

Abstracts of the 10th Workshop on Probiotics and Prebiotics

Spanish Society of Probiotics and Prebiotics (SEPyP)

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Abstracts

Guest Editors

Guillermo Álvarez Calatayud, President of the Spanish Society of Probiotics and Prebiotics (SEPyP), Madrid

Ascensión Marcos, Research Professor at the Spanish National Research Council, Madrid

Luis Peña Quintana, Organizing Committee President of the 10th Workshop on Probiotics and Prebiotics, Las Palmas de Gran Canaria

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The modulation of gut microbiota to improve health has been empirically carried out since ancient times and fermented foods have been used against gastrointestinal infections since the year 76 BC. However, it was not until 1910 that Elie Metchnikoff observed that those inhabitants in the Balkans reached very advanced ages and related this fact with the habitual consumption of a fermented milk. He postulated that the bacteria involved in the fermentation of milk could be responsible for their longevity. Since then, the implication of the alterations of the microbiota in the origin and development of various diseases has promoted great research effort. This fact has been reflected in its clinical application with the use of probiotics and prebiotics. In recent years, techniques have been developed to modulate gut microbiota, with novel applications in medicine, such as faecal microbiota transplantation.

Today, healthcare professionals are increasingly aware of the benefits of a proper diet, which promotes the development of functional foods and, within them, those containing beneficial bacteria. However, despite the effort already made, we are still at the beginning of our knowledge about the microbiota and its influence on health and the emergence of diseases. The research in this field opens before us a very wide range of possibilities of development of new indications and therapeutic procedures, even personalized, that promise significant advances in our perception of what health means.

The Spanish Society of Probiotics and Prebiotics (*Sociedad Española de Probióticos y Prebióticos*, SEPyP) is a scientific non-profit organization founded in 2010, devoted to the development and promotion of scientific knowledge and research, the clinical application and the distribution of the microbiota within the body, together with the effects of probiotics and prebiotics on health. The professionals that form the organization (about a thousand) belong to different disciplines: physicians, pharmacists, veterinarians, microbiologists, basic researchers, immunologists, nutritionists, nurses, midwives, etc. In addition, SEPyP is also supported by the most important companies of this area of knowledge.

Since it was founded, SEPyP annually organizes a workshop with the purpose of promoting the scientific knowledge of probiotics and prebiotics among the health professionals, in addition of being a unique forum for the exchange of the last research advances on the field of the microbiota and microbioma. This year the workshop is held from 6th to 8th February in Las Palmas de Gran Canaria (Canary Islands, Spain).

The scientific programme of the workshop (SEPyP-2019) is included in this special supplement of *Annals of Nutrition and Metabolism*. It includes 2 round tables, 3 extraordinary conferences, 7 workshops, 4 symposia, 20 oral presentations and the abstracts of the posters submitted. During the presentations, compelling topics for the professionals will be discussed, such as our different microbiota, fecal transplantation and sea microbiota, plus other topics of recent update like probiotics. During the Workshop the foundation of the Iberoamerican Society of Microbiota, Probiotics and Prebiotics is planned with the participation of experts on the subject on both sides of the Atlantic Ocean.

In conclusion, we want to express our gratitude to the participating companies for their invaluable help and specially, to the organizing and scientific committees. Also to the speakers, debate moderators of the round tables and workshops that have made this meeting possible.

Guillermo Álvarez Calatayud
Ascensión Marcos
Luis Peña Quintana
Editors

Clinical Uses

INFLUENCE OF BREAST MILK MICROBIOTA ON COLONIZATION, GROWTH AND HEALTH OF INFANTS. EFFECTS OF PROBIOTIC INTERVENTION

J. Fonollá¹, B. Pastor-Villaescusa², J.A. Hurtado³, M. Gil-Campos⁴, J. Uberos⁵, J.L. Leante⁶, L. Affumicato⁷, A. Iglesias-Deus⁸, J.M. Garrido⁹, A.D. Valero¹, C. Rodríguez¹, M.P. Díaz-Ropero¹, J.A. Maldonado-Lobón¹, M. Olivares¹

¹Research Department, Biosearch Life, Granada, Spain. ²Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children's Hospital, Munich, Germany. ³Department of Neonatology, Hospital Materno-Infantil, Granada, Spain. ⁴Department of Metabolism and Pediatrics Research, Hospital Reina Sofía, Córdoba, Spain. ⁵Department of Pediatrics, School of Medicine, Granada, Spain. ⁶Department of Neonatology, Hospital General Universitario Santa Lucía, Cartagena, Spain. ⁷Department of Neonatology, Hospital Regional Universitario, Málaga, Spain. ⁸Department of Neonatology, Hospital Clínico Universitario, Santiago de Compostela, Spain. ⁹Department of Pediatrics, Hospital Universitario, Salamanca, Spain.

Background/Aims: Infant's first years crucial in healthy gut bacteria. The objective of the present study is to evaluate the influence of breast milk microbiota on gut microbiota, growth and health of the infant.

Methods: A randomized double blinded controlled study including 625 women and their children during breastfeeding period was conducted. Women-infant pairs were distributed into two groups: in Lc40 group women received 1 capsule/day containing *L. fermentum* Lc40 3x10⁹cfu; In Control group women received 1 placebo capsule/day containing maltodextrin. The intervention period was 16 weeks. Microbiota in breast milk and infant's feces was analysed and growth and health of infants was followed up during the intervention.

Results: Two hundred and ninety-one pairs of women-infant completed 16 weeks of intervention. A significant correlation was observed between the load of *Lactobacillus*, *Staphylococcus* and *Streptococcus* with the load of *Lactobacillus*, *Staphylococcus*, *Bacteroides* and *E. coli* in infant's feces (p< 0.05). The probiotic intervention modulated this effect. Regarding to growth of infants, superior weight z-scores were observed of infants which mothers had higher values of *Lactobacillus* in milk. *E.coli* load in infant's feces was also related to higher weight and height z-scores (p< 0.05). Load of *Staphylococcus* in breast milk and infant's feces was correlated with higher incidence of respiratory infection. The intervention with *L. fermentum* Lc40 reduce the effect of *Staphylococcus* on infant's health (p< 0.05). Finally, infant colic was significantly more likely to occur in infants which mother had higher level of anaerobes in milk (p= 0.044).

Conclusion: Breast milk microbiota influences infant colonization and is related with parameters of growth and health of the infants. Probiotic modulation of women microbiota might be a useful strategy to promote healthier patters of colonization in infants.

LACTOBACILLUS SALIVARIUS PS7, A PROBIOTIC STRAIN WITH POTENTIAL FOR PREVENTION OF OTITIS

N. Cárdenas¹, V. Martín², R. Arroyo³, M. López⁴, C. Badiola⁵, J.M. Rodríguez³

¹Probisearch SLU. ²Centro Nacional de Microbiología, Instituto de Salud Carlos III. Majadahonda, Madrid. ³Departamento de Nutrición y Ciencia de los Alimentos. Universidad Complutense de Madrid. ⁴Centro de Salud Bermeo. Bermeo, Bizkaia. ⁵Departamento Médico e I&D de Casen Recordati. Pozuelo de Alarcón, Madrid.

Background/Aims: Recurrent acute otitis media (rAOM) in children is a frequent condition with no satisfactory therapy. Recurrent use of antibiotics leads to an increasing antimicrobial resistance of otopathogens; therefore the use of probiotics in infection prevention become a new perspective. There is no evidence on the role of probiotics on rAOM in children. In a previous work, *Lactobacillus salivarius* PS7 was characterized as a potential probiotic strain and showed to exert a high antimicrobial activity against various bacterial species related to rAOM.

Methods: We have conducted a pilot study in 64 otherwise healthy children suffering from rAOM. All children received treatment with one daily dose of 10⁹ CFU of *Lactobacillus salivarius* PS7 with milk or a milk product during 6 months. An external control group of children treated with standard therapy was used for comparison. Outcomes were the incidence and duration of AOM episodes and the pathogen carriage in the external ear canal.

Results: Sixty-one children (mean age 39 months) completed the study. At least one episode of AOM was diagnosed in 36% of treated children, compared to 65% in children treated with standard therapy. The incidence of AOM episodes during the treatment period, compared to the 6-month period before treatment was started, was reduced by 84%. The median duration of AOM episodes in treated children was 4 days, compared to 6 days in children treated with standard therapy. The microbial density in the external ear canal decreased from ≥ 3 log₁₀ CFU at baseline to ≤ 2 log₁₀ CFU at the end of the intervention period.

Conclusion: A strain of *L. salivarius* PS7 has been effective to reduce the incidence and duration of AOM episodes. These promising results should be confirmed in a controlled clinical trial.

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS INFLUENCING THE EFFECTS OF A CHOLESTEROL-LOWERING PROBIOTIC

J. Espadaler, S. Audivert, E. Navarro-Tapia, D. Buj

Innovation Department. AB-BIOTICS SA. Sant Cugat del Valles, Spain.

Background/Aims: Some probiotics have demonstrated cholesterol-lowering activity in randomized trials. However, the effect of demographic and clinical factors on such probiotics and potential interactions of concomitant medications has not been assessed.

Methods: Observational, 12-week prospective study, among patients initiating a probiotic containing strains *L. plantarum* CECT7527, CECT7528 and CECT7529 (1.2×10^9 CFUs u.i.d.). Patients initiating statin treatment, but not those already on statin treatment, were excluded. Multivariate regression was performed to assess the effect of demographic and clinical characteristics (baseline LDL and triglycerides, statin therapy intensity and other concomitant medications) on serum LDL and triglyceride change after 12 weeks, and on reported tolerability issues.

Results: 343 patients, median age 55 (19–85) years, 63% female, baseline LDL and triglycerides of 156.5 ± 43.8 mg/dL and 339.9 ± 227.8 mg/dL, respectively, were available for analysis. Use of statins, fenofibrates, antihypertensive, antidiabetic and antiplatelet medications was 46%, 5%, 57%, 32% and 20%, respectively. LDL reduction averaged 40.8 ± 42.5 mg/dL in the whole cohort, and 34.5 ± 37.5 mg/dL in the non-statin subpopulation (both $p < 0.001$). Baseline LDL ($p < 0.001$) and statin therapy intensity ($p = 0.028$), but not other demographic or clinical characteristics, significantly influenced LDL reduction in the whole cohort (bivariate $R^2 = 0.37$). Reduction of serum triglycerides was also statistically significant in the whole cohort and in the non-statin subpopulation (both $p < 0.001$), and the effect was only dependent on baseline triglycerides ($p < 0.001$). 17% of patients reported tolerability issues (none of them severe), which correlated to antiplatelet use only (OR = 4.18, 95%CI 2.27–7.72; $p < 0.001$).

Conclusions: Probiotic treatment significantly reduced serum LDL cholesterol and triglycerides, regardless of age, gender, antidiabetic, antihypertensive or antiplatelet medication. Higher baseline LDL and triglycerides led to higher efficacy, and combination to statins further increased LDL reduction. Concomitant antiplatelet use increased the incidence of tolerability issues, mostly oily feces and flatulence.

EFFECT OF INTRAPARTUM ANTIMICROBIAL PROPHYLAXIS OVER BIFIDOBACTERIAL POPULATIONS IN HEALTHY FULL-TERM INFANTS: A QUANTITATIVE ASSESSMENT

S. Satorio¹, A. Nogacka^{1,2}, N. Fernández^{2,3}, M. Suárez^{2,3}, G. Solís^{2,3}, C.G. de los Reyes-Gavilán^{1,2}, M. Gueimonde^{1,2}, S. Arboleya^{1,2}

¹Microbiology and Biochemistry Department, Dairy Research Institute of Asturias (IPLA-CSIC), Villaviciosa-Asturias, Spain.

²Diet, Microbiota and Health Group, Health Research Institute of Principado de Asturias (ISPA), Oviedo, Spain. ³Pediatrics Service Department, Central University Hospital of Asturias (HUCA-SESPA), Oviedo, Spain.

Background/Aims: The gut microbiota establishment begins at early life and disturbances on this process may pose implications for the health of the infant and on long-term. The main microbial transfer from mother to baby occurs during delivery, with the exposure to the first colonizers of the gastrointestinal tract, such as bifidobacteria. *Bifidobacterium* is one of the most abundant genus in the infant intestinal microbiota and it is known that this microbial group carries out key functions for maintaining a healthy status. Different external factors may affect the correct gut microbiota establishment. Among these, antibiotic exposure is common, with intrapartum antibiotics being present in over 30% of deliveries; even though its effect over the gut microbiota establishment is not still well-understood. In this context, the aim of this study was to study the effect of the intrapartum antimicrobial prophylaxis (IAP) on the bifidobacteria population establishment.

Methods: Faecal samples from 39 healthy full-term babies were collected at 2, 10, 30, 90 days of life. DNA was extracted and the levels of the most abundant bifidobacterial species (*Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium bifidum*, *Bifidobacterium adolescentis*, *Bifidobacterium catenulatum*, *Bifidobacterium dentium*, *Bifidobacterium angulatum*, *Bifidobacterium animalis*) were determined by q-PCR.

Results: Despite the large inter-individual differences, our results showed a reduction in the levels of the most of bifidobacteria species in babies whose mothers received IAP, with respect to those whose mothers did not. The differences reached statistical significance ($p < 0.05$) for the species *B. longum* and *B. bifidum* during early days.

Conclusions: This study underlines the need to study the effect of the different perinatal factors affecting neonatal microbiota, not only at high taxonomical level but also at species level. This knowledge would help to develop rational strategies for favouring a healthy early microbiota development when this process is challenged.

All authors declare that no competing interest exists.

EFFECTS OF DAILY CONSUMPTION OF THE PROBIOTIC BIFIDOBACTERIUM ANIMALIS SUBSP. LACTIS CECT 8145 ON ANTHROPOMETRIC ADIPOSITY BIOMARKERS IN ABDOMINALLY OBESE SUBJECTS: A RANDOMIZED CONTROLLED TRIAL

E. Chenoll¹, A. Pedret^{2,3}, R.M. Valls^{2,3}, L. Calderón^{2,3}, E. Llauredó^{2,3}, J. Companys^{2,3}, L. Pla^{2,3}, A. Moragas^{5,6,7}, F. Martín-Luján^{5,6,8}, Y. Ortega^{5,6,9}, M. Giral^{2,3}, A. Caimari², P. Martorell¹, F.M. Codoñer^{1,0}, D. Ramón^{1,10}, Ll. Arola^{2,11}, R. Solà^{2,3,4}, S. Genovés¹

¹Biopolis SL/Archer Daniels Midland. R&D Department. Paterna, Valencia, Spain. ²Unitat de Nutrició i Salut. Eurecat, Centre Tecnològic de Catalunya. Reus, Spain. ³Facultat de Medicina i Ciències de la Salut. Universitat Rovira i Virgili. Functional Nutrition, Oxidation, and Cardiovascular Diseases Group (NFOC-

Salut). Reus, Spain. ⁴Hospital Universitari Sant Joan de Reus. Reus, Spain. ⁵Departament de Medicina i Cirurgia. Universitat Rovira i Virgili. Reus, Spain. ⁶Institut Universitari d'Investigació en Atenció Primària-IDIAP Jordi Gol. Tarragona, Spain. ⁷Primary Care Centre Jaume I. Institut Català de la Salut. Tarragona, Spain. ⁸Primary Care Centre El Morell. Institut Català de la Salut. Tarragona, Spain. ⁹Primary Care Centre Salou. Institut Català de la Salut. Tarragona, Spain. ¹⁰Lifesequencing/Archer Daniels Midland. R&D Department. Paterna, Valencia, Spain. ¹¹Facultat de Química. Universitat Rovira i Virgili. Grup de Recerca en Nutrigenòmica. Tarragona, Spain.

Background: The effects of probiotic *Bifidobacterium animalis* subsp. *lactis* CECT 8145 (Ba8145), and those of its heat-killed form (h-k Ba8145) on human anthropometric adiposity biomarkers are unknown. So that, to assess the effect of Ba8145 and h-k Ba8145 ingestion on anthropometric adiposity biomarkers was proposed to be studied.

Methods: A randomized, parallel, double-blind, placebo-controlled trial with abdominally obese individuals was performed. Participants (n= 135) consumed 1 capsule/day containing 10¹⁰ colony forming unit (CFU) of Ba8145, 10¹⁰ CFU of h-k Ba8145, or placebo (maltodextrin) for 3 months.

Results: Ba8145 ingestion decreased waist circumference, waist circumference/height ratio, and Conicity index ($P < 0.05$) versus its baseline. Changes versus the placebo group reach significance ($P < 0.05$) after the h-k Ba8145. Ba8145 decreased body mass index compared to baseline and to placebo group ($P < 0.05$). The decreases in visceral fat area after Ba8145 treatments reached significance ($P < 0.05$) only after h-k Ba8145. When analyses by gender were performed significance remained for women. Diastolic blood pressure and HOMA index decreased ($P < 0.05$) after h-k Ba8145. Gut microbiome analyses showed an increase in *Akkermansia* spp. after Ba8145, particularly in the live form, which was inversely related to weight ($P = 0.003$).

Conclusion: In abdominally obese individuals, the consumption of Ba8145, both as viable and, mainly, as heat-killed cells, improves anthropometric adiposity biomarkers, particularly in women. An increase in the gut *Akkermansia* genus appears as a possible mechanism. Our results support Ba8145 probiotic as a complementary strategy in obesity management.

SYMBIOTICS AS A NEW PERSPECTIVE IN THE TREATMENT OF LACTOSE INTOLERANCE

P. Minale¹, J. Hiller², M.F. Kramer³, M. Heath³, J.L. Justicia³

¹Hospital San Martino. Genoa, Italy. ²Bencard Allergie GmbH. Munich, Germany. ³Allergy Therapeutics. Worthing, United Kingdom.

