乳酸菌

SIBAL – AJLAB 2024

LACTIC ACID BACTERIA IN THE GENOMIC ERA

VI International Symposium on Lactic Acid Bacteria

I Argentinean-Japanese Lactic Acid Bacteria "Tohoku Forum for Creativity" Meeting

8-9 August 2024
Centro Cultural Eugenio Flavio Virla
TUCUMAN - ARGENTINA

健康

CONICET
CERELA

TOHOKU FORUM for CREATIVITY

TOHOKU UNIVERSITY

食料

SIBAL – AJLAB 2024

LACTIC ACID BACTERIA IN THE GENOMIC ERA

VI International Symposium on Lactic Acid Bacteria

I Argentinean-Japanese Lactic Acid Bacteria "Tohoku Forum for Creativity" Meeting

8-9 August 2024
Centro Cultural Eugenio Flavio Virla
TUCUMAN - ARGENTINA

Libro de resúmenes VI International Symposium on Lactic Acid Bacteria & I Argentinean-Japanese Lactic Acid Bacteria "Tohoku Forum for Creativity" Meeting 2024 /
Dr. Lucila Saavedra
Dr. Jean Guy LeBlanc
Dr. Elvira Hebert
Dr. Julio Villena
Dr. Susana Salva
Dr. Guadalupe Vizoso Pinto
Dr. Leonardo Albarracin
Dr. Haruki Kitazawa
Dr. Tomonori Nochi
Dr. Hitoshi Shirakawa
Dr. Jonathan Laiño

Compilación de Jonathan Laiño – 1a edición – San Miguel de Tucumán, Tucumán, Argentina – CONICET-CERELA, 2024
Libro digital, PDF

Organizing Committee

VI International Symposium on Lactic Acid Bacteria

President - Dr. Lucila Saavedra

Vicepresident - Dr. Jean Guy LeBlanc

Secretary - Dr. Elvira Hebert

I Argentinean-Japanese Lactic Acid Bacteria "Tohoku Forum for Creativity" Meeting

Argentina

Dr. Julio Villena
President

Dr. Susana Salva
Vicepresident

Dr. Guadalupe Vizoso Pinto
Dr. Leonardo Albarracin
Secretaries

Japan

Dr. Haruki Kitazawa
President

Dr. Tomonori Nochi
Vicepresident

Dr. Hitoshi Shirakawa
Secretary

Scientific Committee

Dr. Carina Audisio

Dr. Silvia Cazorla

Dr. Silvina Fadda

Dr. Marisa Garro

Dr. Paola Gauffin Cano

Dr. Luciana Gerez

Dr. Jonathan Laiño

Dr. Carolina Maldonado

Dr. Guillermo Marcial

Dr. Lucia Mendoza

Dr. Sandra Quilodrán Vega

Dr. Cecilia Rodriguez

Welcome Message

Welcome to the VI International Symposium on Lactic Acid Bacteria, held in conjunction with the I Argentinean-Japanese Lactic Acid Bacteria "Tohoku Forum for Creativity." This symposium stands as a cornerstone of international collaboration and scientific inquiry, bringing together a diverse community of researchers, professionals, and students dedicated to advancing our understanding of lactic acid bacteria.Over the course of this event, we will delve into pioneering research and innovative applications that highlight the significance of these microorganisms in biotechnology, as well as in human and animal health.

This abstract book showcases the breadth of research in our field, offering insights into the latest studies and developments.

As you explore these abstracts, we hope you find inspiration and foster connections that will drive future collaborations and breakthroughs.

Thank you for being a part of this symposium and contributing to this vibrant scientific event.

Program

Day 1 – Thursday, August 8, 2024

08:00 – 08:30	Registration and Poster mounting	
08:30 – 09:30	Opening Ceremony	
09:30 – 10:30	SESSION 1 - Chair: Jean Guy LeBlanc	HUMAN HEALTH
09:30 – 10:30	S1. *Effects of the extract of rice bran fermented by fungi and lactic acid bacteria on life-style related disease models.* **Dr. Hitoshi Shirakawa**	
10:30 – 11:00	Break	
11.00 – 12.00	SESSION 2 - Chair: Guadalupe Vizoso Pinto	
11.00 – 11.30	S2A. *Microbial therapeutics based on genetically modified lactic acid bacteria.* **Dr. Fu Namai**	
11.30 – 12.00	S2B. *Microbiota-gut-brain axis and its connection with mental health.* **Dr. Katia Sivieri** (on-line)	
12.00 – 12.30	Lunch	
13.30 – 15.00	Poster Session	
15:00 – 17:00	SESSION 3 - Chair: Julio Villena	ANIMAL HEALTH
15:00 – 15:40	S3A. *Anti-viral postimmunobiotics from the porcine small intestine.* **Dr. Haruki Kitazawa**	
15:40 – 16:20	S3B. *Fecal microbiota transplantation to improve microbiota for curing diarrhea in calves.* **Dr. Tomonori Nochi**	
16:20 – 17:00	S3C. *Lactic acid bacteria as silage inoculants: innovations, probiotics and special challenges for goat farming.* **Dr. Roxana Medina**	
17:00 – 17:30	Break	

Program

Day 1 – Thursday, August 8, 2024

17:30 – 18:50	ORAL POSTER PRESENTATIONS - Chair: Alejandra de Moreno	
17:30 – 17:40	HH17. *Oral administration of microencapsulated Limosilactobacillus fermentum CRL1446 improves the main biomarkers of metabolic syndrome in mice.* **C. Tomei**	Human & Animal Health
17:40 – 17:50	HH21. *Lacticaseibacillus rhamnosus CRL1505 and its postbiotic improve the functional properties of circulating neutrophils from immunocompromised mice and subjected to infectious stress.* **B. Vasile**	Human & Animal Health
17:50 – 18:00	AH4. *Intravaginal administration of pharmabiotic-phytobiotic capsules with autochthonous lactic bacteria for the prevention of infections of the bovine reproductive tract.* **M.H. Miranda**	Human & Animal Health
18:00 – 18:10	AH5. *Selection of immunomodulatory lactic acid bacteria from dog's milk and colostrum.* **S. Quilodrán-Vega**	Human & Animal Health
18:10 – 18:20	MP2. *Nitrogen sources as a key factor regulating the expression of gad genes in Levilactobacillus brevis CRL2013.* **M.P. Urquiza Martínez**	Biotechnology - Molecular Biology - Genomics
18:20 – 18:30	AB2. *Effect of selected starter cultures on the microbiological, physicochemical, and sensory characteristics of cured-fermented sausages made with llama (Lama glama) meat.* **L. Nelegatti**	Biotechnology - Molecular Biology - Genomics
18:30 – 18:40	AB8. *Physicochemical and microbiological stability, aroma profile and sensory attributes of Chilto juice fermented with Lactiplantibacillus paraplantarum IBFV-10.* **L. Contreras**	Biotechnology - Molecular Biology - Genomics
18:40 – 18:50	MG3. *Proteomic regulation and gene expression of selenized cells of Fructobacillus tropaeoli CRL2034 in a fermented fruit-based medium.* **L. Crespo**	Biotechnology - Molecular Biology - Genomics

Program

Day 2 – Friday, August 9, 2024

09:00 – 09:30	Poster mounting
09:30 – 10:30	SESSION 4 - Chair: Lucila Saavedra
09:30 – 10:30	S4. *New insights of lactic acid bacteria through large scale metagenomic analysis of food microbiomes.* **Dr. Paul Cotter** (on-line)
10:30 – 11:00	Break
11:00 – 12:00	SESSION 5 - Chair: Elvira Hebert
11:00 – 11:30	S5A. *Role of probiotic derived extracellular vesicles in interkingdom communication.* **Dr. Graciela Lorca** (on-line)
11:30 – 12:00	S5B. *Self-aggregating probiotic bacteria activate enhanced interferon responses via DNA sensing.* **Dr. Jorge Gutierrez-Merino** (on-line)
12:00 – 13:30	Lunch
13:30 – 15:00	Poster Session
15:00 – 16:00	SESSION 6 - Chair: Fernanda Mozzi
15:00 – 15:30	S6A. *Metataxonomic insights on lactic acid bacteria in table olives.* **Dr. Eugenio Parente** (on-line)
15:30 – 16:00	S6B. *Genomic characterization of the potential probiotic strain Limosilactobacillus fermentum CRL2085 isolated from feedlot cattle.* **Dr. Francesca Fanelli**
16:10 – 17:30	SESSION 7 - Chair: María Pía Taranto
16:10 – 16:50	S7A. *Diversity of potassium transporters in lactic acid bacteria and the role of c-di-AMP in potassium homeostasis regulation.* **Dr. Victor Blancato** (on-line)
16:50 – 17:30	S7B. *Designing computational tools to understand the bacterial-human cell communications.* **Dr. Alexis Salas Burgos**
17:30 – 18:50	Closing remarks & award ceremony Best oral & poster presentations - Lucila Saavedra-Julio Villena
19:30	Cocktail Reception

BIOTECHNOLOGY - MOLECULAR BIOLOGY - GENOMICS

Index

Lecture Sessions

Human Health

S1. *Effects of the extract of rice bran fermented by fungi and lactic acid bacteria on life-style related disease models.* 18
Dr. Hitoshi Shirakawa

S2A. *Microbial therapeutics based on genetically modified lactic acid bacteria.* 19
Dr. Fu Namai

S2B. *Microbiota-gut-brain axis and its connection with mental health.* 20
Dr. Katia Sivieri (*on-line*)

Animal health

S3A. *Anti-viral postimmunobiotics from the porcine small intestine.* 21
Dr. Haruki Kitazawa

S3B. *Fecal microbiota transplantation to improve microbiota for curing diarrhea in calves.* 22
Dr. Tomonori Nochi

S3C. *Lactic acid bacteria as silage inoculants: innovations, probiotics and special challenges for goat farming.* 23
Dr. Roxana Medina

Biotechnology – Molecular biology – Genomics

S4. *New insights of lactic acid bacteria through large scale metagenomic analysis of food microbiomes.* 24
Dr. Paul Coter (*on-line*)

S5A. *Role of probiotic derived extracellular vesicles in interkingdom communication.* 25
Dr. Graciela Lorca (*on-line*)

S5B. *Self-aggregating probiotic bacteria activate enhanced interferon responses via DNA sensing.* 26
Dr. Jorge Gutierrez-Merino (*on-line*)

S6A. *Metataxonomic insights on lactic acid bacteria in table olives.* 27
Dr. Eugenio Parente (*on-line*)

S6B. *Genomic characterization of the potential probiotic strain Limosilactobacillus fermentum CRL2085 isolated from feedlot cattle.* 28
Dr. Francesca Fanelli

Index

Lecture Sessions

Biotechnology – Molecular biology – Genomics

S7A. *Diversity of potassium transporters in lactic acid bacteria and the role of c-di-AMP in potassium homeostasis regulation.* **Dr. Victor Blancato** (*on-line*) 29

S7B. *Designing computational tools to understand the bacterial-human cell communications.* **Dr. Alexis Salas Burgos** 30

Oral Posters presentations

Human and Animal Health

HH17. *Oral administration of microencapsulated Limosilactobacillus fermentum CRL1446 improves the main biomarkers of metabolic syndrome in mice.* **C. Tomei** 48

HH21. *Lacticaseibacillus rhamnosus CRL1505 and its postbiotic improve the functional properties of circulating neutrophils from immunocompromised mice and subjected to infectious stress.* **B. Vasile** 52

AH4. *Intravaginal administration of pharmabiotic-phytobiotic capsules with autochthonous lactic bacteria for the prevention of infections of the bovine reproductive tract.* **M.H. Miranda** 62

AH5. *Selection of immunomodulatory lactic acid bacteria from dog's milk and colostrum.* **S. Quilodrán-Vega** 63

Biotechnology – Molecular biology – Genomics

MP2. *Nitrogen sources as a key factor regulating the expression of gad genes in Levilactobacillus brevis CRL2013.* **M.P. Urquiza Martínez** 69

AB2. *Effect of selected starter cultures on the microbiological, physicochemical, and sensory characteristics of cured-fermented sausages made with llama (Lama glama) meat.* **L. Nelegatti** 78

AB8. *Physicochemical and microbiological stability, aroma profile and sensory attributes of Chilto juice fermented with Lactiplantibacillus paraplantarum IBFV-10.* **L. Contreras** 84

MG3. *Proteomic regulation and gene expression of selenized cells of Fructobacillus tropaeoli CRL2034 in a fermented fruit-based medium.* **L. Crespo** 98

Index

Posters

Lactic acid bacteria and human health

HH1. *Oral pathogen inhibition by selected lactid acid bacteria.* **J.R. Naja** 32

HH2. *Effects of probiotic yoghurt administration in a murine metastatic breast cancer model under chemotherapy treatment.* **M.N. Cerasuolo** 33

HH3. *Modulation of lung immune response against Klebsiella pneumoniae by orally administered Lacticaseibacillus rhamnosus CRL1505.* **R. Serda** 34

HH4. *The intestinal fluids of probiotics fed mice trigger immunomodulatory properties on innate immune cells.* **M.J. Martínez Monteros** 35

HH5. *Lacticaseibacillus rhamnosus CRL1505 ameliorates liver injury and inflammation in poly(i:c) induced acute hepatitis.* **M.J. Lorenzo Pisarello** 36

HH6. *Evaluation of a mixture of selected lactic acid bacteria (LAB) in a murine model of levodopa induced dyskinesia (LID).* **P.A. Yánez** 37

HH7. *Administration of Lacticaseibacillus paracasei subsp. paracasei CRL75 modulates humoral and cytokine production during food allergy.* **J. Laiño** 38

HH8. *Oral administration of LP 1518 improves the clinical outcome of l. amazoniensis infection.* **J. Arrieta** 39

HH9. *Lactiplantibacillus plantarum CIDCA83114 as a potential treatment for hemolytic uremic syndrome.* **C. Cancino** 40

HH10. *Probiotics decrease inflammatory bowel damage in aging mice.* **A. Vestani** 41

HH11. *Effects of probiotic yoghurt in a murine breast cancer model under chemotherapy treatment.* **M. N. Cerasuolo** 42

HH12. *Lacticaseibacillus rhamnosus CRL1505 peptidoglycan modulates tissue factor expression and protease activated receptor 1 activation during inflammation-coagulation response triggered by poly(i:c) in the respiratory tract.* **H. Zelaya** 43

Index

Posters

Lactic acid bacteria and human health

HH13. *Effect of riboflavin-overproducing Lactiplantibacillus plantarum CRL2130 on breast cancer cells under treatment with chemotherapeutic drugs.* **R. Levit** 44

HH14. *Effect of probiotics supplementation in re-nutritional diets on the thymus and intestine of malnourished mice.* **M.F. Balcells** 45

HH15. *Evaluation of a riboflavin- overproducing lactic acid bacterium in a murine breast cancer model under treatment.* **R. Levit** 46

HH16. *In vivo study of anti-listeria activity of strains of lactic acid bacteria isolated from fermented foods.* **C. Burgos** 47

HH17. *Oral administration of microencapsulated Limosilactobacillus fermentum CRL1446 improves the main biomarkers of metabolic syndrome in mice.* **C. Tomei** 48

HH18. *Lactiplantibacillus plantarum MPL16 activates p38 MAPK signaling in C. elegans and has a positive impact on longevity and resistance to Enterococcus faecalis infection.* **M.D. Vera** 49

HH19. *Postbiotic nasal priming accelerates the recovery of innate immune response in respiratory mucosa-associated lymphoid tissue in malnourished mice.* **M. Ivir** 50

HH20. *Evaluation of lactic acid bacteria on the nutritional, biochemical and immunological profile of mice fed a high-fat diet.* **A. Márquez** 51

HH21. *Lacticaseibacillus rhamnosus CRL1505 and its postbiotic improve the functional properties of circulating neutrophils from immunocompromised mice and subjected to infectious stress.* **B. Vasile** 52

HH22. *Functional crackers manufactured with LAB-fermented chickpea flour prevents oxidative stress in d-galactose induced aging mice.* **G. Sáez** 53

HH23. *Lacticaseibacillus rhamnosus CRL1505 modulates alveolar macrophages and improve their response to Pseudomonas aeruginosa strains isolated from cystic fibrosis patients.* **L. Arellano-Arriagada** 54

Index

Posters

Lactic acid bacteria and human health

HH24. *Effect of Lactiplantibacillus plantarum cell free culture on the respiratory superinfection induced by bacterial pathogens isolated from cystic fibrosis patients.* **L. Abán** 55

HH25. *Modulation of the Toll-like Receptor 3-mediated intestinal immune response by water kefir.* **S. Dentice Maidana** 56

HH26. *Effect of Lactiplantibacillus plantarum CRL681 on the TLR4-mediated intestinal innate immune response.* **A. Baillo** 57

Lactic acid bacteria and animal health

AH1. *Beer bagasse-enriched medium improves biomass production of probiotic Lactobacillus johnsonii CRL2240.* **J.D. Babot** 59

AH2. *Characterization of surface properties and adhesion of autochthonous lactic acid bacteria isolated from mare's reproductive tract.* **A. Vasquetto** 60

AH3. *Ligilactobacillus salivarius FFIG58 modulates respiratory immunity and confers long-term protection against Streptococcus pneumoniae.* **M. Elean** 61

AH4. *Intravaginal administration of pharmabiotic-phytobiotic capsules with autochthonous lactic bacteria for the prevention of infections of the bovine reproductive tract.* **M.H. Miranda** 62

AH5. *Selection of immunomodulatory lactic acid bacteria from dog's milk and colostrum.* **S. Quilodrán-Vega** 63

AH6. *Effects of Ligilactobacillus salivarius subsp. salivarius A3iob on honey production and health of Apis mellifera bee hives in Argentina.* **A.A. Arroyo Guerra** 64

AH7. *Identification and beneficial characteristics of autochtonous lactic bacteria with probiotic potential for canine puppies.* **N. Carrasco** 65

AH8. *Mixed inoculation using ferulic acid esterase-producing lactobacilli improve nutritional, fermentative and functional properties of corn stover silage.* **E. Andrada** 66

Index

Posters

Metabolism and physiology of lactic acid bacteria

MP1. *Growth of lactic acid bacteria strains isolated from silage in corn plant extract media.* **R. M. Lenz** 68

MP2. *Nitrogen sources as a key factor regulating the expression of gad genes in Levilactobacillus brevis CRL2013.* **M.P. Urquiza Martínez** 69

MP3. *Antimicrobial properties of Levilactobacillus brevis strains isolated from cheese whey of different origins.* **J.C. Alcocer** 70

MP4. *Bile acids modify morphology of a pote tial probiotic lactic acid bacterium.* **C. Moreno** 71

MP5. *RAMAN spectroscopy, an alternative methodology for the identification of lactic acid bacteria.* **A. Ledesma** 72

MP6. *Monitoring the proteolytic activity of lactic acid bacteria using Fourier Transform Infrared Spectroscopy.* **J. Carol** 73

MP7. *Biotransformation of selenium by lactic acid bacteria under different stress culture conditions.* **F.R.E Molina** 74

MP8. *Aromatic profile of wines produced by mixed cultures of yeasts and Oenococcus oeni.* **L.M. Mendoza** 75

Applied biotechnology of lactic acid bacteria

AB1. *Inhibition of enterohemorrhagic Escherichia coli by Lactiplantibacillus plantarum CRL681: aggregation, co-aggregation, and electron microscopy studies.* **A. Baillo** 77

AB2. *Effect of selected starter cultures on the microbiological, physicochemical, and sensory characteristics of cured-fermented sausages made with llama (Lama glama) meat.* **L. Nelegatti** 78

AB3. *Lactic acid bacteria isolated from citrus fruits as starter cultures for the production of fermented lemon juice.* **D.A Barrionuevo** 79

AB4. *Partial characterization of Enterococcus spp. JK06 isolated from regional salami from Tucumán.* **J. Gomez** 80

AB5. *Functional and sensory analysis of Calafate fruit fermented with patagonian lactic acid bacteria.* **B. Sede Lucena** 81

Index

Posters

Applied biotechnology of lactic acid bacteria

AB6. *Pleurotus pulmonarius mushroom as novel resources of prebiotics: effect on the growth and resistance to passage through the gastrointestinal tract of lactic acid bacteria.* **J. Gomez** 82

AB7. *Inhibition of a-glucosidase by lactic acid bacteria through controlled fermentation of Brassica rapa and Undaria pinnatifida.* **B. Martínez Caliari** 83

AB8. *Physicochemical and microbiological stability, aroma profile and sensory attributes of Chilto juice fermented with Lactiplantibacillus paraplantarum IBFV-10.* **L. Contreras** 84

AB9. *In vitro evaluation of the antagonistic potential of Ligilactobacillus murinus 26B1 isolated from mouse against Staphylococcus aureus ATCC 6538P for its application in animal nutrition.* **J. Palma** 85

AB10. *Genetic modification of Lactococcus lactis to produce the anti-inflammatory protein elafin: evaluation of different expression systems.* **R. Levit** 86

AB11. *Influence of temperature, ph and culture media on Salmonella enterica serotype enteritidis biofilm formation.* **S. López Pérez** 87

AB12. *Optimization of the freeze-drying process to obtain more stress tolerant Oenococcus oeni starter cultures.* **E.V. Bentencourt** 88

AB13. *Sensory analysis of the final prototype of a soy food fermented with Lacticaseibacillus paracasei subsp. paracasei CRL207.* **B.L. Nacchio** 89

AB14. *Production of mixed fruit juice enriched with probiotic lactic acid bacteria.* **F. Escobar** 90

AB15. *Impairment of Listeria monocytogenes developed on industrial surfaces by Latilactobacillus curvatus CRL1579 bacteriocin.* **C. Melián** 91

AB16. *Stability of prebiotic galactooligosaccharides during yoghurt manufacture and storage.* **A. Fara** 92

Index

Posters

Applied biotechnology of lactic acid bacteria

AB17. *Evaluation of the proteolytic profile and antioxidants of legume flours fermented with selected lactic cultures.* **A. P. Pezuk** 93

AB18. *Technological strategy to improve the organoleptic characteristics of soy food products: fermentation with selected lactic acid bacteria.* **G.N. Avila Hael** 94

Molecular biology and genomics of lactic acid bacteria

MG1. *Genomic characterization of the honeybee-probiotic strain Ligilactobacillus salivarius subsp. salivarius A3iob.* **M. Elean** 96

MG2. *Functional and genomic characterization of Pediococcus pentosaceus TUCO-3 isolated from cat milk: a potential probiotic strain to combat infections.* **S. Quilodrán-Vega** 97

MG3. *Proteomic regulation and gene expression of selenized cells of Fructobacillus tropaeoli CRL2034 in a fermented fruit-based medium.* **L. Crespo** 98

MG4. *Genomic and proteomic characterization of Lactiplantibacillus plantarum CRL681 focusing on cell surface and secreted proteins.* **A. Baillo** 99

乳
酸
菌

LECTURE SESSIONS

健
康

食
料

S1 EFFECTS OF THE EXTRACT OF RICE BRAN FERMENTED BY FUNGI AND LACTIC ACID BACTERIA ON LIFESTYLE-RELATED DISEASE MODELS

H. Shirakawa[1]

[1]International Education and Research Center for Food and Agricultural Immunology, Graduate School of Agricultural Science, Tohoku University. Sendai, Japan. E-mail: shirakah@tohoku.ac.jp

Japan produces about 8 million tons/year of brown rice. Rice bran is a by-product of rice polishing process, and brown rice consists of 10% rice bran (RB). About 40% of RB is used to produce rice bran oil. RB is also mainly used as livestock feed and fertilizer; however, the use of approximately 25% of RB is not known, and most of it is estimated to be discarded. It is widely known that RB is rich in beneficial substances and has several health benefits. For example, γ-oryzanol, ferulic acid, and tocotrienol are reported to have blood cholesterol-lowering, angiogenesis-inhibiting, anti-tumor, and hypotensive effects. These non-polar components are abundantly present in RB oil and are responsible for most of its beneficial effects. In addition, the residue resulting from the extraction of rice bran oil (defatted rice bran) comprises inositol, phytic acid (inositol 6-phosphate), phytin, and rice bran proteins. These components have been documented to exhibit antitumor, immunostimulatory, antilipidemic, and antidiabetic effects. Although maximum utilization of RB is desirable, it is not fully utilized to the full extent because of its tendency to deteriorate and its characteristic odor. To improve the flavor of RB, we developed a novel fermentation method that employing fungi and lactic acid bacteria sequentially. This fermented rice bran (FRB) exhibited an improved flavor profile and was expected to be enriched in functional components, which are secondary metabolites produced by microorganisms. The beneficial effects of FRB have been verified in several animal models of lifestyle-related diseases. FRB suppressed elevation of blood pressure and improved insulin resistance by enhancing ACE inhibitory activity and elevating adiponectin levels in blood, respectively, in stroke-prone spontaneously hypertensive rats (SHRSP). FRB also suppressed the onset and restoration of dextran sulfate sodium (DSS)-induced colitis in mice and muscular atrophy in streptozotocin (STZ)-induced diabetic rats by inhibiting NFκB activation. Taken together, these results indicate that FRB may be effective in preventing lifestyle-related diseases. We anticipate that FRB will be used in practical applications in the future.

