composition and activity, and short-chain fatty acid (SCFA) production were determined using 16s rRNA gene sequencing and chromatography, respectively.
RESULTS: on the genus level, a dose-dependent significantly increased abundance of *Roseburia* (q = 0.134) was observed after CFE supplementation. A similar trend was observed for *B. eggerthii* (q = 0.184) and *E.ramulus* (q = 0.134), although not significant. Moreover, the relative abundances of *Enterococcus* (q = 0.134) and *L. mucosae* (q = 0.198) were significantly increased after CFE supplementation. Cumulative production of total SCFAs was higher after CFE supplementation compared to control, which was reflected by increased production of acetate.
CONCLUSIONS: in conclusion, CFE supplementation increased abundance of microbes and SCFAs known for anti-inflammatory and anti-obesity effects. These results highlight the potential for supplementation with CFE as an enhancer for gut health.

BIOCHEMICAL AND ANTIBIOTIC INFLUENCE OF GASTROINTESTINAL TRACTS MICROFLORA IN NEONATAL

(1, 2) A. Al-Judaibi, (2) E. AlJudaibi, (2) S. AlShareef

(1) Section of Microbiology, Jeddah University, Jeddah, Saudi Arabia
(2) Section of Biological Science, Jeddah University, Jeddah, Saudi Arabia

OBJECTIVE: colonisation of the neonatal intestinal tract by microbiota may occur as early as the foetal stage, and this colonisation preforms the intestinal microbiota, which is reflected in the intestinal activity and neonate vitality. This study aimed to isolate and identify common bacteria in 19 preterm neonates spending their first weeks of life in the neonatal intensive care unit.
METHODS: first, stool samples were collected, and bacteria were isolated and purified from those samples.
Common bacterial species were investigated regarding their susceptibility or resistance to antibiotics. From 19 stool samples, 15 contained three species in common: *Enterobacter cloacae*, *Enterococcus faecalis* and *Klebsiella pneumoniae*.
RESULTS: differences in the microbial formation and density were correlated with the type of delivery and feeding as well as the administration or no administration of antibiotics to the preterm neonate. Antibiotic susceptibility testing was undertaken, and minimum inhibitory concentrations were determined. The results showed that the minimum level of isolates was affected by several of the most commonly used antibiotics from the following families: aminoglycosides, carbapenems, fluroquinolones, glycylcyclines and polymyxins.
CONCLUSIONS: in the present study, we identified the most common bacterial species present in the intestinal microflora of premature infants during their first days after birth. *Enterobacter cloacae* and *Klebsiella pneumoniae* were the most common gram-negative bacteria, while *Enterococcus faecalis* was the most prevalent gram-positive bacterium. Our microbial susceptibility testing showed that these isolates were sensitive to several of the most commonly used antibiotics from the following families: aminoglycosides, carbapenems, fluroquinolones, glycylcyclines and polymyxins. The analysis revealed a significant level of sensitive towards most tested antibiotics among certain strains isolated from neonates, which raises concern.

AKKERMANSIA MUCINIPHILA ANTIMICROBIAL SUSCEPTIBILITY PROFILE

(1) J. C. Barbosa, (1) D. Machado, (1) D. Almeida, (2) J. C. Andrade, (1) A. C. Freitas, (1) A. M. Gomes

(1) Centro De Biotecnologia e Química Fina (CBQF), Associated Laboratory, School of Biotecnology, Universidade Católica Portuguesa, Porto, Portugal
(2) CESPU, Instituto De Investigação e Formação Avançada em Ciências e Tecnologias Da Saúde, Paredes, Portugal

OBJECTIVE: this study aims to characterize the antimicrobial susceptibility profile of Akkermansia muciniphila DSM 22959, a human commensal and next-generation probiotic candidate, using phenotypic and in silico analyses.
METHODS: phenotypic antibiotic susceptibility assessment: A. muciniphila DSM 22959 was grown in PYGM medium and subcultured at least twice before use. Minimum inhibitory concentration was determined for 8 clinically relevant antimicrobials, as recommended by EFSA-FEEDAP, using broth microdilution and E-test® methods. Both assays were performed at least with three independent replicates and with technical duplicates.
In silico analysis: Antimicrobial resistance genes (ARG), virulence factors (VF), genomic islands (GI) and mobile genetic elements (MGE) were predicted in A. muciniphila DSM 22959 whole genome (accession number: NZ_CP042830.1) using several available databases and bioinformatics tools.
RESULTS: phenotypically, A. muciniphila DSM 22959 shows susceptibility to ampicillin, tetracycline, colistin and fosfomycin and is resistant to gentamycin, kanamycin, streptomycin (aminoglycosides) and ciprofloxacin. Akkermansia muciniphila contains 26 annotated ARG that support the observed resistance profile. Other ARG might not be expressed under the tested conditions. Most ARG and VF are not embedded within GI or MGE. No plasmids were reported for this strain.
CONCLUSIONS: the same susceptibility categorization was obtained in both phenotypic methods. The phenotypic resistance profile is supported by the genomic context. However, there is

no evidence of horizontal acquisition or potential transferability of the identified ARG and VF. Thus, the antimicrobial susceptibility profile of the probiotic candidate A. muciniphila DSM 22959 meets the safety criteria required to be considered for human consumption.

TRANSCRIPTOME AND MITOCHONDRIAL ANALYSIS OF ASD CHILDREN COMPARED TO HEALTHY CONTROLS

(1) M. Cannon, (2) S. Ganeshan, (3) G. Banavar, (3) M. Vuysich

(1) Department of Otolaryngology, Northwestern University, Chicago, US
(2) MitoSwab, Research, Plymouth, USA
(3) VIOME, Research, Los Alamos, USA

OBJECTIVE: Autism Spectrum Disorder (ASD), like many modern society pathologic conditions, is an epigenetically initiated disease. The purpose of this study was to determine the differences in the oral microbiome and mitochondrial health between children from a "blue zone" in Colombia, healthy and A.S.D. children in the U.S.A.
METHODS: the A.S.D. section included 30 children and young adults, ages 6 to 21, who were sampled at three different intervals. The sampling consisted of buccal swabs for Mitoswab testing, and saliva for full transcriptomics, in order to determine the entire virus, bacteria, archaea, and fungus range of species. Dietary and health information was obtained, as were consents and assents per I.R.B. requirements. The Colombia component included 30 children, ages 6-16, who were healthy and within normal behavior standards. Buccal swabbing and salivary sampling was performed only once with this group, as with the typical healthy USA controls. The USA Healthy Control group consisted of children 6-16 who had no history of any medications.
RESULTS: significant differences between each subject and intervention group were demonstrated by the Richness and Shannon Diversity plots. The USA healthy children group had a greater Richness than the other two groups but the Shannon diversity was not significantly different.
CONCLUSIONS: the microbiomes of individuals diagnosed with A.S.D. is significantly different from healthy children in a developing country and healthy children from the USA. Microbiome shifts may have strong epigenetic consequences that may be involved in ASD development.

EVALUATION OF NOMADIC AND NICHE-SPECIALIST LACTOBACILLI AS POTENTIAL VAGINAL PROBIOTICS

(1) C. Cappello, (1) M. Acin-Albiac, (2) D. Pinto, (2) F. Rinaldi, (3) E. Zannini

(1) Food Engineering and Biotechnology, University of Bolzano-Bozen, Bolzano, Italy
(2) Human Microbiome Advanced Project, HMAP, Research & Development, Milan, Italy
(3) School of Food and Nutritional Sciences, University College Cork, Cork, Ireland

OBJECTIVE: the aim of this study is the development of a multi strain probiotic gel to promote lactobacilli-dominated vaginal microbiota in pregnant women and to establish a proper eubiosis on the new-born. Mainly nomadic lactobacilli, isolated from food sources, were screened for functional characteristics and the capability to inhibit *Streptococcus agalactiae, Staphylococcus aureus* and *Candida albicans*, which may lead to adverse pregnancy-related outcomes.
METHODS: one hundred fourteen strains were screened for hydrophobicity, auto-aggregation, peptide hydrolysis, hydrogen peroxide production, and lactic acid isomers quantification. Cell-free supernatants (CFSs) of the candidate strains were co-inoculated with vaginal pathogens for high-throughput inhibition screening. Aiming to evaluate the reduction of the expression of genes involved in the inflammatory cascade the best performing strains were investigated *in vitro* alone and in combination.
RESULTS: fifteen *Lactiplantibacillus plantarum* strains showed outstanding hydrophobicity traits. The auto-aggregation capacity was specie-independent, while the peptide concentration distribution was quite similar among lactobacilli. The production of hydrogen peroxide was strain dependent, with the highest concentrations found for *Lacticaseibacillus paracasei*. *Lb. plantarum* produced both isomers of lactic acid, while *Lb. paracasei* produced only L-isomer. *S. aureus* and *S. agalactiae* were strongly inhibited by a wide range of CFS in different modes of action, whereas *C. albicans* inhibition was less frequent.
CONCLUSIONS: overall, *L. plantarum* had the highest pathogen inhibition score and the best functional traits. Two of the best performing strains showed a reduction on the expression of genes involved in the inflammatory cascade in human keranocytes.

INVESTIGASTIONS OF THE POTENTIAL MECHANISM OF ACTION OF A MULTI-STRAIN PROBIOTIC COMPOSITION AGAINST URO-GENITAL PATHOGENS BY *EX-VIVO* STUDIES

(1) M. Meloni, (2) P. Malfa, (2) D. Piro, (3) L. Brambilla, (4) S. Giardina, (5) S. Lincetti, (6) M. Masciarelli, (2) F. Carlomagno

(1) Vitroscreen, CEO, Milan, Italy
(2) Roelmi HPC, R&D, Origgio, Italy
(3) Vitroscreen, R&D, Milan, Italy
(4) Complife Group, R&D, Pavia, Italy
(5) Complife Group, R&D, Garbagnate Milanese, Italy
(6) Complife Group, R&D, Barcellona, Spain

OBJECTIVE: the urogenital microbiota is dominated by lactobacilli able to counteract pathogens growth. Vaginal infections occur when the urogenital microbiota is unbalanced. The aim of the study was to evaluate the efficacy of SynBalance® Femme, a product containing *L. plantarum* PBS067, *B. animalis* subsp. *lactis* BL050 and *L. rhamnosus* LRH020, to inhibit the adhesion and the growth of pathogens involved in uro-vaginal infections.
METHODS: the antimicrobial and preventive effects of the three probiotic strains and their combination SynBalance® Femme, have been evaluated on a reconstructed bladder epithelium (HBE), infected with *E. coli* and on a reconstructed vaginal human epithelium (VHE, A431 modified) infected with *C. glabrata and C. albicans*, *G. vaginalis*, *N. gonorrheae* and *T. vaginalis*, respectively. In addition, the effects on the viability and the integrity of reconstructed tissues after TEER treatment were also assessed.
RESULTS: a strong antimicrobial activity was observed for *B. lactis* BL050, *L. plantarum* PBS067 and *L. rhamnosus* LRH020, on HBE previously colonized by *E. coli*. For *L. rhamnosus* LRH020 a preventive activity was also observed by SEM analysis. TEER results

showed that none of the strains have negatively influenced the integrity of HBE.
On the vaginal epithelium, SynBalance® Femme and its corresponding strains showed a full inhibition of all tested pathogens, together with a strong reduction of their adhesiveness. The prevention model demonstrated a very strong effect as well.
CONCLUSIONS: these results underling the potential mechanism of action of SynBalance® Femme and their single strains in the prevention and treatment of several urogenital infections.