Objective: Lactose intolerance, characterized by low levels of intestinal lactase, affects approximately 75% of the world population and is defined as a clinical syndrome characterized by pain and abdominal distention, flatulence, and diarrhea that occurs after lactose consumption. The use of specific probiotic strains, in particular those capable of expressing β -galactosidase enzymatic activity, has been proposed as an alternative treatment for subjects with lactose intolerance. The aim of this observational study was to investigate the efficacy of a symbiotic combination of lactic acid bacteria and inulin (SynGut[®]) in patients with lactose intolerance.

Materials and Methods: 38 adult subjects with physician-diagnosed lactose intolerance receiving a symbiotic combination of *Bifidobacterium lactis* W51, *Lactobacillus acidophilus* W22, *Lactobacillus plantarum* W21, *Lactococcus lactis* W19 and inulin (SynGut[®]) were assessed for symptom reduction using the SQLM (symptom questionnaire for lactose malabsorption) and changes in lactose consumption were evaluated. Secondary evaluations involved the changes in the proportion of patients with a positive hydrogen breath test (HBT).

Results: Treatment with SynGut[®] significantly decreased symptoms of lactose intolerance after 3 and 6 months of administration compared to baseline measurement. 71% of lactose intolerance patients reported an improvement of their symptoms after 3 month and 66% patients after 6 month of administration. Moreover, the proportion of patients with a negative HBT increased compared to baseline measurements: 0% at baseline, 65% after 3 months, and 81% after 6 months of administration.

Conclusion: This study shows that the symbiotic supplementation *Bifidobacterium lactis* W51, *Lactobacillus acidophilus* W22, *Lactobacillus plantarum* W21, *Lactococcus lactis* W19 and inulin (SynGut[®]) improves the symptoms of patients with lactose intolerance and opens new perspectives in the use of specific symbiotics in the treatment of lactose intolerance.

Immunonutrition

COMBINING TWO PROBIOTIC STRAINS WITH OLIGOFRACTOSE AND INULINE ABOLISHES THEIR BENEFITS AGAINST SALMONELLA IN PIGLETS

A. Rodríguez-Sorrento¹, L. Castillejos¹, P. Lopez¹, G.C. Cifuentes², M. Rodríguez-Palmero², J.A. Moreno², S.M. Martín-Orúe¹

¹Servicio de Nutrición y Bienestar Animal. Departament de Ciència Animal i dels Aliments. Universitat Autònoma de Barcelona. Bellaterra, Spain. ²Laboratorios Ordesa S.L. Barcelona, Spain.

Background/Aims: The efficacy of two probiotic strains (*Bifidobacterium longum* subsp. *infantis* CECT 7210 (Laboratorios Ordesa S.L.) and *Lactobacillus rhamnosus* HN001) combined or not with a prebiotic mixture of oligofructose and inulin against *Salmonella typhimurium* was evaluated.

Methods: A total of 96 piglets (28 days) were distributed into 32 pens assigned to 5 treatments: one non-challenge (CTR+) and four challenged: same diet (CTR-), or supplemented with probiotic ($> 3 \times 10^{10}$ cfu/kg each, PRO), prebiotic (5%, PRE) or their combination (SYN). After one week, animals were inoculated with *Salmonella*. Feed intake, weight and clinical signs were recorded. On days 4 and 8 post-inoculation (PI), one animal per pen was euthanized and samples collected for *Salmonella* counts, fermentation products, ileal histomorphology and serum TNF- α and PigMAP analysis.

Results: After the challenge, feed intake was decreased but more markedly in the SYN group that showed a lower final weight (Intake: 438^a, 315^b, 293^b, 237^b and 315^b g/d, $P < 0.001$; Final BW: 10.4^a, 9.3^{ab}, 8.9^{ab}, 8.2^b and 9.4^{ab} kg, $P = 0.009$; for CTR+, PRO, PRE, SYN and CTR-, respectively). PRE and SYN groups showed more liquid ileal consistency on day 8 PI ($P = 0.009$). A higher percentage of animals receiving PRO became negative to *Salmonella* in faeces at the end of the study (65% PRO vs 0% CTR- ($P = 0.03$)). Lower amounts of ileal SCFA were observed in SYN ($P = 0.025$) and valerate was increased in PRE and SYN. At day 8PI a significant decrease in the ileal villous/crypts ratio was found in challenged groups except for PRO (1.06^a CTR+, 0.96^{ab} PRO, 0.84^b PRE, 0.84^b SYN, 0.85^b CTR-).

Conclusion: In conclusion, the combined probiotic strains were able to enhance the response of the animals against *Salmonella* with improved ileal histological architecture and a faster clear out of the pathogen from the gut. However, these beneficial effects disappeared when the probiotic was combined with the prebiotic mixture.

RELATIONSHIP BETWEEN FIBER CONSUMPTION, SHORT CHAIN FATTY ACIDS AND CLOSTRIDIA CLASS MEMBERS IN HEALTHY ADULTS

N. Redondo, N. González, L.E. Díaz, A. Gheorghe, B. Villavisencio, S. Gómez-Martínez, A. Marcos, E. Nova.

Immunonutrition group. Metabolism and Nutrition Department. Institute of Food Science, Technology and Nutrition. Spanish National Research Council. Madrid, Spain.

Background: The promotion of beneficial commensal bacteria in the gut, along with an increased short chain fatty acid production (SCFA), have been postulated as potential mechanisms involved in the beneficial effects of a high fiber intake on health.

Objective: To assess the influence of fiber consumption on short chain fatty acid production and the abundance of bacteria belonging to Clostridia class in healthy adults.

Methodology: 261 adults aged between 25-50 y, not suffering chronic disease or following medical treatment were included (51% males). Short chain fatty acid production (acetic, propionic, butyric, isobutyric, valeric and isovaleric) was analysed by gas chromatography, and gut microbiota composition through 16S rRNA gene amplicon sequencing (V3+V4 gene regions. MiSeq 2x270 Illumina) and taxonomic analysis. Fiber consumption (FC) habits were analyzed by a food frequency questionnaire, and the following groups were considered: Low (< 15 g/day), Medium (15-25 g/day) and High (> 25 g/day). General linear models were used to assess fiber consumption effect on the studied variables, with FC groups, gender and BMI-fat groups as fixed factors, and age and energy as covariables. Bonferroni test was used for pairwise comparisons.

Results: The levels of acetic and butyric acid were higher in the high and medium FC groups compared to the low FC group, only reaching statistical significance for the latter (Medium vs. Low: $P = 0.021$ and $P = 0.034$, respectively). On the other hand, the high FC group showed higher levels of *Clostridiaceae* compared to the low FC group ($P = 0.026$), as well as higher *Faecalibacterium prausnitzii* levels ($P = 0.018$). In addition, both acetic and butyric acids were positively correlated to *F. prausnitzii* levels ($P < 0.001$).

Conclusion: Fiber consumption might exert beneficial effects on intestinal health, as observed in the increased levels of butyric acid and *F. prausnitzii*, both associated to gut anti-inflammatory effects.

DETECTION OF RIBOFLAVIN PRODUCTION BY LACTOBACILLUS PLANTARUM STRAINS DURING GROWTH AND ITS GASTROINTESTINAL SURVIVAL

M.L. Mohedano¹, S. Hernandez¹, J. Guy LeBlanc², R. Aznar^{3,4}, T. Requena⁵, P. López¹

¹Centro de Investigaciones Biológicas (CIB-CSIC). Madrid, Spain.

²Centro de Referencia para Lactobacilos (CERELA-CONICET).

Tucuman, Argentina. ³Universidad de Valencia (UVEG). Burjassot,

Valencia, Spain. ⁴Instituto de Agroquímica y Tecnología de

Alimentos (IATA-CSIC). Paterna, Valencia, Spain. ⁵Instituto de

Investigación en Ciencias de la Alimentación (CIAL-CSIC). Madrid, Spain.

Introduction: Certain strains of lactic acid bacteria (LAB) can produce riboflavin, a water-soluble vitamin that belongs to group B. It is produced by plants and many microorganisms but higher animals lack the ability to biosynthesize it.

Methods: We have evaluated riboflavin production by five *Lactobacillus plantarum* strains, isolated from chicha, a fermented alcoholic beverage normally produced from maize, from North-western Argentina, and their isogenic riboflavin-overproducing mutants. Here we describe a method of fluorescence spectroscopy to quantify the production of riboflavin in real time in bacterial

culture supernatants. Thus, the riboflavin overproducing strain *L. plantarum* M5MA1-B2 was selected for further analysis. This strain was fluorescently labeled (mCherry) with the plasmid pRCR12 and its survival to digestive tract stresses in the presence of microbiota in the dynamic multistage BFBL gut model, and in a murine model using conventional adult BALB/c mice was analyzed.

Results: Among the B2 overproducing strains, *L. plantarum* M5MA1-B2 secreted the highest levels of riboflavin. This bacterium was fluorescently labelled by transfer of pRCR12 plasmid. The labelling with mCherry did not affect growth or riboflavin production. This strain, with or without plasmid, is able to adhere to Caco-2 cells and this interaction capacity can be quantified by fluorometry, in the case of the labeled strain. The survival of the isogenic strain M5MA1-B2[pRCR12] has also been demonstrated during in vitro simulation of gastrointestinal tract conditions and by analyzing the BALB/c intestinal content. The results indicated a satisfactory resistance of the strain to gastric and intestinal stress conditions but a low colonization capability. The developed method allows us to quantify the vitamin production, in real time, during bacterial growth, being a rapid and easy test for the selection of B2 vitamin-producing strains.

Conclusion: *L. plantarum* M5MA1-B2 is a suitable probiotic bacteria for the development of functional foods.

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PREDICTING OBESITY FROM GUT MICROBIAL COMPOSITION AND LIPID PROFILE: DOES GENDER MATTER?

T. Fernández-Navarro^{1,4}, I. Díaz³, I. Gutiérrez-Díaz^{1,4}, J. Rodríguez-Carrio^{2,4}, A. Suárez^{2,4}, C.G. de los Reyes-Gavilán^{3,4}, M. Gueimonde^{3,4}, N. Salazar^{3,4}, S. González^{1,4}

¹Department of Functional Biology. Faculty of Medicine. University of Oviedo. Oviedo, Spain. ²Department of Computer Science. Faculty of Science. University of Oviedo. Oviedo, Spain. ³Instituto de Productos Lácteos de Asturias. Consejo Superior de Investigaciones Científicas (IPLA-CSIC). Villaviciosa, Spain. ⁴Metabolism Area. Instituto de Investigación Sanitaria del Principado de Asturias (ISPA). Oviedo, Spain.

Aims: Different fatty acids could drive different changes in the composition and functionality of the intestinal microbiota, contributing to host's lipid metabolism and obesity development. Our aim was to address the connections between gut microbiota and different serum free fatty acids (FFA) in the context of obesity, by analyzing the possible interactions between all factors involved.

Methods: 66 subjects (age 52.7 ± 11.2y) classified according to Body Mass Index (BMI). Total and individual FFA were analyzed by colorimetric enzymatic assay and methyl-tert-butylether-based extraction protocol, respectively. Gut microbiota was determined by qPCR and diet through a food frequency questionnaire. Statistical analyses were performed, and predictive factors for obesity were obtained via classification by decision trees using machine learning methods.

Results: Subjects with higher serum levels of eicosapentaenoic acid (EPA) and higher fecal levels of *Bacteroides* belonged

to normal weight group, with independence of gender. In males, levels of *Faecalibacterium* ≤ 6.456 or > 6.456 (log n° cells / gram of feces) were associated to normal and pre-obesity status, respectively. Furthermore, women with serum palmitic acid > 23.843 µg/mL and *Bifidobacterium* ≤ 6.729 (log n° cells / gram of feces), classified within the overweight group. In the same way, if EPA serum level was > 0.141 µg/mL, or ≤ 0.141 µg/mL running together with *Bifidobacterium* > 8.823 (log n° cells/gram of feces), women were normal weight.

Conclusion: These data point to serum EPA as a significant obesity indicator, independently of the rest of the variables. Furthermore, when the concentration of serum EPA is ≤ 0.235 µg/mL, the interaction between FFA and the gut microbiota seems to be gender-dependent, being associated to *Faecalibacterium* and *Bifidobacterium* for males and females, respectively. These results open the possibility of modifying the obese-linked FFA profile and the altered gut microbiota through dietary interventions attending to gender differences.

SEXUAL DIMORPHISM OF RAT GUT MICROBIOTA: KEY FACTOR IN NUTRITIONAL INTERVENTIONS

M. Massot-Cladera, I. Azagra-Boronat, C. Morales-Ferré, P. Kalousová, À. Franch, M. Castell, M.J. Rodríguez-Lagunas, F.J. Pérez-Cano

¹Secció de Fisiologia. Departament de Bioquímica i Fisiologia. Facultat de Farmàcia i Ciències de l'Alimentació. Universitat de Barcelona. Barcelona, Spain. ²Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB). Santa Coloma de Gramenet, Spain.

Background and Aim: It is well known that there are gender-specific differences of immune system and microbiota composition in human and mice¹. These differences may lead to gender-differential responses in nutritional interventions that act through modulation of microbiota composition (p.e. prebiotics and probiotics). The present study was aimed at analyzing sexual dimorphic differences in gut microbiota and intestinal immunity in adult rats.

Methods: For this purpose, cecal content from 12-week old female and male Wistar rats were collected to characterize microbiota composition by sequencing techniques. Moreover, IgA concentration was determined by ELISA in feces and gut wash obtained at the same time point. Fecal samples were also used to determine the IgA-coating bacteria.

Results: The qualitative metagenomic analysis evidenced that female rats have 1 phylum, 4 families and 13 genera that are not present in male rats. No male-specific colonization was observed. Regarding the quantitative analysis male rats showed significantly higher proportion of Firmicutes phylum which was associated with a higher *Lactobacillaceae* and *Lactobacillus* at family and genus level, respectively (p < 0.05). Female rats had higher proportion of Verrucomicrobia phylum compared to the male rats which was due to a higher presence of *Akkermansiaceae* and *Akkermansia* (p < 0.05). Moreover, female rats have more diversity of microbiota than male rats. No sex-associated differences either on intestinal IgA nor on IgA-coated bacteria were observed.

Conclusion: It can be concluded that there is sexual dimorphism in composition and diversity of microbiota in adult rats fact that has to be considered in the design of preclinical studies.

Reference: 1. Elderman M, de Vos P, Faas M. Role of microbiota in sexually dimorphic immunity. *Front Immunol.* 2018; 9: 1018.

GALACTOOLIGOSACCHARIDE SUPPLEMENTATION AMELIORATES THE ACUTE GASTROENTERITIS PROCESS IN A PRE-CLINICAL MODEL OF DOUBLE ROTAVIRUS INFECTION

M. Massot-Cladera^{1,2}, M. Rigo-Adrover^{1,2}, M.J. Rodríguez-Lagunas^{1,2}, À. Franch^{1,2}, M. Castell^{1,2}, F.J. Pérez-Cano^{1,2}

¹Secció de Fisiologia. Departament de Bioquímica i Fisiologia. Facultat de Farmàcia i Ciències de l'Alimentació. Universitat de Barcelona. Barcelona, Spain. ²Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB). Santa Coloma de Gramenet, Spain.

Background and Aim: Group A rotaviruses (RV) are the leading cause of gastroenteritis among young children worldwide producing limited diarrhoea to severe dehydration and

even death. After RV infection, immunity is not complete and less severe re-infections usually occur. These infections could be modulated by nutritional interventions with bioactive compounds, such as prebiotics. The aim of this study was to evaluate the influence of a particular galactooligosaccharide (GOS) on the RV symptomatology and immune response during two consecutive infections.

Methods: Lewis neonatal rats were inoculated with SA11 (first RV infection) on day 6 of life and with EDIM (second RV infection) on day 17 of life. GOS group was also orally administered with a daily dose of GOS between day 3 and 9 of life. Clinical and immunological variables were assayed during both infective processes until day 28 of life.

Results: During the first infection, the dietary intervention with GOS significantly reduced the incidence, duration and overall severity of the diarrhoea ($p < 0.05$). In addition, it reduced another severity indicator, the faecal weight output, during the diarrhoea period ($p < 0.05$). Second RV infection was not able to induce diarrhoea in any of the groups studied. Immune response during first infection with SA11 was not affected by GOS and had no impact on second infective process, but the dietary intervention significantly increased IFN- γ and TNF- α intestinal levels two weeks after the second infection ($p < 0.05$).

Conclusion: GOS supplementation is able to reduce the incidence and severity of the RV-associated gastroenteritis episode and to modulate the immune response against RV infections.

Microbiology and Veterinarian

IMPACT OF CURATIVE PELVIC RADIOTHERAPY ON THE GUT ENVIRONMENT OF PROSTATE CANCER PATIENTS

L. Guadamuro¹, I. Gutiérrez-Díaz², A.I. Alonso³, M.A. Azcárate-Peril⁴, P. Isidro-Marrón⁵, S. Blanco³, G. Fernández², L. Olay³, G. Juan-Rijo³, S. González², A. Margolles¹, S. Delgado¹

¹Department of Microbiology and Biochemistry of Dairy Products. Instituto de Productos Lácteos de Asturias (IPLA)-Consejo Superior de Investigaciones Científicas (CSIC). Villaviciosa-Asturias, Spain. ²Department of Functional Biology. University of Oviedo. Oviedo-Asturias, Spain.

³Radiation Oncology Service. Hospital Universitario Central de Asturias (HUCA). Oviedo-Asturias, Spain. ⁴Department of Medicine. University of North Carolina (UNC). North Carolina, USA. ⁵Biobank of Principality of Asturias. Hospital Universitario Central de Asturias (HUCA). Oviedo-Asturias, Spain.