S2A MICROBIAL THERAPEUTICS BASED ON GENETICALLY MODIFIED LACTIC ACID BACTERIA

F. Namai[1,2], K. Nishiyama[1,2], H. Kitazawa[1,2], T. Shimosato[3,4]
[1]Tohoku University, Aoba, Aramaki, Aoba-ku, 980-8572 Sendai, Japan. [2]CFAI, Aoba, Aramaki, Aoba-ku, 980-8572 Sendai, Japan. [3]Department of Biomolecular Innovation, Institute for Biomedical Sciences, Shinshu University, 399-4598 Nagano, Japan. [4]Institute for Aqua Regeneration, Shinshu University, 399-4598 Nagano, Japan. E-mail: fu.nama.a3@tohoku.ac.jp

The increasing incidence of inflammatory bowel disease (IBD) in western and rapidly westernizing developing countries is a growing concern. The development of affordable alternatives to conventional IBD therapies is a major challenge. In this study, we explored genetically modified lactic acid bacteria (gmLAB) as an approach to IBD treatment. gmLAB can serve as a carrier to deliver target proteins directly to the intestinal mucosa, as lactic acid bacteria can survive in the intestinal tract. Local delivery of therapeutic proteins using gmLAB is expected to provide a more efficient effect while reducing side effects. Here, we constructed a gmLAB that secretes an interleukin-1 receptor antagonist (IL-1Ra), an inhibitor of IL-1, since the inflammatory cytokine IL-1 is involved in the exacerbation of IBD. IL-1Ra expression vector was introduced into *Lactococcus lactis* NZ9000 to generate gmLAB (NZ-IL1Ra). At the same time, an empty vector was also introduced to generate the control gmLAB (NZ-VC). The IL-1Ra expression in the bacterial cell and secretion in the supernatant were detected using Western blot. The secreted IL-1Ra was purified, and its inhibitory activity against IL-1 signaling was evaluated. The results showed that IL-1Ra secreted by NZ-IL1Ra suppressed IL-2 secretion from IL-1β-stimulated EL4.NOB-1 cells in a concentration-dependent manner. NZ-IL1Ra was then administered orally to mice, and the colon and cecum contents and serum were collected. The concentration of IL-1Ra in each was determined. The concentration of IL-1Ra was significantly increased compared to that in the untreated mice. This indicates that oral administration of NZ-IL1Ra delivers IL-1Ra to the intestinal tract and blood. To evaluate the efficacy of orally administered NZ-IL1Ra in alleviating IBD symptoms, we conducted a study using a mouse model of dextran sulfate sodium (DSS)-induced colitis. Mice given 3% DSS ad libitum experienced weight loss and diarrhea. Oral administration of NZ-VC did not alleviate these symptoms; however, NZ-IL1Ra significantly accelerated their recovery. Analysis of mesenteric lymph nodes revealed a significant reduction of CD4+IL-17A+ cells, induced by IL-1 signaling, in the NZ-IL1Ra group compared to the NZ-VC group. In conclusion, we have successfully constructed a gmLAB that secretes bioactive IL-1Ra with the aim of proposing an approach to IBD. Oral administration of gmLAB transports IL-1Ra to the intestinal tract of mice and suppresses IL-1 signaling in situ, thereby alleviating the symptoms of DSS-induced colitis. We hope that this research will contribute to the improvement of human health.

S2B MICROBIOTA-GUT-BRAIN AXIS AND ITS CONNECTION WITH MENTAL HEALTH

K. Sivieri

S3A POSTIMMUNOBIOTICS FROM PORCINE SMALL INTESTINE

H. Kitazawa[1,2], Y. Imamura[1], H. Yamamuro[1], M. Elean[3], L. Arce[3], F. Namai[1,2], K. Nishiyama[1,2], J. Villena[3]
[1]Tohoku University, Aoba-ku, 9800 Sendai, Japan. [2]CFAI, Sendai, Japan. [3]CERELA-CONICET. Chacabuco 145. 4000 Tucuman, Argentina. E-mail: haruki.kitazawa.c7@tohoku.ac.jp

Infectious diseases are required to be addressed with efficient prevention strategies to protect livestock and human health. Vaccines and antimicrobials can protect livestock from infectious diseases, but their development is labor-intensive and time-consuming. In addition, the drastic increase in antimicrobial-resistant microbes make necessary the development of new measures to prevent infections. Postimmunobiotics, defined as the inanimate microbes and their derived functional molecules with the ability to modulate the immune system, are a promising alternative to combat infectious diseases in livestock. We have focused on the study of postimmunobiotics with the capacity to beneficially modulate anti-viral immune responses. We have selected a postimmunobiotic candidate derived from the probiotic strain *Ligilactobacillus salivarius* FFIG58 strain from our porcine lactobacilli library using and antiviral immunoassay system developed with the originally established porcine intestinal epithelial (PIE) cells. Considering the choline-binding protein (CbpA) as a functional molecule, a *cbpA* knockout (Δ*CbpA*) mutant from the FFIG58 strain was established. The phenotypes and functional activities of wild-type FFIG58 and the Δ*CbpA* strains were compared including their growth ability, their surface characteristics by electron microscopy analysis and their adhesion to PIE cells. The capacity of heat-killed FFIG58 and Δ*CbpA* strains to differentially modulate the Toll-like receptor 3 (TLR3)-mediated innate antiviral immunity and increase the resistance to rotavirus infection was evaluated. In addition, we studied the interaction of heat-killed wild-type FFIG58 and the Δ*CbpA* strains with porcine macrophages, assessing their effect on TLR3-mediated immunity including the modulation of intracellular signaling factors and negative regulators of the TLR signaling pathway. The wild-type FFIG58 strain efficiently adhered to PIE cells, improved TLR3-mediated immunity and enhanced the resistance to rotavirus infection. The strain was also efficiently phagocyted by macrophages and positively modulated their response to TLR3 activation. The immunomodulatory effect of FFIG58 was markedly reduced in the mutant Δ*CbpA*. Additionally, the immunomodulatory activities of *L. salivarius* FFIG58 and the Δ*CbpA* mutant were confirmed *in vivo* using infant mice as a young host model considering the potential application of this strain to improve the resistance to postweaning viral diarrhea in piglets. Heat-killed *L. salivarius* FFIG58 is an interesting candidate to be developed as a postimmunobiotic with the ability to improve antiviral immunity via CbpA. It is expected that our research will lead to the expansion of the food and feed immunological use of postimmunobiotics, which will contribute to the prevention of viral infectious diseases of livestock. This work was supported by BRAIN-JPJ007097, JSPS-19H00965, and JSPS-23H00354 grants.

S3B CROSS FECAL MICROBIOTA TRANSPLANTATION TO TREAT DIARRHEA IN PRE-WEANED CALVES

T. Nochi[1*], J. Islam[1], N. Ohtani[2], Y. Shimizu[2], J. Yamazaki[3], Z. Nakamura[4], H. Tanaka[2]
[1]*Graduate School of Agricultural Science, Tohoku University, Miyagi, Japan.* [2]*Chiba Prefectural Federated Agricultural Mutual Aid Association, Chiba, Japan.* [3]*Hokkaido Federated Agricultural Mutual Aid Association, Hokkaido, Japan.* [4]*Okinawa Prefectural Federated Agricultural Mutual Aid Association, Okinawa, Japan.* 468-1, Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-8572, Japan. E-mail: nochi@tohoku.ac.jp

Calf diarrhea is the most common illness in pre-weaned calves. Effective treatment options to manipulate the gut microbiome environment of calves under commercial operations are of great importance to improve animal health and reduce antimicrobial usage. Fecal microbiota transplantation (FMT) involves transplanting the fecal contents of a healthy donor into a diseased patient to restore gut microbiota to its original state. FMT has been demonstrated to be an effective treatment for calf diarrhea in recent times. However, most of the studies on FMT against calf diarrhea are investigated in a same farm specific manner concerning its safety. However, to introduce FMT as a nationwide positioning, as well as to better design future microbial therapeutics for calf diarrhea, we introduced cross-FMT intervention, in where donors were selected from three different regions within a range of approximately 3,000 km across Japan. We collected 180 feces from healthy calves as donor from three regions of Japan (Hokkaido, Chiba, and Okinawa area, n=60 each), and among them 24 donors were finally selected as potential donors for FMT based on 16S rRNA amplicon sequencing and absence of potential pathogens especially *Clostridium perfringens*, *Cryptosporidium parvum*, *Coccidia*, *Salmonella*, *Rotavirus*, *Coronavirus*, *E. coli* as well as BLDV and BLV. From each potential donor, 3 vials (10 g feces each) were prepared using the original feces and continued to freeze-dry. Finally, prepared donors' feces were sent to three regions and subjected to 72 FMT trials (n=24 recipients/area). Based on the diarrheal severity, pathogen detection, 16S rRNA amplicon sequencing and fecal metabolomics (CE-TOFMS) results, overall FMT efficacy is found 76.39% 7 days after FMT. Based on the area, the FMT efficacy is observed in Hokkaido region (83.34%), Chiba (75.0%) and Okinawa (70.83%). Comparing the donor efficacy, the donor efficacy is observed in Hokkaido (76.67%), Chiba (77.78%) and Okinawa (73.34%). Finally, based on the intra-area donor efficacy, Hokkaido donor showed 100% efficacy in Hokkaido, but 80% for Chiba and 50% for Okinawa. On the contrary, Chiba donors showed similar efficacy (77.78%) in all three areas, while Okinawa donors showed 60% efficacy in Hokkaido and Chiba, but 100% efficacy in Okinawa itself. Fecal microbial community analysis based on 16S rRNA amplicon sequencing revealed an increase in the alpha-diversity indices after FMT and pairwise PERMANOVA analysis from beta diversity confirmed that there is significant difference before and after FMT in successful FMT cases. Furthermore, FMT reduces the gram negative *Proteobacteria* level as fecal amino acids (alanine, leucine, valine, isoleucine, glycine, arginine, and glutamic acid), which may be responsible to restore the gut microbial community to healthy state and lessens the diarrheal severity in successful cases.

S3C LACTIC ACID BACTERIA AS SILAGE INOCULANTS: INNOVATIONS, PROBIOTICS AND SPECIAL CHALLENGES FOR GOAT FARMING

R. Medina[1,2]
[1]CERELA-CONICET. Chacabuco 145, Tucumán, Argentina. [2]Facultad de Agronomía, Zootecnia y Veterinaria, Universidad Nacional de Tucumán, Tucumán, Argentina
E-mail: rmedina@cerela.org.ar

Goat production in Argentina is typically small-scale and for local consumption. It is mainly located in arid and semi-arid regions where overgrazing during the dry season cause environmental degradation. Therefore, sustainable farming needs diversified food sources, for which agricultural by-products can represent a locally available alternative. Corn stover, as other abundant high-fiber by-products, are usually challenging to preserve through ensiling. A strategy to improve its conservation and nutritional properties is using lactic acid bacteria (LAB) that present ferulic acid esterase (FAE) activity. FAE breaks down esterified sugars of plant cell walls, like arabinose, to release hydroxycinnamic acids such as ferulic acid (FA), an antioxidant compound. We isolated LAB with FAE activity from goat feces (*Lactobacillus johnsonii* CRL 2240) and corn silos (*Levilactobacillus brevis* CRL2239, *Lactiplantibacillus plantarum* CRL2241). These strains were selected for studies in corn stover silages, following small farmers' conditions. An uninoculated (UN) silo was used as a control for comparison. Fermentative and nutritional profiles, digestibility, phenolic compounds, antioxidant capacity and metagenomics of bacterial community were evaluated after 60 days of ensiling. *L. plantarum CRL* 2241 reduced gas losses and pH, increased the lactic to acetic acid ratio and FA content, showing characteristics of a first-generation (1G) inoculant with effective FAE activity. *L. johnsonii* CRL 2240 reduced the dry matter loss and modified the fiber composition, acting as a 1G and 3G inoculant. *L. brevis* CRL 2239 strain increased acetate content, increased *in vitro* true digestibility of dry matter and free FA content, acting as a 2G and 3G inoculant. Metagenomic studies showed that inoculated silages had lower diversity and equity at the genus level when compared to UN, while *L. plantarum* CRL 2241 and *L. johnsonii* CRL 2240 strains increased the relative abundance of *Lactobacillus* genus. Subsequently, two mixed inoculants were designed: LPB (CRL 2239 and CRL 2241) and LPBJ (LPB+ CRL 2240). Although both inoculants modulated the ensiling process and increased the free FA content, the inclusion of *L. johnsonii* CRL2240 in LPBJ inoculant reduced the dry matter loss, acid detergent fiber and cellulose content. Furthermore, *L. johnsonii* CRL 2240 presented probiotic properties *in vitro*, for which inoculation of this strain in silages might constitute a probiotic-supply strategy. These can be considered a fourth generation of inoculants.

S4 NEW INSIGHTS OF LACTIC ACID BACTERIA THROUGH LARGE SCALE METAGENOMIC ANALYSIS OF FOOD MICROBIOMES.

P.D. Cotter[1,2]

[1]Teagasc Food Research Centre, Moorepark, Cork, Ireland and the APC Microbiome Ireland, VistaMilk and Food Health Ireland Research Centres; [2]SeqBiome Ltd. E-mail: paul.cotter@teagasc.ie; paulcotter@seqbiome.com

Microorganisms exist along the food chain and impact the quality and safety of foods in both positive and negative ways. Identifying and understanding the behaviour of these microbial communities enable the implementation of preventative or corrective measures in public health and food industry settings. Traditional culture-dependent microbial analyses are time-consuming and target only specific subsets of microbes. However, the greater use of culture-independent metagenomic approaches has the potential to facilitate a thorough characterization of the microbial communities, and of lactic acid bacteria, in particular along the food chain. This has been through an in depth investigation of specific foods, such as cheese and kefir, as well as a broader investigation of a wide range of different food types. Combining this data in a new curated Food Metagenomic database has allowed a variety of different analyses, including by assembly-based (through generating metagenome assembled genomes; over 10,000 high and medium quality MAGs) and non assembly-based, has provided new insights into food associated microorganisms, including many species of Lactic Acid Bacteria, including information regarding their prevalence, distribution and overlap with gut-associated taxa.

S5A ROLE OF PROBIOTIC-DERIVED EXTRACELLULAR VESICLES IN INTERKINGDOM COMMUNICATION

G.L. Lorca
Department of Microbiology and Cell Science. Genetics Institute. Institute of Food and Agricultural Sciences. University of Florida. E-mail: glorca@ufl.edu

We recently showed that *Lactobacillus johnsonii* N6.2 releases EVs with a distinct composition of proteins, and lipids when compared to whole cells. *L. johnsonii* N6.2 is a probiotic bacterium that has been shown to mitigate type 1 diabetes in prone rodents (BBDP rat model) by maintaining euglycemic levels and reducing the inflammatory state. We hypothesized that EVs play a central role in delivering bioactive molecules that may act as mechanistic effectors in immune modulation. We observed that the addition of EVs to the human pancreatic cell line βlox5 reduced cytokine-induced apoptosis. The role of EVs on beta cell function was further evaluated using primary human pancreatic islets. It was found that EVs significantly induced insulin secretion in the presence of high glucose concentrations. Through RNAseq analyses, a significant induction of the AHR and 2', 5'-oligoadenylate synthetase (OAS) pathways were observed. The OAS pathway is part of an innate immune response activated by viral or bacterial RNA. In mammals, the OAS family is composed of three enzymatically active enzymes, OAS1, OAS2 and OAS3, all of which were significantly induced in the presence of EVs, but not by purified membranes from *L. johnsonii* N6.2. We hypothesized that the activation of the OAS pathway by specific effector molecules present in *L. johnsonii* N6.2 secreted EVs could hinder a viral infection *in vivo*. I will discuss our approach to study the role of EVs on viral infection using the murine norovirus (MNV-1) as an RNA virus model of infection.

S5B SELF-AGGREGATING PROBIOTIC BACTERIA ACTIVATE ENHANCED INTERFERON RESPONSES VIA DNA SENSING

S. Solis[1]; E. Maldonado[1]; C. Maluquer de Motes[1]; S. Mukhopadhyay[2]; **J. Gutierrez-Merino**[1*]
[1]School of Biosciences and Medicine, University of Surrey, UK. [2]School of Immunology and Microbial Sciences, King's College London, UK. [*]Corresponding author and speaker. E-mail: j.gutierrez@surrey.ac.uk

The discovery of the human (and animal) microbiome has revolutionized the field of Medicine and Biological Sciences. To date, we have access to endless data informing of the microbial diversity present in different organs and body systems, and how this diversity correlates with many medical conditions. However, we are still unsure which of the many commensal microbes that reside the host are the main drivers that restore or protect health from disease. Much littler the information is concerning the molecules that these key commensals possess to interact with the immune system. This important question has been addressed by our research team as we are trying to reveal the molecules that beneficial bacteria utilize to induce host beneficial responses. Our recent investigations have shown that certain species of lactobacilli significantly activate the production of type I interferon (IFN-I) cytokines in macrophages. IFN-I cytokines are essential to confer protection against microbial infections and auto-immune disorders. For the first time, we have proved that this IFN-I activation is predominantly driven by cGAS, a molecule that activates the cytosolic sensor STING upon the recognition of bacterial DNA. Furthermore, we have observed that lactobacilli encode some surface proteins with the potential to interact with macrophages for subsequent phagocytosis via non-opsonic scavenger receptors. Therefore, we are focused on determining the role that these surface proteins play as a port of entry in macrophages and characterize the IFN-I-mediated intracellular signalling initiated by cGAS. Elucidating these unknown mechanisms will be important to inform on how specific molecules of commensals modulate or stimulate host responses that, in unhealthy individuals, are exacerbated or inhibited. Overall, our studies will provide a better understanding on the molecular crosstalk between the microbiome and mammalian cells, paving the way for major therapeutic discoveries.

S6A METATAXONOMIC INSIGHTS ON LACTIC ACID BACTERIA IN TABLE OLIVES

E. Parente[1], A. Aliano[1], R. Pietrafesa[1], M. Hidalgo[1], A. Ricciardi[1]
[1]Scuola di Scienze Agrarie, Forestali, alimentari ed ambientali. Università degli Studi della Basilicata, Potenza, Italy. E-mail: eugenio.parente@unibas.it

With a global consumption of 2,890x10^3 ton/y (2022/2023, International Olive Council), table olives are among the most popular vegetable fermented foods. Production and consumption are concentrated in the Mediterranean area, where first traces of olive brining date to 7,000 BP. However, they are increasing in other continents, with Peru as leading producer. As for many other vegetable fermentations, removal of the initial microbial contaminants relies only on selection and washing, and starter cultures are rarely used. Selective factors driving the dynamics of the microbiota include stressors such as low nutrient content (due to alkali treatment and washing in alkali treated olives), high phenolic and high (6-10%) NaCl concentration, anaerobic conditions, amensalism and competition. Together with yeasts, lactic acid bacteria (LAB) are major components of table olives microbial communities. Halophilic *Pseudomonadota* (*Celerinatantimonas, Halomonas, Vibrio*) and halophilic and alkaliphilic LAB (HALAB) are also important in alkali treated Spanish style olives and specialties like Picholine. Amplicon targeted metagenomics, often combined with metabolomic approaches have recently replaced culture dependent and low-throughput approaches for the study of the microbiota of table olives. Publicly available data, when combined in properly annotated databases offer potential for quantitative meta-analysis: in the framework of the METAolive project, we combined 11 published studies extracted from the FoodMicrobionet database with two unpublished studies from our lab, thus obtaining a dataset with 525 samples, including raw fruits, olives and brines during fermentation, which is unique in terms of variety and size. We found 24 genera of LAB and HALAB. The latter (*Alkalibacterium, Marinilactobacillus*) were confined to alkali treated olives, and were dominant in the Picholine style. In Spanish style olives large differences exist between varieties, while in Greek style olives *Lactiplantibacillus*, together with *Pediococcus* and *Lentilactobacillus* almost invariably coexist and dominate the fermentation, while the occurrence and the abundance of other genera is more variable. In a study on Nyons olives, LAB prevalence and abundance was very low on contact surfaces and raw materials. Contamination patterns suggest that the dominance of LAB must be due to dispersal from other sources (reinoculum from fermentation vessels) combined with strong selective pressure. METAolive is co-funded by Ministero dell'Università e della Ricerca, PRIN 2022, proposal 2022NN28ZZ, and received funding from the European Union Next-GenerationEU - PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR).

S6B GENOMIC CHARACTERIZATION OF THE POTENTIAL PROBIOTIC STRAIN *Limosilactobacillus fermentum* CRL 2085 ISOLATED FROM FEEDLOT CATTLE

F. Fanelli[1], C. Aristimuño Ficoseco[2], M.E.F. Nader-Macías[2], G.M. Vignolo[2], V. Fusco[1], S. Fadda[2]

[1]ISPA-CNR. Via Amendola 122/O, Bari, Italy. E-mail: francesca.fanelli@ispa.cnr.it

[1]CERELA-CONICET. Chacabuco 145, Tucuman, Argentina. E-mail: sfadda@cerela.org.ar

Limosilactobacillus fermentum is a Gram-positive, heterofermentative species generally considered safe. It is found in sewage, fermented food, and in human and animal gastrointestinal tract. Due to its habitat and its resistance to bile salts this species is recognized as probiotic and introduced in intestinal and vaginal tract diseases treatments. *L. fermentum* CRL2085 was isolated from feedlot cattle in Argentina, selected for its phenotypic traits and administered in a large-scale survey to evaluate the modulation of faecal microbiome in feedlot cattle. To gain more insight into its probiotic potential we performed a genomic characterization of this strain. The genome of *L. fermentum* CRL 2085 is 1.9 Mb in length, has a GC content of 52.2 % and a coding density of 86%. The 1957 predicted genes include 34 carbohydrate-active enzymes, with glycosyl transferases and hydrolases being the most abundant. We identified several probiotic genes involved in adhesion, immunomodulation, temperature, osmotic and oxidative stress responsive genes, complete pathways for folate and riboflavin biosynthesis and tree putative clusters for exopolysaccharides production. *In silico* safety assessment validated the safety of this strains, while the presence of numerous CRISPR-Cas element might prevent the acquisition of horizontally transferred antimicrobial genes. Genomic analysis confirms that *L. fermentum* has an open genome, undergoing a dynamic evolution, with the acquisition of novel unique genes for adaptation to new environments. Genome-based phylogeny supported this evidence, as clustering of the strains only partially correlated with the source of isolation or the geographical origin, thus indicating that some *L. fermentum* strains have been recently introduced and transient in temporary environments. These data confirm the probiotic potential of this strains and its safety and encourage its further characterization to revealing new metabolic attributes that can fully exploit its biotechnological capabilities and optimize its application as probiotic in commercial feedlot.

S7A DIVERSITY OF POTASSIUM TRANSPORTERS IN LACTIC ACID BACTERIA AND THE ROLE OF CYCLIC-DI-AMP IN POTASSIUM HOMEOSTASIS REGULATION

V. S. Blancato[1], G. Acciarri[1], M. E. Taborra[1], F. O. Gizzi[1], C. Magni[1]
[1]IBR-CONICET-UNR. Suipacha 531, Rosario, Argentina. E-mail: blancato@ibr-conicet.gov.ar

Cyclic-di-AMP, a nucleotide second messenger in bacteria and some Archaea, regulates diverse metabolic processes including osmolyte uptake, cell wall homeostasis, antibiotic and heat resistance. Lactic acid bacteria (LAB) possess enzymes for cyclic-di-AMP synthesis and degradation but vary in their receptors. Many cyclic -di-AMP interacting partners identified in bacteria are involved in potassium homeostasis. These include K^+ importers or importer gating components (TrkA/KtrA, TrkH, KupA, KupB, and KimA), two-component systems that regulate K^+ importer expression (KdpD), a K^+ exporter (CpaA), and a riboswitch that regulates K^+ importer gene transcription. LAB exhibit diverse combinations of K^+ transporters, there is also variation between strains of the same species. In our laboratory, KupA and KupB were identified as novel cyclic-di-AMP binding proteins in *Lactococcus lactis*, functioning as high-affinity K^+ transporters inhibited by cyclic-di-AMP binding. Kup transporters are widely distributed among various species of *Lactobacillus* and *Enterococcus* and are also found in some species of *Streptococcus* and *Staphylococcus*. However, no Kup potassium transporter is encoded in the genomes *of B. subtilis*, *L. monocytogenes*, *S. aureus*, or *S. pneumoniae*. In *Enterococcus faecalis*, we demonstrated that Kup and KimA are high-affinity K^+ transporters, with KtrA playing a crucial role under stress conditions. Additionally, we found that the presence of the Kdp system in *E. faecalis* is strain-dependent, being more prevalent in clinical strains compared to environmental, commensal, or food isolates. Our research revealed that *kup* gene regulation in *E. faecalis* is affected by an IS6770 insertion sequence, with a 30% co-occurrence rate in IS6770-positive strains, suggesting its potential role in *E. faecalis* adaptation across diverse niches. These findings highlight the complexity and diversity of cyclic-di-AMP signaling pathways in bacteria, emphasizing the intricate regulatory networks that enable microbial adaptation and survival in different environments. The significance of potassium transporters in LAB is especially important, as they have a crucial role in maintaining cellular homeostasis and ensuring optimal growth and stress response. This is vital for their application in food production and probiotic development.

S7B DESIGNING COMPUTATIONAL TOOLS TO UNDERSTAND THE BACTERIAL-HUMAN CELL COMMUNICATIONS.