COMPREHENSIVE PAN-GENOME ANALYSIS OF *LACTIPLANTIBACILLUS PLANTARUM* COMPLETE GENOMES

(1) F. M. Carpi, (1) M. M. Coman, (2) S. Silvi, (3) M. Picciolini, (1) M. C. Verdenelli, (2) V. Napolioni

(1) Synbiotec Srl, R&D, Camerino, Italy
(2) School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy
(3) Independent Researcher, Gubbio, Italy

OBJECTIVE: the aim of this work was to refine the taxonomy and the functional characterization of publicly available *Lactiplantibacillus plantarum* complete genomes through a pan-genome analysis. Particular attention was paid in depicting the probiotic potential of each strain.
METHODS: 127 complete genome sequence of *L. plantarum* strains, without detected anomalies, was downloaded from NCBI.
RESULTS: Roary analysis of *L. plantarum* pan-genome identified 1,436 core, 414 soft core, 1,858 shell and 13,203 cloud genes, highlighting the "open" nature of *L. plantarum* pan-genome.
Identification and characterization of plasmid content, mobile genetic elements, adaptative immune system and probiotic marker genes (PMGs) revealed unique features across all the *L. plantarum* strains included in the present study.
Considering our updated list of PMGs, we determined that approximatively 70% of the PMGs belongs to the core/soft-core genome.
CONCLUSIONS: the comparative genomic analysis conducted in this study provide new insights into the genomic content and variability of *L. plantarum*. This study provides a comprehensive pan-genome analysis of *L.plantarum*, including the largest number (N = 127) of complete *L. plantarum* genomes retrieved from publicly-available repositories. Our effort aimed to determine a solid reference panel for the future characterization of newly sequenced *L. plantarum* strains useful as probiotic supplements.

IN VITRO INFLUENCE OF *TILIA TOMENTOSA MOENCH* ON SMALL INTESTINE NEUROMUSCULAR FUNCTION

(1) S. Cerantola, (1) S. Faggin, (1) G. Annaloro, (2) F. Mainente, (1) A. Piovan, (3) E. Savarino, (2) G. Zoccatelli, (1) M. C. Giron

(1) Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy
(2) Department of Biotechnology, University of Verona, Verona, Italy
(3) Department of Surgery, Oncological and Gastrointestinal Science, University of Padua, Padua, Italy

OBJECTIVE: irritable bowel syndrome (IBS) is characterized by abdominal pain, bloating and bowel disturbances. IBS therapy is primarily symptomatic, including treatment with herbal remedies. Flower extract of *Tilia tomentosa* Moench (TtM) is occasionally used as an anti-spasmodic in popular medicine. Since its effect on intestinal response is unknown, we evaluated TtM influence on small intestine contractility.
METHODS: Ileal preparations from adult C57BL/6J mice were mounted in organ baths to assess changes in muscle tension, following addition of TtM extract (0.5-36 mcg/mL) or vehicle (ethanol). Ileal segments were pretreated with 12 mcg/mL TtM or vehicle and subjected to: cumulative addition of carbachol (0.001-100 microM); electrical field stimulation (EFS, 0-40 Hz); 10-Hz-EFS with 1 microM atropine + 1 microM guanethidine (non-adrenergic-non-cholinergic-conditions) with/without 0.1 mM L-NAME (pan-NOS inhibitor). The integrity of myenteric plexus was analyzed by immunofluorescence distribution of neuronal markers HuC/D and nNOS and of glial marker S100beta.
RESULTS: increasing addition of TtM induced a marked relaxation in ileal specimens compared to vehicle ($p < 0.001$). Pretreatment with TtM caused a significant reduction of CCh- and EFS-induced contraction compared to related control segments ($p < 0.001$). Following incubation with TtM, a significant reduction in 10 Hz-EFS-mediated relaxation ($p < 0.001$) sensitive to L-NAME was found. In vitro 1-hour-incubation of intestinal specimens with TtM did not affect myenteric plexus neuroglial network.
CONCLUSIONS: our findings show that TtM-induced relaxation on small intestine neuromuscular contraction is mediated by nitric oxide pathways, providing a pharmacological basis for the use of TtM in IBS.

PROBIOTICS AND ACNE: *IN VITRO* TESTING OF NEW PROBIOTIC STRAINS TO COUNTERACT ACNE

(1) M. M. Coman, (2) G. Nannini, (2) A. Amedei, (3) S. Silvi, (1) M. C. Verdenelli

(1) Synbiotec Srl, R&D, Camerino, Italy
(2) Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
(3) School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy

OBJECTIVE: acne is a highly prevalent inflammatory skin condition involving the interaction between skin microbes and host immunity, which lead with the changes in microbial composition and activity disturbing the microbiota balance. Several probiotic strains have been tested for their anti-pathogenic activity against the main pathogens responsible for acne, capacity to fight pathogenic adhesion to HaCaT cells and the *in vitro* down-regulation of innate immunity.
METHODS: the inflammatory response was monitored by immunohistochemistry and ELISA assays, targeting a selection of Innate Immune Markers (IIMs) (IL-6, IL-8, IL-10, IL-17, TGF-b).
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To investigate whether probiotics have direct effects on the growth of *Cutibacterium acne*, *Staphyloccoccus aureus* and *Streptococcus pyogenes*, the antimicrobial activity of live LAB was analysed by a modified cross-streak method. It was also investigated the *in vitro* blockage of pathogens adherence by six probiotic strains to HaCaT cells, under three possible mechanisms: exclusion by adhered probiotics, displacement of adhered pathogens and competition for receptor sites (exclusion test). The inflammatory response was monitored by immunohistochemistry and ELISA assays, targeting a selection of innate immune markers (IL-4, IL-6, IL-8, IL-10, IL-17, TGF-β and IFN-γ).
RESULTS: all strains shown anti-pathogenic activity against the tested pathogens. The inhibition results on HaCaT cells highlights a significant ($P<0.05$) competition of all probiotics against the three pathogens. Every pathogenic strain alone has been shown to lead to up-regulation of innate immune markers, while restoration of the microbiome diversity by probiotics presence suppressed inflammation via down-regulation of innate immunity.
CONCLUSIONS: the results suggest that the probiotics used in the present study could prevent colonization of the skin by relevant pathogens through barrier and interference mechanisms (mainly exclusion and competition), suggesting a successfully use in future conventional therapies of skin disorders (acne).

SACCHAROMYCES CEREVISIAE BASED PROBIOTICS OUTPERFORM LACTOBACILLI IN INHIBITION OF VAGINAL CANDIDIASIS

L. Demuyser, M. Sillen, S. Baldewijns, I. Palmans, P. Vandecruys, P. Van Dijck

VIB-KU Leuven Center for Microbiology, Flanders Institute for Biotechnology, Biology, Leuven, Belgium

OBJECTIVES: although progress has been made to equalize the rights of men and women, female-specific issues, such as vulvovaginal candidiasis (VVC), are still studied less compared to males. This is striking, as VVC affects 75% of all women at least once in their life. In case of recurrent (R)VVC, women experience at least four episodes of infection every year, further increasing the emotional and economic burden.
Especially in case of RVVC, treatment is insufficient, as misdiagnosis, recolonization and resistance impair clearance. Here, we aim to develop a probiotic therapy to target *Candida* infections in the vaginal niche.
METHODS: most of the research on VVC has been performed in non-optimal conditions, using systemic *Candida* isolates and non-representative medium. We used an optimized *in vitro* platform to select promising probiotics against VVC.
RESULTS: 1) in this study, we screened a large set of vaginal *Candida* isolates for virulence properties in the vaginal niche. The isolate representing the highest pathogenicity was used to identify promising probiotic organisms. 2) The use of lactobacilli as probiotic therapy against VVC is debated. We find that certain bacterial strains increase rather than inhibit pathogenicity of *Candida* and point out the role of lactic acid in this process. We also show the potential of *Saccharomyces cerevisiae* strains in inhibiting virulence by *Candida* species. We identified a role for specific fatty acid metabolites in this process.
CONCLUSIONS: by using an appropriate platform, we validated the potential of *S. cerevisiae* in inhibition of *Candida* virulence in the vagina.

ANALYSIS OF THE IMPACT ON HUMAN GUT MICROBIOTA AND OF COLONIZATION ABILITY OF PROBIOTIC MICROBES FROM FERMENTED FOODS THROUGH A SYSTEMATIC APPROACH

C. Devirgiliis, M. Roselli, F. Natella, P. Zinno, B. Guantario, R. Canali, E. Schifano, G. Perozzi

CREA, Council for Agricultural Research and Economics, Research Centre for Food and Nutrition, Rome, Italy

OBJECTIVE: scientific results describing the microbial flow connecting food and gut microbiomes are still fragmented. The aim of the analysis was to provide a state-of-the-art knowledge-base about the scientific literature addressing the connection between foodborne and gut microbiomes, focusing on probiotics added to fermented foods, their possible impact on human gut microbiota composition and their ability to colonize the gut environment. An additional aim was also to highlight experimental approaches and study designs which could be better standardized to improve comparative analysis of published datasets.
METHODS: a systematic literature search for peer-reviewed research articles was carried out on two databases to identify intervention and observational studies analyzing the impact on human gut microbiota composition and colonization ability of probiotic bacteria from fermented foods. Forty-two papers were finally selected.
RESULTS: overall, recurrent gut microbial groups mainly affected by probiotic-added fermented food consumption resulted to be Lactobacilli and Bifidobacteria, whose levels increased following supplementation. Most probiotics were recovered in faecal samples, suggesting colonization ability, although only few studies provided direct evidence of the presence of viable bacterial cells. Moreover, the overall results indicate that colonization was a transient condition, lasting only during the supplementation period.
CONCLUSIONS: further research employing standardized and trans-disciplinary approaches aimed at understanding how probiotic added fermented foods can be tailored to positively influence human gut microbiota and, in turn, host health, are therefore of pivotal importance.