Background: Gastrointestinal symptoms are frequent after pelvic radiotherapy and can greatly affect the quality of life of cancer survivors. The effect of radiation on the intestinal microbiome, and the implications of a radiotherapy-induced dysbiosis and the derived intestinal inflammation have received very little attention.

Aim: To perform a follow-up study in patients with prostate cancer for investigating alterations in gut microbiota and metabolites induced by pelvic radiotherapy and associations with inflammation and dietary changes.

Methods: Fourteen patients with prostate cancer undergoing pelvic radiotherapy were recruited and followed during the anti-cancer treatment until two months after finishing. Four stool samples were collected from each patient and changes in the bacterial communities were investigated by sequencing the V3-V4 region of the 16S rRNA gene with Illumina Technology (Miseq PE250), meanwhile short chain fatty acids (SCFAs) were analysed by gas chromatography. Additionally, calprotectin levels were determined using an ELISA kit and, evaluation of dietary intake was recorded by means of semi quantitative food frequency questionnaires.

Results: The composition of the gut communities changes along the radiation treatment, being the Bacteroidetes, the group more affected ($p=0.008$, Wilcoxon test). Total SCFAs in feces was reduced with pelvic radiation. In particular, statistical differences were observed for butyrate and acetate excretion with respect to basal time. On the contrary, fecal calprotectin increased significantly during radiotherapy ($p=0.016$). In addition, statistical differences in the energy intake were observed before and after two months of radiotherapy.

Conclusions: An impact of pelvic radiotherapy on gut microbiota composition and metabolites was observed in prostate cancer patients. Intestinal inflammation occurs at the same time that the microbiome shifts. The effect of radiation was partially, but not completely, restored after two months of finishing the anti-cancer therapy, with changes in the food ingestion patterns still noticeable at this time point.

A PRELIMINARY STUDY OF BACTERIAL GENETIC DETERMINANTS IN HUMAN GALLBLADDER. A FOCUS ON BILE RESISTANCE

N. Molinero¹, S. Delgado¹, M. Ferrer², A. Margolles¹

¹Department of Microbiology and Biochemistry of dairy products. Dairy Research Institute of Asturias IPLA-CSIC. Villaviciosa, Asturias, Spain. ²Department of Applied Biocatalysis. Institute of Catalysis, CSIC. Madrid, Spain.

Background/Aims: The human gastrointestinal microbiota and its relationship with different physiological states has been characterized in detail in the last years. However, the microbiota of bile and gallbladder has been scarcely studied. Also, the functions of the autochthonous biliary microorganisms and the characteristics that allow the survival in this environment have not been investigated. In this context, we have constructed a metagenomic library from human bile of liver donors, in order to analyse the activities responsible for bacterial survival in presence of bile.

Methods: For the generation of the library we used total DNA isolated from human bile. Fragments with a size of approximately 20-30kb were cloned in the vector pCC1FOS™ (Epicentre®) and transformed into competent *Escherichia coli*. The pool of *E. coli* clones was tested in different concentrations of bile salts in order to detect highly resistant clones.

Results: Growth experiments allowed us to select five clones with a bile resistance phenotype. The selected clones were able to grow in a bile concentration at least 10 times higher than the *E. coli* strain containing the empty vector. The five fosmids were sequenced using Illumina technology and the bioinformatic analysis of the sequences showed the presence of a variety of potential genes that could play a role in bile resistance, coding for putative proteins involved in oxidative stress response, as well as DNA repair proteins, transmembrane pumps and lipopolysaccharide biosynthesis enzymes. Interestingly, the majority of DNA sequences displayed a low homology with genomic sequences of known microorganisms, suggesting that the native biliary microorganisms harbouring the DNA inserts could be new microbial taxa.

Conclusions: Five DNA fragments from biliary microorganisms, able to confer bile resistance in *E. coli*, were identified in our study. Further work is needed in order to unravel the specific DNA sequences responsible for the observed phenotype.

GUT MICROBIOTA AS A NEW PREDICTIVE FACTOR IN ACTIVE RELAPSING-REMITTING MULTIPLE SCLEROSIS

V. Navarro^{1,2}, M.A. Méndez^{1,3}, A. Frías^{1,4}, R. Vela^{1,3}, B. Ruzafa¹, E. Núñez¹, S. Chumillas¹, Y. Marhuenda¹, L. Navarro¹

¹Grupo MiBioPath. Departamento de Medicina Clínica. Universidad Católica San Antonio de Murcia (UCAM). Murcia, Spain. ²Unidad de Enfermedades Infecciosas. Hospital Universitario del Vinalopó. Elche, Spain. ³Servicio de Neurología. Hospital Universitario de Torrevieja. Torrevieja, Spain. ⁴Servicio de Neurología. Hospital Universitario del Vinalopó. Elche, Spain.

Background: Microbiota influence on different illnesses has been studied for over 100 years. However, during the last 5-10 years significant efforts have been focused on demonstrate the relevance of microbiome in neurological diseases. Our recent findings show the effect of gut microbiota composition specifically in multiple sclerosis. In this study we have analyzed the gut microbiota in patients with relapsing-remitting multiple sclerosis and its relationship with the disease evolution.

Methods: The gut microbiota in a cohort of 16 patients with active relapsing-remitting multiple sclerosis and a control group of healthy population was compared by 16s rRNA massive sequencing. After a 24-month follow-up period study, a correlation analysis between microbiota data and the new relapses and new central nervous system injuries was performed in the group of patients with the neurological disease. Besides, we analyzed the correlation of these new relapses or injuries and BMI.

Results: The gut microbiota of patients with multiple sclerosis differs from the healthy population. We have detected significant differences in the levels of some bacteria, including, but not only, the family *Ruminococcaceae*; and the genus *Gemmiger*, *Prevotella*, *Streptococcus*, *Ruminiclostridium*, *Ruminococcus*, *Succinivibrio* and *Sutterella*. Spearman's statistical analysis shows a positive correlation between some of these bacteria with the severity of the disease. On the other hand, we have not found a significant correlation between the BMI and the progression of the disease.

Conclusions: There is a clear difference in the microbiota of patients with active multiple sclerosis from the healthy population. Some of these microorganisms are associated with a worse prognosis, suggesting the role of microbiota composition as a predictive factor of the disease evolution.

EFFECT OF MICROBIOTA MEMBRANE VESICLES ON HUMAN DENDRITIC CELLS ACTIVATION AND DERIVED T-CELL EFFECTOR RESPONSES

N. Díaz, R. Vera, M. Riera, M. Fábrega, R. Giménez, J. Badía, L. Baldomá

Departament de Bioquímica i Fisiologia, Facultat de Farmàcia i Ciències de l'Alimentació. Universitat de Barcelona. Institut de Biomedicina de la Universitat de Barcelona - Institut de Recerca Sant Joan de Déu, Spain.

Background/Aims: Dynamic and complex interactions between the microbiota, the intestinal epithelium and the host immune system are well-known. The influence of gut microbiota on health and disease has been proven. There is scientific evidence that certain gut microbes, especially probiotics have a beneficial role in intestinal homeostasis. However, the microbiota factors that modulate host immune and defense responses have not been always well characterized. All bacteria release extracellular membrane vesicles (MVs) as a mechanism of intercellular communication. The objective of this study was to evaluate the immunomodulatory effect of MVs isolated from *Escherichia coli* strains of intestinal origin on dendritic cells (DCs) and the induced T-cell (LT) effector response.

Methods: MVs from five *E. coli* strains were isolated and tested: the probiotic *E. coli* Nissle strain 1917 (EcN), the derived mutant lacking the outer polysaccharide capsule (EcN: K5) and the com-

mensals ECOR12, ECOR63 and ECOR53. Monocytes isolated from buffy coats from healthy donors were cultured and differentiated into DCs for 7 days, and then incubated with MVs for 24 h. Stimulated DCs were then co-cultured with isolated LT CD4+. DCs and lymphocytes were analyzed by flow cytometry and the supernatants collected for cytokine quantification by ELISA and MULTIPLEX immunoassay.

Results: MVs from all the strains induced maturation of DCs ($p < 0.05$), although with different IL-10, IL-6 and TNF- α secretion profile ($p < 0.05$). After 4-day coculture (mDCs/LTs) MVs induced polarization of LTs towards a TH17 response ($p < 0.05$) with the exception of ECOR53 vesicles ($p < 0.05$). MVs from ECOR12 induced greater polarization to T regulatory response (Treg) than EcN MVs ($p < 0.05$), whereas the others did not cause any significant increase in Treg cells.

Conclusion: Microbiota MVs are sensed by immature DCs and specifically modulate LT cell responses, thus acting as key players in the modulation of the host immune system.

FEEDING TWO BACILLUS STRAINS IN COMMERCIAL SOWS: EFFECTS ON REPRODUCTIVE PERFORMANCE AND GUT MICROBIAL ECOSYSTEM

M. Saladrigas¹, D. Solà-Oriol¹, S. López-Vergé¹, B. Nielsen², J.F. Pérez¹, S.M. Martín-Orúe¹

¹Servicio de Nutrición y Bienestar Animal. Departament de Ciència Animal i dels Aliments. Universitat Autònoma de Barcelona. Bellaterra, Spain. ²Chr. Hansen A/S, Hørsholm, Denmark.

The aim of the present study was to assess in breeding sows the effect of long term administration (three parities) of two different *Bacillus* strains on their reproductive performance and the gut microbial ecosystem. For this purpose, 90 Landrace x Yorkshire dams were selected and randomly allotted into 3 treatments: a control group (CON) fed a standard diet or supplemented with 5×10^8 cfu/kg *B. subtilis* 25841 (PR1) or 5×10^8 cfu/kg *B. amyloliquefaciens* 25840 (PR2). Reproductive parameters were recorded along the three reproductive cycles. Faecal samples ($n = 13$, $n = 11$ and $n = 14$ for CON, PR1 and PR2, respectively) were taken on days 8 and 21 of the third lactation for microbiota analysis by Illumina MiSeq 16S RNA. Both supplemented groups showed higher number of born piglets per litter (18.3, 19.5 and 20.6 for CON, PR1 and PR2, $P = 0.01$) and sows receiving PR2 also presented a higher number of born alive (15.7, 15.6 and 17.4, $P = 0.01$) and weaned piglets (13.9, 13.6 and 14.4; $P = 0.002$). Regarding colonic microbiota, no significant changes were registered in alpha, nor beta-diversity and scarce effects of the diets were found in the community structure (ANOSIM test, $P = 0.08$). However significant changes were observed at phylum level (Bacteroidetes; 22.4, 18.7, 18.6 %, $P = 0.03$) with decreases in the Prevotellaceae (9.4, 7.3, 7.3 %; $P = 0.03$) and increases in the Ruminococcaceae family (16.1, 13.1, 13.7 %; $P = 0.04$). Several genera were also modified by the probiotic strains including, *Prevotella*, *Ruminococcus*, *Megasphaera*, *Oscillospira*, *Dorea*, *Blautia* or *Roseburia*. In conclusion, the addition of *B. subtilis* 25841 and 25840 to the sow diet enhances their

reproductive performance in terms of an increased prolificacy. The strain 25840 also exhibit improvements in the viability of born piglets with increases in the number of weaned piglets per litter. Their administration along three parities demonstrated to have a clear impact on gut microbial ecosystem.

THE EFFECT OF DIETARY MODIFICATIONS ON GUT BACTERIAL MICROBIOME IN GROWING SWINES

C. Roncero¹, F. Codoñer², D. Ramon^{1,2}, A. Cerisuelo³, A.I. Jiménez-Belenguer⁴, M. Cambra-López⁴, P. Ferrer³, S. Calvet⁴, M. Tortajada¹, E. Chenoll¹

¹Biopolis SL/Archer Daniels Midland. R&D Department. Paterna, Valencia, Spain. ²Lifesequencing/Archer Daniels Midland. R&D Department. Paterna, Valencia, Spain. ³Centro de Investigación y Tecnología Animal. Instituto Valenciano de Investigaciones Agrarias. Segorbe, Castellón, Spain. ⁴Universitat Politècnica de València. Valencia, Spain.

Background: Spanish swine industry is the fourth potency in the world and the second potency in Europe (17.4% of European production; source: Ministry of Agriculture, Fisheries and Food). The improvement of the health status and growth performance of swine will revert in important economic benefits in the sector. An

in-depth knowledge of the Spanish swine microbiome, followed by the assessment of the effects of dietary changes (i.e. probiotics and prebiotics) in the microbiota are recognized essential strategies to increase health and performance in pigs. In this study a characterization of Spanish swine gut microbiome and the prebiotic effect of diet have been analyzed.

Methods: A total of 32 Duroc x (LDxLW) growing-finishing pigs were included in the study. Animals were divided in two groups and fed with standard diet and with a potential prebiotic diet containing 24% citrus pulp, respectively. Faecal samples were obtained before the administration of the experimental feeds (time 0) and six weeks after. Data concerning weight and health status were also recorded. DNA was extracted with a modified commercial protocol and libraries obtained. Bacterial microbiome was obtained by MySeq platform (Illumina). Raw sequences were filtered based on quality, merged and annotated.

Results: In all cases, rarefaction curves reached a plateau, showing a complete saturation in the annotation of the sequences. Time 0 microbiome was analyzed to ensure that no significant differences existed before diet modification. The evolution of microbiome with standard and modified diet showed significant differences in genera, highlighting *Clostridium* and *Turicibacter*, which showed different evolution depending on the diet.

Conclusion: The results obtained let us firstly to characterize Spanish swine microbiome. Moreover, citrus pulp-diet showed gut microbiome differences after six weeks of consumption, preliminary pointing to a prebiotic effect of this compound, and support further studies on prebiotic and probiotic diet addition in swine.

Clinical Uses**BENEFICIAL EFFECTS OF PREBIOTIC FORMULATION IN FRAILTY SYNDROME IN A RANDOMIZED AND PLACEBO-CONTROLLED TRIAL: ROLE OF CYTOKINES**

O. Cauli¹, J. Fernández-Garrido¹, C. Buigues¹, R. Navarro-Martínez¹, L. Pruimboom², A.J. Hoogland³, O. Theou⁴, K. Jayanama⁵, K. Rockwood⁴

¹Department of Nursing, University of Valencia, Valencia, Spain. ²University of Groningen, University Medical Center Groningen (UMCG), Groningen, The Netherlands. ³Bonusan BV, Numansdorp, The Netherlands. ⁴Department of Medicine, Dalhousie University and Nova Scotia Health Authority, Halifax, Canada. ⁵Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Background/Aims: Chronic administration of prebiotic formulation containing inulin and fructo-oligosaccharides reduces frailty syndrome in older people. The molecular mechanisms of these effects are unknown and we sought to evaluate the role of pro-inflammatory and anti-inflammatory cytokines in these effects.

Methods: We assessed frailty syndrome by the frail index (FI) score, and pro-inflammatory (TNF-alpha, IL-6 and IL-1beta) and anti-inflammatory (IL-4, IL-10) cytokines in individuals of the treatment (prebiotic formulation administered during 13 weeks) and control group in a randomized and placebo-controlled trial.

Results: At the 13-week follow-up, the placebo group had higher FI levels (preFI 0.23 ± 0.11, 35 postFI 0.24 ± 0.12, p= 0.012) and the intervention group had lower FI levels (preFI 0.22 ± 0.09, 36 postFI 0.20 ± 0.08, p< 0.001). Those individuals that showed an improvement in FI after administration of the prebiotic formulation showed a significant (p< 0.05) higher levels of anti-inflammatory cytokines in blood compared to individuals in the placebo group. In contrast the level of pro-inflammatory cytokines as well as leukocytes counts in blood were unchanged after prebiotic treatment.

Conclusion: Prebiotic administration reduces frailty levels through increasing the concentration of some peripheral anti-inflammatory cytokine levels. This clinical trial opens the possibility to modulate the chronic low-grade inflammation in frailty syndrome in older individuals through prebiotic administration.

LACTOBACILLUS GASSERI LA806 SUPPRESSES VISCERAL HYPERSENSITIVITY AND BARRIER DISRUPTION INDUCED BY CHRONIC STRESS IN RATS

C. Beaufrand¹, S. Holowacz², A. Pérez³, S. Kuyille⁴, V. Theodorou¹

¹Department of Neurogastroenterology, Toxalim, INRA/EI Purpan, Toulouse, France. ²PiLeJe Laboratoire, Paris, France. ³PiLeJe SLU, Sant Just Desvern, Spain. ⁴GENIBIO, Lorp-Sentataille, France.

Introduction: Irritable bowel syndrome (IBS) is a common functional disorder. Underlying mechanisms include gut microbiota unbalance and altered intestinal permeability with subsequent bacterial translocation. Specific probiotic strain ability to adhere well to intestinal cells may play a pivotal role in restoring the microbiota and gut barrier, which might be of significant value in IBS treatment.

Objective: To assess in a model of IBS the efficacy of *Lactobacillus gasseri* LA 806 previously characterized *in vitro* for its capacity to adhere to gut epithelial cells and to reinforce the epithelial barrier.

Methods: Male Wistar rats were treated orally with 1 mL of NaCl 0.9% alone or containing *L. gasseri* LA806 (10⁹ UFC/mL) during 15 days and were submitted to a 4-day water avoidance stress (WAS) session on day 12 of treatment. A group of rats not submitted to WAS and orally treated with the vehicle was used as control (basal condition). Visceral sensitivity was measured (myoelectrical activity in response to colorectal distensions [CRD]) and intestinal permeability assessed using ⁵¹Cr-EDTA as a marker.

Results: *L. gasseri* LA806 reduced significantly WAS-induced hypersensitivity to CRD at the two highest volume of distension (18 ± 3 vs. 31 ± 5 for WAS + vehicle at 0.8 mL and 20 ± 2 vs 29 ± 2 for WAS + vehicle at 1.2 mL). When administered orally at 10⁹ UFC/day during 15 days, *L. gasseri* LA806 reduced significantly WAS-induced hyperpermeability: 1.95 ± 0.14 for WAS + vehicle/LA806 vs. 2.93 ± 0.21 for WAS + vehicle (p= 0.0018). No significant difference was observed between the group no WAS + vehicle and the group WAS + vehicle/LA806.