A. Salas Burgos

乳
酸
菌

LACTIC ACID BACTERIA AND HUMAN HEALTH

健
康

食
料

HH1 ORAL PATHOGEN INHIBITION BY SELECTED LACTID ACID BACTERIA

Naja JR[1,2], Urquiza MP[1], Bulacios GA[1], Cataldo PG[1], Nader MEF[1], Houde V.P[2], Hebert EM[1], Saavedra L[1] and Minahk C[3]

[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. [2]Université Laval, 2420 rue de la Terrasse, Québec, Canada. [3]INSIBIO-UNT-CONICET. Chacabuco 461. 4000 Tucumán, Argentina. E-mail: johanarnaja@gmail.com

Porphyromonas gingivalis is a Gram-negative anaerobic bacterium that plays a significant role in the development and progression of periodontitis. This disease begins when bacteria accumulate on the tooth surface, prompting immune cells to infiltrate the gingival sulcus (dental plaque). This process leads to inflammation, destruction of the supporting tissues of the teeth, bone resorption, and ultimately, tooth loss. Currently, periodontitis is managed through frequent and invasive periodontal therapy, such as scaling and root planning, to remove dental plaque. Therefore, alternative treatments are needed, and lactic acid bacteria (LAB) emerge as a plausible strategy. LAB strains or their metabolites have demonstrated antibacterial, antioxidant, and antifungal properties, as well as immunomodulatory capacities against oral pathogens. Consequently, it can be proposed that LAB may help mitigate the proliferation of periodontopathogens or serve as an adjunct to periodontal treatments. The objective is to study the antibacterial effect of Cell-free supernatant (CFS) of two LAB strains *Lacticaseibacillus rhamnosus* CRL1522 and *Lactiplantibacillus plantarum* CRL1363 against *P. gingivalis* growth. *P. gingivalis* (ATCC 33277) was routinely grown in Todd Hewitt broth (THB) enriched with hemin and vitamin K (THBe) at 37°C in anaerobic conditions. *L. rhamnosus* CRL1522 and *L. plantarum* CRL1363 (CERELA Culture Collection) were grown in MRS medium. Human gingival keratinocytes Htert TIGKs and the macrophage cell line U937 were used as cell models. The LAB strains under study did not exhibit significant antibacterial activity against *P. gingivalis*, although they did slow the pathogen's growth. However, the cell-free supernatant of *L. rhamnosus* CRL1522 and *L. plantarum* CRL1363 significantly reduced the secretion of IL-6 and IL-8 by the keratinocytes and TNF-α and IL-6 by the macrophages, which had been induced by *P. gingivalis*. More importantly, the activity of Arg-gingipain was markedly reduced by both LAB strains. *P. gingivalis* KDP112 was used as negative control. The expression of COX-2, NOD-1, NOD-2, TLR2 and TLR4 was also checked. In summary, *L. rhamnosus* CRL1522 and *L. plantarum* CRL1363 are suitable candidates for combating the periodontopathogen *P. gingivalis*.

HH2 EFFECTS OF PROBIOTIC YOGHURT ADMINISTRATION IN A MURINE METASTATIC BREAST CANCER MODEL UNDER CHEMOTHERAPY TREATMENT

M. N. Cerasuolo[1], J. G. J. LeBlanc[1], A. de Moreno de LeBlanc[1].
[1]CERELA-CONICET. Chacabuco 145. C.P. 4000 Tucuman, Argentina. E-mail: mcerasuolo@cerela.org.ar

Breast cancer (BC) is the most prevalent type of cancer diagnosed in women worldwide, and its metastasis are associated with a high mortality and morbidity rate. Chemotherapy is useful for treating BC metastasis; however, it affects the patients' quality of life leading to discontinuation of therapies. In this sense, the uses of probiotics are a promising alternative as adjuvant. Previously, our group designed a yoghurt made with two strains selected for their immunomodulatory properties. Considering that the host's immune system plays an important role against cancer and metastasis; the aim of this study was to evaluate the effects of the probiotic yoghurt administration in a murine model of BC metastasis undergoing an oral chemotherapy treatment with capecitabine (CAP). The probiotic yoghurt was prepared by fermenting milk with *Streptococcus thermophilus* CRL807 and *Lactobacillus delbrueckii* subspecies *bulgaricus* CRL864 from the CERELA Culture Collection. For BC metastasis, 4T1 BC cells were injected into the upper right mammary gland of 7–8-week-old BALB/c female mice. Tumors were removed after reaching a volume of 0.07 ± 0.02 cm^3, and mice started the treatments 24 hours after the surgery. Animals were randomly assigned into different groups: i) Metastasis control without treatment (received daily saline solution); ii) Yoghurt group administered yoghurt ad libitum; iii) Capecitabine (CAP) group received CAP for 14 days, followed by a 7-days resting period, and restarted the administration for another 14 days. i) Yoghurt + CAP group received yoghurt ad libitum and the described cyclic treatment with CAP. After the second cycle of CAP, mice were euthanized and samples were collected. Blood cell counts, small intestine and lung histology and serum cytokines were evaluated. Results showed that CAP treatment decreased the metastatic areas in the lungs, while this effect was maintained in mice that received probiotic yoghurt, which by itself also was associated with a decrease of metastasis in some animals. CAP treatment was associated with 57 % mortality rate, which was prevented in mice that received yoghurt. Yoghurt administration reduced CAP side effects, improved the blood cell counts, and decreased intestinal mucositis. CAP treatment was associated to decreases of most serum cytokines evaluated; and yoghurt administration increased the regulatory cytokine IL-10 levels in these mice. Yoghurt administration by itself maintained cytokines levels similar to the control but increased levels of IL-10. In conclusion, the probiotic yoghurt has the potential to be administered as immune adjuvant by decreasing undesirable side effects in patients under chemotherapy without affecting the primary treatment.

HH3 MODULATION OF LUNG IMMUNE RESPONSE AGAINST *Klebsiella pneumoniae* BY ORALLY ADMINISTERED *Lacticaseibacillus rhamnosus* CRL1505

R. Serda[1], S. Dentice Maidana[2], L. Albarracín[2], M. Elean[2], M.A. Jure[1], J. Villena[2].
[1]National University of Tucuman, Tucuman, Argentina. [2]CERELA-CONICET, Tucuman, Argentina. E-mail: jcvillena@cerela.org.ar

Orally administered *Lacticaseibacillus rhamnosus* CRL1505 enhances innate respiratory immunity, providing increased protection against respiratory viruses and *Streptococcus pneumoniae*. However, the capacity of the CRL1505 strain to improve respiratory immunity against Gram-negative bacterial infections has not been studied before. The aim of this work was to evaluate whether *L. rhamnosus* CRL1505 was able to beneficially regulate the respiratory innate immune response and enhance the resistance to hypermucoviscous KPC-2-producing *Klebsiella pneumoniae* of the sequence type 25 (ST25). Six-week-old male BALB/c mice were treated with the CRL1505 strain via the oral route (10^8 CFU/mouse/day) during 5 consecutive days and on day 6 nasally challenged with *K. pneumoniae* ST25 strains LABACER 01 or LABACER 27 (10^7 CFU/mouse). Mice without lactobacilli treatment and infected with ST25 strains were used as controls. Bacterial cell counts, lung injuries and the respiratory and systemic innate immune responses were evaluated 2 days after the bacterial infections. Both *K. pneumoniae* strains were able to colonize the lungs of mice while only LABACER 01 was detected in blood samples. LABACER 01 and LABACER 27 increased the levels of albumin and LDH in broncho-alveolar lavages (BAL) that were used as markers of the alteration of the alveolar-capillary barrier and cellular toxicity, respectively ($p<0.05$). The LABACER 01 strain induced a higher lung damage than LABACER 27. The results also showed that *K. pneumoniae* ST25 strains increased the levels of TNF-α, IL-1β, IL-6, IFN-γ, IL-17, KC and MPC-1 ($p<0.01$) in the respiratory tract and blood, as well as the numbers of BAL neutrophils and macrophages ($p<0.05$). Mice orally treated with *L. rhamnosus* CRL1505 had significantly lower *K. pneumoniae* counts in their lungs ($p<0.05$) as well as reduced levels of BAL albumin and LDH ($p<0.05$) than controls. The treatment with the CRL1505 strain avoided the dissemination into blood of *K. pneumoniae* LABACER 01. In addition, reduced levels of inflammatory cells ($p<0.05$), cytokines and chemokines in the respiratory tract and blood ($p<0.01$) were found in CRL1505-treated mice compared to infected controls. Furthermore, higher levels of IFN-γ and the regulatory cytokines IL-10 and IL-27 ($p<0.05$) were found in the respiratory tract and blood of CRL1505-treated mice than controls. These results demonstrate the ability of orally administered *L. rhamnosus* CRL1505 to improve the resistance against *K. pneumoniae* infection and to help with the control of detrimental inflammation in the lungs. Although further mechanistic and clinical studies are necessary, *L. rhamnosus* CRL1505 can be proposed as a candidate to improve patients' protection against hypermucoviscous KPC-2-producing strains belonging to the ST25, which is endemic in the hospitals of the North of Argentina.

HH4 THE INTESTINAL FLUIDS OF PROBIOTICS FED MICE TRIGGER IMMUNOMODULATORY PROPERTIES ON INNATE IMMUNE CELLS

Martínez Monteros MJ[1], Vestani A[1], Perdigón G[1], Maldonado Galdeano C[1,2], Cazorla SI[1,2]
[1]CERELA-CONICET, Chacabuco 145. 4000 Tucumán-Argentina. [2]Cátedra de Inmunología Facultad de Bioq., Qca. y Fcia, UNT. Tucumán-Argentina. E-mail: mariajosemartinezmonteros@gmail.com

In the past decade, it has been proposed that the antimicrobial activity is only one of multiple functions of the antimicrobial peptides. We have previously demonstrated that oral administration of probiotics increases the number of Paneth cells, the 5-α-defensine and the antimicrobial activity of the intestinal fluids against pathogen bacteria. Therefore, our aim was to analyzed whether oral administration of probiotics can modulate the function and survival of key innate-immune cells. Murine peritoneal macrophages and bone marrow derived DC were cultured at 37◦C and 5% CO_2 with LPS, medium alone or the intestinal fluids from mice fed by 7 and 5 days upon a conventional diet or the supplementation with *L. casei* CRL 431 or *L. paracasei* CNCM I-1518, respectively. Twelve hours later, significant increases on the costimulatory molecules CD80 and CD86 were detected on the surface of the antigen presenting cells (APC) stimulated with the intestinal fluids of the mice fed with the probiotics ($p \leq 0.05$) was observed by flow cytometry and confocal assay. Additionally, significant increases in IL-6 and INF-γ were found in the supernatants of the APC stimulated with the intestinal fluids of animals fed with the probiotics, in comparison to the absence of stimuli. Most important, IL-10 counterbalance the increase of the aforementioned proinflammatory cytokines. Intestinal fluids from probiotics fed mice significant promote the migration of macrophages and lymphocytes comparing to those observed in the presence of the chemotactic medium alone (***$p<0.001$), as was observed in an *in vitro* wound-healing and transwell migration assay. Finally, intestinal fluids of probiotics fed animals enables the survival of the macrophages upon exposition to UV, decreasing the number of cells upon early and late apoptosis ($p \leq 0.05$). These results point out the oral administration of Lc 431 and Lp1518 reaches beyond their intestinal antimicrobial effect, modulating the function and survival of key innate-immune effector cells to enhance the clearance of pathogen microbes and the resolution of inflammatory process.

HH5 *Lacticaseibacillus rhamnosus* CRL1505 AMELIORATES LIVER INJURY AND INFLAMMATION IN POLY(I:C) INDUCED ACUTE HEPATITIS

M.J. Lorenzo Pisarello[1], M. Elean[1], L. Albarracín[1], R.E. Serda[1], F. Namai[2], Y. Suda[3], H. Kitazawa[3], J. Villena[1]

[1]CERELA-CONICET. 4000, Tucuman, Argentina. [2]Tohoku University, 980-8572, Sendai, Japan. [3] Miyagi University, 980-8572, Sendai, Japan. E-mail: jcvillena@cerela.org.ar

The immunomodulatory strain *Lacticaseibacillus rhamnosus* CRL1505 regulates the innate antiviral immune responses in the intestinal and the respiratory tracts, allowing a higher resistance to viral challenges and protecting against the inflammatory-mediated tissue damage. The beneficial effects of the CRL1505 strain were not investigated in the context of viral-induced liver inflammation. This work aimed to evaluate the potential protective effect of *L. rhamnosus* CRL1505 in murine model of acute hepatitis induced by the Toll-like receptor 3 (TLR3) viral mimetic, poly(I:C). Male 6-week-old BALB/c mice fed *L. rhamnosus* CRL1505 in the drinking water for 5 consecutive days (10^8 cells/mouse/day). Mice without lactobacilli treatment were used as controls. On day 6, the CRL1505-treated and the untreated control mice received an intraperitoneal injection of 50 µg of poly(I:C) in 100 µl of PBS. Two days after the TLR3 activation, the liver damage, the serum hepatic enzymes ALT and AST as well as the expression of interferons (IFNs) (*IFN-β*, *IFN-γ*, *IFN-λ1*, *IFN-λ2/3*), antiviral factors (*Mx1*, *OAS1*, *RNAseL*, *IFITM3*), inflammatory mediators (*TNF-a*, *IL-1β*, *IL-6*), and regulatory cytokines (*IL-10*, *IL-27*) in the liver tissue were determined. The intraperitoneal administration of poly(I:C) induced inflammatory-mediated liver tissue damage, characterized by elevated levels of ALT and AST ($p<0.05$), increased inflammatory cell infiltration and upregulation of IFNs ($p<0.05$), and the pro-inflammatory factors *TNF-a*, *IL-1β*, *IL-6* ($p<0.05$) compared to basal levels. The supplementation of *L. rhamnosus* CRL1505 resulted in a notable reduction in pro-inflammatory mediators ($p<0.05$), accompanied by a significant elevation in *IFNs*, *IL-10* and *IL-27* ($p<0.01$), as well as *Mx1*, *OAS1*, *RNAseL* and *IFITM3* ($p<0.05$). The differential regulation in the expression of inflammatory and anti-inflammatory factors induced by the CRL1505 strain was clearly reflected in the decrease of poly(I:C)-induced liver damage, which was observed in the improved levels of transaminases. The results of this work demonstrate for the first time that *L. rhamnosus* CRL1505 can exert immunomodulatory and protective effects in the liver in the context of TLR3-induced acute hepatitis. The variations in the immune factors evaluated suggest that the CRL1505 strain would have the ability to enhance liver antiviral defenses and reduce inflammatory-mediated liver damage, making it a promising tool to prevent or reduce the severity of viral hepatitis.

HH6 EVALUATION OF A MIXTURE OF SELECTED LACTIC ACID BACTERIA (LAB) IN A MURINE MODEL OF LEVODOPA INDUCED DYSKINESIA (LID)

P. A. Yánez[1], A. de Moreno de Leblanc[1] J. G LeBlanc[1].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. E-mail: pyanez@cerela.org.ar

Parkinson's disease is the second most common neurodegenerative disease and levodopa is the most widely used drug to treat its signs and symptoms. However, after 5-6 years of use it is associated with complications, such as dyskinesia, that affect patients' quality of life. The possibility that changes in the intestinal microbiota may be associated with these effects is being increasingly studied, and probiotics are being studied as complementary treatments. Previously, in our group, the neuroprotective effect of a mixture (MIX) of three LAB (*Streptococcus thermophilus* (*St.*) CRL808, a folate-producing strain, *Lactiplantibacillus plantarum* CRL2130, a riboflavin overproducing strain, and St. CRL807, a strain with immunomodulatory properties) was demonstrated in a murine parkinsonism model. The aim of this work was to evaluate the potential of this bacterial MIX on the adverse effects associated with levodopa treatment (especially dyskinesia), through a murine model of Levodopa Induced Dyskinesia (LID). C57BL/6 male mice were used as model. After establishing parkinsonism through injections of the neurotoxic drug 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) with intraperitoneal injections of levodopa-benserazide from day 21 were administered with the purpose of inducing dyskinesia. During this period of dyskinesia establishment some groups of animals received the MIX in order to analyze the effect during their development. At day 42, mice that did not receive LAB were separated into two groups that either received or not the bacterial MIX to evaluate the effect once dyskinesia was established. A Motor tests were conducted throughout the model and abnormal involuntary movements (AIMs) of both axial and limbs were evaluated at the end of the experimental period. Mice were sacrificed and serum and brain samples were taken for cytokine determinations. The parkinsonism model was confirmed with the motor tests. Mice received levodopa-benserazide completed the tests in shorter times than parkinsonism control animals. being the group that received LAB MIX from the beginning the one that presented the best results. However, prolonged administration of levodopa was associated with the presence of AIMs, being the MPTP/LEVO group the one that presented the highest score. On the other hand, mice received the LAB MIX from the beginning of the model were the ones with the lowest score, similar to the healthy control animals. Serum cytokines showed the highest levels in the parkinsonism control group. The administration of levodopa decreased most cytokines without significant differences with the other experimental groups. Similar results were observed at the brain level, highlighting that mice received the MIX after the model was established increased IL-10, modulating the immune response at the brain level. In conclusion, the selected LAB MIX has potential to be used as an adjuvant to levodopa, reducing its side effects.

HH7 ADMINISTRATION OF *Lacticaseibacillus paracasei* subsp. *paracasei* CRL75 MODULATES HUMORAL AND CYTOKINE PRODUCTION DURING FOOD ALLERGY

J. Laiño[1]*, M.H. Zelaya[2], M.P. Taranto[1].
[1]CERELA-CONICET, Chacabuco 145, CP 4000, San Miguel de Tucumán, Tucumán, Argentina. [2]Instituto de Bioquímica Aplicada - U.N.T, Balcarce 747, San Miguel de Tucumán, Tucumán, Argentina. *E-mail: jlaino@cerela.org.ar

In general, food allergies are not exclusive to one type of food, but rather many of them have cross-reactions with other foods and/or pollen. Some examples of allergenic foods are shellfish, fish, eggs, soy, peanuts, strawberries, and cow's milk, among others. On the other hand, lactic acid bacteria (LAB) have been demonstrated to be useful in improving immune responses in many different diseases. Their beneficial effect on consumer health is widely reported, as their ability to release and/or produce specific compounds (nutraceuticals) with positive outcomes for the human or animal host. Many exopolysaccharides (EPS) produced by beneficial LAB are powerful immunomodulatory molecules. Previously, it was demonstrated that *Lacticaseibacillus (L.) paracasei* subsp. *paracasei* CRL75 has a potent immunostimulatory effect and produces functional EPS. The aim was to evaluate the ability of *L. paracasei* CRL75 to modulate the immune response during food allergy induction by regulating the humoral immune response and related cytokines. The immunomodulatory capacity of *L. paracasei* CRL75 was evaluated *in vivo* in an OVA-induced intestinal allergy mouse model. Six-week-old BALB/c mice were divided into 3 groups: a) Control (C): non-sensitized animals received a conventional balanced diet and water *ad libitum*; b) OVA: animals sensitized to OVA by bi-weekly intraperitoneal injection; c) *L. paracasei* CRL75 (CRL75): OVA-sensitized animals supplemented with *L. paracasei* CRL75 in drinking water simultaneously to OVA challenge. Two weeks after second injection, the intestinal allergy was induced by feeding an egg white diet for 7 days. Samples were obtained to evaluate: a) cytokines: IL-4, IL-10, IFN-γ, TNF-α, and IL-6; b) OVA-specific antibodies (OD450nm): IgE, IgG, IgG1, and IgG2a; c) bacterial translocation to the liver and spleen. Results demonstrated that administration of *L. paracasei* CRL75 induced the production of IL-10 (pg/ml, C: 89.18±8.11; OVA: 176.3±18.7; CRL75: 371.99±25.49, $p<0.0001$) and IFN-γ, with a significant decrease in IL-4 levels (pg/ml, C: 71.43±18.1, OVA: 941±94.77; CRL75: 545.49±66.92, $p<0.0001$). Regarding humoral response, *L. paracasei* CRL75 induced a significant decrease of OVA-specific IgE levels in serum (OD450nm, C: 0.064±0.017; OVA: 0.717±0.085; CRL75: 0.449±0.085, $p<0.001$), but increased OVA-specific IgG1 and IgG2a compared to the OVA group. No bacterial translocation was detected in any experimental group. In conclusion, *L. parcasei* CRL75 is able to regulate the allergic immune response when administered simultaneously with allergy induction. *L. paracasei* CRL75 developed a Th-1 response, decreasing Th-2 response, with reduced levels of OVA-specific IgE and IL-4. *L. paracasei* CRL75 constitutes a promising option for designing functional foods with beneficial effects on food allergies.

HH8 ORAL ADMINISTRATION OF LP 1518 IMPROVES THE CLINICAL OUTCOME OF *L. amazoniensis* INFECTION

J. Arrieta[1], A. Vestani[1], EM Vélez[1,2], C. Maldonado[1,2], S. Cazorla[1,2]
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. [2]Cátedra de Inmunología Facultad de Bioquímica, Química. y Farmacia, UNT. Tucumán-Argentina. E-mail: jarrieta@cerela.org.ar

Depending on the virulence factors of the parasites and the immune response established by the host, a wide spectrum of disease known as leishmaniasis can appear. One of them, cutaneous leishmaniasis, causes serious disability leaving patients permanently scarred. Neither reliable nor safe vaccine is available. Thus, there is an urgent need for development effective prophylactic and/ or therapeutic approaches against leishmaniasis. It has been proposed that intestinal dysbiosis may cause changes in inflammatory responses and progression of leishmaniasis. Since probiotics contribute to maintain the intestinal homeostasis, we decided to investigate the effect of oral supplementation of *Lacticaseibacillus paracasei* (Lp) CNCM I-1518 on the *L. amazoniensis* infection. Balb/c mice received the bacterium: ten days before the infection (GI); the day of the infection (GII); or at the time the symptoms appeared (GIII). Since the times indicated, all the animals received the probiotics in the drinking water until they were sacrificed. GIV-Infected controls received a conventional diet along all the experience (GIV). Mice were subcutaneous infected in their footpads with 10^6 stationary-phase promastigotes of *L. amazoniensis*. We observed that only the administration of the probiotics before the infection was able to significant decrease the progressive footpad swelling caused by the parasite infection ($p<0.05$). By contrast no significant differences were observed for GII and GIII with respect to infected control animals which developed an ulcerated dermal lesion. Three months after the infection mice were sacrificed and their large intestine, footpads and draining lymph nodes to the site of infection were aseptically excised for parasite load and intestinal and skin microbiota analysis. Unfortunately, no differences were observed in the parasite load neither in the footpad nor in the draining lymph nodes of the animals. By plate count agar on specific selective media, we observed significant increases in the count of total anaerobic bacteria and lactobacilli of GI and GV of large intestine homogenized samples. Interestingly, increases UFC of Enterococcus and Streptococcus were determined in the cutaneous lesions from GI in comparison with the contralateral healthy skin microbiota from the same individuals and from GIV ($p<0.01$). These findings represent the first steps to understand the potential effects of probiotics supplementation in modulating the leishmaniasis. However, further studies are necessary to explore the mechanisms by which Lp CNCM I1518 modify the clinical course of *Leishmania spp.* infection.

HH9 *Lactiplantibacillus plantarum* CIDCA83114 AS A POTENTIAL TREATMENT FOR HEMOLYTIC UREMIC SYNDROME

C. Cancino[1], Y. Carpintero Polanco[1], A. Bernal[1], F. Sosa[1], V. Ramos[1], A. Abraham[2], G. Garrote[2], M. Palermo[1], R. Fernández Brando.

[1]IMEX-CONICET-Academia Nacional de Medicina, José Andrés Pacheco de Melo 3081. 1045 Buenos Aires, Argentina. [2]CIDCA-CONICET-UNLP-CIC, Calle 47 esq. 116. 1900 La Plata, Buenos Aires, Argentina. E-mail: cdcancino@gmail.com

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) O157:H7 is a foodborne pathogen, which can lead to the life-threatening Hemolytic Uremic Syndrome (HUS). There is no treatment to reduce HUS outcome up to date. Growing evidence supports the use of probiotics to treat and prevent intestinal diseases. *Lactiplantibacillus plantarum* CIDCA83114 (CIDCA83114) is a probiotic strain isolated from milk kefir. We have previously demostrated that CIDCA83114 is able to reduce the pathogenicity of STEC *in vitro* by inhibiting its growth, Stx production and activity, adhesion to Caco-2 cells by exclusion as well as modulating the expression of cytokines such as IL8 and TGF_{β}. The aim of this work was to evaluate the preventive or therapeutic capacity of CIDCA83114 on the development of the characteristic symptoms of HUS in a murine model by intragastric inoculation of a STEC strain at weaning. Three protocols were evaluated: pre (inoculating mice 24h before and the same day as STEC), post (inoculating mice daily with CIDCA83114 after STEC infection) and pre-post (PP, combining both treatments). Mice and their food were weighed daily. Blood samples were taken at 72h to determine the leukocyte formula and urea levels as parameters of HUS outcome. Feces samples were collected at 48 and 72h, and then plated onto MacConkey agar to test the excretion of STEC and MRS agar for lactobacilli excretion. Mortality tended to be reduced and/or delayed with the probiotic treatments (median survival: STEC and pre=72h, post and PP=96h, Log Rank test p=0.17), accompanied with an incremented food consumption and mice weight at 72h (mean weight±SD: STEC=6.6±0.2; pre, post and PP=7.7±0.7, Welch's ANOVA p=0.13), as indicators of growth, well-being and an attenuation of the digestive disease. Moreover, urea levels of pre and PP-treated mice showed a tendency to decrease compared to post-treated and STEC mice (mean±SD [mg/dl]: pre: 99.8±16.7, PP: 80.9±13.1, post: 121.2±39.9, STEC: 103.6±15.4; Kruskal-Walis p=0.053) suggesting a reduction of the renal failure characteristic of HUS. Also, STEC excretion in feces was significantly lower in mice treated with CIDCA83114 at 48h (mean±SD [UFC/mg]: STEC=$2.4\pm0.8\cdot10^{9}$, pre=$2.8\pm1.5\cdot10^{4}$, PP=$6.0\pm2.7\cdot10^{6}$; Kruskal-Walis p<0.05). In conclusion, CIDCA83114 accelerated STEC excretion and tended to reduce kidney damage and weight loss along with a higher food consumption, and to alleviate its lethality in mice. These results encourage us to keep testing the ability of this and other LAB strains as potential treatments to reduce HUS outcome after STEC infection.