EFFECTS OF A NOVEL PROBIOTIC COMBINATION ON CROHN'S DISEASE-LIKE ILEITIS MOUSE MODEL

(1) L. Di Martino, (2) A. Osme, (2) T. Pizarro, (3) M. Ghannoum, (1) F. Cominelli

(1) Digestive Health Research Institute, Case Western Reserve University, Cleveland, USA
(2) Department of Pathology, Case Western Reserve University, Cleveland, USA
(3) Department of Dermatology, Case Western Reserve University, Cleveland, USA

OBJECTIVE: we identified beneficial probiotic strains including *S. boulardii*, *L. acidophilus, L. rhamnosus* and *B. breve* that antagonize elevated bacterial pathogens in the inflamed gut. Our aim is to characterize the effect of the probiotic supplement in the murine model of ileitis SAMP1/YitFc (SAMP).
METHODS: two groups of SAMP mice have been used for this experiment. The experimental group was administered with one dose of the probiotic nutritional supplement diluted in sterile PBS (0.25 mg/100uL of PBS) everyday for 60 days through gavage

technique. The control group was administered with sterile PBS. At the end of the treatment, ilea and stool samples were collected for histology and 16S rRNA analysis.
RESULTS: the histology score shows that probiotic treated-mice had a significant decrease of ileitis compared to the control group (unpaired t test: 7.2 ± 0.5 vs. 13.4 ± 2.5; $^{**}P = 0.0069$). Principal component analysis showed that for the bacteriome, mice before the treatment clustered together. In contrast, probiotic treated-samples were widely scattered compared to the limited scattering observed in the control group. 16S rRNA analysis showed that abundance of species belonging to genus *Lactobacillus* was significantly decreased compared to controls ($P < 0.05$). Levels of *Rikenellaceae* were significantly increased in probiotic-treated mice compared to controls ($P < 0.02$).
CONCLUSIONS: the changes followed probiotic use show that the microbiome was positively impacted. In fact, previous studies found that Non-Alcohol Fatty Liver disease patients have significantly higher levels of *Lactobacillus* and lower levels of *Rikenellaceae* compared to healthy subjects.

PRODUCTION OF NATURALLY GAMMA-AMINOBUTIRIC ACID ENRICHED CHEESE FROM PASTEURIZED MILK, USING THE DAIRY STRAIN *LEVILACTOBACILLUS BREVIS* DSM 32386

(1) E. Franciosi, (1) A. Mancini, (1) M. Cid Rodriguez, (2) T. Nardin, R. Larcher, (3) N. Cologna, (3) A. Goss, (1) K. Tuohy, (4) A. Merz

(1) Edmund Mach Fundation, Food Quality and Nutrition, San Michele/adige, Italy
(2) Edmund Mach Fundation, Technology Transfer Center, San Michele/adige, Italy
(3) Trentingrana Consorzio dei Caseifici Sociali Trentini s.c.a., Analysis Laboratory, Trento, Italy
(4) Trentingrana Consorzio dei Caseifici Sociali Trentini s.c.a., Mangement office, Trento, Italy

OBJECTIVE: the cheese-derived strain *Levilactobacillus brevis* DSM 32386 has been reported as able to produce high concentrations of GABA in experimental cheese from raw milk. In this study, we investigated the activity of this strain during the production of experimental cheeses from pasteurized milk, to test its ability to produce GABA before industrial scale-up.
METHODS: *Levilactobacillus brevis* DSM 32386 was tested alone (Lbr) or in combination with commercial proteolytic strains able to increase the amount of free glutamate in cheese, *Lactobacillus helveticus* LH4R and *Lactobacillus delbrueckii* subsp. *bulgaricus* LB1 and was compared with a cheese inoculated only with the starter culture (CTRL) or added with Lbr and glutamate. The GABA concentration was measured by means of UHPLC-HQOMS analysis on milk and cheese samples after 7, and 30 days ripening.
RESULTS: during the ripening, pH of all cheeses remained below 5.5, which is the value required for GABA production. GABA concentration increased during ripening, with the highest concentration in cheeses after 30 days of ripening where *L. brevis* DSM 32386 was added with glutamate (168.92 mg/kg), or with the commercial *L. delbrueckii* subsp. *bulgaricus* (150.89 mg/kg). In CTRL cheeses the GABA concentration was under 100 mg/kg.
CONCLUSIONS: the obtained data supported the hypothesis that *L. brevis* DSM 32386 can be exploited as probiotic culture, improving the *in situ* bio-synthesis of GABA not only in raw milk cheeses but also in pasteurized milk cheeses where the proteolytic activity is led by other lactic acid bacterial strains such as *L. delbrueckii* subsp. *bulgaricus.*

IMPROVED LIPID METABOLISM IN A MOUSE MODEL OF ALZHEIMER'S DISEASE UPON STRATEGIC MODULATION OF GUT MICROBIOTA

C. Gong, L. Bonfilli, M. Cuccioloni, V. Cecarini, M. Angeletti, A. M. Eleuteri

School of Biosciences and Veterinary Medicine, University of Camerino, Camerino (MC), Italy

OBJECTIVE: previous studies demonstrated that probiotics counteract Alzheimer's disease (AD) progression and restore glucose homeostasis in 3xTg-AD mice (Bonfili *et al*. 2017, 2020). Considering the debated role of deregulated lipid homeostasis in AD (McGuinness B *et al*. 2010) and in light of the promising effects of probiotics on energy metabolism, this study aims at elucidating the mechanisms through which gut microbiota manipulation ameliorates impaired lipid metabolism in AD.
METHODS: 8 week-old 3xTg-AD mice and their wild-type counterpart were chronically administered with a multi-strain probiotic formulation and changes in the plasma lipid profile (using lipidomic analyses and enzymatic colorimetric assays), along with the cerebral and hepatic expression levels of key regulators of cholesterol metabolism (through Western blotting and ELISA), were evaluated.
RESULTS: upon probiotics administration, cholesterol biosynthesis was inhibited in AD mice, with the involvement of sterol regulatory element binding protein 1c and liver X receptors mediated pathways. Decreased plasma and brain concentration of 27-hydroxycholesterol and increased brain expression of cholesterol 24S-hydroxylase indicated that alternative pathways of bile acid synthesis are influenced. These data, together with the hypocholesterolemic effects and the ameliorated fatty acids profile, demonstrated that microbiota modulation through probiotics can positively change lipid composition in AD mice, with arachidonic acid representing a hub metabolite in the interactions among probiotic-induced lipid profile changes, insulin sensitivity, and inflammation.
CONCLUSIONS: these data provide important contribution in filling the knowledge gap in the neuroprotective microbiota-lipid-glucose crosstalk in AD and may pave the way for the identification of new therapeutic targets and effective treatments.

ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF CELL FREE SUPERNATANT PRODUCED BY *LACTOBACILLUS REUTERI* DSM 17938

(1) I. Vitale, (1) V. Puca, (1) S. Carradori, (2) A. Di Sotto, (1) Rossella Grande

(1) Department of Pharmacy, G. d'Annunzio University of Chieti-Pescara, Chieti, Italy
(2) Department of Physiology and Pharmacology V. Erspamer, Sapienza University of Rome, Rome, Italy

OBJECTIVE: *Lactobacillus reuteri* colonizes the human gastrointestinal tract, where it can stimulate the host immune system, modulate the microbiota composition, and prevent pathogen colonization thanks to the release of several antimicrobial com-

pounds. The aim of this work was the assessment of the antimicrobial and antibiofilm activity of the Cell Free Supernatant (CFS) produced by *L. reuteri* DSM 17938 *versus Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus mutans.*
METHODS: the CFS was obtained through the centrifugation with centrifugal filter devices of 10 K of *L. reuteri* broth cultures. The Minimum Inhibitory Concentration was determined through the broth microdilution method, the viability assay and Colony Forming Units (CFU) counts, while the Minimum Bactericidal Concentration through CFU counts. The antibiofilm activity was evaluated by assessing the Minimal Biofilm Inhibitory Concentration and the Minimal Biofilm Eradication Concentration through CFU counts, Cristal Violet and a metabolic assay. In addition, the cytotoxicity of CFS was tested on human cell lines.
RESULTS: the CFS showed a satisfactory antibacterial activity toward all the microorganisms tested. Regarding the antibiofilm efficacy the CFS showed MBEC corresponding to 1×MIC *versus P. aeruginosa* and *S. aureus* and corresponding to 2-3 × MIC *versus E. coli*. No effect has been detected toward *S. mutans*. The safety profile toward human cell lines showed promising sureness.
CONCLUSIONS: CFS can be useful for developing alternative therapeutic strategies against bacterial infections associated with biofilm-producer microorganisms. Further studies should be performed to detect the CFS components responsible of the antimicrobial and antibiofilm activities.

HAFNIA ALVEI HA4597 IMPROVES WEIGHT LOSS IN OVERWEIGHT SUBJECTS UNDER HYPOCALORIC DIET: A DOUBLE-BLIND, RANDOMIZED PLACEBO-CONTROLLED STUDY

(1) P. Dechelotte, (1) J. Breton, (2) C. Trotin Picolo, (3) B. Grube, (4) C. Erlenbeck, (4) G. Bothe, (1) S. Fetissov, (2) L. Gregory

(1) Inserm UMR 1073, Rouen University, Rouen, France
(2) TargEDys, R&D, Longjumeau, France
(3) Practice, General medecine, Berlin, Germany
(4) Analyse & Realize GmbH, Clinical, Berlin, Germany

OBJECTIVE: after *Hafnia alvei* HA4597 proved able to produce Caseinolytic peptidase B (ClpB), a peptide mimicking the satiety hormone alpha-MSH and showed promising results on the reduction of food intake and body weight gain in mice models of obesity (Lucas *et al*., 2020, Legrand *et al*., 2020), the objective was to investigate the probiotic strain's efficacy in weight reduction and metabolic health parameters in overweight adults.
METHODS: 236 overweight adults were included in this 12-week prospective study. They received standardized counselling for a -20% hypocaloric diet. Subjects of the HA group received 2 capsules per day providing 100 billion bacteria, while the placebo group received 2 capsules of placebo. The primary outcome was the proportion of responders, defined as subjects who lost at least 3% of baseline body weight at 12 weeks.
RESULTS: the proportion of responders was significantly superior in the HA group (57,7%) compared to the placebo group (41,7%). In addition, the reduction in Body Mass Index (BMI), hip circumference and fasting glycemia were significantly greater in the probiotic group. The feeling of fullness and the global satisfaction were also greater in the HA group.
CONCLUSIONS: a 12 week supplementation with HA4597 significantly improves weight loss, feeling of fullness and glycemia levels in overweight subjects under hypocaloric diet. These data support the use of *Hafnia alvei* HA4597 in the global management of excess weight.