Conclusion: *L. gasseri* LA806 is able to decrease abdominal pain (visceral sensitivity) in part by restoring intestinal epithelial barrier function (intestinal permeability decrease) in a rat model of IBS.

SYMBIOTIC, CONTAINING BACILLUS COAGULANS LMG-S-24828 REDUCES GASTROINTESTINAL ADVERSE EFFECTS IN PATIENTS USING MIGLUSTAT OR TK-INHIBITORS

B. Medrano-Engay^{1,2}, C.J. Gómez-Notario^{2,3}, J. Alcedo^{1,4}, P. Giraldo^{1,3}

¹Instituto de Investigación Sanitaria Aragón. ²Unidad de Investigación Traslacional. Hospital Universitario Miguel Servet. Zaragoza, Spain. ³Fundación Española para el Estudio y Terapéutica de la Enfermedad de Gaucher. ⁴Servicio de Aparato Digestivo. Hospital Universitario Miguel Servet. Zaragoza, Spain.

Introduction: Some tyrosine kinase inhibitors (TK-i) used in the treatment of chronic leukemias and substrate inhibitors (Miglustat), an iminosugar used in lysosomal diseases, can cause gastrointestinal disorders such as diarrhea, distension and abdominal pain. These adverse effects decrease the quality of life related to health and lead to early withdrawal of treatment. Some probiotics have shown an improvement in the symptoms mentioned in patients with func-

tional digestive disorders [1]. Hypothesis: a symbiotic, containing *Bacillus coagulans* LMG-S-24828, minimizes the gastrointestinal adverse effects associated with the use of TK-i or Miglustat.

Objectives: To evaluate in patients treated with TK-i or Miglustat the effect of the controlled administration of the symbiotic for a month, regarding to rhythm and type of stools.

Method: Randomized cross-over trial in which 15 patients with TK-i or Miglustat were blinded to placebo or symbiotic in a daily dose, with a “washing” phase of two months between the administration of each of them. The patients were asked to complete the Bristol Stool Chart (BSC) every day during the study period. This score allows to identify the stool form using seven different images with accompanying written descriptors. The frequency of withdrawal of treatment with TK-i and Miglustat in each group will also be evaluated. The analysis of the results will be carried out using the Student's t-test, considering the statistical significance of the differences with p-value < 0.05. The protocol was approved by the Autonomous Committee of Ethics.

Results: The study period has not yet concluded before deadline. The results and conclusions will be provided in the meeting.

References: 1) Simrén M, Barbara G, Flint HJ, et al. Intestinal microbiota in functional bowel disorders: a Rome foundation report. *Gut*. 2013; 62: 159-76.

EFFECT OF THE ADMINISTRATION OF A PROBIOTIC WITH *LACTOBACILLUS* AND *BIFIDOBACTERIA* ON ANTIBIOTIC-ASSOCIATED DIARRHEA

O. García Trallero¹, L. Herrera Serrano², M. Bibián Inglés², D. Roche Vallés³, J. González Castro³

¹Coordinadora Médica del Servicio de Urgencias, Hospital Universitari Dexeus-Grupo Quirónsalud. Barcelona, Spain.

²Schwabe Farma Ibérica, Departamento Médico. Madrid, Spain.

³Methodex, Compañía de Investigación Clínica. Barcelona, Spain.

Background/Aims: Antibiotics treatment is related to diarrhea (AAD). There is a lack of studies demonstrating the beneficial effect of using a specific probiotic combination: Pearls IC^a (*Lactobacillus acidophilus* NCFM, *Lactobacillus rhamnosus* Lr-32, *Bifidobacterium breve* M-16V, *Bifidobacterium longum* BB536, *Bifidobacterium lactis* BI-04 and *Bifidobacterium bifidum* Bb-02). The aim of the study is to analyze the effect, safety and acceptability of this combination of probiotics on diarrhea associated with treatment with amoxicillin-clavulanic acid (CA).

Methods: Pilot, unicentric, randomized, double-blind, parallel group, placebo-controlled study (probiotic vs. placebo for 30 days). **Target population:** Patients older than 18 years, both sexes treated with CA (850 mg/125 mg every 8 h/orally) for 7 days. **Sample size:** n = 40. Considering a prevalence of the antibiotic effect in the stools of 75% and a reduction by the antibiotic effect of 35% (80% powered and 95% confidence). **Subjects:** Adult patients who attended the Emergency Department (Dexeus Hospital, Barcelona) between January and April of 2018 with prior informed consent with a follow up in primary care at 30 days. **Variables:** The differences between day 0 and day 30 of the number of daily stools and duration of diarrhea were evaluated; Stool consistency according to Bristol Stool

Form, Quality of intestinal life (GIQLI). Subjective evaluation and evaluation of adverse effects of the product through a specifically designed questionnaire. **Statistical methods:** U-Mann-Whitney test. Significance level of 5%. Software R v3.4.2.

Results: Thirty-six subjects were included (18 per group). Pearls IC^a delayed between 4 and 5 days the appearance of the diarrheic episode vs placebo (p < 0.001) with a tendency to decrease the number of daily bowel movements and a better subjective assessment.

Conclusions: Pearls IC^a demonstrated its beneficial effect on DAA by delaying the onset of diarrhea and showed a tendency to decrease the number of daily stools vs placebo.

PATIENT CHARACTERISTICS INFLUENCING INFANT COLIC AMELIORATION UNDER A PROBIOTIC TREATMENT

E. Navarro-Tapia¹, M. Sticco², E. Astó¹, M. Aguilo¹, F. Vallefuoco³, J. Espadaler¹

¹Innovation Department. AB-BIOTICS SA. Sant Cugat del Valles, Spain. ²Pediatric Department. Azienda Sanitaria Locale Caserta, Distretto N°21. SM Capua Vetere. Italia. ³Medical Department. ADL Farmaceutici. Casalnuovo di Napoli. Italia.

Background/Aims: Probiotic interventions are gaining clinical evidence for the treatment of infant colic and other functional gastrointestinal diseases (FGIDs). However, patient characteristics facilitating or preventing response to probiotic intervention in colicky babies have not been studied.

Methods: Prospective, observational trial in babies diagnosed for colic and/or functional constipation, and initiating treatment with an oil suspension containing *B. longum* CECT7894 and *P. pentosaceus* CECT8330 ($\geq 10^9$ cfus daily). Exclusion criteria included preterm delivery, antibiotic or probiotic use within 2 weeks of enrollment, and concomitant acute or chronic medical conditions. Severity of colic, constipation and other FGIDs was rated by pediatricians on a 5-point Likert scale each, at baseline and after 2 weeks. Parental anxiety was measured with the GAD-7 scale. Effect of patient characteristics was assessed by multivariate linear regression.

Results: 36 babies (64% female, 53% cesarean delivery, age range 1-40 weeks) were available for analysis. 85% had moderate-to-severe colic symptoms, 63% had concurrent functional constipation and 45% had other FGIDs. Prevalence of breastfeeding, formula-feeding and mixt feeding were 38%, 38% and 24%. Moreover, 33% of babies had previously failed other treatments for colic symptoms, and 50% of them had at least one parent with anxiety. Colic severity was reduced by 1.4 ± 0.9 points (P < 0.001) and constipation severity was also reduced. Colic improvement was significantly higher in babies with higher baseline scores, and lower in babies having one or more parents with anxiety or also displaying constipation (multivariate R²= 0.55, individual factors p-values ranging 0.049 to < 0.001). Type of feeding, mode of delivery, gender, body weight and previous failure of other colic interventions did not influence the change in colic severity in babies taking this particular probiotic formula.

Conclusions: Baseline severity, concomitant FGIDs and parental anxiety can influence treatment response in babies receiving a probiotic intervention for infant colic.

ITS-SEQUENCING REVEALS ALTERATIONS ON THE ABUNDANCE OF SPECIFIC GUT *BIFIDOBACTERIUM* POPULATIONS IN PREMATURE BABIES

S. Arboleya^{1,2}, C. Milani³, N. Fernández^{2,4}, M. Suárez^{2,4}, C.G. de los Reyes-Gavilán^{1,2}, G. Solís^{2,4}, M. Ventura³, M. Gueimonde^{1,2}

¹Microbiology and Biochemistry Department, Dairy Research Institute of Asturias (IPLA-CSIC), Villaviciosa-Asturias, Spain.

²Diet, Microbiota and Health Group, Health Research Institute of Principado de Asturias (ISPA), Oviedo, Spain. ³Department of Life Sciences, University of Parma, Parma, Italy. ⁴Pediatrics Service Department, Central University Hospital of Asturias (HUCA-SESPA), Oviedo, Spain.

Background/Aims: The correct gut microbiota colonization at the beginning of life is a key event for the foundation of early and future health. This process is compromised in premature babies due to different aspects: immaturity, long hospital stay, medications, difficulties for oral feeding, etc. Several studies have identified the alterations on this process, being *Bifidobacterium* one of the most affected microbial groups. *Bifidobacterium* is one of the first colonizers and dominant in the intestinal microbiota of breast-fed healthy babies. Moreover, some strains are widely used as probiotics, with premature babies being among the population groups that could benefit more from the development of probiotics for promoting a correct microbiota establishment. Here we aimed at characterizing the colonization and development of bifidobacterial microbiota in premature babies exposed to different perinatal factors.

Methods: Faecal samples were collected at 2, 10, 30 and 90 days of life from 40 premature- and 40 full term-babies (for comparison). DNA was extracted, used for PCR-amplification of the ITS region, submitted to next-generation-sequencing and the sequences were annotated against an improved bifidobacterial ITS database (Milani et al. 2014).

Results: We found noticeable differences in the abundance of bifidobacterial species between premature and full term babies along the first three months of life. Among the different perinatal factors studied, delivery mode and feeding, further affected the composition of bifidobacteria in premature babies.

Conclusions: ITS region allowed to monitor the gut bifidobacteria colonization during the first months of life. This work confirm that different perinatal factors affect the microbiota development in preterm babies and extends this observation to the specific influence on the bifidobacterial microbiota. This would allow improving the development of bifidobacteria as probiotic for promoting a correct gut microbiota colonization in these infants.

SURVEY ABOUT KNOWLEDGE AND USE OF PROBIOTICS AND PREBIOTICS BY PEDIATRICIANS

C. Rodríguez, M. Zeferino, S. de Lucas, L. Torres, G. Álvarez-Calatayud, C. Sánchez, M. Tolín, C. Miranda, J. Pérez-Moreno

Pediatric Gastroenterology, Hepatology and Nutrition Unit. Gregorio Marañón University Hospital. Madrid, Spain.

Introduction: The contribution of probiotics, prebiotics and symbiotics to the organism allows to reinforce a healthy balance between microorganisms that form intestinal microbiota. Therefore, given their beneficial effects on nutrition and health, health professionals tend to use them more frequently. The aim of the study was to establish the general knowledge of physicians and nurses specialized in pediatrics about prebiotics, probiotics and symbiotics, as well as to determine their use in routine clinical practice.

Method: The Spanish Society of Probiotics and Prebiotics (SEPyP) developed an online survey in November 2017 that consisted of 14 questions about the relationship between breast milk modulatory nutrients, infant milk formulas and intestinal microbiota. The survey was sent by email to 6134 pediatricians and pediatric nurses registered in the database of the course sponsored by Nutricia: "Nutrition in Pediatrics", obtaining a total of 537 responses, received in two rounds. All data were analyzed with the statistical package SPSS.

Results: 75% of the surveyed professionals were pediatricians, of which 52% belonged to primary care. 86% knew correctly the definition of probiotics and a 74% knew the one of prebiotics. However, 5% confused the two terms. Up to 31% of professionals were unaware that both compounds are present in breast milk. An 87% of the participants used probiotics in their clinical practice. The most commonly used forms of administration were pharmacological preparations and infant formulas (30% and 21% respectively). 52% used prebiotics frequently, mainly pharmacological preparations. Only 40% of the participants used symbiotics in their clinical practice, though just 79% of the participants could identify them correctly.

Conclusion: Despite the fact that most of professionals dedicated to Pediatrics know, distinguish and use probiotics and prebiotics, there is still a great lack of knowledge about the fundamental concepts of the relationship between the microbiota and health, as is the case with breastmilk. As a positive data, 99% of these professionals consider it necessary to continue receiving training on intestinal microbiota, probiotics and prebiotics.

Conflict of interest: This work has been funded by the company Nutricia-Danone.

THE INVOLVEMENT OF THE PEDIATRICIAN IN EMERGENCIES, ESSENTIAL IN THE ADHERENCE TO THE RECOMMENDATION OF PROBIOTICS

M.I. Lostal¹, R. Fernando¹, R. Garcés¹, A.K. Andrés¹, M. Baranguan¹, B. Castán¹, P. Caudevilla¹, R. García Romero²

¹Pediatric Emergency Department. Hospital Royo Villanova. Zaragoza. Spain. ²Pediatric Gastroenterology Department. Hospital Miguel Servet. Zaragoza. Spain.

Background: Probiotics are not funded products, whose acquisition depends on the recommendations of the pediatrician, being very important their training and involvement for information to families. Despite the scientific evidence, its recommendation is not done systematically, and the consequences are worse health outcomes, increased use of human resources and health care cost. The objective of the study is to verify the adherence to the recom-

mendation of probiotics, in a pediatric emergency service, participating in a project to improve the quality of care by systematically recommending them.

Methods: Quality improvement project.

- All children 0-14 years old, attended in Pediatric Emergency, for infectious pathology that equals oral antibiotherapy and GEA. Recommendation in writing of probiotics.
- Professionals: 7 pediatricians of the Emergency Department.
- Start of the project: Noviembre 2017, continues in active.
- Verification of adherence to the recommendation through a telephone survey of 192 randomly selected families.

Results:

- Probiotic acquisition 173/192 (90,1%). 19 cases have not acquired the most frequent cause not consider it necessary or unavailable in pharmacy. The price was the cause at 26%.
- In 90.1% the recommended probiotic was acquired. In 11 cases, another was acquired on the recommendation of the pharmacist. The price was only in 1 cases the reason of the change.
- 18% did not complete the treatment. The most frequent cause (71%) was not considered necessary and 29% difficulties for its administration to the child.

Conclusions:

- High adherence to the recommendation (90%).
- Good filling (82%).
- False idea that the price hinders the acquisition of the product.
- The involvement of pediatricians in the recommendation of probiotics is essential to improve the quality of care in the emergency department.

USE OF GUTALIVE® AND ITS IMPACT ON THE STANDARDIZATION OF DOWNSTREAM MICROBIOTA-BASED STUDIES

N. Martínez¹, C. Hidalgo^{1,2}, A. Margolles^{1,3}, S. Delgado^{1,3}, B. Sánchez^{1,3}

¹Microviable Therapeutics, SL. Oviedo, Asturias, Spain.

²Department of Food, Bioprocessing and Nutrition Science, North Carolina State University, Raleigh (NC, USA).

³Departamento de Microbiología y Bioquímica de Productos Lácteos. Instituto de Productos Lácteos de Asturias. Consejo Superior de investigaciones Científicas. Villaviciosa, Spain.

Aim: The aim of this work was to evidence that the use of different stool collection strategies has a deep impact on the viability and diversity of the fecal microbiota that is recovered in the laboratory, notably if oxygen toxicity is not considered. This is particularly critical if the purpose of the sample is to envisage personalized biotherapeutic purposes, such as autologous fecal microbiota transplant or designing personalized biotherapeutics.

Methods: In order to analyze differences in the viability of fecal microbial populations during the whole delivery procedure, the same fecal specimens were sampled in conventional stool containers and GutAlive® devices, which minimize exposure of fecal microbiota to oxygen. Samples from five healthy donors were used and 150 differential colonies between the two devices were recovered. Differences included those associated to the two collection devices

and individuals. All colonies were identified by 16S rRNA gene sequencing. Complete genomes of two extremely oxygen sensitive (EOS) bacteria recovered were sequenced with the Illumina MiSeq Sequencing System.

Results: Microbial diversity obtained was notably higher using GutAlive®. This device was able to maintain the viability of EOS species such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii* and a possible new member of the *Clostridiales* order. These obligate anaerobes were not recovered using the conventional stool container. Remarkably, GutAlive® allowed culturing and identifying an anaerobic isolate which may represent a new lineage within *Clostridiales*. This shows the importance of a personalized approach in microbiota-based therapies, as this novel isolate was recovered only from feces of one of the donors.

Conclusion: Using GutAlive® for stool sampling and transport allowed higher recovery of viable EOS bacteria by limiting oxygen exposure during the whole process. By standardizing the sampling and transport of the fecal specimens to the lab, GutAlive® can be applied for the normalization of microbiota-based studies, analysis and developments.

Competing interests: NM is a full-time employee of Microviable Therapeutics; CH-C, SD, AM and BS are co-founders and SAB members of Microviable Therapeutics, and also co-inventors on at least one patent regarding microbiome stool collection kit and applications.

SCREENING OF LACTOBACILLI STRAINS OF HUMAN ORIGIN CANDIDATES FOR THE PREVENTION OF URINARY TRACT INFECTIONS

E. Simón¹, E. Astó², E. Navarro²

¹Marketing Department. Stada. Sant Just Desvern, Spain.

²Innovation Department. AB-Biotics SA. Sant Cugat del Valles, Spain.

Background/Aims: Intestinal and vaginal pathogen reservoirs are thought to play a key role in recurrent urinary tract infections (rUTIs) in women. Preventive probiotic interventions are gaining interest to avoid excessive antibiotic use, and disruption of the normal microbiota. We sought to identify and characterize candidate probiotic strains capable of reducing pathogen reservoirs for the prophylaxis of rUTIs.