HH10 PROBIOTICS DECREASE INFLAMMATORY BOWEL DAMAGE IN AGING MICE

A. Vestani[1], J.G. Arrieta[1], E.M. Velez[1,2], C. Maldonado Galdeano[1,2], S.I. Cazorla[1,2].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. [2]Catedra de Inmunología Facultad de Bioquímica, Química y Farmacia, UNT. Tucumán-Argentina. E-mail: avestani@cerela.org.ar

Inflammatory bowel disease (IBD) is a chronic, multi-causal condition characterized by progressive structural and functional damage of the gastrointestinal tract. Evidence from recent epidemiological data suggests that the patient population with IBD is chronologically aging. There are several treatments to IBD including immunomodulators, aminosalicylates, antibiotics, among others. However, many patients do not respond to such therapies or lose responsiveness over time. Probiotics arise as adjuvants to these conventional therapies. Balb/c mice from 120 days old were feeding during 60 days with the probiotics ($1x10^8$) *Lacticaseibacillus paracasei* CNCM I-1518 (Lp1518) or a Mix of probiotic bacteria (Bio-Kult Protexin) in the drinking water. Supplementation was administered in intervals of 10 days; followed by 5 days of rest, in which animals received only a conventional diet. An intestinal inflammation was induced by two subcutaneous injections of 7.5mg/kg/day of indomethacin (I) on the last two days of the experiment. Experimental groups are as follow: G1: indomethacin (I); G2: Lp1518+I; G3: Mix+I. Controls groups are: G4: normal control (NC); G5: Lp1518; G6: Mix. Controls animals received 2 injection of PBS. Mice were euthanized by cervical dislocation and blood, intestinal fluids and small and large intestinal were taken. We observed a significant body weight loss in (I) compared to (NC) ($p<0.05$). In contrast, mice with indomethacin-induced inflammation and supplemented with the probiotics showed an increase in body weight compared to their pre-indomethacin injection values. Interestingly, while (I) mice showed a significant reduction in their hematocrit ($p<0.05$), Lp1518+I and Mix+I presented similar values than NC mice. In hematoxylin-eosin staining of small intestine sections, damage of the intestine architecture was observed. (I) animals showed dilated crypts, villi that did not run parallel and an important shortening of the intestinal villi, compared to NC and Lp1518+I and Mix+I mice ($p<0.05$). Finally, Lp 1518 supplementation restore the large intestine microbiota shifts induced by indomethacin, increasing the total lactobacilli and anaerobes population. These results highlight probiotics as an effective supplement to face aging-related disorders in IBD, thereby helping to avoid adverse clinical outcomes among older community dwellers.

HH11 EFFECTS OF PROBIOTIC YOGHURT IN A MURINE BREAST CANCER MODEL UNDER CHEMOTHERAPY TREATMENT

M. N. Cerasuolo[1], J. G. LeBlanc[1], A. de Moreno de LeBlanc[1].
[1]CERELA-CONICET. Chacabuco 145. C.P. 4000. Tucuman, Argentina. E-mail: mcerasuolo@cerela.org.ar

Breast cancer (BC) remains the most commonly diagnosed cancer globally in women. Surgical removal of the tumor is still the best treatment; however, adjuvant treatments are usually required to eliminate possible remaining tumor cells. Chemotherapy is the most common treatment in these cases, which is not specific for tumor, affecting other highly replicative cells and the patients' quality of life. In this sense, probiotics have been proposed as candidates, not only to decrease the severity of non-desired side effects, but also because their immune modulating properties. Previously our group developed a probiotic yoghurt with two starter bacteria selected for their immunomodulatory properties demonstrated in models of intestinal inflammation and colon cancer. The aim of this study was to evaluate the effects of probiotic yoghurt administration in a murine model of breast cancer under oral chemotherapy treatment with capecitabine (CAP). The probiotic yoghurt was prepared by fermenting milk with *Streptococcus thermophilus* CRL807 and *Lactobacillus delbrueckii* subspecies *bulgaricus* CRL864. For BC induction, 4T1 cells were injected into the upper right mammary gland of 7–8-week-old BALB/c female mice. When tumors reached a diameter of 0.3 ± 0.1 cm, mice started receiving different treatments. Animals were randomly assigned into different groups: i) a BC control without treatment received unfermented milk ad libitum; ii) Yoghurt group received yoghurt ad libitum; iii) CAP group received unfermented milk and daily oral CAP administration during 14 days; iv) Yoghurt + CAP group received yoghurt ad libitum and CAP. Tumor volume and body weight was assessed during the whole experiment. After 14 days, mice were euthanized and samples were collected. Blood cell counts, small intestine histology, and serum cytokines were evaluated. Results showed that probiotic yoghurt by itself decreased the tumor growth. When it was administered in mice treated with CAP, there was a decrease in side effects without affecting the anti-tumor treatment. Mice that received the probiotic yoghurt showed less intestinal inflammation, reduced weight loss, and a higher survival rate. Serum cytokines showed that yoghurt administration was associated with a modulation of the immune response increasing the levels of IL-10 (a regulatory cytokine). In conclusion, probiotic yoghurt administration did not interfere with the cancer chemotherapeutic treatment, was able to reduce its side effects and modulated the host immune response, making it an ideal candidate for adjunct therapies.

HH12 *Lacticaseibacillus rhamnosus* CRL1505 PEPTIDOGLYCAN MODULATES TISSUE FACTOR EXPRESSION AND PROTEASE ACTIVATED RECEPTOR 1 ACTIVATION DURING INFLAMMATION-COAGULATION RESPONSE TRIGGERED BY POLY(I:C) IN THE RESPIRATORY TRACT

H. Zelaya[1,2], L. Arellano-Arriagada[2], K. Fukuyama[3], K. Matsumoto[3], G. Marranzino[2,4], F. Namai[3], S. Salva[2], S. Alvarez[1,2], G. Agüero[1], H. Kitazawa[3], and J. Villena[2,3]
[1]Institute of Applied Biochemistry, Tucuman University, Tucuman, Argentina. [2]Laboratory of Immunobiotechnology, CERELA-CONICET, Tucuman, Argentina. [3]Graduate School of Agricultural Science, Tohoku University, Sendai, Japan. [4] Universidad del Norte Santo Tomás de Aquino (UNSTA), Tucuman, Argentina. E-mail: jcvillena@cerela.org.ar

Previously, we demonstrated that nasally administered viable or non-viable *Lacticaseibacillus rhamnosus* CRL1505 as well as its peptidoglycan (PG-Lr1505), beneficially modulates TLR3-mediated inflammation-coagulation relationship in mice. In this work, to deep in the possible mechanisms underlying this positive effect, we investigated the capacity of PG-Lr1505 to modulate the tissue factor (TF) expression and protease activated receptor 1 (PAR1) activation as part of immuno-coagulative response triggered by the viral pathogen-associated molecular pattern poly(I:C) in the respiratory tract. Adult BALB/c mice were nasally treated with PG-Lr1505 (8 µg/mL in 50 µL of PBS) for two days. Treated and untreated control mice were then nasally challenged with poly(I:C) (250 µg/day). Mice received three doses of poly(I:C) with a 24 h rest period between each administration. The immuno-coagulative response was studied after the last administration of poly(I:C). The challenge with poly(I:C) significantly increased blood and respiratory pro-inflammatory mediators and induced hemostatic changes in plasma as well as TF, TF pathway inhibitor (TFPI), and thrombomodulin (TM) expressions in the lungs. However, PG-Lr1505-treated mice differentially modulated the hemostatic parameters in plasma and lungs. The study of TF in lungs samples and in specific lung myeloid populations ($CD45^+$, $Gr1^+$, $Gr1^{low}$, $Gr1^{high}$, $F4/80^+$ and, $F4/80^+MHCII^+$ cells), showed that poly(I:C) significantly increased TF expression in all the lung cell populations evaluated, in both experimental groups. No significant differences were observed between the groups for the distinct myeloid populations, except for $F4/80^+TF^+$ ($p<0.01$) and $F4/80^+MHCII^+TF^+$ ($p<0.05$) lung cells, which were significantly lower in the PG-Lr1505 group than controls. Considering thet PARs activation by coagulation proteases contributes to the increase of inflammation, we evaluated the activation of the thrombin receptor PAR1 in lungs. Poly(I:C) induced a significant increase in PAR1 activation in total lung samples, in lung $CD45^+$, $Gr1^+$, $Gr1^{low}$, and $Gr1^{high}$ neutrophils as well as in $F4/80^+$, and $F4/80^+MHC\text{-}II^+$ cells from both groups. However, PG-Lr1505-treated mice evidenced a trend toward decreased total PAR1 activation in the lung ($p=0.057$). These results indicate that the peptidoglycan from *L. rhamnosus* CRL1505 is able to regulate TF expression modulating the procoagulant state which could contribute to less PAR1 activation in lungs in the context of the activation of TLR3 signaling pathways, contributing to a beneficial modulation of inflammation-hemostasis crosstalk. The CRL1505 peptidoglycan could be used as a nasally administered postbiotic to reduce inflammation-coagulation alterations during the course of respiratory viral infections.

HH13 EFFECT OF RIBOFLAVIN-OVERPRODUCING *Lactiplantibacillus plantarum* CRL2130 ON BREAST CANCER CELLS UNDER TREATMENT WITH CHEMOTHERAPEUTIC DRUGS

R. Levit[1], M. N. Cerasuolo[1], J. G. LeBlanc[1], A. de Moreno de LeBlanc[1].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. E-mail: rlevit@cerela.org.ar

Breast cancer (BC) is the deadliest cancer in women. Patients with early BC are normally treated with chemotherapeutic agents that produce undesirable side effects such as gastrointestinal toxicity that can cause the discontinuation or delays of therapy. Therefore it is necessary to explore new strategies that may improve oncological therapies and/or attenuate side effects. A riboflavin-overproducing strain, *Lactiplantibacillus* (*L.*) *plantarum* CRL2130, was selected because of its anti-inflammatory properties shown in mucositis and colitis models. The aim of this study was to evaluate *L. plantarum* CRL2130 *in vitro* using a murine BC cell line exposed to chemotherapeutic drugs. The effect of immune cells stimulated with *L. plantarum* CRL2130 was also analyzed. Murine BC cell line 4T1 was cultured in the presence of bacterium cell extract and then treated with capecitabine (CAP) or 5-Fluorouracil (5-FU), evaluating viability by MTT assay, and the production of reactive oxygen species (ROS) by using 2',7'–dichlorofluorescin diacetate dye. *L. plantarum* CRL725 (strain from which CRL2130 was derived that does not overproduce riboflavin) and commercial riboflavin were used as controls. To study the immunomodulatory potential, spleen cells from BALB/c mice (n=9) fed by gavage for thirteen days with *L. plantarum* CRL2130 (10^8 CFU/mL) were cultured and the conditioned medium (CM) obtained was evaluated on the viability of tumor cells exposed to CAP. The levels of cytokines (IL-10, TNF-α and IL-6) in CM were also determined by cytometric bead array. The results showed that *L. plantarum* CRL2130 extract significantly ($p<0.05$) decreased the viability of tumor cells even in the absence of CAP (71±3 %), and had a synergetic effect in its presence (56±2 %). *L. plantarum* CRL2130 extract also exerted an antioxidant effect by a significantly ($p<0.0001$) reduction in the production of ROS both in presence (76±1 %) and in absence (68±1 %) of 5-FU. CM from splenocytes from mice that received *L. plantarum* CRL2130 decreased the viability of tumor cells even in the absence of CAP, without interfering with the effect of this drug. The cytokines analysis showed in the CM a lower level of the pro-inflammatory cytokine TNF-α than in the CM of splenocytes from mice that did not receive the bacterium. In conclusion, *L. plantarum* CRL2130 showed an anti-tumor effect in the *in vitro* model of BC without interfering with the chemotherapeutic treatment. This beneficial effect is, at least in part, due to its antioxidant properties (associated with the production of riboflavin) and its immunomodulatory potential.

HH14 EFFECT OF PROBIOTICS SUPPLEMENTATION IN RE-NUTRITIONAL DIETS ON THE THYMUS AND INTESTINE OF MALNOURISHED MICE

MF Balcells[1,2], E Velez[1,3], S Cazorla[1,3], G Perdigón[1], S Feliu[4] y **C Maldonado[1,3*]**
[1]CERELA-CONICET, Chacabuco 145. 4000 Tucumán-Argentina. [2] Cátedra de Microbiología y Parasitología, Nutrición. UNSTA. Tucumán-Argentina.[3]Cátedra de Inmunología Facultad de Bioq., Qca. y Fcia, UNT. Tucumán-Argentina. [4]Cátedra de Nutrición, Facultad de Fcia. y Bioq. UBA. C.A.B.A. Buenos Aires-Argentina. E-mail: cmaldo@cerela.or.ar

Malnutrition is a major public health problem, affecting millions of people in our country and around the world. Nutritional imbalances compromise the immune system causing various degrees of immunodeficiency, affects the intestinal barrier, especially the intestinal epithelial cells (IEC) and the thymus, increasing the susceptibility to acute infections and leading to the development of more severe forms of disease. Studies have shown that the addition of nutrient recovery diets allows for partial or even full restoration of the thymus functionality. Our group has demonstrated that probiotic supplementation recovers the intestinal barrier, histological alterations, and the mucosal and systemic immune functions even though nutrient intake remained insufficient. Our aim was to study the effect of nutritional recovery diets supplemented with different strains of probiotics in a mouse malnutrition model. Adult Balb/c mice were divided into eight groups: A) NC: Normal Control; B) DSN: Malnutrition Control; C) DSN+F: DSN+ conventional food; D) DSN+M: DSN+Milk; E) DSN+Lp: DSN+ *Lacticaseibacillus Paracasei* 1518; F) DSN+PM: DSN+ commercial probiotic MIX; G) DSN+F+M: DSN+ conventional food + Milk; H) DSN+F+Lp: DSN+ conventional food + *Lp* 1518; and I) DSN+F+PM: DSN+ conventional food + commercial probiotic MIX. Samples of thymus and IEC were cultured to measure IL-6, IFN-γ, TNF-α, IL-12 and IL-10 levels. Also T lymphocyte population was analyzed in thymus. Fatty acid profile was analyzed in serum samples. DSN group showed an elevated proinflammatory cytokines level in Thymus and IEC culture supernatant. Re-nutrition diets in all malnourished mice significantly decreased levels of proinflammatory cytokines and increased IL-10 values compared to DSN mice, especially in DSN mice receiving Lp and PM. Re-nutrition diet with probiotic supplementation recovers LT $CD4^+$ and LT $CD4^-/CD8^-$ population in thymus. PM supplementation increased the percentage of LT CD4+ and Lp normalized the percentage of the LT CD4+/CD8+ population. Re-nutrition diet in combination with Lp or PM improve the percentage of some omega 3 fatty acids (alpha linolenic, EPA, DHA) and the omega 6 family (Linoleic and arachidonic) and oleic, diminished in DSN mice. Our research revealed that the re-nutrition diet with probiotics promote a better recovery of the immune system and improved fatty acid profile compared to re-nutrition diet alone. These findings suggest that the combination of a re-nutrition diet with probiotics may be an effective strategy to improve health and lifespan in malnourished individuals.

HH15 EVALUATION OF A RIBOFLAVIN- OVERPRODUCING LACTIC ACID BACTERIUM IN A MURINE BREAST CANCER MODEL UNDER TREATMENT

R. Levit[1], M. N. Cerasuolo[1], J. G. LeBlanc[1], A. de Moreno de LeBlanc[1].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucuman, Argentina. E-mail: rlevit@cerela.org.ar

Breast cancer (BC) is the most common cancer in women and is the leading cause of cancer-related death worldwide. Chemotherapy is widely used for the treatment of BC; however, it produces adverse effects in patients. The search for adjuvant agents that minimize the toxicity of oncological drugs without reducing their anti-tumor efficacy has become an important issue. Previously, *Lactiplantibacillus* (*L.*) *plantarum* CRL2130, a riboflavin- overproducing strain, showed an anti-tumor effect in an *in vitro* model of breast cancer without interfering with the chemotherapeutic treatment. The aim of this study was to evaluate the effect of *L. plantarum* CRL2130 in an *in vivo* model of breast cancer under treatment with capecitabine (CAP). BALB/c mice were injected with 4T1 cells into a mammary gland. When the tumor reached a diameter of 0.4 ± 0.1 cm, mice received an oral treatment with CAP (cyclically for two weeks with one week of rest) and were separated into groups that received: *L. plantarum* CRL2130, *L. plantarum* CRL725 (strain from which CRL2130 was derived that does not overproduce riboflavin) (100 µl/day for one week, concentration of $1x10^8$ CFU/ml), commercial riboflavin (in a concentration equivalent to that produced by *L. plantarum* CRL2130), or physiological solution (FS). Other groups received only the bacteria, the riboflavin or FS. The tumor volume and weight of the mice were measured every two days. Blood samples were taken to measure serum cytokine levels by flow cytometry. Animals that received *L. plantarum* CRL2130 not only did not inhibit treatment with CAP but also improved tumor volume decrease. These animals also presented a better general condition with less weight loss associated with CAP treatment. Mice that received CAP and *L. plantarum* CRL2130 showed a modulation of systemic cytokines modified by both tumor growth and CAP treatment. In conclusion, the administration of *L. plantarum* CRL2130 was associated with an anti-tumor effect in the BC model. Additionally, the riboflavin- overproducing strain was able to reduce some side-effects associated with chemotherapy without affecting its primary anti-tumor activity.

HH16 *IN VIVO* STUDY OF ANTI-LISTERIA ACTIVITY OF STRAINS OF LACTIC ACID BACTERIA ISOLATED FROM FERMENTED FOODS

C. Burgos[1], B. Vasile[1], P. Castellano[1*], S. Salva[1*].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucuman, Argentina. E-mail: cburgos@cerela.org.ar

Listeria monocytogenes is an intracellular foodborne pathogen which causes the severe disease listeriosis in immunocompromised individuals. Macrophages play a dual role during *L. monocytogenes* infection by both promoting dissemination of *L. monocytogenes* from the gastrointestinal tract and limiting bacterial growth upon immune activation. Some strains of lactic acid bacteria (BAL) are able to modulate the mucosal immune system and increase resistance to infections. This study evaluated the effect of oral administration of 12 LAB isolated from fermented foods on the functionality of peritoneal macrophages and resistance to *L. monocytogenes* infection in a murine model. First, safety and immunobiotic potential of the selected strains were evaluated. Different groups of 6-week-old C57BL/6 mice were fed with each of the isolated bacilli (10^8 CFU/mL/day) for 7 days. On the eighth day, both treated and untreated control mice were evaluated for hepato/splenomegaly and phagocytic activity of peritoneal macrophages by flow cytometry. The results showed that none of the bacilli studied induced hepato/splenomegaly. More importantly, the phagocytic capacity of peritoneal macrophages was significantly higher in mice preventively treated with two strains of bacilli (SC411 and SC076) compared to the control group ($p<0.05$). Then, a murine model of listeria infection was achieved. On the eighth day, both treated mice and untreated control mice received one intraperitoneal dose of cyclophosphamide (150mg/kg) that induced immunosuppression. On day 11, mice were infected orally with *L. monocytogenes* (10^9 UFC/mice). The infection resistance and innate immune response were evaluated after the listerial challenge. The untreated group showed a high susceptibility to listerial infection, an impaired innate immune response in peritoneal lavage and a decrease of phagocytic cells. Interestingly, SC411 and SC076 treatments significantly increased peritoneal lavage neutrophils and macrophages, blood neutrophils and peroxidase+ cells with respect to the untreated mice. This, in turn, significantly reduced the translocation of the pathogen to liver and spleen compared with the untreated mice. In conclusion, SC411 and SC076 lactobacilli strains may be particularly suitable for food-based applications to reinforce the host immune system and induce a more efficient local innate immune response against *L. monocytogenes*.

HH17 ORAL ADMINISTRATION OF MICROENCAPSULATED *Limosilactobacillus fermentum* CRL1446 IMPROVES THE MAIN BIOMARKERS OF METABOLIC SYNDROME IN MICE

C. Tomei[1], A. Márquez[1], M. Russo[1], E. Andrada[1], R. Medina[1], P. Gauffin-Cano[1].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. E-mail: ctomei@cerela.org.ar

Metabolic syndrome (MS) is one of the most relevant health problems in the world due to the increased consumption of high-fat diets. The probiotic *Limosilactobacillus fermentum* CRL 1446 has been scientifically described for its ability to improve biomarkers of MS. Microencapsulation has become an appropriate technological alternative to protect probiotic microorganisms from drastic conditions of the gastrointestinal tract or the technological process. The work aim was to evaluate the effectiveness of oral administration of microcapsules containing *Limosilactobacillus fermentum* CRL1446 (CRL1446) on improving nutritional, biochemical, and inflammatory parameters in a mice pre-clinical model in MS. Adult male C57BL/6 mice were fed for 15 weeks and divided into the following groups (n=6). (a) Control group (Cont; mice fed a standard diet [SD]; (b) Metabolic Syndrome (MS; mice fed a high-fat diet [HFD]); (c) MS + Bacteria-free empty microcapsules (Mic); (d) MS + microcapsules containing CRL1446 strain (MicPro; mice fed a HFD plus a daily microcapsule dose in drinking water [$1x10^9$ CFU/day/mouse]). Body weight was measured weekly. At the end of the study, animals fasted for 12 hours and were subsequently anesthetized and sacrificed. The adiposity index was determined. The following metabolic and inflammatory parameters were evaluated. (a) Lipidic profile: total cholesterol (TC), high-density lipoprotein cholesterol, low-density cholesterol (LDL), and triglycerides (TG) by enzymatic methods. (b) Glycemic profile: plasma glucose levels (enzymatic methods), Insulin (ELISA Test), and Oral glucose tolerance tests (OGTT). (c) Pro-inflammatory profile: TNF-α and IL-6 cytokines (flow cytometry) and (d) Leptin levels (ELISA Test). The results show that the administration of MicPro did not influence the decrease in body weight or adiposity index. However, metabolic parameters were significantly improved. MicPro induces a significant reduction in TG, TC, and LDL. Likewise, we observed a decrease in glucose and insulin, which allowed significant improvement in OGTT. Also, a significant reduction in the levels of leptin and pro-inflammatory cytokines was observed in response to the treatment. This probiotic strain maintains its hypoglycemic and hypolipidemic properties after the microencapsulation process, which could facilitate their incorporation into different food matrices.

HH18 *Lactiplantibacillus plantarum* MPL16 ACTIVATES P38 MAPK SIGNALING IN *C. elegans* AND HAS A POSITIVE IMPACT ON LONGEVITY AND RESISTANCE TO *Enterococcus faecalis* INFECTION

M.D. Vera[1], J. Sacur[1,3], H. Kitazawa[3], J. Villena [2,3*] y M.G. Vizoso Pinto [1,3*].
[1]Infection Biology Lab, INSIBIO (CONICET-UNT) and Faculty of Medicine of the National University. Av. Gral. Roca 2070. 4000 Tucumán, Argentina. [2] CERELA-CONICET. Chacabuco 145. 4000Tucumán, Argentina. [3]Tohoku University, Aoba-ku, 9800 Sendai, Japan. 3CFAI, Sendai, Japan. E-mail: mgvizoso@fm.unt.edu.ar

Lactiplantibacillus plantarum strains are good probiotic candidates. Research on *Caenorhabditis elegans* provides compelling evidence that probiotics can positively influence lifespan and health through various mechanisms, including immune modulation. Our objective was to study *L. plantarum* MPL16 and CRL1506 for their probiotic properties, with special focus on innate immune modulation, lifespan increase, and resistance to *Enterococcus faecalis* infection. We fed *C. elegans* with probiotics or *E. coli* Op50 as control and monitored the survival rate daily for 25 days. For immune modulation assays, we extracted RNA from the lactobacilli fed worms after 24h and performed a RT-qPCR for genes involved in microbial-associated molecular patterns recognition (*tlr-1*), the PMK-1 pathway (p38 mitogen-activated protein kinase analog pathway, *pmk-1*), effector proteins (lysozymes: *lys-1, lys-3, lys-5, lys-8*), and the tight junction protein claudin-like in *Caenorhabditis* (*clc-1*), mainly expressed in the epithelial cells of digestive tube. To evaluate the protective effect of probiotics on the susceptibility to *E. faecalis* infection, we fed worms during 24h with *L. plantarum* MPL16 or CRL1506 and then challenged them with *E. faecalis* IBL102. Nematodes at the L4 stage were extracted from NGM plates with *E. coli* Op50 using a washing solution. New NGM plates were seeded with 50 µl of a 16-hour culture of strains MPL16 and CRL1506, or Op50 as control. We placed 15 to 20 nematodes per plate and determined survival every 24h and assessed bacterial count to test colonization for 7 days. We examined intestinal distention using optical and transmission electron microscopy. Feeding *C. elegans* with *L. plantarum* MPL16 or CRL1506 increased worms' lifespan by approximately 20%. Both strains increased the expression of the effector *lys-5,* significantly. *L. plantarum* MPL16 enhanced also *tir-1*, *pmk-1* and *clc-1* expression. *E. faecalis* infection reduced worms' survival and affected their offspring. Pre-treatment of nematodes with CRL1506 or MPL16 delayed the onset of infection-related lethality (L50) by 24h and 48h, respectively, compared to the control group. MPL16 treatment resulted in a 1 log reduction in enterococci CFU/mL and restored reproductive capacity affected by enterococcal infection. Both strains increased longevity and improved resistance to *E. faecalis* infection but *L. plantarum* MPL16 was better at controlling infection. This strain also modulated the expression of genes involved in the p38 MAPK signaling pathway analog and *clc-1*. The upregulation of clc-1 has been related to a strengthening of tight junctions, which may improve the gut barrier function. These findings in *C. elegans* offer promising insights that could be applicable to other organisms, including humans, although further research is needed to confirm these effects in higher organisms. These results lead us to conclude that *L. plantarum* MPL16 is a promising candidate for developing probiotic supplements, and that modulating innate immunity pathways, such as the PMK-1 pathway, and improving the gut barrier function are part of their mechanisms of action.