GENOME-GUIDED CREATION OF NEXT-GENERATION PROBIOTICS

T. Hitch, N. Kumar, T. Clavel

Functional Microbiome Research Group, University Hospital RWTH Aachen, Aachen, Germany

Metagenomic analysis has provided detailed taxonomic and functional insights into host-associated microbiota. Using this wealth of information, consortia of microbes that confer health benefits to their hosts can be developed, termed 'next-generation probiotics'.
Host-associated microbiota consist of hundreds of species, each containing thousands of protein families (Pfams), making them functionally complex. While a few species dominate most microbiota profiles, species occurring at lower abundances are known to contribute important functions. Due to this, consortia based on dominant species in an environment are likely to miss key or essential functions. To solve this, we developed an automated system for the creation of representative synthetic communities, such as next-generation probiotics, called MiMiC (Minimal Microbial Consortia), based on the functional repertoire of an input microbiome.
MiMiC predicts functionally representative minimal consortia using an iterative scoring system based on maximal match-to-mismatch ratios of Pfams between a database of genomes (generic or isolate specific) and input metagenomes. Reduction of metagenomes, and genomes to the presence and absence of Pfams was confirmed to retain resolution and allow metagenomic profiles between six environmental and host-derived microbial communities to be distinguished. Furthermore, when looking at microbiome from pigs or humans from different countries, significant differences were observed within their predicted consortia. MiMiC represents a step forward in the automated development of synthetic communities and can be applied to generate next-generation probiotics for both generic use (replacement of FMT or neonatal probiotics), or personalized treatments (supplementation of missing functions to improve gut health).

EFFECT OF FIBER TYPE ON THE METABOLISM OF *EX VIVO* HUMAN GUT MICROBIOTA

(1, 2) Z. Huang, (1) V. Fogliano, (1) E. Capuano, (2) J. Wells

(1) Food Quality and Design Group, Wageningen University, Wageningen, Netherlands
(2) Host-Microbe Interactomics Group, Wageningen University, Wageningen, Netherlands

Bioactive metabolites produced by the gut microbiota are important for human health. Short-chain fatty acids (SCFAs) produced by carbohydrate fermentation and indole derivatives from tryptophan catabolism are well recognized for their impact on host physiology and can also have effects beyond the gut by entering the circulation. Here, we used an *ex vivo* experimental framework to investigate the effect of fiber type on the production of short-chain fatty acids (SCFAs) and indole derivatives. Pectin and inulin were chosen as examples of plant fibers to fer-

ment with bacteria from the proximal colon (PC) and distal colon (DC) compartments of Simulator of Human Intestinal Microbial Ecosystem (SHIME®). Fermenter samples were collected to measure effects on human microbiota composition and diversity as well as metabolite production. Pectin and inulin fermentation resulted in distinct metabolic profiles in the PC and DC. Inulin yielded higher concentrations of SCFAs than the pectin after 24 h fermentation. The relative concentration of indole derivatives varied depending on the fiber used, with higher concentrations of indole-3-acetic acid and indole-3-aldehyde for pectin, and a higher concentration of indole-3-propionic acid for inulin. When PC and DC were supplied with the same amount and type of fibers, microbiota in the DC produced more SCFAs than the PC. Indole derivatives were largely produced in the DC. Fermentation with pectin or inulin suppressed microbiota catabolism of tryptophan in the PC. Taken together, our results suggest that the type of fiber must be considered in the formulation of functional foods for intestinal health benefits.

INVESTIGATING THE SUSCEPTIBILITY OF THE NEXT GENERATION PROBIOTIC *FAECALIBACTERIUM PRAUSNITZII* UNDER STRESS CONDITIONS

(1) D. Machado, (1) M. Domingos, (1) D. Almeida, (1) J. C. Barbosa, (2) J. C. Andrade, (1) A. C. Freitas, (1) A. M. Gomes

(1) Department of Biotechnology, Universidade Católica Portuguesa, Centro de Biotecnologia e Química Fina (CBQF), Associated Laboratory, Porto, Portugal
(2) CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Paredes, Portugal

OBJECTIVE: *Faecalibacterium prausnitzii* is a multi-skilled intestinal bacterium proposed as a next generation probiotic. However, detailed information addressing the safety of this novel probiotic (in terms of antimicrobial susceptibility) and its technological fitness is still lacking. These data are important when developing probiotic products. This work aimed to evaluate *F. prausnitzii* DSM17677 susceptibility when exposed to selected antimicrobials, oxygen, acidic pH and bile.
METHODS: antimicrobial susceptibility of *F. prausnitzii* DSM17677 to ampicillin, vancomycin, gentamicin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline and chloramphenicol was assessed following European Food Safety Authority guideline. *Faecalibacterium prausnitzii* DSM17677 cultures were exposed to: 1) ambient air up to 5-minutes; 2) acidic pH (3 and 5) during 2-hours; 3) bile concentrations (0.1, 0.25 and 0.5 %) up to 3-hours. Viability was determined by colony-forming units plating (CFU) at defined time-points.
RESULTS: *Faecalibacterium prausnitzii* DSM17677 was susceptible to vancomycin, clindamycin, tetracycline and chloramphenicol. Moreover, this strain exhibited high viability reductions (> 4 log CFU/ml) after 1-minute of aerobic exposure of inoculated plates, and after 1-hour exposure to pH 3 and in all bile concentrations tested. However, this strain tolerated well the exposure to pH 5 for 2-hours.
CONCLUSIONS: given high *F. prausnitzii* DSM17677 sensitivity to aerobic atmosphere, pH 3 and bile, our data revealed the need to develop delivery systems able to promote the viability and stability of this bacterium when subject to such environmental stresses, envisaging its future application as a probiotic strain. Furthermore, this work contributes to the establishment of *F. prausnitzii* DSM17677 antimicrobial susceptibility profile.

INVESTIGASTIONS OF THE POTENTIAL MECHANISM OF ACTION OF A MULTI-STRAIN PROBIOTIC COMPOSITION AGAINST URO-GENITAL PATHOGENS BY *EX-VIVO* STUDIES

(1) M. Meloni, (2) P. Malfa, (2) D. Piro, (3) L. Brambilla, (4) S. Giardina, (5) S. Lincetti, (6) M. Masciarelli, (2) F. Carlomagno

(1) Vitroscreen, CEO, Milan, Italy
(2) Roelmi HPC, R&D, Origgio, Italy
(3) Vitroscreen, R&D, Milan, Italy
(4) Complife Group, R&D, Pavia, Italy
(5) Complife Group, R&D, Garbagnate Milanese, Italy
(6) Complife Group, R&D, Barcellona, Spain

OBJECTIVE: the urogenital microbiota is dominated by lactobacilli able to counteract pathogens growth. Vaginal infections occur when the urogenital microbiota is unbalanced. The aim of the study was to evaluate the efficacy of SynBalance® Femme, a product containing *L. plantarum* PBS067, *B. animalis* subsp. *lactis* BL050 and *L. rhamnosus* LRH020, to inhibit the adhesion and the growth of pathogens involved in uro-vaginal infections.
METHODS: the antimicrobial and preventive effects of the three probiotic strains and their combination SynBalance® Femme, have been evaluated on a reconstructed bladder epithelium (HBE), infected with *E. coli* and on a reconstructed vaginal human epithelium (VHE, A431 modified) infected with *C. glabrata and C. albicans*, *G. vaginalis*, *N. gonorrheae* and *T. vaginalis*, respectively. In addition, the effects on the viability and the integrity of reconstructed tissues after TEER treatment were also assessed.
RESULTS: a strong antimicrobial activity was observed for *B. lactis* BL050, *L. plantarum* PBS067 and *L. rhamnosus* LRH020, on HBE previously colonized by *E. coli*. For *L. rhamnosus* LRH020 a preventive activity was also observed by SEM analysis. TEER results showed that none of the strains have negatively influenced the integrity of HBE.
On the vaginal epithelium, SynBalance® Femme and its corresponding strains showed a full inhibition of all tested pathogens, together with a strong reduction of their adhesiveness. The prevention model demonstrated a very strong effect as well.
CONCLUSIONS: these results underling the potential mechanism of action of SynBalance® Femme and their single strains in the prevention and treatment of several urogenital infections.

PREBIOTIC LACTULOSE AS EFFICACIOUS MICROBIOTA AND METABOLITE MODULATOR IN CIRRHOSIS ENVIRONMENT

(1) A. Mancini, (2) S. Larsen, (3) F. Campagna, (2) P. Franceschi, (3) P. Amodio, (4) C. Pravadelli, (2) M. Pindo, (1) K. Tuohy

(1) Food Quality and Nutrition, Fondazione Edmund Mach, San Michele all'Adige, Trento, Italy
(2) Computational Biology Unit, Fondazione Edmund Mach, San Michele all'Adige, Trento, Italy
(3) Department of Medicine-DIMED, University of Padova, Padua, Italy
(4) Gastroenterology Unit, Santa Chiara Hospital, Trento, Italy

OBJECTIVE: gut microbiota has a fundamental role in the pathogenesis of liver cirrhosis as well as their complications as in the case of hepatic encephalopathy (HE). Current HE clinical treatment is mainly based on manipulating the gut microbiota and ammonia production/absorption using prebiotic lactulose, antibiotic rifaximin and probiotic VSL#3.

METHODS: here we investigated the modulation of gut microbiota, in terms of microbial composition and metabolism, upon fermentation of lactulose, rifaximin and VSL#3, using in vitro-24hours anaerobic pH-controlled batch cultures inoculated with faecal microbiota of cirrhotic patients.
RESULTS: over time, cirrhotic microbiota responded dynamically to the treatments. In particular, significant differences (PERMANOVA Weighted/Unweighted/Bray-Curtis estimators) were observed after 24 h. The main taxa associated with decompensated cirrhosis, were reduced with lactulose. At the same time, taxa associated with healthy conditions, such as *Lachnospiraceae*, *Ruminococcaceae*, *Blautia* and *Bifidobacterium*, were promoted as confirmed by the Indicator Species Analysis. Lactulose alone or in combination with the probiotic VSL#3 led to an increase production of SCFA and decrease in ammonia production. These shifts in metabolite production are indicative of carbohydrate fermentation and are consistent with improved gut health and reduced risk of HE.
CONCLUSIONS: we demonstrated that lactulose is able to significantly increase the relative abundance and absolute numbers of bifidobacteria, which was associated with an increased production of SCFA and a reduction in ammonia content. Future investigations should assess the molecular pathways that are involved in the modulation of gut microbiota and its metabolic reprogramming while translational studies should examine the clinical potential of this *in vitro* observations.