Methods: Screening for survival in in vitro simulated gastric (pH 3), intestinal and vaginal conditions, antagonistic capacity against urinary pathogens, presence of bacteriocins genes and resistance to specific antimicrobial agents was performed on *Lactobacillus* isolates from human origin and compared with *L. rhamnosus* ATCC 55826 reference strain (vaginal isolate).

Results: *L. plantarum* strains CECT8675 and CECT8677 displayed the best characteristics. They showed similar growth to *L. rhamnosus* ATCC55826 strain under simulated vaginal and intestinal conditions, but increased survival to simulated gastric conditions ($p < 0.05$ and $p < 0.10$, respectively). A significant decrease in growth was observed for rUTI pathogens *E. coli* UPEC, *P. mirabilis* S. saprophyticus, *K. pneumoniae* and *E. faecalis* when co-cultured with the supernatants of candidate probiotic

strains, both unadjusted and adjusted to the same pH as control media (pH 6). The pathogen inhibition profile differed for these two candidate probiotic strains. Our results showed inhibition not only due to lactic acid, but also to the presence of different plantaricin genes: *plnEF* in *L. plantarum* CECT8675 and *plnW* in *L. plantarum* CECT8677. Moreover, CFU/mL counting of *E. coli*, *S. saprophyticus*, *P. mirabilis* and *K. pneumoniae* after co-incubation showed not only a bacteriostatic effect, but also bactericidal activity after 24 hours of co-incubation (higher than *L. rhamnosus* ATCC55826). Finally, both *L. plantarum* strains showed a safe antibiotic resistance profile according to ISO 10932:2010 IDF 2010.

Conclusions: Candidate strains *L. plantarum* CECT8675 and CECT8677 deserve further evaluation in clinical studies for rUTI prophylaxis.

SELECTION AND VALIDATION OF LACTOBACILLUS RHAMNOSUS CECT 8800 AS AN OPTIMAL PROBIOTIC STRAIN FOR VAGINAL APPLICATIONS

E. Chenoll^{1*}, I. Moreno^{2,3*}, M. Sánchez¹, I. Garcia-Grau^{2,4}, A. Silva¹, M. González-Monfort³, S. Genovés¹, F. Vilella², C. Simón^{2,3,4,5}, C. Seco-Durbán⁶, D. Ramón¹

¹Biopolis SL/Archer Daniels Midland. R&D Department. Paterna, Valencia, Spain. ²Igenomix Foundation-Instituto de Investigación Sanitaria Hospital Clínico (INCLIVA). Valencia, Spain. ³Igenomix SL, Research Department. Paterna, Spain. ⁴Department of Obstetrics & Gynecology, University of Valencia. Valencia, Spain. ⁵Department of Obstetrics & Gynecology, School of Medicine, Stanford University. Palo Alto, CA, USA. ⁶Ferring SAU. Madrid, Spain. *These authors contributed equally to this work and are considered joint first authors.

Background/Aims: Bacterial strains (normally lactobacilli) vaginally administered have been extensively tested on the treatment of different vaginal conditions, although the selection and characterization of these bacterial strains are commonly based on poor criteria. The aim of these work was to perform a comprehensive analysis of the optimal strain to be selected for this purpose.

Methods/Results: Forty-four strains were obtained out of fourteen samples of healthy women's vaginal swabs. Molecular identification of each strain was performed by RAPD and 16S rRNA sequencing. Six of these strains were lactobacilli from species *L. casei*, *L. jensenii* and *L. rhamnosus*. In addition, collection strains *L. crispatus* CECT 4840 and *L. iners* DSM 13335 were included in this study. First, pH reduction was measured in pure and co-cultures of these strains and strains *L. rhamnosus* CECT 8800 and *L. casei* BPL013 were selected for further studies. Both strains are producers of short chain fatty acids (SCFAs), being *L. rhamnosus* CECT 8800 the most efficient. Further studies with *L. rhamnosus* CECT 8800 regarding protection from pathogens infection of endometrial primary cultures (*G. vaginalis*, *A. vaginae*, *P. acnes* and *S. agalactiae*), quantification of secreted pro-inflammatory cytokines, antibiotic resistance, and resistance to pharma products for IVF treatment and toxicology were performed. *L.*

rhamnosus CECT 8800 resulted as an efficient protector from main vaginal pathogens, reducing the pro-inflammatory cytokines levels produced by a non-lactobacilli dominated microbiota, no carrying genetic information for antibiotic resistance and being resistant to progesterone treatment. *In vitro* cytotoxicity test and *in vivo* retarded hypersensitivity and vaginal mucosa irritation tests were performed with the selected strain showing an optimum safety profile to be used vaginally.

Conclusion: *L. rhamnosus* CECT 8800, is an optimal candidate for vaginal microbiota restorer treatments. The strain has shown to be the most efficient from the group studied in terms of SCFAs production, high pH reduction phenotype, anti-inflammatory and anti-infective properties and excellent toxicological and safety profile.

CREATION OF AN ONLINE PLATFORM FOR THE SPREAD OF KNOWLEDGE ABOUT THE USE OF PROBIOTICS AND PREBIOTICS IN CLINICAL PRACTICE (ELPROBIOTICO.COM)

C. Masdeu¹, X. Tapounet¹, E. Moreno², F. Guarner³, G. Álvarez-Calatayud⁴, J. Pérez Moreno⁴

¹Dpto. Médico., Profármaco S.L. Barcelona, Spain. ²Dpto. Médico., Laboratorios Zambon. Barcelona, Spain. ³Hospital Vall d'Hebron. Barcelona, Spain. ⁴Hospital General Universitario Gregorio Marañón. Madrid, Spain.

Introduction: El Probiótico (www.elprobiotico.com) is a website launched in January 2014. The purpose was to spread information for health professionals about the evidence and clinical practice of probiotics and prebiotics.

Material and methods: Under the coordination of the Scientific Committee (led by the doctors Francisco Guarner and Guillermo Álvarez Calatayud), are published in the portal weekly contents of free access in relation to the topicality of microbiota, probiotics and prebiotics. Since the launch, the offer of programs accredited by the Commission for continuing training of SNS and CCFCPS has been extended periodically, with three major accredited courses currently available: "Probiotic therapy in Gastroenterology", "Scientific evidence and clinical practice guidelines for the use of probiotics, prebiotics and symbiotics" and "Probiotics and prebiotics in health and disease" as well as other monographic review topics.

Results: Five years after the launch of the Web, 123 short articles, 8 review topics and 19 clinical cases, as well as multimedia content and other formats have been published. 25,151 users have been registered on the platform and about 6,200 different people visit each month, with an average time per visit of 4 minutes and 17 seconds. The most consulted contents are those of the formative area, with more than 15,000 evaluation tests carried out among all the users since the launch.

Conclusions: The increase observed in registration and participation statistics the training activities of the portal confirms the efficacy of the Web probiotic as a tool to spread knowledge about the microbiota and the clinical use of probiotics and prebiotics among health professionals and suggests an increase in the interest of this group for these issues in recent years.

EVALUATION OF PROBIOTIC POTENTIAL IN STRAINS ISOLATED FROM HUMAN MILK AND INFANT FECES

Y.N. Correa Holguín¹, O.I. Montoya Campuzano², A. Velásquez Restrepo³

¹Biotechnology Master's. National University of Colombia, Sciences Faculty. Medellín, Colombia. ²Associate Professor. National University of Colombia, School of Biosciences, Sciences Faculty. National University of Colombia. Medellín, Antioquia, Colombia. ³Undergraduate student in Biological Engineering, Sciences Faculty, National University of Colombia. Medellín, Colombia.

Background/Aims: Breast milk is a component of high food value due to the presence of essential nutrients and elements such as: immune cells, immunoglobulins, antimicrobial peptides and microorganisms both diners and beneficial, among these, the Lactic acid Bacteria (BAL) of the genera *Streptococcus*, *Enterococcus*, *Lactococcus*, *Lactobacillus*, *Weissella*, *Bifidobacterium* and *Leuconostoc*, recognized for having probiotic potential, that is to say, that administered in adequate quantities are capable of influence the health of the individual. In Colombia, mainly in Antioquia there are few studies that characterized the natural microbiota of biological fluids such as breast milk and the feces of exclusively breastfed infants, so from the realization of a study in this population, obtained 34 isolates.

Methods: They underwent in vitro tests to evaluate their probiotic potential and were identified using biochemical and molecular techniques.

Results: It was verified that the BAL isolates of both milk and feces have good probiotic potential, resisting hostile conditions of acid pH and bile salts at 0.3%, in addition to good susceptibility to antibiotics and as antagonists against microorganisms pathogens. Of the 10 molecularly identified strains, the best bacteria with probiotic potential of 100% were: *Enterococcus faecium* isolated from breast milk, *Enterococcus faecalis* isolated from the feces of infants from which also isolated a *Lactobacillus rhamnosus* with a potential of 93.75%.

Conclusion: The consumption of breast milk as a first food is very important not only from the nutritional point of view but also microbiological due to the presence of BAL with probiotic capacity but it is recommended to use molecular tests for a total trace of probiotics in both biological sources.

CHANGES CONCERNING TO THE LEVEL OF KNOWLEDGE AND PRACTICES ABOUT PROBIOTICS AMONG ARGENTINE DOCTORS IN THE LAST 5 YEARS

C. Boggio Marzet

Pediatric Gastroenterology & Nutrition Section. Pediatrics Division. Hospital Gral. de Agudos "Dr. I. Pirovano". Buenos Aires, Argentina.

Introduction: Practices about probiotics have changed drastically in the last decade due to increasing knowledge on scientific evidence. In Argentina there are no published data about this topic.

Aim: To evaluate knowledge, attitudes and practices of physicians about probiotics in two periods of time.

Methods: A closed-ended structured questionnaire was implemented in two periods of time (2012-2017) to physicians in the city of Buenos Aires. Target and sample size: 120 and 95 doctors were interviewed (Pediatricians and Gastroenterologists).

Results: More than 50% of doctors are familiar with probiotic use, showing statistically differences between 2012 and 2017 survey (44% vs 69,4%; p 0.0003 Z proportion Test). Probiotic definition according to FAO/WHO criteria was correct in both groups (71,8% vs 75,6%), showing better recognition as a yeast in the 2017 group (p 0.002). Probiotics characteristics were better recognized as to adhering to the mucosa and alive microorganisms in the second group (p 0.01) and *Saccharomyces boulardii/Lactobacillus casei* have been proven to work in acute infectious diarrhea and antibiotic associated diarrhea (39,7% and 21,2%; p 0.004). Although doctors recommend probiotics to their families (42,10%) and themselves (29,4%), this tendency was increased in the last 5 years in both groups (67,3% and 70,5% respectively; p 0.0001). There is a high level of confidence in both groups (83% and 82,2%) considering probiotics safety (80% and 69,3%) and being used in children (66% and 68,3%) with no statistical differences between groups. Reasons for not recommending or prescribing probiotics are: no official guidelines (20,3% and 23%) and not having enough experience (26,8% and 28%). Overall 87% and 82% doctors in both groups read at least 1 article/year about probiotics. Information resources are: pharmaceutical industry (23%), congresses (20,5%) and journals (20,1%) in the first group and congresses (31,5%), internet (20,2%) and journals (18,4%) in the second one, showing statistically differences between the groups (p 0.002; Z proportion Test)

Conclusions: Most doctors feel well informed about probiotics with high level of confidence on their safety. More than 70% recommend probiotics for their relatives or themselves. Lack of information is a key obstacle for not prescribing probiotics even though more than 80% read at least 1 article/year related to probiotics. Continuous medical education is key to promote the use of them and to increase awareness among the scientific community.

Immunonutrition & Veterinarian

COMBINING TWO PROBIOTIC STRAINS WITH OLIGOGALACTOSE ABOLISHES THEIR BENEFITS AGAINST F4+ETEC IN PIGLETS

A. Rodríguez-Sorrento^{1*}, L. Castillejos¹, P. Lopez¹, G.C. Cifuentes², M. Rodríguez-Palmero², J.A. Moreno², S.M. Martín-Orúe¹

¹Servicio de Nutrición y Bienestar Animal, Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona. Bellaterra, Spain. ²Laboratorios Ordesa S.L. Barcelona, Spain.

Background/Aims: This study evaluates the efficacy of combining two probiotic strains (*Bifidobacterium longum* subsp. *infantis* CECT7210 (Laboratorios Ordesa S.L.) and *Lactobacillus rhamnosus* HN001) with oligogalactose to improve their efficacy against enterotoxigenic *Escherichia coli* (ETEC) F4+.

Methods: A total of 96 piglets of 21 days were distributed into 32 pens assigned to 5 treatments: one non-challenge treatment (CTR+) and four challenged: same diet (CTR-), or supplemented with probiotic strains ($> 3 \times 10^{10}$ cfu/kg each) (PRO), prebiotic (5%) (PRE) or their combination (SYN). After one week, piglets were orally inoculated with F4+ ETEC (1.2×10^{10} cfu). Feed intake, weight gain, fecal consistency and rectal temperature were recorded. On days 4 and 8 post-inoculation (PI), one animal per pen was euthanized and samples of blood, feces and tissues collected. Enterobacteria and coliform counts, fermentation products, ileal histomorphology and serum TNF- α and PigMAP were analyzed.

Results: Group PRO performed better after the challenge than SYN, reducing the number of enterobacteria and coliforms in ileal mucosa scrapes. Fermentation was scarcely modified by the treatments. The challenge promoted an important decrease of villous height at day 4 PI similar in all diets. At day 8 PI, PRO fed animals showed improved recovery of villi height (337^a CTR+, 292^{ab} PRO, 272^b PRE, 269^b SYN, 267^b CTR-; $P < 0.001$). Acute phase protein PigMAP was markedly increased by SYN treatment at day 8 PI (0.51^b CTR+, 0.57^b PRO, 0.56^b PRE, 2.43^a SYN, 0.62^b CTR- mg/ml, $P = 0.003$). Pro-inflammatory cytokine TNF- α was also increased by SYN at day 4 PI (86^{ab} CTR+, 98^{ab} PRO, 75^b PRE, 118^a SYN, 77^b CTR- mg/ml, $P = 0.013$).

Conclusion: To conclude, results show that animals receiving the probiotic combination performed better against the F4+ challenge showing a higher recovery of ileal mucosa. However, these benefits disappeared when probiotics were combined with oligogalactose leading to detrimental effects on growth rate and pro-inflammatory markers.

THE USE OF PROBIOTICS IN SWINE: AN ALTERNATIVE FOR PREVENTING DISEASES

R.M. Montoya Moreno¹, T. Pérez Sánchez¹, H. Fuertes Negro¹, J.V. Díaz Cano², I. Badiola Saiz³

¹Enfermedades Infecciosas, Facultad de Veterinaria. Universidad de Zaragoza. Zaragoza, Spain. ²Nutrición Animal. Pentabiol S.L. Galar, Navarra. ³Centre de Recerca en Sanitat Animal (CRESA). Universidad Autònoma de Barcelona. Barcelona, Spain.

The use of probiotics is an alternative to the therapeutic treatment of pathological problems in pigs. The source of many of these disorders lies in an alteration of the intestinal microbiota that may be associated with management changes, such as weaning, since the passage from a liquid to a solid diet besides the suppression of the mother's immune contribution constitute the beginning of any of these alterations.

The mechanisms of action of the probiotic strains are based on competitive exclusion phenomena between these and the invading pathogens, the stimulation of the immune response, as well as the optimization of the use of the nutrients received through the diet.

This study aims to make a first approach to the knowledge of the use of probiotics in the porcine species. For that purpose, a series of experimental tests were designed to be carried out in livestock farms that house the different stages of production (lactation and transition), in order to determine the benefits they bring, such as improvement of production rates or decrease in classic medical treatment.

The said components were administered by means of the formulation of a commercial fermented feed that incorporates these ingredients and that was applied in the aforementioned phases. For sampling, different moments were established according to the objectives set.

The results obtained in the trials conclude that the use of product tested can have positive effects on piglet in peri-weaning by reducing the possible inflammatory effects of the lipopolysaccharides of the Gram-negative bacteria and also increased the natural immune response against the Gram-positive bacteria. Finally, the integrity of the intestinal mucosa was favoured, as seems to be inferred from the increase in the height of the intestinal villi.

FERMENTED DAIRY FOODS: IMPACT ON INTESTINAL MICROBIOTA AND HEALTH-LINKED BIOMARKERS

S. González^{1,3}, T. Fernández-Navarro^{1,3}, C.G. de los Reyes-Gavilán^{2,3}, N. Salazar^{2,3}, M. Gueimonde^{2,3}

¹Department of Functional Biology, Faculty of Medicine. University of Oviedo. Oviedo, Spain. ²Instituto de Productos Lácteos de Asturias, Consejo Superior de Investigaciones Científicas (IPLA-CSIC). Villaviciosa, Spain. ³Metabolism Area. Instituto de Investigación Sanitaria del Principado de Asturias (ISPA). Oviedo, Spain.

Aims: The intake of fermented foods is gaining increasing interest due to their health-promoting benefits. Among them, fermented dairy foods have evidenced association with obesity prevention, and reduction on the risk of metabolic disorders and immune-related pathologies. Fermented foods could lead to these health benefits by providing to the consumer with both, easily metabolizable nutrients and beneficial microorganisms. Our aim was to evaluate the possible relationship between the consumption of fermented dairy products and the intestinal microbiota, serum lipid profile and the pro-oxidant/inflammatory status.

Methods: 151 healthy adults (age 57.9 ± 17.4 y) were evaluated. Dietary fermented food intake was assessed by an annual

food frequency questionnaire (FFQ), including 26 fermented dairy products. Levels of the major phylogenetic types of the intestinal microbiota were determined by qPCR. Serum glucose and lipid profile, as well as serum malondialdehyde (MDA), C-reactive protein (CRP) and leptin levels were determined by standardized protocols.