HH19 POSTBIOTIC NASAL PRIMING ACCELERATES THE RECOVERY OF INNATE IMMUNE RESPONSE IN RESPIRATORY MUCOSA-ASSOCIATED LYMPHOID TISSUE IN MALNOURISHED MICE

M. Ivir[1], B. Vasile[1], F. Gutiérrez[1], S. Avarez[1*], S. Salva[1*].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucuman, Argentina. E-mail: hivir@cerela.org.ar

Lymphoid tissue of the upper respiratory tract is responsible for immunosurveillance of inhaled respiratory pathogens. The hosts immunosuppressed by malnutrition are known to be particularly susceptible to *Streptococcus pneumoniae*. The nasal priming of malnourished mice with the peptidoglycan (PG) from *Lacticaseibacillus rhamnosus* CRL1505 (Lr) is as effective as viable strain for improving systemic and respiratory immune response. But the impact of these treatments on mucosa-associated lymphoid tissue is unknown. Hence, the immune stimulation of nasopharynx-associated lymphoid tissue (NALT) and cervical lymph nodes (CLN) induced by Lr or PG on the resistance to infection with a respiratory pathogen (*S. pneumoniae*) was studied in malnourished mice under repletion treatments. The cytokine profiles that induced the innate immune response in the infectious process were investigated. Weaned Swiss mice were malnourished with a protein-free diet (PFD) for 21d. Malnourished mice received a balanced conventional diet (BCD) during 7d (BCD group) or BCD with nasal supplementation with Lr (10^8 cells/mouse/d) or PG (8 µg/mouse/d) during the last 2d of treatment (Lr or PG groups). Malnourished control mice (MNC) received PFD while the well-nourished control group (WNC) consumed BCD. On d8, all groups were infected with *S. pneumoniae* (10^7 cells/mouse). Before infection, MNC showed a significant decrease of the total cells, T and B lymphocytes in NALT and CLN as well as the macrophages, myeloid and dendritic cells in NALT. In addition, the MNC showed an increase of NALT Gr-1+ cells % and the microbial load in nasal washes. BCD treatment was not able to normalize these parameters. However, the Lr and PG groups improved the total cells, B and T cells counts in NALT and CLN. Challenge with *S. pneumoniae* increased the numbers of neutrophils and macrophages and TNF-α, INF-γ, IL-6, IL17 and IL-10 levels in nasal washes. The values were lower in MNC than in WNC. However, unlike the BDC group, Lr and PG groups showed values of NALT phagocytes, and lymphocytes, T CD4+ cells in CLN and NALT similar to WNC mice. Moreover, IL-10 and TNF-α levels in nasal washes were higher in Lr or PG groups. NALT is a target for postbiotics administration to improve respiratory immunity in immunocompromised malnourished hosts.

HH20 EVALUATION OF LACTIC ACID BACTERIA ON THE NUTRITIONAL, BIOCHEMICAL AND IMMUNOLOGICAL PROFILE OF MICE FED A HIGH-FAT DIET

A. Márquez[1], C. Tomei[1], E. Andrada[1], M. Russo[1], R.Medina[1], P. Gauffin-Cano[1].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. E-mail: amarquez@cerela.org.ar

Currently, the use of probiotic lactic acid bacteria (LAB) as nutritional therapy are a promising option to reverse the metabolic markers that characterize diet-induced obesity (DIO). The objective of this work was to comparatively evaluate the administration of 3 strains of LAB on body weight gain (BWG), biochemical, and inflammatory parameters in mice with DIO. C57BL/6 mice were fed for 10 weeks different diets and divided into the following groups (n=6): Control: standard diet (SD); Obese (Ob): high-fat diet (HFD); Ob+CRL1446: HFD + *Limosilactobacillus fermentum* CRL1446; Ob+CRL1449: HFD + *Lactiplantibacillus plantarum* CRL1449 and Ob+CRL1472: HFD + *Lactiplantibacillus plantarum* CRL1472. The dose of LAB used was 10^8 CFU/day/mouse. BWG was evaluated weekly. After 10 weeks of feeding, the livers were extracted for histological analysis, and the following determinations were made in plasma: cytokines by CBA, and glucose and lipid profile by enzymatic methods. Cardiovascular risk indices (CRI) were calculated the following: Total cholesterol-HDL/HDL; LDL/HDL and Triglycerides/HDL. The results were compared with the Ob group. BWG decreased by 20, 14, and 15% in Ob+CRL1446, Ob+CRL1449, and Ob+CRL1472 groups, respectively. HFD significantly increased TNF-α, MCP-1, and IL-6, while no significant differences were observed in IL-10 levels, demonstrating a pro-inflammatory effect of this diet compared to SD. Supplementation with the different strains tended to restore the pro-inflammatory cytokines; however, this difference was not significant concerning the Ob group. The animal groups that received LAB showed a lower fat infiltration in the hepatocytes, and the histology of the livers of CRL1446 group was similar to the Control group. Levels of glucose values decreased in groups fed with CRL1446 (36%), CRL1449 (34%) and CRL1472 (22%). CRI of treated groups was similar to the Control group. Interestingly, although total cholesterol and LDL cholesterol values did not change notably, the administration of the LAB increased HDL values in obese animals, thus improving atherogenic indices. In conclusion, the administration of LAB significantly improves the metabolic and inflammatory markers present in obesity, with a strain-dependent effect. *Limosilactobacillus fermentum* CRL1446 turned out to be the most suitable to be used as an adjuvant in the nutritional treatment of this pathology.

HH21 *Lacticaseibacillus rhamnosus* CRL1505 AND ITS POSTBIOTIC IMPROVE THE FUNCTIONAL PROPERTIES OF CIRCULATING NEUTROPHILS FROM IMMUNOCOMPROMISED MICE AND SUBJECTED TO INFECTIOUS STRESS

B. Vasile[1], M. Ivir[1], F. Gutiérrez[1], S. Avarez[1], S. Salva[1*].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucuman, Argentina. E-mail: bvasile@cerela.org.ar

Polymorphonuclear neutrophils are the first line of defense against pathogens. Blood neutrophils number depends on the balance between bone marrow granulopoiesis and apoptosis in peripheral tissues. Many current chemotherapeutic drugs have cytotoxic side effects that impair the life quality of the patients conditioning their treatment. We previously showed that both *Lacticaseibacillus rhamnosus* CRL1505 and its cell wall (CW) were able to improve bone marrow emergency myelopoiesis and protection against respiratory pathogens in mice undergoing chemotherapy. In this work we studied the functional properties of circulating neutrophils from immunocompromised mice fed with strain CRL1505 or its CW, and subjected to infectious stress. Adult Swiss-mice were orally treated with *L. rhamnosus* CRL1505 or CW during 16 consecutive days. On day 6, treated and untreated mice received one intraperitoneal dose of cyclophosphamide (Cy-150mg/kg). On day 9, mice were infected with *Streptococcus pneumoniae* (10^7 UFC/mice). The microbicidal activity, expression of integrins and formation of extracellular trap of neutrophils (NETs) were evaluated after the pneumococcal challenge. The Cy group showed a decrease of number of peroxidase positive cells in blood, a low expression of CXCR4 and CD62L in Gr1+Ly6G+Ly6C- cells in bone marrow. The reduced neutrophil activation was consistent with reduced NETs formation in Cy-treated mice. However, *L. rhamnosus* CRL1505 and CW treatments were effective to significantly increase lung neutrophils and macrophages, blood neutrophils and peroxidase+ cells with respect to the Cy group. Besides, the treatment with CW was more effective than *L. rhamnosus* CRL1505 to decrease retention signals in the BM cells. Furthermore, mice treated with CRL strain and CW showed a higher expression of SYTOX positive neutrophils compared to the Cy group, evidencing a recovery of the ability to form NETs. In conclusion, *L. rhamnosus* CRL1505 and its cell wall are able to modulate neutrophil homeostasis, key pieces for the resolution of a respiratory infectious process. In this way, the scientific bases are provided for future technological developments of products intended for this purpose.

HH22 FUNCTIONAL CRACKERS MANUFACTURED WITH LAB-FERMENTED CHICKPEA FLOUR PREVENTS OXIDATIVE STRESS IN D-GALACTOSE INDUCED AGING MICE

G. Sáez[1], G. Zárate[1,2].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. [2]Universidad de San Pablo Tucumán, Argentina. E-mail: gzarate@cerela.org.ar

Legumes are economically relevant food crops worldwide. In addition to providing nutrients such as carbohydrates, proteins, dietary fiber, minerals and vitamins, pulses also contain diverse phytochemicals, which confer numerous physiological and health benefits. The consumption of pulses has been associated with the prevention of many non-communicable diseases such as obesity, metabolic syndrome, diabetes, cardiovascular disease, among others. In view of their benefits, the researches on novel applications of legumes have significantly raised in the last years. Fermentation has been proposed as an effective biotechnological strategy for improving nutri-functional properties of legumes as it has proven to remove antinutrients with the simultaneous release of bioactive compounds. In previous studies we selected 2 strains of lactic acid bacteria (LAB) isolated from legumes for improving by fermentation the legume flours quality. In the present study functional crackers manufactured with LAB-fermented chickpea flour were fed to D-galactose induced aging mice in order to assess the prevention of oxidative stress. With this aim chickpea flour was fermented for 24 h at 37°C with *Lactiplantibacillus plantarum* CRL 2211 and *Weisella parameseteroides* CRL 2182 (≅ 10^6 CFU/g). Fermented and unfermented (control) doughs were incorporated to crackers and fed C57/6BL mice for 9 weeks. The oxidative stress associated to aging was induced by a daily subcutaneous injection of 150 mg/kg of D-galactose to mice during the whole test period. The animals were euthanized and oxidative damage to lipids and proteins were determined on livers, kidneys and brains by spectrophotometric methods (TBARS and DNPH, respectively) whereas relevant groups of the colonic microbiota were determined by qPCR. Long administration of D-galactose, significantly increased malondialdehyde and carbonyl groups in the organs and decreased the population of lactobacilli, bifidobacteria and bacteroides in the colon. Consumption of fermented crackers did not produced any structural alteration of the organs and prevented lipid peroxidation and the oxidative damage of proteins in the livers and brains. These effects could be due to the high concentration of phenolic compounds and the antioxidant activity observed in the aqueous extracts of fermented crackers. In addition, fermented crackers prevented the decrease of colonic lactobacilli and bifidobacteria. Our results demonstrate the potential benefits of including fermented legume flours in the development of new functional foods useful to protect the host against physiological alterations related to chronological senescence.

HH23 *Lacticaseibacillus rhamnosus* CRL1505 MODULATES ALVEOLAR MACROPHAGES AND IMPROVE THEIR RESPONSE TO *Pseudomonas aeruginosa* STRAINS ISOLATED FROM CYSTIC FIBROSIS PATIENTS

L. Arellano-Arriagada[1], M. Elean[2], J.N. Argañaraz Aybar[3], L. Albarracín[1], S. Orosco[4], J.C. Valdéz[3], H. Kitazawa[2], N. Gobbato[3], J. Villena[1]
[1]Tohoku University, 980-8572, Sendai, [2]CERELA-CONICET. 4000, Tucuman, Argentina. Japan. [3]University of Tucuman. Argentina. [4]Niño Jesus Children Hospital, SIPROSA, Argentina. E-mail: jcvillena@cerela.org.ar

Lacticaseibacillus rhamnosus CRL1505 can modulate respiratory immunity improving the resistance to respiratory virus and *Streptococcus pneumoniae.* However, its beneficial effect in the context of Gram-negative bacteria-mediated respiratory infections was not investigated in depth. Previously, we isolated pathogenic *Pseudomonas aeruginosa* (Pa) from cystic fibrosis (CF) patients and showed that the multi-resistant (PaR) strain had a higher capacity to induce lung damage and inflammation than the antibiotic-sensitive (PaS) strain. This work evaluated whether nasally administered *L. rhamnosus* CRL1505 can modulate the response of alveolar macrophages to pathogenic PaS and PaR strains. Adult BALB/c mice (6-weeks-old) were nasally primed with viable (LV1505) or heat-killed (HK1505) *L. rhamnosus* CRL1505 (10^8 CFU/mouse/day) during 2 consecutive days while control mice received no treatment. On day 3, alveolar macrophages were isolated from mice, cultured (10^5 CFU/well) and stimulated *in vitro* with LPS ((50 ng/ml), PaS, or PaR (10^6 CFU/well). One day after the challenges, the levels of TNF-α, IL-1β, IL-6, IFN-γ, IL-10, IL-27, CCL2, CXCL2, and CXCL10 were determined in alveolar macrophages supernatants by ELISA kits. The results showed that the challenge of alveolar macrophages significantly increased the levels of all the inflammatory factors in cells stimulated with LPS, PaS and PaR although the concentrations of TNF-α, IL-1β, IL-6, CCL2, and CXCL10 were higher in the LPS group ($p<0.05$) than in groups infected with PaS or PaR. Alveolar macrophages obtained from LV1505-treated mice produced higher levels of IFN-γ, IL-6, and IL-27 ($p<0.05$) and lower levels of TNF-α, CCL2, CXCL2, and CXCL10 ($p<0.05$) compared to controls, for the three LPS, PaS and PaR challenges. No differences were observed between LV1505-treated and control macrophages when IL-1β and IL-10 were analyzed. Of note, when HK1505 was evaluated, it was observed that its effect was comparable to the observed for LV1505, except for IFN-γ levels that were higher in the LV1505 group than in HK1505. The results presented here show that the nasal priming with *L. rhamnosus* CRL1505 can modulate alveolar macrophages response to pathogenic *P. aeruginosa* strains isolated from CF patients. Interestingly, the non-viable CRL1505 strain was as efficient as the viable bacteria to modulate alveolar macrophages´ function, which could be an important advantage for its application in patients with compromised immune system or lung functionality, such as patients with CF.

HH24 EFFECT OF *Lactiplantibacillus plantarum* CELL FREE CULTURE ON THE RESPIRATORY SUPERINFECTION INDUCED BY BACTERIAL PATHOGENS ISOLATED FROM CYSTIC FIBROSIS PATIENTS

L. Abán[1], S. Orosco[2], J.N. Argañaraz Aybar[3], L. Albarracín[4], A. Venecia[5], L. Perret[6], S. Ortiz Mayor[7], K. Nishiyama[8], J.C. Valdéz[3], H. Kitazawa[8], J. Villena[4], **N. Gobbato[3]**
[1]CONICET-Salta-Jujuy (Argentina), [2]Niño Jesus Children Hospital (Argentina), [3]University of Tucuman (Argentina), [4]CERELA-CONICET (Argentina), [5]Maternity and Gynecology Hospital (Argentina), [6]Tucuman Ministry of Health (Argentina), [7]Centro de Salud Hospital (Argentina), [8]Tohoku University (Japan). E-mail: nadiagobbato@hotmail.com

Lactiplantibacillus plantarum ATCC10241 cell free supernatant (LpCFS) can reduce the lung damage and inflammation induced by pathogenic *Pseudomonas aeruginosa* (Pa) strains isolated from cystic fibrosis (CF) patients, in mice. Considering that the co-infection with *Streptococcus milleri* group (SMG) can enhance the respiratory damage in Pa-infected CF patients, this work aimed to investigate the LpCFS ability to modulate lung inflammation and damage induced by the respiratory superinfection with Pa and SMG. A multi-resistant (PaR) and an antibiotic-sensitive (PaS) Pa strains, with the capacities to induce respiratory infections of different severities were used. Adult BALB/c mice (6-week-old) were nasally challenged with PaS or PaR (10^6 CFU/mouse), for 2d. After 5d, animals were infected with SMG (10^4 CFU/mouse) for 3d. Nebulization of mice with LpCFS started 1d after SMG challenge and was performed for 1d, 3d or 5d, 5 minutes by day using a compressor/nebulizer connected to a hermetically sealed plastic container. LpCFS was obtained from the ATCC10241 strain cultured in MRS medium for 16h at 37°C, centrifugated for 30 min at 30,000g at 10°C and filtered through a millipore filter (0.22 µm). Mice nebulized with PBS were used as controls. Results showed that the Pa-SMG superinfections induced significantly higher lung damage, inflammation and bacterial colonization for both PaR and PaS, than the previously reported for infections induced by PaR and PaS alone. The PaR-SMG superinfection was more severe that the induced by PaS-SMG. The nebulization with LpCFS for 5d significantly reduced PaR and PaS loads in lungs ($p<0.05$) and the inflammatory-mediated tissue damage as demonstrated by the lower levels of elastase release and leukocytes counts in broncho-alveolar fluid (BAL) samples ($p<0.05$) and the histological examination. Nebulization with LpCFS for 1d did not induce changes in the resistance of mice against the Pa-SMG superinfection, while 3d treatment was less efficient that the 5d LpCFS administration. This work demonstrated that the severity of Pa respiratory infection is exacerbated by SMG in mice and that the treatment with LpCFS can reduce the lung damage and inflammation induced by the superinfection. Although further clinical and mechanistic studies are necessary, the results show that the LpCFS could be an adjuvant alternative to treat Pa-SMG respiratory superinfections in CF patients.

HH25 MODULATION OF THE TOLL-LIKE RECEPTOR 3-MEDIATED INTESTINAL IMMUNE RESPONSE BY WATER KEFIR

S. Dentice Maidana[1], M. Elean[1], L. Albarracín[1], R. Ortiz Moyano[1], F. Namai[2], Y. Suda[3], H. Kitazawa[2], J. Villena[1]

[1]CERELA-CONICET. 4000, Tucuman, Argentina. [2]Tohoku University, 980-8572, Sendai, Japan. [3] Miyagi University, 980-8572, Sendai, Japan. E-mail: jcvillena@cerela.org.ar

Kefir, a fermented beverage containing lactic acid bacteria (LAB), has been associated to beneficial effects on the host´s health. The previous works examining the impact of kefir on the immune system focused on milk kefir or the exopolysaccharides and LAB strains derived from it, while water kefir has not been evaluated. Furthermore, studies have focused on kefir's ability to modulate immune system hemostasis and exert anti-inflammatory effects, while its specific action on antiviral immunity has not been investigated. Then, the aim of this work was to examine the potential immunomodulatory effects of water kefir on the intestinal innate antiviral immunity mediated by Toll-like receptor (TLR)-3. Male 5-week-old BALB/c mice fed water kefir *ad libitum*, diluted 1:5, 1:10, or 1:20 in the drinking water, for 6 consecutive days. Mice without water kefir treatment were used as controls. On day 7, the treated groups and the untreated control mice received an intraperitoneal injection of 100 µl of PBS containing 30 µg of the TLR3 agonist poly(I:C). Two days after the TLR3 activation, the intestinal damage and the innate immune response were studied. The intraperitoneal administration of poly(I:C) induced inflammatory-mediated intestinal tissue damage, characterized by increased inflammatory cell infiltration and upregulation of interferons (IFNs) (IFN-β, IFN-γ) and pro-inflammatory mediators (TNF-α, IL-1β, IL-15, IL-6). The histological analysis of small intestinal samples showed that mice receiving water kefir 1:5 exhibited reduced damage and a lower inflammatory cell infiltration. Kefir treated mice had significantly lower levels of serum LDH, AST, and ALT ($p<0.05$). In addition, lower levels of TNF-α, IL-15, and IL-6 ($p<0.01$), and higher concentrations of IFN-β, IFN-γ and IL-10 ($p<0.05$) were found in intestinal and serum samples of water kefir 1:5-treated mice compared to controls. The treatment with 1:10 of water kefir reduced intestinal damage and modulated cytokines but its effect was significantly lower than the 1:5 treatment. The administration of water kefir 1:20 did not modify the parameters evaluated compared to control mice. These results indicate that water kefir exert its immunomodulatory effects in a dose dependent manner. The *in vivo* studies allow to speculate that water kefir can induce two beneficial effects on the intestinal TLR3-mediated immune response: the enhancement of antiviral defenses and the protection against the inflammatory-mediated tissue damage. These protective effects of water kefir require further exploration to understand how water kefir, or its specific molecules/strains can influence the immune response and to determine the extent of its protection against a real viral challenge.

HH26 EFFECT OF *Lactiplantibacillus plantarum* CRL681 ON THE TLR4-MEDIATED INTESTINAL INNATE IMMUNE RESPONSE

A. Baillo[1], M. Elean[1], R. Ortiz Moyano[1], S. Fadda[1], J. Villena[1]
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucuman, Argentina. E-mail: jcvillena@cerela.org.ar

Previously, we demonstrated that the oral administration of *Lactiplantibacillus plantarum* CRL681 increase the resistance to enterotoxigenic *Escherichia coli* infection and that this protective effect was related to its capacity to modulate intestinal immunity. In this work, we aimed to further characterize the immunomodulatory capacities of the CRL681 strain evaluating its effect on the Toll-like receptor (TLR)-4-mediated intestinal innate immune response. Female 5-week-old BALB/c mice were fed *L. plantarum* CRL681 in the drinking water for 5 consecutive days (10^8 cells/mouse/day). Mice without lactobacilli treatment were used as controls. On day 6, the CRL681-treated and the untreated control mice received an intraperitoneal injection of 60 µg of lipopolysaccharide (LPS) in 100 µl of PBS. On day 6 (before LPS challenge) and 2 days after the TLR4 activation, the phagocytosis of peritoneal macrophages (PMs) and the levels of TNF-α, IL-6, IFN-γ and IL-10 in intestinal fluid and serum samples were determined. The CRL681 increased the phagocytic activity of PMs ($p<0.05$) while no significant differences were detected in the levels of intestinal and serum cytokines when CRL681-treated and control mice were compared at basal conditions. The intraperitoneal administration of LPS induced an increase in the PMs phagocytic activity as well as in the levels of TNF-α, IL-6, IFN-γ and IL-10 in both experimental groups. However, mice treated with the CRL681 strain had levels of PMs phagocytic activity ($p<0.05$), intestinal and serum IL-6 and IFN-γ ($p<0.01$) as well as intestinal IL-10 ($p<0.05$) that were significantly higher than controls. In a second set of experiments, mice were orally treated with the CRL681 as described before. On day 6, these mice and controls were sacrificed, the PMs were collected, cultured and stimulated *in vitro* with LPS. Twenty-four hours after the challenge, the levels of TNF-α, IL-1β, IL-6, IFN-γ, IL-12, CSF2, CSF3, CCL2, CCL8, IL-27, and IL-10 were determined in culture supernatants. Results showed that PMs from CRL681-treated mice were able to produce significantly higher levels of IFN-γ, TNF-α, IL-6, CSF3, and IL-27 ($p<0.05$), and lower levels of IL-1β, CSF2, CCL2, and CCL8 ($p<0.05$) than PMs from control animals. No differences were detected in the levels of IL-12 and IL-10 when the two groups were compared. The results show that *L. plantarum* CRL681 can exert immunomodulatory effects in the gut in the context of TLR4-induced inflammation by modifying the cytokine profile produced by peritoneal macrophages. The variations in the immune factors evaluated suggest that the CRL681 strain can increase immune defenses and regulate inflammation induced by TLR4 activation, making it a promising tool to prevent or reduce the severity of not only pathogenic *E. coli* infections, but also infections induced by other Gram-negative bacteria.

乳
酸
菌

LACTIC ACID BACTERIA AND ANIMAL HEALTH

健
康

食
料

AH1 BEER BAGASSE-ENRICHED MEDIUM IMPROVES BIOMASS PRODUCTION OF PROBIOTIC *Lactobacillus johnsonii* CRL2240

J. D. Babot, M. Obregozo, E. Andrada, R. Medina, J.G. Le Blanc
CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. E-mail: jbabot@cerela.org.ar

Ferulates play an important role in inhibiting ruminant digestion of plant cell walls. Therefore, the ferulic acid esterase-producing *Lactobacillus johnsonii* CRL2240 has been studied as a silage inoculant and goat probiotic. In order to perform farm-scale experiments, a cost-effective conservation strategy must be developed, for which biomass production is the first step. Cultures of this strain in MRS or LAPTg broths normally reach sub-optimal counts for a conservation process (2×10^8 CFU/mL). Therefore, the aim of this work was to design a low-cost, animal protein-free medium that allows a higher biomass yield of *L. johnsonii* CRL2240. To this end, protein or fiber-rich industrial by-products were used. *L. johnsonii* CRL2240 was inoculated into different culture media, incubated at 37 °C, and CFU/mL and pH of the cultures were determined at regular intervals. The following culture media and incubation conditions were tested: 1) LAPTg broth, static, free pH; 2) MM broth (containing texturized soybean flour), static, free pH; 3) MM broth, with stirring (75 rpm), free pH; 4) MM broth, with stirring (75 rpm), pH fixed at 6.50; 5) MM broth + $FeSO_4$, static, free pH; 6) BSG broth (containing beer bagasse) with 1.5% yeast extract, static, free pH; 7) BSG broth with 1% yeast extract, static, free pH; and 8) BSG broth with 1% yeast extract, centrifuged, static, free pH. Counts reached 2×10^8 CFU/mL and pH 4.3 at 8 h when biomass was produced in LAPTg broth, while growth in MM broth in a bioreactor with stirring or without it led to similar counts but lower pH (3.8) for the latter condition. Similar results were obtained by adding $FeSO_4$ to MM broth, although cultures reached the stationary phase after only 4 h of incubation. Remarkably, counts rose to around 1×10^9 CFU/mL and pH reached 3.8 after 6 h in BSG with 1.5% yeast extract. To further lower the cost of the broth, yeast extract concentration was reduced to 1% without affecting biomass production. Finally, eliminating excess beer bagasse flour by centrifugation after autoclaving BSG broth led to similar cell counts and pH results. In conclusion, a low-cost, animal protein-free broth where *L. johnsonii* CRL2240 reaches counts four times higher than LAPTg broth (1×10^9 vs 2×10^8 CFU/mL), and in a shorter incubation time, was designed. Moreover, a valorization of a residue of the beer industry is described.