SPECIALIZED FOOD PRODUCTS MAY ENHANCE THE EFFICACY OF ISOCALORIC DIET IN THE TREATMENT OF NON-ALCOHOLIC STEATOHEPATITIS

(1) S. Morozov, (1) A. Sasunova, (2) I. Vorobyova, (2) V. Vorobiova, (2) V. Sarkisyan, (2) A. Kochetkova, (1) V. Isakov

(1) Department of Gastroenterology, Hepatology and Nutrition, Federal Research Center of Nutrition and Biotechnology, Moscow, Russian Federation
(2) Laboratory of Biotechnology and Specialized Food Products, Federal Research Center of Nutrition and Biotechnology, Moscow, Russian Federation

OBJECTIVE: recommendations on diet modification for patients with non-alcoholic fatty liver disease (NASH) are predominantly restrictive. We aimed to assess the efficacy of the approach that enreached isocaloric diet (ID) with active ingredients provided in the form of newly developed specialized food product (SFP) for patients with NASH.
METHODS: formula of SFP was developed according to literature data on the efficacy of nutrients for NASH. Product (code name "SPP1") contained (%RDA): proteins-8%; fats-7% (including ω-3 PUFA-40%); soluble dietary fiber-160%; phospholipids-25%; alpha-lipoic acid-33%; betaine-10%; minerals-13%-44%; vitamins (A, E, D3, K1, C, B1, B2, B6, B12, PP, Folic acid, Pantothenic acid, Biotin) 24%-140%. Patients enroled to the study (NCT04308980) had confirmed NASH (per EASL), gave informed consent. They were randomized into the group received ID with 2 portions of SFP dayly 2 weeks, or ID only. Repeated (baseline and on 15th day) clinical evaluations, body composition (InBody, Korea), blood chemistry measurement were performed.
RESULTS: twelve subjects were enrolled to ID + SFP and 8 in ID group. Groups didn't differ by age, sex, and BMI. The product was well tolerated. In contrast to ID group, those received SFP demonstrated significant decrease of weight and loss of body fat (**table I**). In both groups, we found a trend for ALT and AST decrease; however, it was significant in ID + SFP group only.
CONCLUSIONS: new foods in combination with isocaloric diet may be beneficial in the treatment of NASH. Our study showed greater body fat loss and improvement of laboratory markers when new food product was used.

Table I. The influence of new specialized food product in combination with isocaloric diet and isocaloric diet alone on body composition and blood chemistry in patients with NASH.

	ID+SFP, n = 12		P	Isocaloric diet (control), n = 8		P
	Baseline, Mean ± SD	EOT, Mean ± SD		Baseline, Mean ± SD	EOT Mean ± SD	
Weight, kg	110.6 ± 16.1	107.8 ± 15.5	0.002	106.7 ± 22.1	103.7 ± 20.8	0.07
BMI, kg/m2	38.7 ± 5.4	37.7 ± 5.1	0.003	38.9 ± 7.2	37.9 ± 7.3	0.08
Body fat, kg	50.2 ± 10.7	48.5 ± 10.8	0.002	48,9 ± 11.4	46,8 ± 11.6	0.07
ALT, U/L	81.1 ± 28.2	73.4 ± 38.1	0.05	60.0 ± 26.3	43.8 ± 30.1	0.1
AST, U/L	61.5 ± 29.2	42.6 ± 16.3	0.04	41.8 ±20.1	32.4 ±15.6	0.07
Cholesterol, mmol/L	5.6 ± 1.2	5.4 ± 1.3	0.6	5.8 ± 2.1	5.7±1.8	0.3
Alkaline phosphatase, U/L	130.7 ± 99.5	117.0 ± 89.0	0.04	142.1 ± 95.2	139.1 ± 99.4	0.09

LOCAL APPLICATION OF PROBIOTICS PROMOTES EXCISIONAL WOUND HEALING IN RATS

(1) M. Moysidis, (1) G. Stavrou, (1) V. Birba, (2) J. K. Tsetis, (1) A. Ioannidis (1), G. Tsaousi (1), K. Kotzampassi (1)

(1) Department of Surgery, Aristotle University of Thessaloniki, AHEPA University Hospital, Thessaloniki, Greece
(2) Uni-Pharma S.A., Pharmacist, Athens, Greece

OBJECTIVE: excisional wounds are one of the most used wound-healing models and are considered to resemble acute clinical wounds requiring healing by second intention. In such wounds, skin barrier function must be promptly restored for further damage or infection to be prevented and an aesthetically acceptable scar to be achieved. Nowadays, there is ongoing knowledge that bacteria residing on the skin are involved in the complex process of defense against pathogens and tissue healing. Hence, the objective of this preliminary study on a rat model of excisional wounds was to evaluate the influence of a locally applied probiotics compound on wound healing rates.
METHODS: thirty-two Wistar rats were randomly allocated into probiotics (*L. rhamnosus*, *B. longum*, 10^{11} cfu/gr) and control groups. Six excisional full-thickness wounds were created on each dorsum by using a sterile, 8-mm circular punch (registration 227933(934)). Probiotics or saline were applied, and wounds covered with sterile adhesive dressing; the same treatment applied

every 4 days. On days 0, 4, 8, 16 wound healing area (mm^2) was assessed, by means of photography (Cannon EOS-50D, EF-100 mmf/2.8 L Macro lens) and digital planimetry (Image J, Bethesda, MD) on each time-point. ANOVA analysis was then applied.
RESULTS: probiotics-treated rats experienced a rapid decline of the wounded area [mm^2] in relation to controls, p = 0.0001, at every time point; D4: 42.05 ± 12.65 *vs* 49.37 ± 10.63; D8: 14.71 ± 4.51 *vs* 26.33 ± 3.86; D16: 2.43 ± 1.00 *vs* 11.06 ± 2.92.
CONCLUSIONS: these preliminary results clearly demonstrate that the local application of probiotics significantly promotes the wound healing process. Further studies are in process.

IMPACT OF FERMENTED HEMPSEED BRAN ON THE HUMAN DISTAL COLON MICROBIOTA WITH MICODE *IN VITRO* MODEL

L. Nissen, F. Casciano, A. Gianotti

DiSTAL-Department of Agricultural and Food Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy

OBJECTIVE: the use of hemp seed bran (HB) in industrial food application has not been tackled yet, and hemp bran has traditionally been discarded during hemp seed powder processing. Knowledge on the functional capabilities of HB is very limited. For example, it is not known the impact of HB on intestinal microbiota, in particular on that of large intestine, were the vegetable fibers are fermented and degraded.
METHODS: in this work, we investigated in depth the prebiotic potential of HB and transformed HB in comparison to fructooligosaccharides (FOS) underwent human distal colonic fermentation using the *in vitro* colon model MICODE (multi-unit in vitro colon gut model). During the 24 h of fermentation at different time points, volatilome analysis (SPME GC/MS), and microbiota analyses (MiSeq and qPCR) were performed.
RESULTS: the results indicated that HB transformed samples in an healthy ecological condition of the human colon are able to: i) preserve microbial eubiosis; ii) increase the abundance of beneficial bacterial groups, such as *Bifidobacterium* and *Akkermansia*; iii) produce bioactive low organic fatty acids; iv) reduce detrimental compounds, such as p-cresol; v) generate a striking value of prebiotic index; vi) limit opportunistic and proteolytic bacteria (*Collinsella* and *Desulfovibrio*).
CONCLUSIONS: our study evidenced the prebiotic role of transformed HB through a critical evaluation of its functionalities on the gut microbiota, thereby valorizing the use of hemp seed by-product, as a food supplement.

LACTOBACILLUS RHAMNOSUS GG THERAPEUTIC SUPPLEMENT IN MILD-MODERATELY ACTIVE ULCERATIVE COLITIS PATIENTS: RESULTS FROM A DOUBLE BLIND RANDOMIZED CLINICAL TRIAL

(1) C. Pagnini, (1) M. C. Di Paolo, (2) F. De Angelis, (2) M. Mattana, (1) R. Urgesi, (1) L. Pallotta, (1) M. G. Graziani, (2) G. Delle Fave

(1) Gastroenterology and Digestive Endoscopy, AO S. Giovanni Addolorata, Rome, Italy
(2) Gastroenterology, Sapienza University of Rome, Rome, Italy

BACKGROUND: therapeutic administration of probiotic bacteria in ulcerative colitis (UC) patients appears rational and attracting, but consistent dishomogeneity exists in published studies. We have previously demonstrated that Lactobacillus rhamnosus GG (LGG) has favourable properties such as adhesion to the colonic mucosa and anti-inflammatory and immunomodulatory effect.
AIM: to investigate the potential clinical application of therapeutic supplement of LGG (ATCC 53103) in mild-moderately active UC patients, evaluating efficacy and safety.
MATERIALS AND METHODS: UC patients with mild-moderately active disease (Clinical Mayo score ≥ 2) despite oral treatment with oral mesalamine, after a wash-out period of 2 weeks from mesalamine, were randomized to assume a regular (1.2×10^{10} CFU/day) or a double (2.4×10^{10} CFU/day) dose of LGG for 1 month. Clinical activity before and after treatment were compared and clinical response was defined as a reduction of Clinical Mayo score ≥ 1 point. Primary end-points were the improvement of clinical symptoms and the safety evaluation, and secondary end-points was comparison between the two dosages of LGG. Patients who had a disease flare stopped LGG supplement and went back to regular therapy. Intention-to-treat (ITT) and per protocol (PP) analysis were performed. The trial has been registered to ClinicalTrials.gov (NCT04102852).
RESULTS: 40 patients were preliminarily included in the study (M/F = 21/19), and 31 (78%) completed the treatment period. In the ITT analysis: 19/40 (48%) patients showed clinical response, 12/40 (32%) remained stable, and 8/40 (20%) had a disease flare. In the PP analysis, 20/32 (63%) had a clinical response, and 12/32 (37%) remained stable. The mean reduction of Clinical Mayo score was 0.6 points (p = 0.004). No serious adverse event was recorded. No significant difference in efficacy and safety was observed between the two different doses of LGG.
CONCLUSIONS: in the present interim analysis of a double-blind randomized clinical trial, LGG administration was effective and safe in UC patients with mild-moderate clinical activity.