Results: Among fermented dairy foods, whole natural yogurt (71.0 ± 99.5 g/day), flavoured skimmed yogurt (5.9 ± 32.6 g/day) and flavoured fermented milk (2.8 ± 23.0 g/day) were the most consumed. While whole natural yogurt showed a positive association with fecal levels of *Akkermansia* and *Bacteroides* group, skimmed yogurt intake was inversely related to every analysed microbial group. Yogurt consumers showed higher levels of *Akkermansia* and *Lactobacillus* group than non-consumers. Furthermore, CRP serum levels were significantly lower in yogurt consumers.

Conclusion: Yogurt consumption was associated with higher fecal levels of certain microorganisms such as *Bacteroides* and *Akkermansia*, for which health benefits have been reported. Also, yogurt consumers showed lower CRP concentrations pointing to the need of exploring, through human intervention studies, the possible anti-inflammatory effect of these foodstuffs.

MODULATION BY *LACTOBACILLUS RHAMNOSUS* GG OF MICROBIOTA AND INNATE IMMUNE FUNCTIONS IN CHRONOLOGICAL OLD AND ADULT PREMATURE AGEING MICE

T. Requena¹, P. Hernández², B. Arauzo¹, N. Ceprian^{2,3}, M. De la Serna², M.C. Martínez-Cuesta¹, C. Peláez¹, M. De la Fuente^{2,3}

¹Department of Food Biotechnology and Microbiology. Institute of Food Science Research (CIAL-CSIC). Madrid, Spain.

²Department of Genetics, Physiology and Microbiology. Faculty of Biology. Complutense University of Madrid (UCM). Madrid, Spain.

³Institute of Research Hospital 12 de Octubre. Madrid, Spain.

Background and Aims: Ageing is characterized by an impairment of the homeostatic (nervous and immune) systems and by changes in the intestinal microbiota. The objective of the study was to analyse differences in the gut microbiota and in several innate immune functions in old and prematurely ageing mice (PAM) when compared with adults, and the modulatory effects of supplementing with *Lactobacillus rhamnosus* GG.

Methods: Prematurely ageing mice (PAM), old and adult mice (females ICR-CD1) were divided into two groups (N = 8-10), which received LGG (10^9 CFU/mice/day; lyophilized in skim milk and added to drink water) or skim milk without the probiotic LGG (controls). After 4 weeks of LGG supplementation, mice were analysed for several innate immune parameters (chemotaxis, phagocytosis and natural killer activity). Faecal samples were collected for microbiota analysis at 0, 2 and 4 weeks of LGG supplementation.

Results: Ageing in ICR-CD1 female mice was related with increase of *Lactobacillus* and *Blautia coccoides/Eubacterium rectale* and decrease of *Akkermansia*. LGG supplementation was related

with changes in *Bifidobacterium*, *Faecalibacterium* and *Ruminococcus* counts. PAM and old mice showed lower values of the immune functions analysed than adult mice. After 4 weeks of LGG ingestion all these functions improved, achieving values similar to those in adults.

Conclusion: *L. rhamnosus* GG can be considered a probiotic supplement useful for upgrading the immune system in ageing and, consequently, for reaching a healthy longevity.

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IMPROVEMENT IN PRODUCTIVE AND HEALTH INDICATORS IN IBERIAN PIGS SUPPLEMENTED WITH INGUBAL®

M. Bravo^{1,2}, R. Cerrato², W.L. García-Jiménez², D. Risco², P. Gonçalves², V. Arenas², J. Femia², J. Rey¹, P. Fernández-Llario²

¹Animal Health Department, Faculty of Veterinary, University of Extremadura, Spain. ²Innovación en Gestión y Conservación de Ungulados & Ingulados Research S.L., Spain.

Background: During the last years, the introduction of probiotics into the field of animal production is receiving great interest, given the constant changes in the livestock sector. Thus, the need to enhance the productivity to satisfy the increasing demand in the animal products together with the emergence of infectious diseases negatively affecting the production and the restrictions on the use of antimicrobials has led to the search for new approaches. Ingubal® is a fermented supplement containing yeast and different strains of lactic acid bacteria with proven probiotic properties and granted QPS status.

Aim: The objective was to assess the effect of Ingubal® as an alternative to the use of antimicrobials in animal feed.

Methods: Two homogeneous groups of Iberian pigs were formed according to age, sex and health status. The study group was fed with a standard feed supplemented with Ingubal® from birth to weaning and the control group received only the standard feed. Whole blood and serum were collected for complete blood count and biochemical parameters determination. Faecal swabs were collected for counting total mesophilic and lactic acid bacteria. Iberian pigs were weighed at different time points to estimate the effect on final weight and average daily gain (ADG).

Results: Our most important findings show that ADG was significantly higher in supplemented Iberian pigs. In this group, higher haematocrit, haemoglobin count, mean corpuscular volume and mean corpuscular haemoglobin were found but there were no differences in the erythrocyte count regarding the control group. A lower white blood cell count was also found in the study group.

Conclusions: These results show that the use of fermented feed with probiotic action (Ingubal®) has led to an improvement in the productive and health indicators, thereby favouring an improvement on farm profitability.

**LACTOBACILLUS RHAMNOSUS MP01 AND
LACTOBACILLUS PLANTARUM MP02 PREVENT
GASTROINTESTINAL INFECTIONS IN CANINE
PUPPIES AFTER WEANING**

L. Fernández¹, R. Ramos^{2,3}, M. Pérez³, R. Arroyo³, J.M. Rodríguez³

¹Sección Departamental de Tecnología Alimentaria. Facultad de Veterinaria. Universidad Complutense de Madrid. Madrid, Spain.

²Clínica Veterinaria Galileo. Madrid, Spain. ³Departamento de Nutrición y Ciencia de los Alimentos. Facultad de Veterinaria. Universidad Complutense de Madrid. Madrid, Spain.

The probiotic potential of *Lactobacillus rhamnosus* MP01 and *Lactobacillus plantarum* MP02, two strains isolated from canine milk, was evaluated through different assays, including survival to conditions similar to those found in the canine gastrointestinal tract, production of antimicrobial compounds, adherence to intestinal

mucin, degradation of mucin, and pattern of antibiotic sensitivity. Globally, both strains showed a high *in vitro* probiotic potential. Subsequently, acute and repeated doses toxicity was tested in a rat model, which confirmed the safety of the strains. Finally, a clinical trial was performed to evaluate the potential of the strains to prevent episodes of infectious gastroenteritis when administered for 2 months to 1-month-old puppies. A group (n= 12, 6 males and 6 females) of German shepherd puppies received *L. rhamnosus* MP01 while a second group (n= 12, 6 males and 6 females) received *L. plantarum* MP02. The same trial was performed with Yorkshire puppies and one placebo group for each breed (German shepherd, n= 12; Yorkshire, n= 12) was also included. The results showed that administration of the strains was associated to a significant reduction of gastroenteritis episodes in both breeds. This preventive effect was associated to statistically-significant increase in the populations of lactobacilli and Faecalibacterium spp., and in the fecal concentration of butyrate, acetate and propionate. Probiotic treatment had no statistically-significant effect neither on the body weight of the puppies nor on the fecal IgA concentration, when compared with the same parameters in the control group.

Microbiology

MICROBIOLOGIC TECHNOLOGY WORKS FOR CLEANING AND DISINFECTING

C. Castrillo, E. Vianna Sosa

Alter-Entorn SL. Mora de Ebro, Tarragona, Spain.

Aims: Microbiologic technology works for cleaning and disinfecting.

Methods: Trials were performed in triplicate and for each of the microorganisms separately. The chemical cleaning product was sodium hypochlorite, which is used routinely in daily cleaning. After performing each application methodologies for products section C, we proceeded to the collection of samples from the surfaces of the tiles in order to proceed to the quantitative evaluation of the presence of the pathogenic strains inoculated in each case and the remaining microorganisms. The culture media and conditions used, were the traditional environmental tests in microbiology and medium surfaces. The method for evaluating the presence of microorganisms were by contact plates or plates Roda.

Results: Microbes cleaners are good for cleaning and disinfecting. When cleaning with microbes' cleaners you have obviously more microbes left (10) in the surface than cleaning with a chemical (4) in time 0. But in time 24H once you introduce pathogens, the growth is more important in the chemical (30) cleaned surface than the one with microbes (12).

Conclusion: We learn that even using chemicals surface is never 100% free of microbes. After 24 h microbiology content on the surface are in all cases higher than before. Pathogens develop quicker when the surface has been previously cleaned by a chemical than a microbe cleaning product. It means that pathogens find less resistance and then develop quicker. The persistence of bacteria of the genera *Lactobacillus* or *Bacillus* Kocuria (included in the cleaner product formulation) does not determines a negative effect. When doing a recount, we need to use a system for detecting bacteria that facilitates screening of these groups of microorganisms considered capable of controlling pathogens, as has been shown in studies in vivo even with both men and animals.

Study done by Dr. Maria Angel Calvo (Barcelona University, Microbes Department).

COMPARISON OF DIFFERENT MODES OF REGULATION IN EXPRESSION OF DEXTRANSUCRASE FROM *LEUCONOSTOC LACTIS* AV1N AND *LACTOBACILLUS SAKEI* MN1 STRAINS

N. Besrouir^{1,2}, M.L. Mohedano¹, I. Fhoula², K. Zarour¹, A. Prieto¹, A. Najjari², I. Ouzari², P. López²

¹Department of Microbiological and Plant Biotechnology. Biological Research Center (CSIC). Madrid, Spain. ²LR03E503 Laboratoire Microorganismes et Biomolécules Actives, Faculté des Sciences de Tunis, Université de Tunis El Manar, Tunis, Tunisia.

Introduction: Lactic acid bacteria (LAB) dextransucrases, encoded by *dsr* genes, synthesise dextrans. The demand for "natural food" has led to increased use of dextrans for the development of functional foods, as they improve food texture and have immunological and antiviral properties.

Methods: In this work, the dextran-producing *Leuconostoc lactis* AV1n, isolated from avocado, was investigated. The polymer was purified from culture supernatants, its structure determined by methylation, FTIR and GC analyses, and quantified by the phenol-sulphuric method. The putative *P_{dsrLL}* promoter and *dsrLL* (pRCR20) or *P_{dsrLL}* alone (pRCR21), were cloned into the pRCR plasmid upstream of *mrfp*, the mCherry coding gene. Plasmids were established in *Lactococcus lactis*, transferred to other LAB by electroporation, and mCherry fluorescence was then measured spectrophotometrically.

Results: Upon transfer of pRCR20 to the dextran non-producing *Leuconostoc mesenteroides* CM70, the bacterium became red due to the mCherry expression and produced dextran, confirming that the *dsrLL* encodes the AV1n dextransucrase. The production of dextran in AV1n was temperature-dependent and in the presence of sucrose, reached ten-fold higher levels at 20°C than at 37°C. Influence of temperature and carbon source in gene expression was monitored by measurement of the mCherry levels in AV1n[pRCR21]. Furthermore, expression from the *P_{dsrLS}* promoter of the dextran-producing *Lactobacillus sakei* MN1, was investigated. Thus, AV1n carrying the pRCR15 plasmid, with the *P_{dsrLS}-mrfp* fusion, was also analysed. The results confirmed an induction of expression from *P_{dsrLL}-mrfp* at low temperature in the presence of sucrose, glucose, maltose or fructose. Sucrose also induced expression from *P_{dsrLL}*. However, the fusion *P_{dsrLS}-mrfp* was activated by increasing the temperature (20-37°C) in the presence of sucrose, though no influence of temperature was detected when exposed to other sugars.

Conclusion: Two types of regulation of expression of LAB dextransucrases have been identified, suggesting two different mechanisms for environmental adaptation.

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CHARACTERIZATION OF PROBIOTIC PROPERTIES OF *LACTOBACILLUS SALIVARIUS* PS7

N. Cárdenas¹, V. Martín², R. Arroyo³, C. Badiola⁴, J.M. Rodríguez³

¹Probisearch SLU. ²Centro Nacional de Microbiología, Instituto de Salud Carlos III. Majadahonda, Madrid. ³Departamento de Nutrición y Ciencia de los Alimentos. Universidad Complutense de Madrid. ⁴Departamento Médico e I&D de Casen Recordati. Pozuelo de Alarcón, Madrid.

Background/Aims: *Lactobacillus salivarius* is a well-characterized bacteriocin producer frequently isolated from human gastrointestinal tracts (GIT), milk, female genital tract and other sources. Several strains have gained attention as promising probiotics due to their ability to modulate gut microbiota, produce antimicrobial substances, stimulate protective immune response, inhibit faecal enzymatic activity and produce short chain fatty acids allowing an advisable acidification of the gut, among others.

Hence, the aim of this study was to isolate, identify and characterize a *Lactobacillus salivarius* strain with potential probiotic activity.

Methods: *In vitro* studies including survival after transit through an *in vitro* gastrointestinal model, assays of adhesion to epithelial cell lines, production of beneficial molecules and antimicrobial activity were performed. Furthermore, a safety characterization, including metabolic activities, antibiotic resistance and animal toxicity assays in rat models were conducted.

Results: The strain identification was confirmed by sequencing the 16S rRNA and by MALDITOF. *L. salivarius* PS7 was deposited in the Colección Española de Cultivos Tipo (CECT) and named *L. salivarius* CECT 9422. The viability of the strain after exposition to conditions simulating those found in the gastrointestinal tract was high. The strain was susceptible to antibiotics and did not produce histamine, tyramine, putrescine or cadaverine. The *in vivo* acute and repeated dose ingestion study demonstrated a lack of mortality and morbidity after the inoculation of rats with the PS7 strain. This strain has shown to exert a high antimicrobial activity against *Alloiococcus otitis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*, regular causal agents of acute otitis media (AOM). PCR analysis revealed that this strain contains structural genes of bacteriocin Abp-118 in its genome.

Conclusion: Our results suggest that *L. salivarius* PS7 should be considered as probiotic strain and a potential alternative for the prevention of AOM.

ANALYSIS OF SOCIAL AND MICROBIOLOGICAL FACTORS IN THE MEDELLÍN MILK BANK POPULATION TO STRENGTHEN PUBLIC LACTATION POLICY

D.F. García Vanegas¹, O.I. Montoya Campuzano², Y.N. Correa Holguín³

¹Undergraduate student in Political Science, Faculty of Human and Economic Sciences, National University of Colombia. Medellín, Colombia. ²Associate Professor. School of Biosciences, Faculty of Sciences. National University of Colombia. Medellín, Antioquia, Colombia. ³Master in Biotechnology. Faculty of Sciences, National University of Colombia, Medellín, Colombia.

Introduction: In the early stages of life, in Colombia, malnutrition and morbidity-mortality is an evident problem, therefore, it is appropriate to conduct impact research, thus generating a synergy academy-society. For the integral development of the individual in the physiological and psycho-social, lactation is critical, for the nutrients that strengthen the digestive and immune system, and provides a beneficial microbiote to the intestine of the infant, including Lactic Acid Bacteria.

Background: The last report of the bank Hospital General de Medellín (HGM), demonstrated the effectiveness of lactation by reducing the incidence of Necrotic Enteritis from 3.33% in 2015 to 1.8% in 2017, evidencing the importance of orienting public policies that allow to increase the indicators of breastfeeding.

Objective: To relate breastfeeding with sociocultural and biological mother-child factors that allow identifying critical variables that contribute to the discussion on public policies, being the experience of the Brazilian network of Human Milk Banks a reference.

Methods: The population and microbiological parameters of 67 donors and their infants were used, both external and those of the HGM bank, carried out by researchers from the National University of Colombia-Medellín. The databases were analyzed with the variables obtained through surveys, with the R software.

Results: The critical variables were socioeconomic factors, exclusive breastfeeding for 3 months and vaginal births in 63%, evidence the decrease of cases in these variables to the detriment of breastfeeding.

Conclusion: It was proven that social and microbiological factors do influence lactation and consequently in the formulation of public policies, highlighting milk banks as a facilitating tool for the fulfillment of the goals established in the different national plans for health promotion and prevention.

A FECAL-CULTURE MODEL, MONITORING GAS PRODUCTION, FOR ASSESSING PREBIOTICS' FERMENTABILITY IN NORMAL-WEIGHT AND OBESE SUBJECTS

A. Nogacka^{1,2}, N. Salazar², A. Endo³, A. Suárez², C. Martínez-Faedo², C.G. de los Reyes-Gavilán^{1,2}, M. Gueimonde^{1,2}

¹Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias (IPLA-CSIC). Villaviciosa, Spain. ²Instituto de Investigaciones Sanitarias del Principado de Asturias (ISPA). Oviedo, Spain. ³Department of Food and Cosmetic Science, Tokyo University of Agriculture. Abashiri, Japan.

Background/Aims: The gut microbiota is altered in different conditions, which leads to the interest in prebiotics to restore it and to identify the best suited compounds. The use of fecal culture models provides an interesting strategy, allowing to identify prebiotics with suitable fermentation profiles, and appropriate microbiota-modulation properties, for each population. Here we aimed at comparing the fermentability and microbiota-modulation ability of different prebiotics, including the novel prebiotic 1-Kestose, in normal-weight and obese adults.

Methods: Fresh fecal samples from 9 normal-weight (BMI < 25 kg/m²) and 10 obese (BMI > 40) volunteers were collected, transported to the laboratory (under anaerobic conditions), homogenized, diluted (10% v/v) into a carbohydrate-free basal medium and stabilized at 37°C in an anaerobiosis cabinet. The prebiotics tested (Actilight, Synergy, P95, Inulin, GOS and 1-kestose) were added (0.3%, w/v) to the stabilized fecal cultures and incubated at 37°C for 24 hours. Gas production along incubation was monitored in real-time by using the ANKOM RF System. Samples were taken at 0 and 24 hours of incubation for pH measurements, determination of short-chain-fatty-acids by gas chromatography and gut microbiota analyses by qPCR.