AH2 CHARACTERIZATION OF SURFACE PROPERTIES AND ADHESION OF AUTOCHTHONOUS LACTIC ACID BACTERIA ISOLATED FROM MARE'S REPRODUCTIVE TRACT

A. Vasquetto[1], J.A. Silva[1], H. Mouguelar[2], N. Pereyra[3], J. Aguilar[4], M.E.F. Nader-Macías[5], M.S. Pellegrino[1].

[1]UNRC, FCEFQyN, Microbiology and Immunology Dept. -INCIVET-CONICET, Route 36 Km 601. 5800 Córdoba, Argentina. [2]UNRC, FAyV, Anatomy Department. [3]UNRC, FAyV, Animal Pathology Dept. [4]UNRC, FAyV, Animal Production Dept. [5]CERELA-CONICET, Chacabuco 154. 4000 Tucumán, Argentina. E-mail: mpellegrino@exa.unrc.edu.ar

Endometritis is an inflammatory disease of the endometrium and is the most common cause of subfertility in mares, causing great economic losses in equine breeding. Since there are currently no established protocols for the treatment and/or prevention of this disease, alternative and innocuous preventive measures, including probiotic formulas with beneficial autochthonous Lactic Acid Bacteria (LAB), are proposed. The aim of this work was to evaluate surface-adhesive properties of LAB isolated from mare's reproductive tract. Twenty-nine LAB were evaluated for: *biofilm formation, using the microplate method with crystal violet staining and spectrophotometric quantification; *exopolysaccharide production (EPS), where LAB were inoculated in MRS with different carbohydrates (glucose, sucrose and skimmed milk), seeded in spots and their phenotype (mucoid/ropy) observed; **in vitro* adhesion of LAB to equine epithelial cells (EEC), for which EEC and LAB suspensions were co-incubated in equivalent proportions at 37°C for 1 h, transferred to smears, stained with Haematoxylin-Eosin and the Percentage of Adhesion (PA) calculated by microscopic observation; *and adhesion and permanence of LAB in *in vivo* assay, in which mare's vagina was inoculated with three preselected LAB (10^{10} CFU/ml): *Pediococcus pentosaceus, Enterococcus hirae* and *Weissella cibaria*, and at 24-96 hours post-inoculation, vaginal swabs taken for LAB count in MRS agar medium and vaginal biopsies evaluated by scanning electron microscopy. All the LAB studied showed to form biofilm on MRS broth, in higher amount on media without surfactant. Forty-eight percent of the LAB produced EPS, most of them with a ropy phenotype in all the condition, except two strains with mucoid phenotype in sucrose-added medium. Twenty-six LAB strains exhibited medium to high PA. Referred to the *in vivo* inoculation assay, an increase, although not significant ($p>0.05$), was observed in CFU LAB numbers (~4.4 $\log_{10}$ CFU/ml) at day 4 post-inoculation. After one day of inoculation, an increase in the number of bacteria was observed, with similar morphology to those inoculated, on the surface of epithelial tissue in the treated group. The results obtained support the characterization of these native LABs, for their selection and subsequent inclusion in the design of a homologous tract specific probiotic formula to prevent equine endometritis.

AH3 *Ligilactobacillus salivarius* FFIG58 MODULATES RESPIRATORY IMMUNITY AND CONFERS LONG-TERM PROTECTION AGAINST *Streptococcus pneumoniae*

M. Elean[1], L. Arellano-Arriagada[2], L. Albarracín[1], F. Namai[2], H. Kitazawa[2], J. Villena[1]
[1]CERELA-CONICET. 4000, Tucuman, Argentina. [2]Tohoku University, 980-8572, Sendai, Japan. E-mail: jcvillena@cerela.org.ar

Ligilactobacillus salivarius FFIG58 was isolated from the intestine of wakame-fed pigs. This strain was selected based on its ability to modulate the innate immune responses triggered by the activation of Toll-like receptor (TLR)-3 or TLR4 signaling pathways in the intestinal mucosa. This work aimed to evaluate whether nasally administered *L. salivarius* FFIG58 can modulate the innate immune response in the respiratory tract and confer long-term protection against the respiratory pathogen *Streptococcus pneumoniae*. Infant mice (3-weeks-old) were nasally primed with *L. salivarius* FFIG58 (10^8 CFU/mouse/day) during 2 consecutive days and then stimulated with the TLR3 agonist poly(I:C) for three days. Five (t1) or thirty (t2) days after the last poly(I:C) administration mice were infected with pneumococci serotype 6B (10^6 CFU/mouse). Animals without lactobacilli treatment and challenged with poly(I:C) and *S. pneumoniae* were used as controls. Experiments with the non-immunomodulatory strain *L. salivarius* FFIG79 were performed in parallel for comparison. Mice were sacrificed 2 days after the pneumococcal challenge to evaluate the resistance to the infection and the respiratory innate immune response. Results of t1-experiments demonstrated that *L. salivarius* FFIG58 could enhance the protection against the secondary pneumococcal infection as shown by the reduced lung and blood bacterial cell counts ($p<0.05$) and the lower levels of the biochemical parameters of broncho-alveolar lavages that evaluate lung damage ($p<0.05$) compared to control and FFIG79-treated mice. The effect of the FFIG58 strain was related to its ability to modulate the alveolar macrophages-mediated respiratory immune response. *L. salivarius* FFIG58 improved the ability of alveolar macrophages to produce IL-6, IFN-γ, IFN-β, TNF-α, IL-27, CCL2, CXCL2, and CXCL10 ($p<0.05$) in response to the pneumococcal challenge compared to controls and FFIG79-treated mice. Of note, when the resistance to secondary pneumococcal infection and the innate immune response were evaluated in t2-experiments, the effect of the FFIG58 was comparable to that observed in t1-experiments. The results presented in this work show that the nasal priming of infant mice with *L. salivarius* FFIG58 protected the animals against secondary pneumococcal infection for at least 30 days after the stimulation with poly(I:C), The long-term phenotypic changes in alveolar macrophages induced by the FFIG58 strain suggest that nasally administered immunomodulatory lactobacilli would be able to stimulate trained immunity in the respiratory tract.

AH4 INTRAVAGINAL ADMINISTRATION OF PHARMABIOTIC-PHYTOBIOTIC CAPSULES WITH AUTOCHTHONOUS LACTIC BACTERIA FOR THE PREVENTION OF INFECTIONS OF THE BOVINE REPRODUCTIVE TRACT

MH Miranda, MEF Nader Macías.
CERELA-CONICET. Chacabuco 145. 4000 Tucuman, Argentina. E-mail: hmiranda@cerela.org.ar

The postpartum bovine reproductive tract is susceptible to infections that are treated with antibiotics and hormones, or a combination of them. The growing problem of the transmission of resistance to antimicrobials requires to reduce their use and look for alternative therapies, such as probiotics and phytobiotics. The objective of this work is to advance in the design of pharmabiotics/phytobiotics formulas with bovine beneficial autochthonous lactic acid bacteria (BBALB) for the prevention of reproductive tract infections. Hard gelatin capsules containing six different spray-dried BBALB strains ($3x10^{10}$ CFU), individually or combined with phytoderivatives (Malva and Lapacho) were administered intravaginally to cows (n=30). Two doses were applied with an interval of 15 days, before the probable date of delivery, and two after the postpartum. The modification of the cultivable autochthonous microbiota from vaginal washings and the permanence/colonization of the inoculated BBALBs were evaluated, and the safety of the designed formulas was determined through nutritional-clinical and hematological-biochemical parameters in blood and serum. All the females of the different experimental groups (EG) remained healthy before parturition (5±0.0 RS). During the postpartum period, two cows from the BBALB MG (mammary strains group) and one Control showed signs of reproductive infections. The females of the different EG maintained or slightly decreased their postpartum weight (304.5 ± 4.95 KgLW). All the animals showed normal hematocrit (36.44±1.08%), and leukocyte formula within bibliographic reference values (RV) in the BBALB Vg, BBALB VG (Vaginal strains)+VE (Vegetal extracts) and BBALB MG+VE groups, no significant differences were obtained with Control. Glycemia was lower than RV=40-88.2 mg/dl, with no significant differences between EG and Control at the same sampling time. Metabolic parameters (glycemia, proteinemia, albuminemia and C-reactive protein) were normal in all the animals throughout the protocol. The cultivable microbiota of the bovine vagina was slightly modified after the administration of the capsules. Total aerobic mesophylls increased slightly in all the EGs after parturition. Enterobacteriaceae remained at 2.50±0.05 logUFC/ml during the assay in all EGs, except in BBALB Vg+VE. Lactic bacteria increased at postpartum (BBALB Vg:2.15±0.07, BBALB Vg+VE:2.00±0.02 and BBALB MG:1.91±0.05 logUFC/ml). The results indicate that the intravaginal administration of pharmabiotic/phytobiotic capsules is safe and do not produce adverse effects when administered to pre- and postpartum cows.

AH5 SELECTION OF IMMUNOMODULATORY LACTIC ACID BACTERIA FROM DOG'S MILK AND COLOSTRUM

S. Quilodrán-Vega[1], C. Muñoz[1], L. Albarracín[2], J. Toledo[1], J. Villena[2].
[1]University of Concepción, Concepción, Chile. [2]CERELA-CONICET, Tucuman, Argentina.
E-mail: squilodran@udec.cl

Probiotic microorganisms are an alternative to prevent and treat infectious enteropathies in dogs. Previously, we isolated lactic acid bacteria (LAB) from canine milk and colostrum and characterized potential probiotic strains according to their functional properties, including their resistance to gastrointestinal conditions, inhibitory effect against dog´s pathogens (*Escherichia coli*, *Salmonella* and *Clostridium perfringens*), and intestinal adhesion. In this study we aimed to further characterize dog´s LAB strains assessing their immunomodulatory activities. For these studies, experiments were performed *in vitro* in the canine macrophage cell line (DH82 cells) and *in vivo* in murine models of *E. coli* or *Salmonella* intestinal infections. For *in vitro* experiments, canine macrophages (10^6 cells/well) were stimulated for 12 hours with the different canine LAB strains using four doses (10^6, 10^7, 10^8 or 10^9 CFU/mL). Negative controls without LAB treatment were included. The expressions of the pro-inflammatory cytokines *TNF-a* and *IL-8*, and the pattern recognition receptors *TLR2* and *NOD2* were determined by real-time PCR. Among the studied LAB, *Lactiplantibacillus plantarum* TUCO-16 (from canine colostrum) and *Lacticaseibacillus rhamnosus* TUCO-17 (from canine milk) strains induced a significant increase in the expression of *TNF-a*, *IL-8*, and *TLR2* ($p<0.05$) in canine macrophages, with the higher dose evaluated. These two strains were selected for studies in mice. Female 5-week-old BALB/c mice were orally treated with strains TUCO-16 and TUCO-17 (10^8 cells/mouse/day) for five consecutive days. On day 6, mice were challenged with a mouse adapted enterotoxigenic *E. coli* (ETEC) K88 strain (10^9 cells/mouse) or *Salmonella typhimurium* (10^7 cells/mouse). Two days after the challenges the resistance to the infections and the innate immune responses were evaluated. The oral administration of TUCO-16 and TUCO-17 strains to mice significantly augmented their resistance to pathogenic *E. coli* or *Salmonella* intestinal infections. Both canine strains reduced intestinal damage and pathogen counts in the liver and spleen ($p<0.05$) and avoided their dissemination into the bloodstream. These protective effects were related to the ability of TUCO-16 and TUCO-17 strains to augment the production of IFN-γ, IFN-β, IL-6, and IL-10 ($p<0.05$) and reduce TNF-α, KC, MCP-1, and IL-15 ($p<0.05$) in the intestinal mucosa. *L. plantarum* TUCO-16 and *L. rhamnosus* TUCO-17 are potential probiotic candidates for improving intestinal health in dogs, particularly for their ability to inhibit the growth of Gram-negative pathogens common in gastrointestinal infections and modulate the animal's immune response. Further studies are required to effectively demonstrate the beneficial effects of TUCO-16 and TUCO-17 strains in dogs.

AH6 EFFECTS OF *Ligilactobacillus salivarius* subsp. *salivarius* A3iob ON HONEY PRODUCTION AND HEALTH OF *Apis mellifera* BEE HIVES IN ARGENTINA

A.A. Arroyo Guerra[1], J. Villena[2], M.C. Audisio [1,3,4]

[1] INIQUI-CONICET. [2] CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. [3] Fac. de Cs. Exactas. [4] Fac. de Ingeniería. UNSa. Av. Bolivia 5150. 4400 - Salta, Argentina. E mail: arroyoguerraagustin@gmail.com

The honeybee (*Apis mellifera*) plays a key role in the pollination of crops and wild plants globally, significantly contributing to ecosystem balance. In Argentina, their importance is noteworthy, positioning the country as the third-largest honey producer and second-largest exporter worldwide. However, beehive health is threatened by the *Varroa* sp. mite, which feeds on bees' fat bodies and spreads various infectious agents, weakening colonies and reducing honey production. This study, analysed the effects of *Ligilactobacillus salivarius* subsp. *salivarius* A3iob, a strain isolated from the gut of a worker bee, in experimental hives under field conditions. The strain was administered monthly to each hive from June to October 2022. Five hives per group received the following treatments: 1) viable bacteria at a concentration of 10^9 cfu/mL and 2) thermal-treated bacteria (98°C for 10 minutes). These groups were compared to a control group treated with sterile MRS broth. The parameters studied were i) honey production, ii) abdomen weight, and iii) incidence of *Varroa* sp. Statistical evaluation was carried out using a non-parametric method (Kruskal-Wallis), considering significant differences as those with a p-value < 0.05. Hives treated with viable bacteria produced significantly more honey (14.2 ± 6.06 kg, p=0.0381) than the control hives (3.55 ± 2.42 kg). The thermal-treated group showed similar performance to the control group (5.80 ± 3.9 kg). Additionally, a significant increase in abdomen weight was observed in bees treated with live bacteria (58.70 mg ± 16.33) compared to the control group (40.6 mg ± 12.5, p=0.0003). The thermal-treated group showed similar results to the control (48.79 mg ± 14.08). Regarding varroa infestation rates, no significant differences were found between the treated groups at the end of the trial (p= 0.1399). However, the viable bacteria group had a higher incidence of the parasite (2.20% ± 3.36) compared to the thermal-treated (1.20% ± 1.26) and control groups (0.68% ± 1.05). These results indicate that A3iob improves honey production and abdomen weight in treated bees. The hives with the viable bacteria produced considerably more honey (14.2 ± 6.06 kg, p=0.0381) than the control group (3.55 ± 2.42 kg). The abdomen weight of bees treated with live bacteria was significantly higher than the control group (58.70 mg ± 16.33, p=0.0003). However, the higher varroa incidence in the live bacteria group suggests the need for further studies to better understand these interactions.

AH7 IDENTIFICATION AND BENEFICIAL CHARACTERISTICS OF AUTOCHTONOUS LACTIC BACTERIA WITH PROBIOTIC POTENTIAL FOR CANINE PUPPIES

N. Carrasco, R. Levit, C. Aristimuño Ficoseco, A. de Moreno de LeBlanc, J.G LeBlanc, M.E.F. Nader-Macias.
CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. E-mail: ncarrasco@cerela.org.ar

Probiotics are frequently applied for the restoration of the autochtonous microbiota and prevention of infections, being of main importance the host and mucosa of isolation. For the selection process of new homologous probiotic microorganisms, functionality and technological aspects must be evaluated. This work is aimed to evaluate different beneficial properties and to identify lactic acid bacteria (LAB) isolated from mother's milk and faeces of different dog breeds, in order to advance in the design of a homologous LAB probiotic formula to restore the dog gut microbiota and protect them from infections. 100 different strains were isolated from dogs: 17 from mother's milk, 83 from faeces, and were evaluated in the production of beneficial enzymes (protease, lipase, amylase, cellulase and feruloyl esterase) and H_2O_2 production. Thirty strains were selected for sharing beneficial properties and genetically identified. The ability to adheres to intestinal cells was evaluated in 10 strains. Protease was determined in agarized skim milk, lipase in MRS-milk cream, amylase in MRS-starch agar medium (revealed with Indole), feruloyl esterase in 1% in methanol (w/v) ethylferulate 1g/L added to MRS agar without glucose, cellulase in MRS-carboxymethyl cellulose agar (revealed with lugol). H_20_2 production was detected using the tetramethyl benzidine-MRS agar (TMB-MRS) plate method. Genetic identification was performed by sequencing the 16s RNA gene. The ability of LAB strains to adhere to the gut epithelium was assessed in vitro using the Caco-2 human colon carcinoma epithelial cell line. The survey of beneficial enzymes showed 24% strains had protease activity, 20% cellulase activity and 19% feruloyl esterase activity, 39% produced H_20_2 (12% weak, 9% strong and 18% very strong). The genetic identification indicated: 4 *Lactobacillus johnsonii*, 2 *Ligilactobacillus salivarius*, 2 *Lactiplantibacillus plantarum*, 2 *Streptococcus thoraltensis*, 2 *Pediococcus acidilactici*, 7 *Enterococcus* (*E.*) *canintestini*, 4 *E. faecalis*, 6 *E. faecium* and 1 *E. durans*. 10 selected LAB strains evidenced adhesion ability close to 70%. This work supports the selection of LAB strains with the best characteristics, and to define those that share properties, in a way to decide their optimal combinations, to advance in the design of probiotic formulas with homologous strains for the health of canine puppies.

AH8 MIXED INOCULATION USING FERULIC ACID ESTERASE-PRODUCING LACTOBACILLI IMPROVE NUTRITIONAL, FERMENTATIVE AND FUNCTIONAL PROPERTIES OF CORN STOVER SILAGE

E. Andrada[1,2], N. Suarez[1,2], M. C. Abeijón-Mukdsi[1], **R. Medina**[1,2].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. [2]Facultad de Agronomía, Zootecnia y Veterinaria, Universidad Nacional de Tucumán, Tucumán Argentina. E-mail: rmedina@cerela.org.ar

Mixed inoculants combine different species of lactic acid bacteria to potentiate its effects, and currently dominate the silage market. Ferulic acid esterase producing (FAE+)-inoculants can potentially enhance the fiber digestibility by ruminants, therefore enhancing their productive performance. Up to date, mixed FAE+ inoculants are scarcely studied. We have previously studied three FAE+ lactobacilli that induced positive and different fermentative and nutritional modifications in corn stover when used as single-strain inoculants. In this work, we aimed to evaluate the effects of the mixed inoculation of these strains. Therefore, an uninoculated control (UN) group was compared to the inoculation of two formulae: *Lactiplantibacillus plantarum* CRL2241 and *Levilactobacillus brevis* CRL2239 (Mix LPB), or these strains plus the addition of *Lactobacillus johnsonii* CRL2240 (Mix LPBJ). Inoculants were applied (10^6 CFU/g fresh matter) and vacuum-sealed. In 60-day corn stover silos, fermentative (soluble carbohydrates, organic acids and ethanol), nutritional (fiber digestibility) and functional (total phenolic compounds, concentration of hydroxycinnamic acids, antiradical activity) parameters were determined. Likewise, a metagenomic analysis of the bacterial community was carried out. The studies showed that both mix formulae reduced dry matter losses (2% LPB vs UN), and increased the free ferulic acid content (10X vs UN) and antioxidant activity of corn stover. Metagenomic analysis revealed a strong lactobacilli dominance in the inoculated silages. When compared to LPB, LPBJ further reduced dry matter loss (4% vs UN), and cellulose content (2% vs UN). In conclusion, beneficial modifications were induced by mixed inoculants when considering their fermentative, nutritional and functional parameters.

乳酸菌

METABOLISM AND PHYSIOLOGY OF LACTIC ACID BACTERIA

健康

食料

MP1 GROWTH OF LACTIC ACID BACTERIA STRAINS ISOLATED FROM SILAGE IN CORN PLANT EXTRACT MEDIA

R. M. Lenz[1], M. C. Audisio[1,3,4], C. Ibarguren[2,3].
[1]INIQUI-CONICET. [2]Fac. de Cs. de la Salud. [3]Fac. de Cs. Exactas. [4]Fac. de Ingeniería. UNSa. Av. Bolivia 5150- 4400 - Salta, Argentina. E-mail: rominalenz22@gmail.com

Biological additives, mainly lactic acid bacteria, are one of the best options for controlling and improving the silage fermentation process. In silage, the water-soluble carbohydrates are fermented under anaerobic conditions, creating an acidic environment that inhibits the proliferation of undesirable microorganisms. The aim of this work was to determine the growth capacity of lactic acid bacteria strains, isolated from corn and sorghum silages, in a corn plant extract medium, to determine their potential as silage inoculants. We worked with 6 out of 28 isolates obtained from sorghum and corn silages samples, selected mainly for their acidifying capacity. These strains were phylogenetically characterized as *Lactiplantibacillus plantarum* R3EA, *L. plantarum* F1M, *Pediococcus pentosaceus* R1M1, *P. pentosaceus* A1B2, *P. pentosaceus* B2M and *Limosilactibacillus reuteri* AUM1. A corn extract medium (MEM) was used as culture medium, obtained by maceration of a chopped fresh corn plant in sterile distilled water (10% w/v) for 10 min, with subsequent filtration and sterilization in an autoclave (121°C, 15 min). Growth of each strain was evaluated by inoculating (1% v/v) a suspension in PBS buffer. These suspensions were obtained by recovering of the pellet of a fresh overnight culture in MRS broth, centrifuging and double rinsing with PBS buffer, and then resuspending in the same buffer. The cultures were incubatedat 37°C under microaerophilic conditions. The viability of each strain was determined by plate counting using MRS agar at 0, 2, 4, 6 and 24 h. Based on these results, a strain of each genus was selected. Subsequently, the growth of *L. plantarum* R3EA, *P. pentosaceus* A1B2 and *L. reuteri* AUM1 in a mixed culture in MEM was evaluated, with and without addition of glucose (0.2, 0.5, 1% w/v) as additional carbon source. All strains maintained their viability in MEM. Regarding the growth of each individual strain, the cultures maintained a viability *ca.* 7-7.5 log cfu/ml during 24 h, except for *L. plantarum* F1M, which recorded a viability of *ca.* 5.5 log cfu/ml at 24 h. In the case of mixed cultures, the best growth profile was observed in the MEM + 0.5% w/v glucose, with a viability of *ca.* 7.6 log cfu/ml after 24 h, while in media without added glucose they *ca.* 6.5-7 log cfu/ml. These assays allowed the selection of 3 over 6 strains with suitable properties as potential inoculants for corn silage, according to their capacity for development in a medium with reduced nutrients. Furthermore, the addition, of an alternative carbon source to the plant substrate could optimize silage fermentation process.

MP2 NITROGEN SOURCES AS A KEY FACTOR REGULATING THE EXPRESSION OF GAD GENES IN *Levilactobacillus brevis* CRL 2013

M.P. Urquiza Martínez, P.G. Cataldo, G.A. Bulacios, J.R. Naja, L. Saavedra, E.M. Hebert. CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. E-mail: purquiza@cerela.org.ar

Gamma-aminobutyric acid (GABA) is a non-protein amino acid that primarily functions as an inhibitory neurotransmitter in the central nervous system. Interest in GABA-enriched foods has surged in recent years due to its antihypertensive, anti-inflammatory and antidepressant effects. Given the low natural abundance of GABA in food products, microbial fermentation emerges as a highly promising approach for producing this valuable bioactive compound. The conversion of glutamate to GABA in lactic acid bacteria (LAB) is carried out by the GAD system, which consists of the transcriptional regulator (GadR), the glutamate:GABA antiporter (GadC) and the enzyme glutamate decarboxylase (GadB and/or GadA). *Levilactobacillus brevis* CRL 2013, an efficient producer of GABA, harbors two gad genes encoding glutamate decarboxylase enzymes, *gadA* and *gadB*. Notably, *gadB* is located adjacent to *gadC*, with the transcriptional regulator *gadR* identified upstream of gadC. To elucidate the regulation of GABA production in *L. brevis*, we analyzed the transcriptional organization of the *gad* genes and evaluated the effect of different nitrogen sources in a chemically defined medium (CDM) on GABA production. Transcriptional analyses of *gad* related genes in *L. brevis* CRL 2013 identified two transcriptional units; one comprising the *gadR* and *gadC* genes, and the other encompassing *gadC, gadB*, and gltX genes. To further investigate the physiology of GABA production, we optimized a CDM and supplemented it with glutamate to analyze its impact on GABA production. While glutamate supplementation alone did not activate GABA synthesis in CDM, the addition of nutritional factors beyond the natural amino acids present in CDM, such as yeast extract (YE), significantly enhanced GABA production. YE contains peptides, nucleotides, proteins, amino acids, sugars, and various trace elements. Then, to determine whether nitrogenous components activate GABA synthesis in *L. brevis*, we analyzed growth, pH, and GABA production in CDM containing different amounts (0.5% to 5%) of casitone (digested casein) and vegetable peptone. In 2% casitone, GABA production was similar to that obtained in CDM supplemented with 1% YE, where maximum production was observed. Although 2% vegetable peptone also showed GABA production, the levels were not comparable to those observed with Casitone. Transcriptional analysis confirmed a substantial increase in *gadB* and *gadR* expression upon casitone supplementation, further supporting their role in activating the GABA production pathway.