TUNING GUT MICROBIOTA THROUGH A PROBIOTIC BLEND IN GEMCITABINE TREATED PANCREATIC CANCER XENOGRAFTED MICE

(1) C. Panebianco, (2) F. Pisati, (3) M. Ulaszewska, (3) A. Andolfo, (1) A. Villani, (4) F. Federici, (4) L. Manna, (4) E. Rizzi, (5) A. Potenza, (6) T. P. Latiano, (1) F. Perri, (7) C. Tripodo, (1) V. Pazienza

(1) Gastroenterology Unit, Fondazione IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Foggia, Italy
(2) Histopathology Unit, Cogentech S.C.a.R.L, Milan, Italy
(3) Proteomics and Metabolomics Facility (ProMeFa), IRCCS San Raffaele Scientific Institute, Milan, Italy
(4) Sintal Dietetics S.R.L., Castelnuovo Vomano, Teramo, Italy
(5) Dietetic and Clinical Nutrition Unit, Fondazione IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Foggia, Italy
(6) Oncology Unit, Fondazione IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Foggia, Italy
(7) Department of Health Sciences, Tumor Immunology Unit, University of Palermo, Palermo, Italy

OBJECTIVE: pancreatic cancer (PC) is an aggressive and chemotherapy-resistant cancer. Gemcitabine treatment shifts the intestinal microbiota of PC mice towards an inflammatory profile which may worsen side effects. We investigated whether a specific probiotic blend, by rebalancing microbiota, could reduce gemcitabine-induced inflammation and side effects.
METHODS: probiotics were administered to PC xenografted mice. Histopathological stainings were performed on cancer sections to evaluate morphology, proliferation, DNA damage, colla-

gen deposition and epithelial-mesenchymal transition. Intestinal sections were stained with HE, Ki67 and Alcian Blue/PAS. Fecal DNA underwent 16S rRNA sequencing to analyze microbiota, blood samples were collected to assess blood cell count, biochemical parameters and to perform serum metabolomics. Cell-free supernatants (CFSs) prepared from single probiotic strains were assayed on BxPC-3 cells and their composition was analyzed by metabolomics approach.
RESULTS: mice receiving probiotics displayed a retardation trend in tumor growth, with tumor showing a decreased stromatogenesis and mesenchymal phenotype. A milder intestinal damage, an improved blood count, an increase in fecal species richness and in short chain fatty acids-producing bacteria were also observed. Serum levels of amino acids, choline and pyruvic acid significantly dropped upon probiotics consumption. CFSs-derived from *Bifidobacterium bifidum* and *Bifidobacterium breve* were the most effective in inhibiting cell migration, affecting cell cycle and inducing apoptosis. Amino acids, nitrogenous bases, vitamins, pyruvate and butyrate metabolism resulted among the most represented pathways in CFSs.
CONCLUSIONS: these results suggest that specific probiotics administration could help relieving some adverse effects of gemcitabine in the setting of PC treatment by restoring a favorable microbiota.

PRONEUROGENIC AND NEUROPROTECTIVE EFFECT OF A MULTI STRAIN PROBIOTIC MIXTURE IN A MOUSE MODEL OF ACUTE INFLAMMATION: INVOLVEMENT OF THE GUT-BRAIN AXIS

(1) C. Petrella, (1) G. Strimpakos, (1) A. Torcinaro, (1) S. Middei, (1) V. Ricci, (2) G. Gargari, (2) D. Mora, (1) F. De Santa, (1) S. Farioli Vecchioli

(1) Biomedical Sciences, Institute of Biochemistry and Cell Biology - IBBC-CNR, Rome, Italy
(2) Department of Food Environmental and Nutritional Sciences DeFENS, University of Milan, Milan, Italy

OBJECTIVE: neuroinflammation can severely affect brain homeostasis and adult hippocampal neurogenesis with detrimental effects on cognitive processes. Brain and gut are connected via the "gut-brain axis", a bidirectional communication system, whose modulation through probiotics could represent an intriguing approach for the prevention or even the cure of several diseases. In the present study we evaluated the putative neuroprotective effect of prolonged consumption of a multi-strain probiotic formulation (named OttaBac) based on food-associated strains and human gut bacteria in a mouse model of acute inflammation.
METHODS: mice were gavaged with OttaBac (10^9 CFU/mouse/day) for 15 days before a single intraperitoneal injection of LPS (0.1 mg/kg). We sacrificed the animal after 2 and 24 hours from LPS treatment and we evaluated physiological and behavioral changes, proliferation and differentiation of the new neurons within the hippocampal dentate gyrus, the neuroinflammatory response, the modifications of intestinal permeability and of the inflammatory state in OttaBac versus vehicle-administered mice.
RESULTS: the results indicate that the administration of OttaBac not only prevents the LPS-dependent increase of pro-inflammatory cytokines in specific regions of the brain (hippocampus and cortex) and in the gastrointestinal district, but also triggers a potent proneurogenic response capable of enhancing hippocampal neurogenesis. This effect is accompanied by a potentiation of intestinal barrier, as documented by the increased epithelial junction expression in the colon.
CONCLUSIONS: our hypothesis is that pre-treatment with the multi-strain probiotic formulation helps to create a systemic protection able to counteract or alleviate the effects of LPS dependent acute pro-inflammatory responses.

CANOLA MEAL FERMENTATION WITH PROBIOTIC LACTOBACILLI: IMPACT OF PHENOLIC ACIDS ON ANTIMICROBIAL ACTIVITY

V. Pham, M. Gänzle

Agriculture, Food and Nutritional Science, University of Alberta, Edmonton, Canada

OBJECTIVES: feed fermentations in animal production deliver high cell counts of probiotic lactobacilli as well as bioactive compounds. Antimicrobial phenolic compounds are abundant in canola meal but can be converted to derivatives with different antimicrobial activity during lactic fermentation. This study aimed to quantify phenolic acids in canola meal fermented with probiotic lactobacilli, and to investigate the antimicrobial activity of fermented and unfermented canola meal against intestinal bacteria in chickens *in vitro*.
METHODS: canola meal was fermented for 24 h with *Lactiplantibacillus plantarum* TWM1.460, *Furfurilactobacillus rossiae* FUA3583 or its mutant FUA3583 delta*par1*delta*par2*, or *Limosilactobacillus reuteri* FUA3536. Unfermented and chemically acidified samples were incubated in the same condition. Phenolic acids were quantified via high-performance liquid chromatography. Antimicrobial activity against *Fructilactobacillus sanfranciscensis* FUA3024, *Limosilactobacillus reuteri* FUA3613, and *Salmonella* FUA10060 was assessed using a critical dilution assay.
RESULTS: sinapic acid was the major phenolic acid in canola meal with a concentration of 1498 ± 3.1 mg/kg. After fermentation with *Lp. plantarum* TMW1.460, *Ff. rossiae* FUA3583 and FUA3583 delta*par1*delta*par2*, *Lm. reuteri* FUA3536, sinapic acid concentrations were reduced to 193 ± 8.1 mg/kg, 178 ± 18 mg/kg, 494 ± 197 mg/kg, 604 ± 114 mg/kg, respectively. Unfermented canola meal was most inhibitory to *Fl. sanfranciscensis*; *Lm. reuteri* and *Salmonella* were more resistant. The inhibitory activity of unfermented and fermented canola meal was generally similar but fermentation with *Lp. plantarum* decreased the antimicrobial activity.
CONCLUSIONS: lactic metabolism decreased the antimicrobial activity of phytochemicals in canola meal but this effect was not related to metabolism of sinapic acid. This result could provide insight on improved canola fermentations for enhanced gut health.

INNOVATIVE PERSPECTIVES ON THE DETOXYFING EFFECTS OF *LACTOBACILLUS* PROBIOTIC STRAINS

(1) S. Rapacioli, (1) S. Stasi, (2) A. Visciglia, (2) A. Amoruso, (2) M. Pane

(1) Bict S.R.L., Innovation Development, Lodi, Italy
(2) Probiotical Research S.R.L., R&D, Novara, Italy

OBJECTIVE: food safety is a major concern for Health Agencies and consumers worldwide. Poor quality or contaminated food

can constitute a significant risk factors for Human health. The objective of this study was to evaluate whether strains of probiotics of the genus *Lactobacillus* are capable of degrading or sequestering chemical contaminants such as heavy metals, glyphosate and biogenic amines.
METHODS: five *Lactobacillus* strains were evaluated. At this purpose, the microorganisms were grown *in vitro* and exposed to different concentrations of contaminants. Through validated analytical methods and statistical analysis, the concentration of contaminants in the culture broth was evaluated and their level of sequestration or degradation by probiotics was quantified.
RESULTS: data analysis showed that a strain of *L. plantarum* was able to degrade glyphosate in an amount of 12% in respect to the initial quantity added. A degree of uptake of several heavy metals was also observed: in particular cadmium is sequestered up to 72% and chromium up to 20%. Three strains showed degradative activity towards amines by degrading up to 50% of Tryptamine, Spermidine and Spermine.
CONCLUSIONS: in this investigation it has been demonstrated how some strains belonging to the genus *Lactobacillus* are able to sequester or degrade food contaminants. The population of the Human gut with these bacteria could lead to a decreased risk of absorption of these contaminants, thus introducing a novel perspective of the beneficial effects of probiotics on Human health.

EFFECTS OF A PREBIOTIC INTERVENTION WITH A HIGHLY PURIFIED EXTRACT OF BLACK ELDERBERRY: RESULTS FROM THE ELDERGUT TRIAL

(1) S. Reider, (1) J. Längle, (2) N. Przysiecki, (2) A. Pfister, (2) A. Zollner, (3) S. Sturm, (4) S. Plattner, (2) H. Tilg, (1) A. Moschen

(1) Christian Doppler Laboratory for Mucosal Immunology, Johannes Kepler University of Linz, Linz, Austria
(2) Department for Internal Medicine 1, Medical University of Innsbruck, Innsbruck, Austria
(3) Department of Pharmacognosy, Leopold-Franzens University of Innsbruck, Innsbruck, Austria
(4) IPRONA AG/SPA, Lana, Bolzano, Italy

BACKGROUND: the intestinal microbiome is a major contributor to human health and disease. Influencing the microbiome potentially improves gastrointestinal symptoms. Prebiotics are one way to influence the microbiome and pre-existing microbiome configuration is influencing their effectivity. Better characterization of determinants for efficacy of prebiotics is needed. We aimed to characterize the interaction of a highly purified black elderberry extract rich in polyphenols with the intestinal microbiome and host physiology.
METHODS: the ELDERGUT Trial was a longitudinal cohort trial in 30 healthy participants with 3 periods of 3 weeks. Prior to the intervention period patients were characterized for 3 weeks, and the intervention period was followed by a 3 week wash-out phase. Patients completed weekly symptoms questionnaires and provided a weekly biosample set. 16S amplicon sequencing was applied to fecal DNA and metablomics data were generated from urine samples by nuclear magnetic resononance spectroscopy (NMR).
RESULTS: while no effects on clinical symptoms were observed, microbiome analysis revealed a sharp increase in a-diversity both at the beginning and after the end of the prebiotic intervention. A similar pattern was observed in an analysis of beta-diversity (unweighted unifrac index), indicating prebiotic-induced changes of intestinal microbiome composition. On the genus level, changes in multiple taxa including *Lactobacillus* and *Akkermansia* could be observed.
CONCLUSIONS: the ELDERGUT trial reveals a rapid effect of a prebiotic intervention with black elderberry extract. After initial perturbation of community structures, counterregulatory responses seem to establish a new stable equilibrium accompanied by various changes in the taxonomic composition and metabolite output of the microbiome.