Results: In both volunteers' groups kestose resulted the more fermentable prebiotic as indicated by the largest accumulation

of gas and the lowest pH after incubation. On the contrary, inulin was the less fermentable compound. Interestingly, intestinal microbiota from obese individuals tended to show a lower ability to produce gas than that from normal-weight volunteers. The different prebiotics were able to induce changes in microbiota composition in both volunteer groups, although showing differences among them.

Conclusions: The fecal culture model used, with real-time monitoring of gas production, constitutes a fast and easy method for assessing the fermentability of prebiotics in different population groups. 1-kestose showed good characteristics suggesting its applicability as a readily fermentable prebiotic substrate for intestinal microbiota modulation.

IMPROVING ROBUSTNESS OF PROBIOTIC *LACTOBACILLUS PENTOSUS* STRAINS BY ADAPTATION TO EDIBLE OILS

E. Alonso García¹, B. Pérez Montoro¹, J.J. Rodríguez de la Fuente¹, N. Caballero Gómez¹, S. Castillo-Gutiérrez², M.D. Estudillo-Martínez², N. Benomar¹, H. Abriouel¹

¹Área de Microbiología, Departamento de Ciencias de la Salud, Facultad de Ciencias Experimentales, Universidad de Jaén, Jaén, Spain. ²Área de Estadística e Investigación Operativa, Departamento de Estadística e Investigación Operativa, Facultad de Ciencias Experimentales, Universidad de Jaén. Jaén, Spain.

Background/Aims: Probiotic *Lactobacillus pentosus* were isolated throughout fermentation process of naturally fermented Aloreña green table olives. These strains exhibited high tolerance to low pH and high concentrations of bile salts as well as antimicrobial activity against several pathogens. To enhance their probiotic activity against several stresses, pre-adaptation of *L. pentosus* strains was done using edible oils.

Methods: In this study, we determined the effect of edible oils on survivability and stress tolerance of adapted *L. pentosus* strains such as acid and bile tolerance. Further, we analysed stress gene expression in adapted *L. pentosus* strains versus non-adapted *L. pentosus* strains by qRT-PCR.

Results: Seven *L. pentosus* strains were adapted to different edible oils (i.e., sunflower, olive and argan). We determine that survival and growth of probiotic *L. pentosus* strains in the presence of vegetable-based edible oils was dependent on the strain tested and the oil used. However, pre-adaptating the strains to the corresponding oils significantly increased their cell viabilities. As such, we examine whether pre-adaptating probiotic *L. pentosus* strains with oils will improve probiotic properties of *L. pentosus* strains such as tolerance to acids or bile. Improvements in stress resistance were observed in some pre-adapted strains with oils such as acidic and bile conditions; further, pre-adaptations with some oils induced stress gene expression for moonlighting proteins involved in several stress responses and other functions.

Conclusions: These results showed that pre-adaptation with vegetable edible oils may represent a novel approach to enhance robustness of probiotic bacteria improving their stability in probiotic products.

LACTIC ACID IS PARTLY RESPONSIBLE OF THE ANTIPROLIFERATIVE EFFECT OF *L. ACIDOPHILUS* OVER CACO-2 AND HT29 CELLS

B. Sánchez García

IPLA-CSIC. Villaviciosa, Asturias, Spain.

Background: Several independent studies have addressed the beneficial role of lactic acid bacteria over different cancer types, both *in vitro* and *in vivo*. However, and to our knowledge, no molecular study has been performed to understand how this inhibitory effect may take place.

Methods: Different fractions from several lactic acid bacteria and bifidobacteria were obtained in order to test their effect over two well-known epithelial cell lines, Caco-2 and Ht29, using the RTCA device. Effect of this extract over cell cycle and the process of necrosis/apoptosis was checked by flow cytometry. Molecular mechanism of action was elucidated by RNASeq.

Results: The most active fraction was obtained from *Lactobacillus acidophilus*, and RNASeq analysis revealed an activation of several detoxification/xenobiotic pathways in epithelial cells. Nuclear magnetic resonance revealed a quite complex extract with the presence of alanine and lactic acid. Alanine had no effect over proliferation but buffering of the extract suggested that some of the observed inhibitory effect was due to lactic acid. The antiproliferative effect was visible from 300 mg/L lactic acid, and 2,7 g/L were enough to induce complete culture death in Ht29 and Caco-2.

Conclusion: Although it remains to know the role of other compounds in the anti-proliferative effect of the *L. acidophilus* extract, we have shown for the first time that Caco-2 and Ht29 cell lines are sensible to lactic acid concentrations lower than those observed for instance in yoghurt (9 g/L), suggesting that carcinogenic cells may be more sensitive to lactic acid than normal cells. Production of lactic acid in the colon is therefore a molecular mechanism of action by which lactic acid bacteria exert their beneficial role in the colon.

ALPHA DIVERSITY STUDY IN PATIENTS WITH *CLOSTRIDIUM DIFFICILE* INFECTION AND ASYMPTOMATIC CARRIERS

E. Núñez¹, P. Sánchez^{1,2}, B. Ruzafa¹, S. Chumillas¹, Y. Marhuenda¹, J.A. Férrez^{1,2}, L. Navarro¹, V. Navarro^{1,2}

¹Grupo MiBioPath. Departamento de Medicina Clínica. Universidad Católica San Antonio de Murcia (UCAM). Murcia, Spain. ²Unidad de Enfermedades Infecciosas. Hospital Universitario del Vinalopó. Elche, Spain.

Background: *Clostridium difficile* (CD) is a gram-positive, anaerobic, spore-forming bacillus, considered the leading cause of nosocomial diarrhea and pseudomembranous colitis in hospitalized patients. The pathogenicity is associated with the use of antibiotics and a decreased of the immune response, due to age and comorbidities. Intestinal colonization of CD can lead to a situation such

as the absence of symptoms to mild-moderate diarrhea or pseudomembranous colitis cases. The aim of this study is to contrast the alpha diversity of intestinal microbiota in patients diagnosed with symptomatic *CD* infection (CDI) versus asymptomatic colonized by *CD*. The hypothesis is that the colonization of certain groups of key bacteria can be related to the overgrowth of *CD* or the transition from asymptomatic carrier (AC) to patients with CDI.

Methods: Alpha diversity was compared at the genus level using the Shannon and Simpson indexes and the Chao1 and Abundance-based Coverage Estimator (ACE) richness estimators of two groups of patients composed of 15 patients with CDI and 15 asymptomatic carriers of *CD*. The composition of the intestinal microbiota was obtained by amplification of bacterial gene DNA 16S, its massive sequencing and taxonomic allocation of the obtained sequences.

Results: Alpha diversity indexes showed both groups with a medium-low diversity without significant differences (Shannon: CDI = 2.1 vs AC = 1.9; Simpson: CDI = 0.8 vs AC = 0.7). There were no statistically significant differences in terms of species richness (Chao1: CDI = 47.2 vs AC = 51.6; ACE: CDI = 47.2 vs AC = 51.6). Additionally, these results have been compared with a group of healthy people.

Conclusions: Due to similarities in terms of a decreased alpha diversity between CDI and AC groups, it is possible that colonization would pose a risk in the progression from a carrier to an infected state of *CD*.

SUSCEPTIBILITY OF *SERRATIA MARCESCENS* ISOLATES CAUSING NEONATAL SEPSIS TO THE PREDATORY BACTERIA *BDELLOVIBRIO BACTERIOVORUS*

C. Saralegui¹, C. Herencias², J. Rodríguez-Beltrán¹, M. Ponce-Alonso¹, I. Cortés-Prieto³, F. Baquero¹, Á. San Millán¹, A. Prieto², R. del Campo¹

¹Servicio de Microbiología y Parasitología. Fundación Ramón y Cajal de Investigación Sanitaria (IRYCIS). Hospital Ramón y Cajal. Madrid, Spain. ²Centro de Investigaciones Biológicas (CIB). Centro Superior de Investigaciones Científicas (CSIC). Madrid, Spain. ³Facultad de Farmacia. Universidad Complutense de Madrid (UCM). Madrid, Spain.

Background/Aims: *Serratia* sp. are emerging as a leading cause of sepsis in neonatal intensive care units (NICU), usually presenting resistance to multiple antibiotics. The predatory bacteria *Bdellovibrio bacteriovorus* can feed from a broad range of human pathogens, as it has been proven in mammalian cell lines and animal models, being non-toxic and non-pathogenic for host cells. We aimed to assess the susceptibility of *S. marcescens* isolates causing neonatal sepsis to *B. bacteriovorus* predation in order to explore ecological antimicrobial alternatives to antibiotics.

Methods: Eight *S. marcescens* clinical isolates obtained from the NICU of a tertiary hospital were used as preys. Whole genome sequencing was applied to assess the phylogenetic relationship and genotypic determinants of antimicrobial resistance of the isolates. Also, antimicrobial susceptibility testing was performed by the MicroScan Walkaway system. *B. bacteriovorus* HD100 strain was used as predator. Co-culture of prey and predator were performed using a

96-well Bioscreen plate. Thirty μ L of *Bdellovibrio* suspension cells (10^8 or 10^9 PFU/mL) were added to prey cultures in HEPES broth and incubated at 30°C for 48h with shaking. The capability to prey was evaluated by quantifying the amount of reduction in optical density at 600 nm of the cultures. *Pseudomonas putida* KT2440 was used as a positive predation control.

Results: *S. marcescens* isolates were found to be genetically closely related, but not identical. Antimicrobial resistant determinants were found in their genomes, some within mobile genetic elements. Phenotypically, the strains exhibited different patterns of resistance. All *S. marcescens* strains showed similar significant reductions in their population density. However, residual cells of prey were detected in all experiments.

Conclusion: *B. bacteriovorus* efficiently predated *in vitro* *S. marcescens* isolates recovered from preterm neonatal patients affected with late-onset sepsis, regardless their antibiotic resistance profile. Predatory bacteria might be explored as a novel adjuvant to antibiotherapy.

MICROBIOTA OF PRE AND POST-PASTEURIZED HUMAN MILK AND FECES OF INFANTS FROM A MILK BANK

Y.N. Correa, S. Roldán, O.I. Montoya

Microbiología, Investigación y Extensión. Universidad Nacional de Colombia.

Background/Aims: Human milk is considered as an ideal and complete nourishment for newborns and children in the early stages of life. It contains a potential probiotic microbiota derived from the digestive system of the mother that is transmitted by the entero-mammary pathway, which helps with the metabolism of nutrients, maturation of the immune system, competition or antagonism against pathogens, and is also present in the infant feces. However, in some cases infants can't receive it for many reasons, which is why the Human Milk Banks are in charge of the promotion, protection, collection and processing of the aliment, that is donated by mothers both internal and external to the hospital, to be administered to those infants who need it. The microbiota of the pre and post pasteurized human milk from donors of a milk bank and the feces of their infants, was studied.

Methods: 40 samples of breast milk were collected at each processing step in the bank and also 40 samples of infant feces. For the isolation of the microorganisms, serial dilutions were made and then were inoculated in selective culture media according to each of the microorganisms studied.

Results: The isolates with probiotic potential were identified by biochemical tests or molecular analysis. *Lactobacillus brevis* from infant feces and *Enterococcus faecium* from post-pasteurized milk were identified. A reduction in milk microbiota including the potential probiotics was observed after pasteurization.

Conclusion: It was found that breast milk contains an autochthonous microbiota that includes commensal and potential probiotic microorganisms. In addition, it is necessary to implement faster and safer analysis for the identification of Acid Lactic Bacteria (LAB) in human milk to avoid unnecessary discarding of milk in banks.

CHARACTERIZATION OF MICROBIOTA OF HUMAN MILK AND INFANT FECES

Y.N. Correa, P.A. Moreno, O.I. Montoya

Microbiología, Investigación y Extensión. Universidad Nacional de Colombia.

Background/Aim: Human breast milk is a very complex food, enriched by a wide diversity of nutrients and other elements that favor the immune and gastrointestinal system development, among them, a native microbiota coming from the mother's gastrointestinal system through the enteromammary pathway and which will be part of the gastrointestinal tract microbiota of the infant, among these beneficial microorganisms lactic-acid bacteria (LAB), which have potential probiotic species. Having in mind the importance of a good nutrition at the first years of human life and the strong scientific evidence that supports the probiotics as a natural alternative to enhance the nutrient metabolism, the development of the immune system and the antagonism versus pathogens both in nurslings and children under five years old, the probiotics are presented as a better alternative to reduce the antibiotic consumption and thus counteract the increasing resistance to them. The aim is characterize the microbiota of human breast milk and the feces of infants.

Methodology: 27 breast milk and 27 infant feces samples were studied by means of cultivation in selective agars for the microbiota of interest. For the isolates identification, a conventional microbiologic characterization, molecular and biochemical tests and probiotic potential tests were made. The ability to behave as probiotics was evaluated.

Results: The presence with probiotic potential of *Lactobacillus rhamnosus* in colostrum, *Lactobacillus plantarum* in mature breast milk and *Enterococcus faecalis* in infant feces were identified.

Conclusion: The studied strains in this investigation were viable facing the probiotic potential tests, for their ability to resist in vitro gastrointestinal conditions, besides having a good antimicrobial activity versus various pathogens and susceptibility to widely used antibiotics. This opens the door to brand new biotechnological works with probiotic strains obtained from human sources for the benefit of the consumer.

ANTIMICROBIAL ACTIVITY AGAINST HUMAN PATHOGENIC STRAIN OF *STAPHYLOCOCCUS AUREUS* CECT4013 BY *LACTOBACILLUS GASSERI* STRAINS

G.C. Cifuentes, M. Rodríguez-Palmero, J. Jiménez, J.A. Moreno

Basic Research Department, Laboratorios Ordesa S.L. Barcelona, Spain.

Background/Aims: The objective of this work was to isolate, identify and characterize *Lactobacillus* strains from human samples (human milk and feces of breast feeding babies and their mothers) as potential probiotics with antimicrobial activity against *Staphylococcus aureus* CECT4013.

Methods: Three strains of *Lactobacillus gasseri* (ORD0529, ORD0713 and ORD0714), isolated from feces of breast feeding

babies, were selected to assess this activity, and compared with *Lactobacillus salivarius* CECT5713 (positive control), a strain with antibacterial activity against *S. aureus*. Broth inhibitory assays were carried out in order to assess this antibacterial activity using neutralized cell-free culture supernatants (CFCS) and so demonstrate that the antibacterial activity was not due to the production of organic acids. The antimicrobial activity was measured by optical density (OD) and plate counting expressed as CFU/ml.

Results: More than three hundred probiotic strains from human samples were isolated, identified and characterized through several molecular biology techniques, including sequencing of gene 16S RNA, Random Amplification of Polymorphic DNA (RAPD) and also biochemical tests (API 50CH, API ZYM, catalase test, oxidase test, animopeptidase test and Gram stain). Neutralized supernatants of all three selected probiotics showed a significant reduction of growth of *S. aureus* CECT4013 expressed as CFU/ml along the time, being significant at 3 and 6 hours with the neutralized supernatant of *L. gasseri* (ORD0529) compared with all strains tested, included the positive control.

Conclusions: The three strains of *Lactobacillus* isolated from feces of breastfeeding babies, and particularly *L. gasseri* (ORD0529) possess antibacterial activities that results in a reduction of growth of *S. aureus* CECT4013.

GAMMA-AMINOBUTYRIC ACID PRODUCTION BY *BIFIDOBACTERIUM* STRAINS FROM HUMAN ORIGIN

G.C. Cifuentes¹, M. Rodríguez-Palmero¹, D. Bellido², I. Casals², J. Jiménez¹, J.A. Moreno¹

¹Basic Research Department, Laboratorios Ordesa S.L. Barcelona, Spain. ²Unitat de Tècniques Separatives i Síntesi de Peptids. Centres Científics i Tecnològics of Barcelona University. Barcelona, Spain.

Background/Aims: The gut-brain axis (GBA) consists of bidirectional communication between the central and the enteric nervous system, linking emotional and cognitive centers of the brain with peripheral intestinal functions, gut microbiota can influence these interactions. The objective of this research work is detecting probiotic bacteria able to synthesize biochemical compounds capable to interact with the brain through this gut-brain axis. For this purpose, we evaluate the ability to produce gamma-aminobutyric acid (GABA) of a probiotic strain collection, belonging to *Bifidobacterium* and *Lactobacillus* genera, from human milk and feces of breast feeding babies and their mothers.

Methods: A simple test for screening of bacteria with the key enzyme Glutamate decarboxylase (GAD) was made and the positive strains for this enzyme test were grown in medium containing different concentrations of monosodium glutamate (MGS) (0, 20, 30 and 50mg/ml). This experiment was carried out two times in triplicate. GABA production was measured by mass spectrometry.

Results: More than three hundred probiotic strains from human origin were isolated, identified and characterized through several molecular biology techniques and also biochemical tests. 16 strains of *Bifidobacterium* genus were positive for the GAD enzyme, but only 6 strains were able to export GABA, including species of *Bifidobacterium angulatum* and *Bifidobacterium adolescentis*.

Conclusions: GABA production was strain-dependent. *B. angulatum* was the best producing strain of GABA among the range of strains tested. These results provide novel opportunities to consider these strains as functional ingredients.

SCREENING OF GABA PRODUCTION IN MEMBERS OF THE GENUS *BIFIDOBACTERIUM*

L. Ruiz¹, P. Ruas-Madiedo¹, S. Duranti², C. Milani², G.A. Lugli², M. Ventura², A. Margolles¹

¹Department of Microbiology and Biochemistry of Dairy Products. Instituto de Productos Lácteos de Asturias (IPLA-CSIC). Villaviciosa, Spain. ² Department of Chemistry, Life Sciences and Environmental Sustainability. University of Parma. Parma, Italy.