MP3 ANTIMICROBIAL PROPERTIES OF *Levilactobacillus brevis* STRAINS ISOLATED FROM CHEESE WHEY OF DIFFERENT ORIGINS

J.C. Alcocer[1,2], R.M. Lenz[1] N. Álvarez[1], C. Coronel[1], H. Cristóbal[1], C. Ibarguren[1,2], M.C. Audisio[1,3,4].
[1]INIQUI-CONICET. [2]Fac. de Cs. de la Salud. [3]Fac. de Cs. Exactas. [4]Fac. de Ingeniería. UNSa. Av. Bolivia 5150. 4400 - Salta, Argentina. alcocerjimena20@gmail.com

Industrial cheese whey (ICW) and artisanal cheese whey (ACW) are differentiated by the use of lactic bacteria cultures and commercial rennet in the former, and natural rennet or "panchera" in the latter. The aim of this work was to evaluate the antimicrobial spectrum of previously isolated strains from ICW and ACW, and to characterize them phylogenetically. A total of 23 isolates (5 strains from ICW and 18 from ACW) were grown in MRS broth, with glucose replaced by lactose (MRSL), at 37°C for 24h, under microaerophilic conditions. Then, a screening was carried out using the agar diffusion technique against indicator strains (*Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* and *Salmonella* sp.), to select those strains with the best antimicrobial activity. Subsequently, the effect of direct contact of cell-free supernatants (CFS) of two strains of *Lactobacillus* sp., at pH 4 and after the neutralization with 1M NaOH, on the viability of a cell-suspension of *S.* Typhimurium ATCC14028 was evaluated. The viability of the pathogenic strain was determined at 0, 1, 2 and 24 h by plate count in BHI agar medium. A suspension of the pathogen in BHI broth was used a pathogen growth control. Finally, the isolates with the best antimicrobial profile were selected and characterized phylogenetically by sequencing the 16S rRNA subunit (Macrogen, Seoul, Korea) and performing an online search for similarities using the BLAST program in GenBank. The agar diffusion technique showed that the strains with an inhibition zone ≥ 6 mm against the indicators tested were those identified as LS91 and LS94 from ICW and ACW, respectively. On the other hand, the direct contact with *S.* Typhimurium ATCC14028, at acidic pH. LS91 produced a total inhibition after 24 h, while LS94 generated a decrease of 4 log orders (*ca.* 6 log cfu/mL) during the 24 h of contact. On the other hand, the SLC at neutral pH together with the control maintained a viability between 7 and 9 log cfu/mL. The analysis of the 16S rRNA subunit revealed that LS91 and LS94 exhibited 97% and 99% homology *with Levilactobacillus brevis*, respectively. In conclusion, the isolated strains from ICW and ACW presented antimicrobial activity, mainly due to the production of organic acids. The phylogenetic identification allowed to corroborate that the isolates correspond to bacterial species that are present in artisanal cheeses and cheese whey, and are usually used in industrial fermentations.

MP4 BILE ACIDS MODIFY MORPHOLOGY OF A POTENTIAL PROBIOTIC LACTIC ACID BACTERIUM

C. Moreno[1], N. Gómez[1], A. Ledesma[1,2], M. P. Taranto[3], **A. Y. Bustos**[1,4,5]
[1]CIBAAL-UNSE-CONICET. Ruta Nacional 9 Km 1125, Villa El Zanjón. 4206 Santiago del Estero, Argentina. [2]Facultad de Ciencias Exactas y Tecnología, Universidad Nacional de Santiago del Estero. Av. Belgrano (s) 1912. 4200 Santiago del Estero, Argentina. [3]CONICET-CERELA. Chacabuco 145. 4000 Tucumán, Argentina. [4]Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero. Av. Belgrano (s) 1912. 4200 Santiago del Estero, Argentina. [5]Facultad de Ciencias Sociales, Humanidades y de la Salud, Universidad Nacional de Santiago del Estero. Av. Belgrano (s) 1912. 4200 Santiago del Estero, Argentina. E-mail: yaninabustos17@gmail.com

Bile acids (BAs) are the main endogenous modulators of the composition and metabolic activity of the intestinal microbiota. In the present work, the effect of conjugated [taurodeoxycholic (TDCA)] and free [cholic acid (CA) and deoxycholic acid (DCA)] BAs, on the survival, structural and surface properties of *Lentilactobacillus (L.) parabuchneri* CB12 was evaluated applying viability assays and Scanning Electron Microscopy (SEM). Our results evidenced that free BAs were more toxic than conjugated, with CA being significantly more harmful than DCA). SEM showed that untreated bacterial cells exhibit well-defined morphology characteristic with short rod shape and a smooth surface. No bacterial aggregates were observed. With TDCA addition, the cell surface appeared rougher and irregular with some depressions. Granular precipitate corresponding to the DCA released by the action of the CB12-bile salt hydrolase enzyme was observed. Treatment with free BAs such as DCA and CA caused significant alterations at lower concentrations. Thus, bacterial cells treated with 2 mM DCA, and 0.05 mM CA showed significant surface distortions and many bacteria presented depressions and fold formation on the surface. In general, cells were longer than control cells and interrupted septa as signal of incomplete cell division were also observed. SEM micrographs showed bacterial aggregates, which would be related to modifications in their surface properties by exposure to BAs. Remarkably, exposure to CA revealed pore-like depressions. In general, the magnitude of the effects founded was dependent to the type of BA and its concentration, being more evident in the presence of CA even at low concentrations, which would explain its greater inhibitory effect. This work provides useful information about the effects of BAs on lactic acid bacteria strains, and it would allow the development of strategies to positively modulate the composition of the microbiota.

MP5 RAMAN SPECTROSCOPY, AN ALTERNATIVE METHODOLOGY FOR THE IDENTIFICATION OF LACTIC ACID BACTERIA

A. Ledesma[1,2], **J. Carol[1]**, Y. Bustos[1,3,4]
[1]CIBAAL-UNSE-CONICET. Ruta Nacional 9 Km 1125, Villa El Zanjón. 4206 Santiago del Estero, Argentina. [2]Facultad de Ciencias Exactas y Tecnología, Universidad Nacional de Santiago del Estero. Av. Belgrano (s) 1912. 4200 Santiago del Estero, Argentina. [3]Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero. Av. Belgrano (s) 1912. 4200 Santiago del Estero, Argentina. [4]Facultad de Ciencias Sociales, Humanidades y de la Salud, Universidad Nacional de Santiago del Estero. Av. Belgrano (s) 1912. 4200 Santiago del Estero, Argentina. E-mail: carolpazjuanjose@gmail.com

Conventional methods of bacterial identification usually rely on biochemical and molecular tests, which are often time-consuming and expensive. In this context, Raman spectroscopy can be a valuable and attractive tool in bacterial identification, because it allows obtaining single-cell spectra within a population and describes the DNA, protein and lipid composition of the cell. The sample is illuminated from the incident laser, it scatters the light with the vibrations of the molecular bonds providing information which defined the identity of cell kind, and it allows discriminations and classification of microorganisms at level of a single cell. For this reason, the objective of this work was to obtain Raman spectra of a group of 17 lactic acid bacteria (LAB), previously isolated by our group and identified by Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) Mass Spectrometry (MS) and 16S gene sequence. For this purpose, cells from a stationary phase culture were resuspended in 100 mM sodium phosphate buffer (pH 7.4) before processing. The spectra were obtained using a confocal Raman spectrometer with a 532 nm laser excitation source. A single bacterial cell was focused using a 100 X objective. Each spectrum consisted of an average of 10 measurements per strain, with an exposure time of 5 seconds in the range of 100 to 2000 cm^{-1}. The profile of characteristic bands attributed the major cell components (proteins 1660 and 533 cm^{-1}, carbohydrates 980 cm^{-1}, lipids 887, cm^{-1} and nucleic acid 1081 and 391 cm^{-1}) observed for each analysed strain, allowed us to differentiate the three groups of genera identified as *Leuconostoc mesenteroides*, *Lentilactobacillus parabuchneri* and *Lactiplantibacillus plantarum*. For the last genus three subgroups were differentiated. This could be due to differences between the molecular composition at the strain level. In addition, statistical treatment of spectra through principal component analysis and partial least squares discriminant confirms it. In conclusion, Raman spectroscopy served to identify certain LAB, at least, at the genus and species level, positively correlating with the results obtained through genetic sequencing and mass spectrometry. However, it is still necessary to obtain more readings per cell to confirm if this methodology allows differences in bacteria at the strain level.

MP6 MONITORING THE PROTEOLYTIC ACTIVITY OF LACTIC ACID BACTERIA USING FOURIER TRANSFORM INFRARED SPECTROSCOPY

J. Carol[1], M. Ibarra[1], A. Ledesma[1,2], Y. Bustos[1,3,4].
[1]CIBAAL-UNSE-CONICET. Ruta Nacional 9 Km 1125, Villa El Zanjón. 4206 Santiago del Estero, Argentina. [2]Facultad de Ciencias Exactas y Tecnología, Universidad Nacional de Santiago del Estero. Av. Belgrano (s) 1912. 4200 Santiago del Estero, Argentina. [3]Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero. Av. Belgrano (s) 1912. 4200 Santiago del Estero, Argentina. [4]Facultad de Ciencias Sociales, Humanidades y de la Salud, Universidad Nacional de Santiago del Estero. Av. Belgrano (s) 1912. 4200 Santiago del Estero, Argentina. E-mail: carolpazjuanjose@gmail.com

During fermentation of milk to produce cheese or yogurt, the microorganisms used as starters progressively modify the casein structure and chain length, giving rise to oligopeptides and free amino acids. The study of the changes induced by the complex proteolytic system of microorganisms requires procedures that, in general, are not very sensitive. In fact, there are few routine instrumental methods for the study of conformational changes of complex proteins. Thus, the objective of this work was to monitor the proteolytic activity on casein of the lactic acid bacteria (LAB) strains *Lactiplantibacillus (L.) plantarum* CB2, *Lentilactobacillus (L.) parabuchneri* CB7 and *L. plantarum* CB11, by Fourier transform infrared spectroscopy. In addition, total proteins and free amino acids were quantified using the Bradford and OPA methods, respectively. For this purpose, cell suspensions prepared from stationary phase cultures of the strains were incubated with 5 mg/mL casein at 37 °C and samples were taken at baseline, 16 and 20 hours. In the supernatants, the intensities of the amide I and II bands of the proteins were recorded between 1500 and 1700 cm^{-1}, which refer to the tension and bending of the carbonyl and amine groups. Comparison of the spectra obtained with the control casein showed that both the amide I and amide II bands presented notable differences after incubation with the different strains. The results showed a significant decrease, proportional to the incubation time, in the intensity of both bands for casein incubated with *L. plantarum* CB2 and *L. parabuchneri* CB7 strains. Remarkably, with *L. plantarum* CB11 at 20 h, the intensity of the amide II band increases and shifts to higher wavelengths, indicating possible formation of other compounds. In relation to protein quantification, CB2 and CB7 strains decreased the initial casein concentration by 21.13 and 43.43 %, respectively. On the other hand, *L. plantarum* CB11 showed no significant protein consumption. Amino acid quantification shows an increase for the three strains evaluated, with a higher value observed for CB11. From these tests it can be concluded that infrared spectroscopy served as a highly sensitive methodology to study the degree of hydrolysis of three LAB, whose proteolytic activity could serve to increase the nutritional value of fermented foods in terms of peptides and amino acids content.

MP7 BIOTRANSFORMATION OF SELENIUM BY LACTIC ACID BACTERIA UNDER DIFFERENT STRESS CULTURE CONDITIONS

F.R.E Molina[1], L.G. Ruiz Rodríguez[1], R.R. Raya[1], F. Mozzi [1]
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. E-mail: fmolina@cerela.org.ar

Selenium (Se) is an essential micronutrient for most living organisms. Se is ingested through food consumption of animal and plant sources, which absorb this metalloid from the soil. Se concentration, redox state, and bioavailability are highly variable and depend on the geographic region. Se is known for its antioxidant, anti-inflammatory, and antiviral activities and its deficiency in humans is associated with viral infections, male infertility, different types of cancer, and aging. Some lactic acid bacteria (LAB) can biotransform inorganic Se (iSe) into seleno-amino acids and seleno-nanoparticles (SeNPs), which are more bioavailable than inorganic Se salts. This work aimed to evaluate the ability of *Weissella cibaria* 25 and *Lactiplantibacillus paraplantarum* CRL 2051 to grow and bioaccumulate Se in the presence of this metalloid under different stress culture conditions. Both strains were grown in MRS with sodium selenite (5 ppm) as iSe for 24 h under the following culture and stress conditions: i) 30 and 37 °C, ii) pH 4.0, 5.5, and 6.6, iii) 0.05% (v/v) H_2O_2, and iv) 4 and 6% (w/v) NaCl. Microbial growth was determined by OD_{600}; SeNPs formation was positively visualized by the reddish color of the colonies on MRS agar after incubation at 30 °C for 48 h. The intracellular bioaccumulation of iSe was indirectly determined in the culture medium supernatant by the di-amino naphthalene colorimetric method. The results showed that the presence of Se did not significantly affect the microbial growth of *W. cibaria* 25 and *L. paraplantarum* CRL 2051 under the majority of the assayed conditions. LAB colonies, typically white, presented a reddish color in the presence of Se indicating their ability to reduce sodium selenite to elemental Se (Se^0) and form SeNPs. Both strains CRL 25 and CRL 2051 showed the highest microbial growth (ΔDO: 0,40 and 0,57), and iSe intake (97 and 76%), respectively, when cultured at 30 °C and pH 6.6. A higher temperature, lower pH, and the presence of H_2O_2 and NaCl caused a decrease in Se accumulation and microbial growth in both strains. At pH 4.0 and 5.5 the intracellular Se decreased 13% in both cases. The addition of 6% NaCl affected Se accumulation the most in both LAB, being *L. paraplantarum* the most sensitive strain. Under oxidative stress culture conditions, a decrease of 31 and 47% intracellular Se in *W. cibaria* and *L. paraplantarum,* respectively, was recorded. In summary, both assayed strains could grow in the presence of and biotransform Se under different stress culture conditions; however, they could not cope with the presence of H_2O_2. In most of the studied conditions, Se helped to counteract the detrimental effect caused by the assayed stress factors.

MP8 AROMATIC PROFILE OF WINES PRODUCED BY MIXED CULTURES OF YEASTS AND *Oenococcus oeni*

L.M. Mendoza[1], M. Flores[2], C. Belloch[2]
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. [2]IATA-CSIC. Avda. Agustín Escardino 7. 46980 Paterna, Valencia, España.
E-mail: lmendoza@cerela.org.ar

During wine-making process, the alcoholic fermentation conducted by yeasts is the main reaction transforming grape sugars into ethanol. However, the malolactic fermentation (MLF) is a critical step to obtain high quality wines. The MLF consists in the conversion of malic acid into lactic acid and it is desirable because decreases total acidity and improves the stability and sensorial characteristics of wines. Among wine lactic acid bacteria, *Oenococcus oeni* is the best adapted species and is almost exclusively used for the induction of MLF. In addition, it was descripted that some strains of *O. oeni* are able to produce several enzymes (glycosidases, esterases, phenolic acid decarboxylases, citrate lyases) that modify the wine aroma profile. The aim of this study was to evaluate the potential to produce aroma volatile compounds by different strains of *O. oeni* in combination with wine yeasts. Three strains of *O. oeni* (X2L, ST and Sb10) were evaluated in mixed cultures with *Saccharomyces cerevisiae* Cf8, *Hanseniaspora vineae* mc1 and *Metschnikowia pulcherrrima* T2. Bacteria were inoculated at 24 h of alcoholic fermentation and at the end of this fermentation in synthetic wines. Samples were incubated at 25 and 15 °C for 14 and 25 days, respectively. Volatile compounds were analyzed using a gas chromatograph with a quadrupole mass detector. Regardless of the studied strain, the inoculation of *O. oeni* modified the concentrations of esters, alcohols and diacetyl in comparison to wines produced without MLF, being their content higher at 25 °C than 15 °C. The differences between the two temperatures could be related to better bacterial growth at 25 °C. *O. oeni* X2L showed the greatest modifications in the aromatic profile with high 2-phenylethanol and aldehyde concentrations but low ethyl hexanoate content, whereas strains ST and Sb10 increased ethyl octanoate concentration. Based on these results, it could be indicated that each *O. oeni* strain showed a differential effect on wine volatile composition. Further studies with grape must are needed to know whether *O. oeni* inoculation and the observed changes in volatile compounds improve wine aroma.

乳
酸
菌

APPLIED BIOTECHNOLOGY OF LACTIC ACID BACTERIA

健
康

食
料

AB1 INHIBITION OF ENTEROHEMORRHAGIC *Escherichia coli* BY *Lactiplantibacillus plantarum* CRL681: AGGREGATION, CO-AGGREGATION, AND ELECTRON MICROSCOPY STUDIES

A. Baillo[1], S. Fadda[1], J. Villena[1],
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucuman, Argentina. E-mail: abaillo@cerela.org.ar

Lactiplantibacillus plantarum CRL681 (Lp681) inhibits the growth of enterohemorrhagic *Escherichia coli* (EHEC) in various matrices. The cell surface is crucial to compete with pathogens for specific binding sites. Therefore, good self-aggregation and co-aggregation with pathogens are desirable features in potentially probiotic strains. This study compared the inhibitory capacity of three *Lactiplantibacillus plantarum* strains (Lp681, Lp1506 and a mutant of Lp1506) against EHEC in a chemically defined medium (CDM), in addition to evaluating their ability to self-aggregate and co-aggregate with EHEC. Finally, the interaction of Lp681 with *E. coli* was observed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Growth curves were performed for each strain in 100 mL of CDM, inoculating 10^6 CFU/mL of the strains and 10^4 CFU/mL of EHEC, incubated at 30°C for 48 hours. Samples were taken at different times to determine pH and viability. Aggregation assays were performed by inoculating 4 mL of CDM with a concentration of 10^8 CFU/mL for each bacterium and incubated at 30°C for 24 hours without shaking. For co-aggregation assays, 2 mL of each LAB culture with 10^8 CFU/mL was mixed with 2 mL of the EHEC culture with 10^8 CFU/mL, incubated at 30°C without shaking for 6 hours. In both assays, optical density (OD) was measured at 600 nm. The results showed that Lp681 inhibits EHEC growth at 24 hours of incubation. No viable counts were observed at 48 hours when EHEC were co-cultured with Lp1506. No inhibitory activity was detected for LpMut. In self-aggregation assays, significant differences were observed at 6 hours of incubation, with Lp681 achieving 80% self-aggregation, compared to 27% and 36% for Lp1506 and LpMut, respectively. At 24 hours, Lp681, Lp1506 and LpMut reached values between 92% and 74%, with Lp681 and Lp1506 being the most self-aggregating. In coaggregation tests with EHEC, Lp681 showed significantly higher values than the other strains, reaching 49% at 6 hours. SEM and TEM images showed that Lp681 surrounds EHEC cells, evidencing cell lysis with disruption of the cell membrane and cell wall. The results show that *L. plantarum* CRL681 can inhibit EHEC in a chemically defined medium by cell lysis. In addition, it has high self-aggregation and co-aggregation abilities, suggesting its potential to bind to epithelial mucosa and prevent the adhesion of pathogens such as EHEC. Lipoteichoic acid is important for aggregatory and inhibitory capacity, as seen by comparing strain Lp1506 and its mutant. *L. plantarum* CRL681 proved to be more efficient than Lp1506 in inhibiting EHEC and coaggregating, making it a strain of interest for probiotic applications.

AB2 EFFECT OF SELECTED STARTER CULTURES ON THE MICROBIOLOGICAL, PHYSICOCHEMICAL, AND SENSORY CHARACTERISTICS OF CURED-FERMENTED SAUSAGES MADE WITH LLAMA (*Lama glama*) MEAT

L. Nelegatti[1], A. Baillo[1], M. Torino[1], S. Fadda[1]
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucuman, Argentina. E-mail: sfadda@cerela.org.ar

Llama meat (LM) is a viable alternative to beef and pork in the Andean regions. Its high protein and low fat content make it a promising product for the innovative food market. The formulation of specific starter cultures (SC) for the fermentation of LM constitutes an interesting biotechnological strategy for its comprehensive utilization. This approach will facilitate the diversification and preservation of this raw material while obtaining additionally products with added value. The objective of this work was to produce fermented sausages with LM using SC, composed of previously selected Lactic Acid Bacteria (LAB) and a Coagulase-Negative *Staphylococcus* (CNS). Three batches (B) were obtained: B1 (control) with spontaneous fermentation; B2 inoculated with *L plantarum* CRL681 and *S. carnosus* F833; and B3 with *L. sakei* CRL2243 and *S. vitulinus* GV318. Handmade cured sausages of the *salamín* type were produced and evaluated at 0 (initial time), 3 (fermentation), and 17 days (end of maturation) through microbiological, physicochemical (pH, water activity -a_w-, lactic acid, color, and free amino acid -FAA-content), and sensory analysis. The results confirmed the competence of LAB and CNS in the meat curing environment, as at 17 days and in all three batches, LAB reached a count of approximately 9 log CFU/g, while CNS reached between 7-9 log CFU/g. Additionally, during the fermentation stage (3rd day), a significant decrease in pH was observed in the batches with SC compared to the control, demonstrating a higher acidifying capacity of the LAB strains of the SC. B2 recorded the highest lactic acid production at the end ripening (7.78 g/g). The typical drying process of these products was evidenced by a decrease in a_w, from 0.94 to 0.85±0.01 in B2 and B3, while B1 showed a slightly smaller decrease (a_w 0.89). The color parameters (L*, a*, and b*) also varied at the final time. The increase in L* and a* in the 3 batches suggests brighter and more intensely red-colored sausages. The more pronounced decrease in b* in B2 and B3 resulted in products with less yellow hue. Increase in FAA was observed due to various metabolic processes such as proteolysis. However, the higher content of FAA in B2 and B3 stands out. The hygienic quality of all final products was adequate, with no pathogenic bacteria detected. Sensory analysis with trained judges revealed that sausages with SC scored higher in overall uniformity, spice aroma, spicy, acidic, and *umami* flavors, and chewiness. On the other hand, B1 recorded higher scores in less favorable attributes such as greasy texture, rancidity, moldy and strange odor, bitter taste, and pasty mouthfeel. This work demonstrates that the use of selected SC produces safer and improved quality Llama meat fermented sausages.

AB3 LACTIC ACID BACTERIA ISOLATED FROM CITRUS FRUITS AS STARTER CULTURES FOR THE PRODUCTION OF FERMENTED LEMON JUICE

D.A Barrionuevo[1,2], F. Escobar[1], A.S Isas[1], F.R.E Molina[1], C.P Van Nieuwenhove[1,3]
[1]CERELA-CONICET. Chacabuco 145, 4000, Tucumán, Argentina. [2]Facultad de Bioquímica Química y Farmacia-UNT, Batalla de Ayacucho 471, S.M. de Tucumán, Argentina. [3]Fundacion Miguel Lillo, Miguel Lillo 251, S.M. de Tucumán, Argentina. E-mail: dbarrionuevo@cerela.org.ar

Lemon (*Citrus limon* L.) is widely cultivated in Tucumán, representing one of the main economies of the region. The juice possesses bioactive compounds of interest for the prevention of diseases such as obesity, diabetes, and cardiovascular disorders, with a high content of polyphenols (mostly flavonoids) and vitamin C. Lactic acid bacteria (LAB) isolated from fruits can adapt to the fruit matrix and be used as starter cultures to obtain new fermented beverages with longer shelf life. The objective of this work was to genetically characterize five LAB strains isolated from citrus fruits and evaluate their potential use as starter cultures into a pasteurized lemon juice (LJ: 2.0° Brix; pH 4.6 adjusted with sodium carbonate). For identification, a fragment of the 16S rRNA gene was amplified by PCR and using a specific primer (plb16) from the bacterial DNA, then purified and sequenced. Afterwards, sequences were compared with others deposited in the GenBank database. The LAB were identified as *Leuconostoc citreum* (three strains) and *Weissella bombi* (two strains). Subsequently, *Lc. citreum* M5C1 and *W. bombi* M6C2 were selected as starter cultures and inoculated at 2% (v/v) in LJ. Fermentation was carried out at 30°C for 48 h, then stored at 4°C for 28 days. Aliquots of samples were collected during fermentation (0; 24; and 48 h) and at the end of shelf life (28 days) to evaluate bacterial growth (by plating on agar media), pH, total phenolic content (TPC) and antioxidant activity (TEAC, FRAP and DPPH methods). After fermentation, both strains achieved 1 log unit growth (*Lc. citreum* M5C1: from 7.4 ± 0.12 to 8.79 ± 0.10; *W. bombi* M6C2: 6.90 ± 0.13 to 8.55 ± 0.08 CFU/mL), remaining constant after 28 days at 4°C. Despite the microbial growth, the pH of the juices slightly increased from 4.6 to 4.8-5.0 after fermentation, possibly attributed to citric acid metabolism. Both strains increased TPC around 40-50% after 48 h of fermentation ($p < 0.05$), with a consequent increase in the antioxidant activity. The viability of both LAB remained at similar values after storage. However, there was a significant decrease on TPC and antioxidant activity ($p < 0.05$). The results demonstrated the ability of *L. citreum* M5C1 and *W. bombi* M6C2 to tolerate the citrus matrix and increase the functionality of the beverages after fermentation. The uses of autochthonous starter cultures allow greater adaptation to adverse substrates and enhances the functional properties of lemon juice.