THE PROTECTIVE ROLE OF TOMATO AND OLIVE MICRONUTRIENTS IN THE DEFENSE AGAINST PERSISTENT ORGANIC POLLUTANTS-INDUCED TOXICITY

(1) E. Rubini, (1) M. Minacori, (1) F. Altieri, (1) G. Paglia, (1) M. Eufemi, (2) P. G. Natali

(1) Department of Biochemical Sciences A. Rossi-Fanelli, Sapienza University of Rome, Rome, Italy
(2) Department of Medicine and Aging Sciences, Center for Advanced Studies and Technology (CAST), G. D'Annunzio University, Chieti, Italy

BACKGROUND: persistent Organic Pollutants (POPs) such as lindane, hexachlorobenzene, DDT, dioxin etc. belong to a large class of diffused organic compounds well-known for their toxic effects on human health. Among POPs, particular attention has been focused on the β-hexachlorocyclohexane (β-HCH), the more stable and persistent isomer of the hexachlorocyclohexanes family. In fact, despite its worldwide environmental distribution, β-HCH effects on human health have not been largely investigated. Previous cellular and molecular studies performed on prostate cancer cells demonstrated that b-HCH activates a wide range of signaling pathways and acts as an endocrine disruptor, promoting cellular processes related to carcinogenesis, tumor progression and chemoresistance. In spite of its small size, b-HCH has a relevant impact on cellular homeostasis, making it mandatory to explore defense strategies against its multifaceted biological effects.
METHODS: for this purpose, a screening of natural substances was carried out on the above enlisted cell targets to test their capability to counteract b-HCH actions by performing many different biochemical and cellular assays.
RESULTS: among a wide array of selected compounds, extracts containing micronutrients from tomato and olive show a dose-dependent significant chemoprotective activity in the considered cell lines by contrasting b-HCH-induced intracellular responses such as anti-apoptotic and pro-metastasizing events, increase in ROS production and DNA damage.
CONCLUSIONS: these experimental outcomes identified the chemoprotective effects of tomato and olive-derived micronutrients, recommending the development and testing of tailored enriched formulations for exposed individuals. Investigations along this line are ongoing.
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VARIABILITY OF ANTIMICROBIAL AND ANTIFUNGAL EFFECT OF *LACTOBACILLUS PLANTARUM* AND *LACTOBACILLUS ACIDOPHILUS*

O. Rybalchenko, O. Orlova, V. Kapustina

Medical Department, Saint Petersburg State University, Saint Petersburg, Russian Federation

INTRODUCTION: an essential point in the prevention and complex bacteriotherapy of dysbiosis is the level and spectrum of antagonistic activity of probiotic bacteria.
AIM: to reveal the nature of the relationship between industrial strains of lactobacilli (LB) and opportunistic microorganisms (UPM) at the ultrastructural level.
MATERIALS AND METHODS: industrial strains *Lactobacillus plantarum* 8RA-3 and *L. acidophilus* D75. Clinical isolates: 8 strains of *S. aureus* producing ά-hemolysin, 20 strains of *E. coli* Hly +, 12 strains of *C. albicans* were detected using transmission electron microscopy on a JEM-100C (JEOL, Japan).
RESULTS: with the manifestation of antagonistic activity of LB in relation to UPB of various types, significant changes were found in all interacting cells. Thus, extensive invaginations of intracytoplasmic membrane structures appeared in LB cells, the formation of which indicated the activation of metabolic processes in them. Electron microscopic examination of co-grown cultures of LB with UPB and *C. albicans* in places of close cell contact revealed significant destructive changes in the cells of LB themselves. The main differences were in the nature of the destruction of cell walls by the type of desquamation of small layer-by-layer fragments of peptidoglycan layers. Along with destructive changes in the cell wall, a specific change in the ultrafine structure of the protein-ribosomal complex of the cytoplasm of lactobacilli was noted.
CONCLUSIONS: ultrastructural changes revealed during the joint cultivation of LB with *S. aureus*, *E. coli* Hly +, and *C. albicans* testified to the strict specificity of the interaction of these microorganisms.

TEXTURED SOY PROTEIN MODULATES GUT MICROBIOTA AND SHORT-CHAIN FATTY ACIDS METABOLISM

(1) Catarina Teixeira-Guedes, (2) Teresa Sánchez-Moya, (2) Gaspar Ros-Berruezo, (1) Cristina Pereira-Wilson, (2) Rubén López-Nicolás

(1) Centre for the Research and Technology of Agro-Environmental and Biological Sciences, Department of Biology, University of Minho, Braga, Portugal
(2) Human Nutrition and Food Science, Faculty of Veterinary Sciences, University of Murcia, Murcia, Spain

OBJECTIVE: texturized soy protein has been widely used as a meat analogs and garnering attention due to its nutritional advantages when compared to conventional animal proteins. The present work aims to compare the impact of textured soy with traditional food containing pasta and meat, on pH shift, intestinal microbiota, and metabolism of short-chain fatty acids (SCFA) using a batch of human fecal culture fermentation.
METHODS: prior to the fermentation, samples were in vitro digested, passing through mouth, gastric, and small intestine simulation phases, and then in vitro fermented for 6, 24, and 48 h. The shift of pH, gas, and short-chain fatty acids (SCFAs) production, as well as changes in gut microbiota, were evaluated along the fermentation time.
RESULTS: a significant decrease was observed in pH over time in media with fermentable sources when compared with negative control. SCFA concentration increased over time and it was significantly higher for both textured soy and potato:meat when compared with inulin (positive control). For potato:meat and inulin, acetate was the major SCFAs produced during fermentation time whereas for textured soy was butyric acid. Butyric acid production was 10-fold higher in medium containing textured soy when compared with potato:meat and inulin. Textured soy showed a significant increase in Bifidobacterium and Lactobacillus when compared to the remaining fermentable sources.
CONCLUSIONS: textured soy presented a strong prebiotic effect and significant increase butyric acid production that plays an important role in the prevention of colorectal cancer. These results suggest that consumption of textured soy used alone or as an ingredient of novel functional foods, may contribute to improving intestinal health and therefore human health promotion.

MULTISPECIES PROBIOTICS PROMOTE PERCEIVED HUMAN HEALTH AND WELLBEING: INSIGHTS INTO THE VALUE OF RETROSPECTIVE STUDIES ON USER EXPERIENCES

L. van de Brugwal, A. van der Geest

Athena Institute, Vrije University, Amsterdam, Netherlands

When taking a broader perspective on the societal impact of probiotics, engagement of end-users is important to discover unmet needs, define relevant health benefits and identify key considerations for successful implementation in daily practice. This study therefore takes a retrospective approach and analyses a database of user experiences to review the effects of four multispecies probiotic formulations. The user experiences were analysed in a dependent sample manner (without control group) and complement previous randomized controlled trials that have been performed with the formulations. The database consisted of 584 evaluable user experiences regarding the impact of probiotic supplementation on perceived quality of life (QoL), gastrointestinal (GIT) symptoms and reported stool consistency after two weeks of consumption. Two different scales were used (N = 344 in a 5-point scale; N = 240 in a 10-point scale), which are presented as separate analyses. In the combined population of the 5-point-scale questionnaire, a significant increase in perceived QoL and a significant reduction in perceived GIT symptoms was observed. Descriptive summaries also indicate that diarrhoea- and constipation-like stool patterns are reduced following supplementation. Moreover, half of participants indicated that probiotic supplementation had a positive effect on their unmet medical need, and 64% of users were likely to continue using the product. Similar results were observed in the 10-point scale questionnaire. Considering the clinical relevance of probiotic supplementation in specific target groups, subgroup analyses were performed on participants who consumed the products for diarrhoea, constipation, Inflammatory Bowel Disease, Irritable Bowel Syndrome, and antibiotic usage. Overall, findings support the potential of probiotics to advance perceived human health and support the daily wellbeing of users. This systematic analysis of user experiences thereby contributes to the external validity of studies evaluating clinical effects of probiotics and increases knowledge on their societal impact. In conclusion, this study showed the potential value of retrospective studies on user experiences. However, the question remains whether and to what extent user expe-

rience-based knowledge is perceived useful by stakeholders in microbiota innovation, and whether it has potential to support clinical decision making.

INADEQUATE SAFETY REPORTING IN RCTs IN IRRITABLE BOWEL SYNDROME A SYSTEMATIC REVIEW: PHARMACEUTICAL INTERVENTIONS VS PROBIOTIC INTERVENTIONS

A. van der Geest, L. van de Burgwal

Athena Institute, Vrije University, Amsterdam, Netherlands

Randomised clinical trials (RCTs) offer a unique opportunity to obtain controlled efficacy and safety data to support clinical decisions. However, most RCTs have a stronger focus on efficacy rather than safety. In connection to this paper, a meta-analysis was conducted and published to evaluate the efficacy of probiotics compared to that of pharmaceuticals in Irritable Bowel Syndrome (IBS). To compare the burden to benefit ratio between probiotic as well as pharmaceutical interventions, we aimed to identify the safety profile of both intervention types. RCTs including participants (> 16 years old) with IBS comparing probiotic or pharmaceutical interventions with placebo were identified by systematic searching of MEDLINE (January 2015 – November 2020). Although inclusion criteria were similar for both intervention types, substantial differences between safety profiles for both pharmaceutical and probiotic control groups were identified. Several inconsistencies in safety reporting were identified between and within pharmaceutical and probiotic studies, that could be categorized by: didn't report on safety; only reported Adverse Reactions (ARs) or Adverse Events (AEs) with a certain severity; didn't report the total number of AEs; didn't split in the control or experimental arm; didn't specify AEs; and used different thresholds for "common" AEs. Hence, it is difficult to compare safety data from pharmaceutical and probiotic RCTs across and between different studies. In conclusion, based on the current approaches to safety reporting we could not establish an unambiguous safety profile for probiotic and pharmaceutical interventions in IBS. Therefore, a critical comparison of the benefit to burden ratio was not possible.