Gamma-amino butyric acid (GABA) is the main inhibitor neurotransmitter in the central nervous system, regulates multiple physiological processes in the human body and its dysfunctions has been linked to anxiety and depression disorders. In recent years, dietary supplementation with GABA has been associated with antihypertensive, analgesic and antidepressant properties and thus there is an increasing interest in identifying probiotic strains with GABA production capabilities, which could act as delivery vectors of this neurotransmitter in the human gut. Several lactic acid bacteria, including lactobacilli and streptococcal strains, have been reported capable to produce GABA, though this activity has been scarcely explored in members of the genus *Bifidobacterium*.

In this work the GABA production capability was studied among members of the genus *Bifidobacterium*. First, an *in silico* analysis was performed on available *Bifidobacterium* genomes, to determine how widespread GABA production genes are within this group of bacteria. Based on the results, a screening of GABA production capability was performed in a collection of 58 *Bifidobacterium* strain, mainly comprised of members of the species *B. adolescentis*. For this purpose, the strains were grown overnight in the presence of the GABA precursor, monosodium glutamate and then, GABA and residual monosodium glutamate concentrations were determined through high-performance liquid chromatography in cell-free supernatants. The presence of the *gadB* and *gadC* genes, encoding a glutamate decarboxylase and a glutamate/GABA antiporter system, respectively, in the GABA producing strains was determined through PCR.

The *gadB* and *gadC* genes were commonly detected in strains belonging to the species *B. adolescentis*. Besides, 25 % of the analyzed strains were capable to transform almost all monosodium glutamate provided, to GABA. In conclusion, GABA production is a relatively common trait in *B. adolescentis* strains, which could represent potential candidates for GABA delivery *in vivo*.

LACTOBACILLUS SALIVARIUS LPM01 (DSM 22150) REDUCES INFLAMMATION IN CELLULAR MODELS OF INTESTINAL EPITHELIUM

R. Vera¹, N. Diaz¹, M.L. Ormeño², M. Acevedo², R. Gimenez¹, J. Badia¹, L. Baldomà¹

¹Departament de Bioquímica i Fisiologia. Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona; Insitute de Biomedicina de la Univesitat de Barcelona – Institut de Recerca Sant Joan de Déu, Spain. ²Inversiones Wellness Technologies Ltda. Concepción, Chile.

Background/Aims: Gut dysbiosis is associated with pathologies that occur with inflammation. Administration of probiotics is a therapeutic strategy to modulate microbiota composition. *Lactobacillus salivarius* LPM01 is a probiotic isolated from human breast milk. Previous studies performed *in vitro* or *in vivo* models have shown that this strain inhibits growth of pathogenic bacteria. Administration of this probiotic also improves health status in immunocompromised people. At present no information is available about the anti-inflammatory effects on human intestinal epithelial cells, an important issue to reinforce its role as a probiotic.

Methods: The intestinal epithelial cell line HT29 was seeded (1×10^5 cells/mL) in 12-well plates and grown for 7 days. Two models were set up to mimic an inflammatory Background: (i) addition of TNF- α (10 ng/ml, 2-h incubation), and (ii) incubation with enteropathogenic *Escherichia coli* (EPEC, MOI 100) for 3h. The probiotic was grown, washed and added to HT-29 culture at a concentration of 1×10^7 UFC/mL. The secreted pro-inflammatory cytokine IL-8 was quantified by ELISA. Probiotic-free supernatant and isolated membrane vesicles (MV) have also been tested. MVs were visualized by Cryo-TEM.

Results: *L. salivarius* LPM01 reduce IL-8 secretion induced by TNF- α when HT-29 cells were first incubated with this cytokine followed by incubation with the probiotic, indicating that this strain may have a curative effect. The analysis performed with HT-29 cells simultaneously incubated with *L. salivarius* and EPEC, showed that this probiotic also significantly reduces the EPEC-induced IL-8 secretion, confirming the protective effect on pathogen-associated inflammation. Addition of MVs or bacteria-free supernatant instead of *L. salivarius* did not reduce IL-8 levels. This effect could not be attributed to a secreted factor.

Conclusion: *L. salivarius* LPM01 displays anti-inflammatory effects. This probiotic reduces IL-8 secretion by 45% when HT-29 cells were treated with EPEC and by 50% when inflammation was induced by TNF- α .

TAXONOMICAL AND FUNCTIONAL CHARACTERIZATION OF THE INTESTINAL MICROBIOTA ASSOCIATED TO OBESITY

M. Gil Fernández¹, R. Del Campo², C. Peláez¹, T. Requena¹, M.C. Martínez-Cuesta¹

¹Department of Food Biotechnology and Microbiology. Institute of Food Science Research (CIAL-CSIC). Madrid, Spain. ²Microbiology Service, Hospital Universitario Ramón y Cajal, IRYCIS. Madrid, Spain.

Background and Aims: The intestinal microbiota has been pointed out as a key factor in obesity, since it contributes to meta-

bolic and immunological homeostasis in the host. The dysbiosis of the gut microbiota linked to obesity has been related to a higher Firmicutes to Bacteroidetes ratio, a lower gene richness and an alteration of the metabolic functions of the gut microbiota [1, 2, 3]. The main objective of this work was to outline the significance of the compositional and/or functional changes in the obese-associated intestinal microbiota.

Methods: The taxonomic composition of the fecal microbiota of obese (Ob) and normal weight (Np) individuals was determined by high-throughput sequencing analysis using the Illumina Seq platform. Amplification of the 16S rRNA gene was carried out using as primers sequences directed towards the V3-V4 regions of the gene. Functional characterization was determined by measuring short chain fatty acids (SCFAs) by High Performance Liquid Chromatography (HPLC) and the ammonium concentration spectrophotometrically.

Results: Data analysis did not show significant differences between Ob and Np individuals in the dominant phyla Bacteroidetes (13%), Firmicutes (76%), Actinobacteria (10%) and Proteobacteria (1.8%). Nevertheless, the proportion of Verrucomicrobia was significantly higher in normal weight individuals (Np). At genus level, *Collinsella*, *Alistipes*, *Clostridium*, *Clostridium* XIVa, *Romboutsia* and *Oscillibacter*, among others, showed significant differences between both groups of individuals. Bacterial diversity according to the Chao1 index was significantly higher in Np individuals. Regarding the functional characterization, the fecal samples of Ob individuals showed the highest concentrations of acetic and butyric acids.

Conclusion: In spite of the great interindividual variability, the microbiota of obese individuals is characterized by a higher or lesser proportion of certain microbial groups and a higher formation of SCFAs in relation to the microbiota of normal weight individuals.

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INTRODUCTION OF MILK CHANGE LACTIC BACTERIA AND PROTEIN METABOLITES IN INFANTS OUTGROWING COW'S MILK ALLERGY

S. Delgado¹, L. Guadamuro¹, M. Díaz¹, S. Jiménez², C. Molinos³, D. Pérez⁴, C. Bousoño², M. Gueimonde¹, A. Margolles¹, J.J. Díaz²

¹Department of Microbiology and Biochemistry of Dairy Products. Instituto de Productos Lácteos de Asturias (IPLA)-Consejo Superior de Investigaciones Científicas (CSIC). Villaviciosa, Asturias, Spain. ²Pediatric Gastroenterology, Hepatology and Nutrition Section. Hospital Universitario Central de Asturias (HUCA). Oviedo, Asturias, Spain. ³Department of Pediatrics. Hospital Universitario de Cabueñes. Gijón, Asturias, Spain. ⁴Pediatrics Service. Hospital Universitario San Agustín. Avilés, Asturias, Spain.

Background: Cow's milk protein allergy (CMPA) is very common in infancy. Currently, the only therapeutic option is a dairy elimination diet. Standardized oral milk challenges are performed each 6 months to determine possible tolerance acquisition.

Aim: To analyze the intestinal changes in feces of infants with non-IgE mediated CMPA after successful milk challenges and introduction of dairy product in their diet.

Methods: Twelve allergic children (between 1 and 2 years old) that were initially consuming extensively hydrolyzed formulas provided stool samples before oral milk challenges, and a week and a month after. Changes in the intestinal microbiota populations were determined by high-throughput sequencing of the 16S rRNA gene, meanwhile diverse microbial metabolites (short chain fatty acids and indoles) were quantified by chromatographic methods.

Results: The introduction of milk in infants with outgrowing non-IgE CMPA increased significantly the levels of fecal lactic acid bacteria, in particular the genus *Lactococcus*. Microbial metabolites derived from the catabolism of proteins, such as escatol (produced from tryptophan) enhanced, meanwhile branched chain fatty acids diminished.

Conclusions: The introduction of dairy products is accompanied by modifications in the infant gut environment through changes in the microbiota and protein metabolic end-products according to this kind of dietary change.

DOES MATERNAL PSYCHOSOCIAL DISTRESS INFLUENCE HUMAN MILK MICROBIOTA?

M. Aparicio¹, P.B. Brown², C. Hechler², R. Beijers², J.M. Rodríguez¹, C. de Weerth², L. Fernández³

¹Sección Departamental de Nutrición y Ciencia de los Alimentos. Facultad de Veterinaria. Universidad Complutense de Madrid. Madrid, Spain. ²Developmental Psychology, Behavioural Science Institute. Radboud University. Nijmegen, The Netherlands.

³Sección Departamental de Tecnología de los Alimentos. Facultad de Veterinaria. Universidad Complutense de Madrid. Madrid, Spain.

Background: Human milk microbiota participates in the correct development of newborn infants as it is the source of a diverse population of commensal bacteria. The microbiota present in human milk depends on different maternal factors such as gestational age, antibiotic therapy, diet, geographical origin and health status. Maternal distress postpartum affects a relatively high number of women. The objective of this work was to assess the evolution of the composition of human milk microbiota and to determine whether maternal psychosocial distress affects this bacterial community.

Methods: Participants (n= 51) of the BINGO study collected milk samples at weeks 2, 6, and 12 and completed questionnaires about maternal stress, anxiety and depression at week 6 postpartum. Bacterial composition and diversity in milk samples was determined by 16S rRNA sequencing (V3-V4 region) using Illumina MiSeq technology.

Results: There was a wide variation in the bacterial profile of individual milk samples. At the phylum level, *Firmicutes* and *Proteobacteria* were found in every sample. *Firmicutes* had also the

highest relative abundance (72% at week 2 and 45% at week 12). Globally, the bacterial diversity in milk samples, measured as the Shannon diversity index, increased over time, but differences were found between women with low and high psychosocial distress. In samples from women with high psychosocial distress, there was no change in bacterial diversity with time, in contrast to the increase registered in women with low psychosocial distress. These changes were not related to differences in the relative abundance of the main bacterial genera.

Conclusions: There was a progressive and significant increase in bacterial diversity during the first three months postpartum. The relative abundance of *Firmicutes*, in particular *Staphylococcus* but not *Streptococcus*, decreased during this period. Low maternal psychosocial distress was associated to higher bacterial diversity (Shannon diversity index) in milk samples taken at week 12.

PROBIOTIC BIOFILMS CAN MODULATE THE ADHESION OF PATHOGENS

P. Bustamante¹, J. Jara¹, L. Fernández¹, J.M. Rodríguez², B. Orgaz¹

¹Sección Departamental de Tecnología Alimentaria. Facultad de Veterinaria. Universidad Complutense de Madrid. Madrid, Spain.

²Sección Departamental de Nutrición y Ciencia de los Alimentos. Facultad de Veterinaria. Universidad Complutense de Madrid. Madrid, Spain.

Background: Biofilms are microbial communities that grow attached to surfaces. Their formation on medical devices is a common source of hospital-acquired infections. Especially concerning are those found inside the nasogastric enteral tubes that are used for feeding preterm children whose suckling ability is not yet developed. The type of biofilms described in such devices are quite complex, including members of the *Enterobacteriaceae* family, *Pseudomonas* spp., staphylococci, lactic acid bacteria and *Bifidobacterium* spp. Very often these communities become dominated by *Enterobacteriaceae*, leading to neonatal infections. We hypothesize that the outcome could be different if the surfaces were previously colonized with probiotic strains.

Methods: Biofilms of probiotic microorganisms isolated from human milk and feces of healthy lactating children were developed in multi-well PVC microplates. Attached population and biomass (cell + matrix) were measured over time. Biofilms 3D structure was visualized by CLSM. To study the role of the presence of probiotic strains in pathogen adhesion, PVC surfaces were first conditioned with the former biofilms and then were used as adhesion substratum for *Serratia marcescens*, a ubiquitous microorganism frequently isolated from NICU environments and an increasing cause of preterm sepsis.

Results: All the tested strains were able to form dense biofilms although at a different pace. Several strains of *Bifidobacterium* spp. and *Lactococcus lactis* were the faster biofilm formers. When their biofilms were conditioning the PVC surfaces, *S. marcescens* adhesion was strongly reduced.

Conclusions: Probiotic biofilms could modulate the adhesion of pathogens to nasogastric enteral tubes, being good candidates as first colonizers in these medical devices.

TARGET-SPECIFIC PROBIOTICS: A NEW ALTERNATIVE FOR THE CONTROL OF HYPERURICEMIA AND THE PREVENTION OF GOUT?

M. Garranzo¹, J. Jara¹, B. Orgaz¹, J. Segura², J.M. Rodríguez², L. Fernández¹

¹Sección Departamental de Tecnología Alimentaria. Facultad de Veterinaria. Universidad Complutense de Madrid. Madrid, Spain.

²Departamento de Producción Animal. Facultad de Veterinaria. Universidad Complutense de Madrid. Madrid, Spain. ³Sección Departamental de Nutrición y Ciencia de los Alimentos. Facultad de Veterinaria. Universidad Complutense de Madrid. Madrid, Spain.

Background: Uric acid is a final product of purine catabolism that plays a key role as antioxidant in human plasma. Absorption at intestinal level of purines from ingested food may also contribute to uric acid levels in plasma. Uric acid concentrations above normal levels in plasma are referred to as hyperuricemia, and are associated with the development of gout. At present, pharmacological treatment for hyperuricemia is the standard option but it produces undesirable side-effects. The aim of this work was to assay the ability of a collection of lactic acid bacteria and bifidobacteria to transport and metabolize purines and uric acid.

Methods: The intake and intracellular transformation of inosine and guanosine and the production of xanthine, hypoxanthine and guanine after incubation at 37°C was evaluated in a collection of 17 strains (*Lactobacillus*, *Bifidobacterium*) isolated from human milk of healthy women. The concentration of purine metabolites was carried out using HPLC. Other probiotic properties (pH and bile resistance, antibiotic sensibility, antimicrobial activity and biofilm formation kinetics) were also determined *in vitro*.

Results: A preliminary screening revealed that most *Lactobacillus* isolates had high ability to uptake inosine and guanosine (> 95% of the concentration available in the media), in contrast to *Bifidobacterium* isolates. Selected strains from this initial screening (*Lactobacillus plantarum* MP05 and four strains of *Lactobacillus salivarius* (MP07, MP49, MP71 and MP312) showed a complete transformation of the inosine and guanosine or uric acid added. *L. salivarius* MP07, MP49 stood out by having the highest acid and bile resistance, respectively, and the fastest biofilm formation kinetics.

Conclusions: Nucleoside intake and transformation by *L. plantarum* MP05 and *L. salivarius* MP07, MP49, MP71 and MP312, as well as resistance to acid pH and bile salts, antimicrobial activity and biofilm formation ability, make them good candidates for the control of hyperuricemia.

A PEA OLIGOSACCHARIDE PREPARATION EXERTS IN VITRO BIFIDOGENIC PROPERTIES ON INFANT FECAL MICROBIOTA

M.C. Marín-Manzano¹, M. Díez-Municio², R. Olías¹, F.J. Moreno², A. Clemente¹

¹Instituto de Investigación en Ciencias de la Alimentación (CIAL-CSIC). Madrid, Spain. ²Estación Experimental del Zaidín (EEZ-CSIC). Granada, Spain.

Background: There is a growing interest in identifying dietary plant-derived carbohydrates capable of modulating the composition and metabolic activities of the infant gut microbiota. Non-digestible carbohydrates, including α -galacto-oligosaccharides (α -GOS) from plant sources like pea, are resistant to hydrolysis and absorption in the small intestine, and might contribute to microbiota modulation.

Methods: Gas chromatography with flame ionization detection (GC-FID) was used for the separation and quantitation of the main pea α -GOS from a commercial preparation named AlphaGOS®. By using fecal homogenates of six infant donors (from 6 months to 2 years old), *in vitro* incubation studies of pea α -GOS in comparison with commercially available β -galacto-oligosaccharides derived from lactose (GOS-La) were carried out. α -GOS metabolism during the fermentation period (0-24 h) and their modulatory effect on infant fecal microbiota was evaluated.

Results: Pea α -GOS composition was mainly comprised by manninotriose (Gal-(α 1 \rightarrow 6)-Gal-(α 1 \rightarrow 6)-Glc, 49% on dry matter) and verbascotetraose (Gal-(α 1 \rightarrow 6)-Gal-(α 1 \rightarrow 6)-Gal-(α 1 \rightarrow 6)-Glc, 43% on dry matter), followed very distantly by melibiose (Gal-(α 1 \rightarrow 6)-Glc, 4% on dry matter). Quantitative analysis demonstrated that pea α -GOS were extensively and rapidly fermented by infant fecal microbiota. A decrease of pH in fermentation media accompanied by a statistically significant growth of bifidobacteria similar than GOS-La was observed; pea α -GOS showed a selective significant increase of *Bifidobacterium longum* and *Bifidobacterium catenulatum/pseudocatenulatum*.

Conclusion: These data support a potential use of plant-derived α -GOS as prebiotic compounds in infant formula.