INTESTINAL EPITHELIAL PROTEASES CONTROL MUCOSAL BIOFILMS FOR BETTER, FOR WORSE, IN THICKNESS OR HEALTH

N. Vergnolle

Inserm, U1220, Toulouse, France

Just like dysbiosis and decreased microbiota diversity have been associated with intestinal pathologies, changes in proteolytic homeostasis at mucosal surfaces have been observed in gut pathological situations such as infection, chronic inflammation, functional disorders or even cancer. Studies have demonstrated in the gut that lumenal proteases are for the most part from host origin. We have postulated that the intestinal epithelium is a major source of proteases and protease inhibitors, which together exert controls on mucosal microbiota biofilms. Our objectives were to i) investigate which epithelial proteases are controlled by the presence of microbiota, ii) investigate the effects of identified proteases on human intestinal biofilms in health and disease, iii) determine the contribution of such proteases to microbial biofilm homeostasis or dysbiosis.
Using activity-based probes, we have identified several proteases that are produced and secreted in an active form, by human intestinal epithelial cells. Among such proteases, thrombin, elastase-2 and trypsin-1 and -2 were released in large amounts on the luminal side of the epithelium. Germ-free mice lack the expression of such proteases in the epithelium, but their expression was up-regulated upon microbiota transfer. This indicated that the expression of these epithelial proteases is controlled by the presence of microbiota.
Further, we have investigated the effects of protease exposition to human intestinal biofilms (from healthy donors and from inflammatory bowel disease: IBD patients). Results showed that thrombin exposure reduced bacterial survival and decreased biofilm biomass of healthy individual biofilms, while biofilms from Crohn's disease patients seemed to be resistant to the effects of thrombin. Thrombin exposure also increased the virulence (translocation) of bacteria detached from healthy biofilms. Elastase-2 exposure increased healthy biofilm bacterial survival and conferred a pro-invasive phenotype to biofilm detached bacteria. However, in Crohn's disease patient biofilms, Elastase-2 increased biofilm bacteria survival. Finally, Elastase exposure of human biofilms from healthy or Crohn's disease individuals seemed to increase firmicute and proteobacteria abundance, while the same dose of Elastase in biofilms from Ulcerative Colitis disease patients decreased proteobacteria and increased firmicute abundance.
Taken together these results demonstrate that epithelial proteases are controlled by the presence of microbiota and conversely can control microbial biofilms in terms of their composition, their growth and their virulence. Such interactions seemed to be very complex and will require further investigation, but our results clearly point to proteolytic homeostasis as a major component of microbiota behavior in the gut.

YOGURT ENRICHED WITH FIBERS AND PROBIOTIC BACTERIA INCREASED THE ABUNDANCE OF *BIFIDOBACTERIUM ANIMALIS* SUBSP. *LACTIS* BB-12 IN HUMAN GUT MICROBIOME

E. Viiard, M. Jaagura, N. Part, J. Kazantseva, K. Adamberg

Center of Food and Fermentation Technologies, Food Research, Tallinn, Estonia

OBJECTIVE: to evaluate the effect of a probiotic- and fiber-enriched symbiotic yogurt on human gut microbiota, digestive comfort, and selected blood markers. In addition to starter bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*), the yogurt contained probiotic bacteria *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus acidophilus* LA-5, and a mixture of soluble and insoluble fibers (resistant starch, polydextrose, high polymerization inulin, and xylooligosaccharides) in total 4.9 g/100 g.
METHODS: the test subjects (N = 81, double-blind controlled study) consumed 200 g of enriched yogurt for 14 days. Fecal and blood samples were collected before and after the intervention. The subjects filled in a 3-day food frequency questionnaire describing the consumption of main food groups. Standardized blood analyses were performed and metagenetic sequencing of 16S rRNA gene amplicons was used to determine the composition of fecal bacterial communities.
RESULTS: both control and test yogurts reduced fasting blood lipid and glucose levels, while the fiber-enriched yogurt induced a

remarkable increase of *B. animalis* BB-12 levels in the fecal samples. Increased fiber intake did not cause major digestive discomfort and helped maintain regular bowel movement during the study period.
CONCLUSIONS: enrichment of yogurt with a mixture of fibers significantly promoted the growth of *B. animalis* BB-12 in the human gut during two weeks of daily consumption and helped maintain a healthy digestive pattern. Regular consumption of symbiotic foods can be an effective strategy to modulate the human gut microbiota, improve the plasma lipids' profile and glucose levels.

SUPPLEMENTS ACTIVE ON CHOLESTEROL ABSORPTION

F. Visioli

Molecular Medicine, University of Padova, Padua, Italy

OBJECTIVE: cholesterol control is mainly achieved via pharmacological treatment and some new compounds are being developed to further lower LDL concentrations and cardiovascular risk in high-rick patients. However, due to the rather recent availability of *ad-hoc* formulations, patients often independently self-administer supplements and functional foods without medical input, either inappropriately or in situations in which no significant advantage can be gained.
METHODS: this lecture will outline the effects of the most frequently occurring cholesterol-lowering substances in functional foods or in supplements, with particular attention being paid to the inhibitors of cholesterol absorption.
RESULTS: there are two major classes of molecules most active in inhibiting cholesterol absorption. Phytosterols (plant sterols and stanols) inhibit the intestinal absorption of cholesterol, which is partly derived from foods (300–500 mg/day), and largely from the bile (1000 mg/day), in a dose-dependent way, contingent upon their total intake with food or supplements. In order to obtain a significant cholesterol-lowering effect, at least 1.5 g of phytosterols must be consumed per day after the main meal.
Both dietary and supplementary intakes of fibre is also effective in the control of plasma LDL cholesterol levels, likely attributable to the increase of faecal excretion of cholesterol, bile acids or other dietary fats. Examples include beta glucan, glucomannan, psyllium, and chitosan. A positive side effect of such fibers is their prebiotic action.
CONCLUSIONS: despite being freely available for purchase, these products should be used following shared agreement between the caring physician and the patient. Moreover, patients should consider the practicality of sustaining treatment costs over time, considering that such a treatment is often lengthy and in theory life-long. Yet, well-characterized and titrated formulations containing inhibitors of cholesterol absorption should be critically considered in the framework of a moderate risk cardiovascular patient in need of lowering his/her cholesterol concentrations.

ANTICANCER EFFECTS OF *LACTOBACILLUS RHAMNOSUS* GG (LGG) SUPERNATANT

(1) S. Vivarelli, (1) R. Salemi, (2) L. Falzone, (1) M. Santagati, (1) M. Libra

(1) Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy
(2) Epidemiology and Biostatistics Unit, IRCCS Istituto Nazionale Tumori Fondazione G. Pascale, Naples, Italy

OBJECTIVE: cancer represents the second leading cause of death worldwide. Gut microbiota plays a dual role in cancer, as certain pathogens elicit tumorigenesis, whereas a number of beneficial probiotics can ameliorate immune defense against tumors. *Lactobacillus rhamnosus GG* (LGG) administration mitigates therapy-related intestinal damage and elicits the host's immune system. Additionally, LGG might help to directly arrest cancer growth, although the mechanism underneath remains yet elusive. The objective of this study was to assess the effect of LGG culture supernatant (LGG-SN) on the growth of a selected panel of cancer cells.
METHODS: cell-free LGG-SN was obtained by growing LGG at steady conditions in a culture medium compatible with human cells. LGG-SN was administered to four cancer cell lines (both local colorectal cancer and distal melanoma). To assess the effects of LGG-SN on cancer growth, several complementary approaches were used including: MTT metabolic assay, Trypan blue cell death count, BrdU proliferation assay, Propidium iodide cell cycle analysis and cleaved-Caspase-3 apoptotic readout.
RESULTS: LGG-SN significantly reduces the viability of all the cancer cell lines in a concentration-dependent manner. Moreover, LGG-SN when administered in combination with cytotoxic drugs show a synergic effect. Importantly, LGG-SN inhibits cancer cell proliferation and specifically induces a cell cycle G2/M arrest, without promoting apoptosis.
CONCLUSIONS: overall, these results suggest the potential use of LGG-SN as adjuvant of anti-cancer therapies. Future studies are needed to identify the active molecule(s) contained in LGG-SN and to validate these findings in translational models (*i.e.*, patient-derived tumor organoids).

A NOVEL SMALL INTESTINAL MICROBIOME ASPIRATION (SIMBA) CAPSULE DEVICE TO DETECT AND SAMPLE PROBIOTICS RELEASE IN THE HUMAN SMALL INTESTINE

(1) G. Wang, (2) C. Andrews, (2) L. Wilsack, (2) R. Rehak, (3) L. Lou, (4) J. Auger, (4) O. Matieu, (1) S. Bruehlmann, (1) S. Menon, (5) Q. Tang, (5) G. Jin, (5) B. Wang

(1) Nimble Science Ltd, Canada
(2) Cumming School of Medicine, The University of Calgary, Calgary, Canada
(3) EFW Radiology, Calgary, Canada
(4) Lallemand Health Solutions, Rosell Institute for Microbiome and Probiotics, Montreal, Canada
(5) Department of Gastoenterology, The Tianjin Medical University, Tianjin, China

OBJECTIVE: the SIMBA capsule is a novel ingestible device aiming to sample luminal fluid in the small intestine. We aim to test performance characteristics of SIMBA in healthy volunteers and demonstrate SIMBA's safety and efficacy in tracking the microbiome profile's change in small intestine during oral probiotics ingestion in real time.
METHODS: 20 healthy volunteers ingested 2 SIMBA capsules after fasting and underwent abdominal X-rays every 30 mins out to 210 min to assess capsules' location and deployment. Capsules were independently collected and returned with a stool sample. One week later, 2 further SIMBA capsules were ingested simultaneously with a dual strain probiotic and collected when passed.

Endpoints: sampling location at baseline, capsule sample and stool microbiota analysis using 16S sequencing, qPCR probiotic strain detection, safety, and subject usability assessment for both capsule sets (total 80 capsules).
RESULTS: 78/80 SIMBA capsules were successfully retrieved for analysis. 65/66 selected SIMBA capsules had sufficient DNA for 16s sequencing, which showed clearly different microbiota composition between SIMBA samples and stools, and between baseline and intervention SIMBA samples. Absolute quantification using probiotic strain-specific qPCR results showed SIMBA capsules detect an increase of the probiotics concentration in the small intestine after oral ingestion of the probiotics. The rest 12 capsules were sent for metabolomic analysis and results will be published in future.
CONCLUSIONS: the SIMBA capsule appears safe and reliable for collection of SI content which can be used for tracking spatial and temporal microbiome change in small intestine without the need for deep endoscopy.