AB4 PARTIAL CHARACTERIZATION OF *Enterococcus* SPP. JK06 ISOLATED FROM REGIONAL SALAMI FROM TUCUMÁN

J. Gomez, F. Gharzia, **K. Gianni de Carvalho**
PROIMI-CONICET. Avenida Belgrano y Pasaje Caseros. 4000 Tucuman, Argentina. E-mail: ka20_04@hotmail.com

The food industry is always looking preservatives that are highly effective, long-lasting, and nowadays, of natural origin. Additionally, those capable of inhibiting various pathogens such as *Listeria monocytogenes*, *Salmonella* spp., and *Escherichia coli* are preferred. In this regard, bacteriocins produced by lactic acid bacteria (LAB) represent an effective, natural method that can also provide benefits when supplied together with the producing strain, acting as a probiotic. This work aimed to identify an LAB isolate from regional salami from Tucumán, determine its safety and probiotic characteristics, evaluate the production of antimicrobial compounds, and assess its application in a food model for biopreservation. The LAB isolate named JK06 was identified by 16S rRNA gene sequencing and Gram staining. The presence of virulence factors was evaluated using phenotypic assays. The ability to grow on MRS agar with bile and resistance to the gastrointestinal tract (GIT) were evaluated at pH values of 1.5, 2, 2.5, and 3 (gastric juice, GJ) for 120 min and at pH 8 (enteric juice, EJ) for 24 h. Cell-free supernatants (CFS) were evaluated for their inhibitory capacity against *Listeria innocua*, *L. monocytogenes*, *E. coli*, *Enterococcus faecalis*, *E. faecium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella enterica*, and *S. typhimurium*. The active compound causing inhibition was determined by neutralization, heating to 100°C, and treatment with proteinase K, catalase, and lipase. The inhibitory capacity of selected LAB and/or bacteriocins on *L. monocytogenes* Scott A was evaluated during hamburger storage for 15 days at 4°C. Based on the position of isolate JK06 in various phylogenetic trees obtained, it was concluded that the LAB belongs to the *Enterococcus* genus. *Enterococcus* sp. JK06 was found to be non-hemolytic and did not exhibit lipase or gelatinase activity. Additionally, it was capable of growing in MRS medium supplemented with 3 g/L of bile salts. The ability of *Enterococcus* sp. JK06 to withstand simulated GIT conditions was evaluated, and no viable cell count was observed under the most extreme conditions (pH = 1.5 and 2) after 30 min of exposure to simulated GJ. However, the strain recovered its viability when treated with simulated EJ for 1 h. It was also observed that the inhibitory activity of the CFS was unaffected by heat treatment or by the action of lipase and catalase but was affected by proteinase K. As for the food model, it was observed that after 5 days of storage at 4°C, there was a complete elimination of *Listeria*, while in control hamburgers, the count remained stable (10^3 CFU/mL). The active compound responsible for antimicrobial activity is of a protein nature, possibly a bacteriocin. Given the results observed throughout the study, it can be concluded that *Enterococcus* sp. JK06 and/or its bacteriocin have high potential for use as a probiotic or food additive, respectively.

AB5 FUNCTIONAL AND SENSORY ANALYSIS OF CALAFATE FRUIT FERMENTED WITH PATAGONIAN LACTIC ACID BACTERIA

B. Sede Lucena[1], **L. Crespo**[2], O. Ordoñez[1], J. Burini[3], M. Bruzone[3], F. Mozzi[2], M. Pescuma[1]
[1]Centro de Investigación y Extensión Forestal Andino Patagónico (CIEFAP), Ruta 259 Km 16,24 - CC 14 (9200) Esquel, Chubut, Argentina.[2]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. [3]Centro de Referencia en Levaduras y Tecnología Cervecera (CRELTEC), Instituto Andino Patagónico de Tecnologías Biológicas y Geoambientales (IPATEC), CONICET Universidad Nacional del Comahue, Quintral 1250 (CP8400), San Carlos de Bariloche, Argentina. bsede@correociefap.org.ar

Calafate (*Berberis microphylla*) is a native Patagonian barberry used in culinary and medicinal practices. This study aimed to analyze the ability of lactic acid bacteria (LAB) isolated from calafate fruits and flowers to modify the phenolic compound concentration and antioxidant capacity of calafate juice. Additionally, the inhibition of metabolic syndrome-related enzymes by the fruit juices was tested, and the sensory attributes of the selected fermented juice were analyzed. The total phenolic compounds in juice supernatants were determined using the Folin-Ciocalteu method, and antioxidant activity was measured by four methods (ABTS•+, DPPH, FRAP, and ILP). The inhibition of α-glucosidase, α-amylase, and lipase was expressed as a percentage relative to the control (enzyme activity without juice supernatants) based on the total phenolic compound concentration in the juice's supernatants (mg GAE/ml). Sensorial analysis was conducted by the trained sensory panel of IPATEC according to ASBC methods. *Leuconostoc citreum* F2, *Lacticaseibacillus paracasei* B4, *Fructobacillus fructosus* B7, and *Latilactobacillus curvatus* B34 grew in the calafate juices (1.33 ± 0.03-2.61 ± 0.30 log CFU/ml at 24 h). *F. fructosus* B7 consumed glucose and fructose the most (2.30 ± 0.45 g/L and 3.73 ± 0.44 g/L, respectively) producing the natural sweetener mannitol (3.89 ± 0.77 g/L). All juices exhibited antioxidant capacity although, the juice fermented with *L. paracasei* B4 showed the highest total phenolic compound concentration (2662.58 ± 344.51 mg GAE/100 ml) and antioxidant capacity as measured by ABTS•+(38916.42 ± 2157.52 µmol TE/100 ml). The juices inhibited metabolic syndrome-related enzyme activities, with the lowest IC_{50} values for α-glucosidase observed in juices fermented by *F. fructosus* B7 and *L. paracasei* B4 (0.56 ± 0.10 and 0.64 ± 0.05 mg GAE/ml, respectively), and for α-amylase in juices fermented by *L. curvatus* B34 and *L. paracasei* B4 (0.34 ± 0.01 and 0.37 ± 0.06 mg GAE/ml, respectively). The non-fermented juice IC_{50} values were 0.47 ± 0.05, 0.77 ± 0.02 and 0.20 ± 0.02 for α-amylase, α–glucosidase and lipase, respectively. Based on antioxidant and enzyme inhibitory activity, the *L. paracasei* B4 fermented juice was selected for sensory evaluation and compared with the non-fermented one. Both juices had a fruity aroma, but the *L. paracasei* B4 fermented juice had distinctive lactic acid and diacetyl notes. Both samples were sweet and fruity, with the *L. paracasei* B4 juice also having higher acidity and lactic acid content. *L. paracasei* B4 could be suitable for producing a functional fermented calafate juice.

AB6 *Pleurotus pulmonarius* MUSHROOM AS NOVEL RESOURCES OF PREBIOTICS: EFFECT ON THE GROWTH AND RESISTANCE TO PASSAGE THROUGH THE GASTROINTESTINAL TRACT OF LACTIC ACID BACTERIA

J. Gomez[1], A. Ledesma[1,2], M. Taranto[3], **A. Bustos**[1,4,5]
[1]CIBAAL-UNSE-CONICET. Ruta Nacional 9 Km 1125, Villa El Zanjón. 4206 Santiago del Estero, Argentina. [2]Facultad de Ciencias Exactas y Tecnología, Universidad Nacional de Santiago del Estero. Av. Belgrano (s) 1912. 4200 Santiago del Estero, Argentina. [3]CONICET-CERELA. Chacabuco 145. 4000 Tucumán, Argentina.[4]Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero. Av. Belgrano (s) 1912. 4200 Santiago del Estero, Argentina. [5]Facultad de Ciencias Sociales, Humanidades y de la Salud, Universidad Nacional de Santiago del Estero. Av. Belgrano (s) 1912. 4200 Santiago del Estero, Argentina. E-mail: yaninabustos17@gmail.com

Prebiotics improve host health by promoting the growth and metabolic activity of beneficial strains such as probiotic lactic acid bacteria (LAB). Due to their numerous benefits, the search for and characterization of new prebiotics is an area of growing interest. In this sense, the *Pleurotus (P.) pulmonarius* mushroom is rich in non-digestible carbohydrates that could act as prebiotics favoring the growth and activity of beneficial bacteria. The objective of this work was to extract potential prebiotic oligosaccharides from *P. pulmonarius* and evaluate their efficiency to modulate the growth and resistance of probiotic LAB applying simulated gastrointestinal tract (SGIT) conditions. For this, the relative growth rate (RGR), prebiotic activity (PA), prebiotic index (PI) and survival through SGIT of selected LAB strains in the presence of mushroom oligosaccharides was evaluated. The strains were inoculated in MRS broth supplemented with fungal carbohydrates as the only carbon source or with glucose or commercial FOS as controls. Our results showed that fungal carbohydrates exert a positive effect on the growth of selected LAB strain compare to glucose and FOS. So, *Levilactobacillus (L.) brevis* ATCC 14869, *Lactiplantibacillus (L.) plantarum* CB11 and *Lentilactobacillus (L.) parabuchneri* CB12 presented RGR and PA > 1. On the other hand, *Bifidobacterium longum* DSM 20219, and *Limosilactobacillus reuteri* ATCC 23272 did not grow in the presence of fungal carbohydrate or FOS. A PI of 0.47-0.55 was obtained indicating that the extract was selectively utilized by beneficial microorganisms in relation to glucose. After gastric passage, no significant difference was observed compared to the initial count. After incubation in intestinal juice, the colony-forming capacity of *L. brevis* and *L. plantarum* CB11 decreased approximately 0.5 log units when glucose was used as a carbon source, while in the presence of fungal oligosaccharides, viability was maintained at initial levels of 8 log CFU/mL. Our results indicate that components of *P. pulmonarius* could stimulate the growth and resistance of certain probiotic strains during the SGIT passage, which would allow its use in the design of efficient symbiotic prototypes.

AB7 INHIBITION OF A-GLUCOSIDASE BY LACTIC ACID BACTERIA THROUGH CONTROLLED FERMENTATION OF *Brassica rapa* AND *Undaria pinnatifida*

B. Martínez Caliari[1], R. Parada[2], F. Sosa[1,2], M Vallejo[1], **K. Gianni de Carvalho**[3]

[1] Laboratorio de Biotecnología Bacteriana, Fac. de Cs. Naturales y Cs. de la Salud (Sede Trelew) -Univ. Nac. de la Patagonia San Juan Bosco. 9 de julio 25, (9100) Chubut, Argentina. [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina. [3] PROIMI-CONICET. Avenida Belgrano y Pasaje Caseros. 4000 Tucuman, Argentina. E-mail: ka20_04@hotmail.com

Type 2 diabetes mellitus (T2DM) is a metabolic disorder associated with insulin resistance and dysfunction of the hormone-producing beta cells of the pancreas. T2DM is increasing in prevalence worldwide and today represents 95% of diabetes cases, becoming a serious public health problem, especially in developing countries. Fruits and vegetables are natural sources of active ingredients that promote health. Specifically, brassicas serve as a model for determining the influence of biochemical processes during fermentation on the contribution of metabolites related to nutrition and health. Previous studies have demonstrated an increase in bioactive compounds in fermented vegetables and the functional properties exhibited by seaweed, particularly *Undaria pinnatifida*. This study aimed to develop new functional foods through two-step controlled fermentations using isolated autochthone strains of the genus *Leuconostoc* spp. and *Lactiplantibacillus* spp, whose consumption can inhibit α-glucosidase activity, an enzyme linked to T2DM. The inhibitory effects of extracts from spontaneous and controlled fermentation of *Brassica rapa* (Chinese cabbage) and *U. pinnatifida* were assayed. The fermentation process was conducted at 18 °C for 30 days. Then, samples were dehydrated, and water and methanol extracts were prepared using a solid-to-liquid ratio of 1:10 w/v. Alpha-glucosidase activity was assayed using maltose as a substrate, and the amount of glucose liberated was measured using the glucose oxidase-peroxidase method (GOD-POD). The reaction rate of α-glucosidase activity and enzyme inhibition by water and methanol extracts was analyzed by Michaelis-Menten kinetics using Lineweaver-Burk plots. The Michaelis constant (*Km*) and maximal velocity (*Vmax*) were determined by the Lineweaver-Burk plots equation. No significant difference was detected between the *Vmax* results of control, spontaneous, and controlled fermentation in both types of extracts. On the other hand, *Km* values mM were 1.94, 2.95, and 7.98 for control, spontaneous, and controlled fermentation, respectively, when water extracts were assayed. In the case of methanol extracts, *Km* values mM were 3.12, 3.27, and 7.04 for control, spontaneous, and controlled fermentation, respectively. These results suggest that metabolites from the fermentation of Chinese cabbage and *U. pinnatifida* exert competitive inhibition of α-glucosidase activity, with an enhanced effect in controlled fermentation. Controlled fermentation could thus be a potential method for developing anti-diabetic foods that can improve diabetes management.

AB8 PHYSICOCHEMICAL AND MICROBIOLOGICAL STABILITY, AROMA PROFILE AND SENSORY ATTRIBUTES OF CHILTO JUICE FERMENTED WITH *Lactiplantibacillus paraplantarum* IBFV-10

L. Contreras[1], E. Salguero[1], M.I. Isla[1,2], **S. Torres**[1,2].
[1]INBIOFIV, CONICET-UNT. S. Martin 1545. 4000 Tucumán, Argentina. [2]FCN e IML, UNT. Miguel Lillo 205. 4000 Tucumán, Argentina. E-mail: storres@csnat.unt.edu.ar

The fruits of chilto (*Solanum betaceum*), a plant native to South America, are natural functional foods rich in health-beneficial bioactive compounds (phenolics, flavonoids, anthocyanins, and carotenoids). However, fresh chilto fruits and their products, such as juice, have a short shelf life. Producing fermented chilto juice with probiotic lactic acid bacteria offers a promising alternative to extend shelf life and enhance its functional, nutritional, and organoleptic qualities. This study aimed to assess the microbiological and physicochemical stability, sensory properties, and aroma compounds profile of fermented chilto juice using previously selected *Lactiplantibacillus paraplantarum* IBFV-10, a potentially probiotic strain autochthonous from chilto fruits. Fermented chilto juice (FJ) was prepared by fermenting (24 h; 37 °C) pasteurized juice (64 °C; 30 min) with IBFV-10 strain and then stored for 30 days at 8°C. Unfermented pasteurized chilto juice (CJ) stored under the same conditions was used as a control. The microbiological quality of juices during storage was studied by investigating the presence of total mesophiles, fungi, yeasts, enterobacteria, and lactic acid bacteria (LAB). Also, the physicochemical parameters titratable acidity, pH, total soluble solids, browning index, and color were evaluated in both juices. The aroma characterization was investigated by GC–MS, and a sensory evaluation was conducted through pairwise comparison analysis and acceptability testing (7-point hedonic scale) using untrained panels. The FJ showed no growth of contaminating microorganisms during the 30-day storage period at 8°C. In contrast, the CJ exhibited contamination with various bacteria, including total mesophiles, LAB, and enterobacteria. The physicochemical parameters of the FJ were maintained throughout refrigerated storage. Contrarily, the CJ experienced a decrease in the pH values, an increase in the titratable acidity and the browning index, and color changes, all indicative of deterioration. The fermentation altered the volatile profile of chilto juice, resulting in changes in aroma compounds and sensory differences compared to the CJ. Certain aroma compounds that are characteristic of chilto fruits (methyl butanoate, ethyl butanoate, methyl hexanoate, (Z)-3-hexen-1-ol, and eugenol) decreased after the fermentation, while others like ethyl acetate and acetic acid, increased. In line with this, the sensory analysis showed different perceptions of sensory attributes between the FJ and the CJ. However, panelists showed satisfactory acceptability of both juices, with no significant preference between them, expressing willingness to consume and purchase the FJ product. This study demonstrates that fermenting chilto juice with *L. paraplantarum* IBFV-10 boosts the shelf life of the juice. Further, distinctive new organoleptic characteristics of the FJ could promote its acceptance as a functional product by the non-dairy probiotic food market.

AB9 *IN VITRO* EVALUATION OF THE ANTAGONISTIC POTENTIAL OF *Ligilactobacillus murinus* 26B1 ISOLATED FROM MOUSE AGAINST *Staphylococcus aureus* ATCC 6538P FOR ITS APPLICATION IN ANIMAL NUTRITION

J. Palma[1], I. Sandoval[1], A. Llorente[2], C. García[3], **S. Fadda[4]**, C. Mercado[3], C. Soto[3]
[1]Posgrado, FESC Cuautitlán, UNAM, San Sebastián Xhala, Cuautitlán Izcalli 54714, México. [2]Ingeniería y Tecnología, FES Cuautitlán, UNAM, San Sebastián Xhala, Cuautitlán Izcalli 54714, México.[3] Ciencias Biológicas, FES Cuautitlán, UNAM, San Sebastián Xhala, Cuautitlán Izcalli 54714, México. [4]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. Email: llorente@unam.mx

Due to their ability to produce various metabolites with antagonistic activity, lactic acid bacteria (LAB) are being focus of study for their probiotic potential for the control of pathogens of sanitary concern. In this project, the objective was to demonstrate the antagonistic activity of *Ligilactobacillus murinus* 26B1 (*L. murinus*) isolated from the mouse intestinal tract, through bacterial inhibition tests and *in situ* zymography, against *Staphylococcus aureus* (*S. aureus*), one of the main causal agents responsible of foodborne diseases. *L. murinus* 26B1 was isolated from the intestinal contents of lactating mouse. Molecular characterization (16S rRNA) was performed, and the antagonistic activity of its culture supernatants was evaluated. Proteins were quantified by the Bradford method, by means of the *Spot on the Lawn* technique *and in situ* zymography, inhibitory and lytic activity were evaluated, respectively; the relative molecular mass was determined (SDS-PAGE). The biofilm formation capacity of both strains, *L. murinus* and *S. aureus* ATCC 6538P, was evaluated by crystal violet staining. In compliance with the requirements for new probiotics, established by FAO-WHO-WHOSA (2022), the identification results (16S rRNA) showed that strain 26B1 belongs to the genus *Ligilactobacillus* and species *L. murinus*. Using the *spot of the lawn* technique, inhibition zones of up to 9 mm were obtained with viable *L. murinus* cells against *S. aureus*. Zymography with *M. lysodeikticus* revealed a lytic band around 108 kDa, whereas three bands were identified with *S. aureus*, with relative molecular masses of 108±4.80, 92±0.22, and 23±1.11 kDa. The amino acid sequence of the 23±1.11 kDa protein with lytic activity was obtained by peptide fingerprinting (UHPLC). The inhibition kinetics determined a MIC (minimal inhibitory concentration) was 0.07±0.01 mg/mL and a reduction of 3 log CFU/mL of *S. aureus* at 24. *L. murinus* showed moderate biofilm production at 4°C and 25°C, and strong biofilm production at 37°C, whereas *S. aureus* exhibited moderate biofilm production at 4°C and strong biofilm production at 25°C and 37°C. Applying the FAO-OMS-OMSA criteria (2022) our results confirm that *L. murinus* 26B1 strain from murine origin was identified at the genus and species level; its antagonistic potential against *S. aureus* ATCC 6538P and the 23±1.11 kD band showed similarity to a protein fragment with aminopeptidase function.

AB10 GENETIC MODIFICATION OF *Lactococcus lactis* TO PRODUCE THE ANTI-INFLAMMATORY PROTEIN ELAFIN: EVALUATION OF DIFFERENT EXPRESSION SYSTEMS

R. Levit[1], A. Aucouturier[2], P. Langella[2], L. Bermúdez-Humarán[2], A. de Moreno de LeBlanc[1], J. G. LeBlanc[1].

[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. [2]Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350, Jouy-en-Josas, Francia. E-mail: rlevit@cerela.org.ar

Lactococcus (*L.*) *lactis* is the model lactic acid bacteria (LAB) for genetic modification for the production of heterologous proteins of therapeutic interest. Our group constructed two strains of *L. lactis* capable of expressing and administering the anti-inflammatory protein elafin using two different expression systems. In the first, *L. lactis* LL-thy-elafin produces elafin through the NICE (Nisin-Inducible Controlled Expression) system, a system based on the autoregulation of nisin biosynthesis. In this strain, the elafin expression cassette (under the control of the nisin-inducible promotor) is located on the bacterial chromosome and it was modified to make it thymidine auxotrophic, contributing to its safety. In the second, *L. lactis* LL-elafin produces elafin trough the SICE (Stress-Inducible Controlled Expression) system, a system based on a vector carrying an expression cassette under the transcriptional control of a stress-inducible promotor. In this system, the expression of the protein of interest is induced after the administration of LAB to the host in which it finds different conditions than in culture. The aim of this study was to evaluate and compare the anti-inflammatory potential of *L. lactis* LL-thy-elafin and *L. lactis* LL-elafin in vitro using cancerous and non-cancerous intestinal cell lines, and in vivo in a murine model of intestinal mucositis (IM). In vitro: the effect of the recombinant strains on the efficacy of 5- Fluorouracil (5-FU) was evaluated with Caco-2 and IEC-18 cells exposed to 5-FU and/or the recombinant bacteria and measuring viability by MTT assay. In vivo: BALB/c mice were injected daily with 5-FU to induce IM and orally administered with the recombinant strains (10^8 CFU/mL). *L. lactis* carrying the empty plasmid vector (LL-empty) and the respective wild-type strains (LL) were used as controls. Diarrhea onset, small intestine morphology and histopathology, and serum cytokines levels were assessed. In vitro assays demonstrated that the elafin-producing strains enhanced the cytotoxic effects of 5-FU on colon cancer cells (Caco-2) and improve the survival of non-cancerous intestinal epithelial cells (IEC-18) in the presence of 5-FU. In vivo assays showed that elafin-producing strains were able to reduce the onset of diarrhea, with an increase in the villus height/crypt depth ratio, and a reduction of intestinal inflammation. Recombinant strains reduced serum levels of pro- inflammatory cytokines with increases of the anti-inflammatory cytokine IL-10. In conclusion, although both systems were effective, *L. lactis* expressing elafin with the SICE system showed the best results. This system has the additional advantage that the presence of regulatory genes is not required nor does not require chemical induction before their use.

AB11 INFLUENCE OF TEMPERATURE, PH AND CULTURE MEDIA ON *Salmonella enterica* SEROTYPE ENTERITIDIS BIOFILM FORMATION

S. López Pérez[1], C. Melián[1], P. Castellano[1].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucuman, Argentina. E-mail: slopez@cerela.org.ar

Biofilms are defined as complex microbial communities established on a wide range of surfaces and embedded in a self-produced matrix of extracellular polymeric substances. Adhesion of pathogenic and/or spoilage microorganisms to equipment materials and biofilm development constitute a potential chronic source of microbial contamination threatening the safety and quality of food products, resulting in food-borne disease and economic losses. Particularly, *Salmonella enterica* serotype Enteritidis one of the most important agents of foodborne disease in several countries forms biofilms to evade the action of traditional disinfectants, environmental stress and the host's immune system. Food products including contaminated meat and eggs have caused outbreaks of human salmonellosis. In addition, biofilm formation is a multi-step process starting with attachment to a surface then formation of micro-colony that leads to the formation of three-dimensional structure and finally ending with maturation followed by detachment. This process depends on an interaction among bacterial cells, the attachment surface and environmental conditions. In this context, the aim of this study was to determine the biofilm production capacity of *S. enterica* serotype Enteritidis at two different temperatures of incubation, pH and four culture media. The pathogenic strain was isolated from poultry and identified by using conventional standard biochemical and serological tests. 200 µl of a culture (16 h of incubation) were added to each well of the microplate. The following culture media were used separately: triptein soy broth (TSB) + 0.5% yeast extract (YE), Luria-Bertani (LB) both with pH 7.0 and TSB + 0.5% YE + 0.5% NaCl at pH 5.5. Two temperatures were tested, 25 °C to promote the expression of the components of the extracellular matrix and 10 °C as the temperature of meat and poultry processing lines. Microplates incubated at 25 °C were examined at 24 and 48 h and those incubated at 10 °C at 3 and 6 days. Biofilm formation was determined by crystal violet staining and classified as weak, moderate or strong based on the established cut-off line. In microplates incubated at 25 °C, *S. enterica* biofilm formation was greater in the TSB+YE+NaCl medium, with weak and moderate formation at 24 and 48 h, respectively. At 10 °C, a greater increase in pathogen biofilm formation was detected in all culture media at 6 days of incubation, with moderate formation profiles. At 3 days, a similar biofilm formation pattern was only observed in TSB+YE+NaCl and LB media. The contribution to the Knowledge of factors that influence in the formation and dispersion of *S. enterica* serotype Enteritidis biofilms could allow the design of prevention strategies to avoid microbial contamination, controlling an important public health problem.

AB12 OPTIMIZATION OF THE FREEZE-DRYING PROCESS TO OBTAIN MORE STRESS TOLERANT *Oenococcus oeni* STARTER CULTURES

E.V. Bentencourt[1], R.R Raya[1], L.M. Mendoza[1]
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucuman, Argentina. E-mail: ebentencourt@cerela.org.ar

Malolactic fermentation (MLF) is an important process in wine production, as it contributes to improving the quality and organoleptic characteristics of wine by reducing acidity and increasing microbiological stability. *Oenococcus oeni* is the best species adapted to winemaking conditions and it is almost exclusively used as an MLF starter culture. Therefore, achieving success in this fermentation has led to increased emphasis on developing methods for the production and conservation of starter cultures. Freeze-drying is widely used in the production of starter cultures; however, this process can cause damage to the cell membrane, denaturation of proteins and DNA, resulting in a significant decrease in cell survival. For this reason, cryoprotectants are used to enhance cell viability. The objective of this study was to evaluate the viability of *O. oeni* strains pre-treated with glutathione (GSH) or amino acids as stress protective agents and later subjected to freeze-drying process using different cryoprotectants. Two strains of *O. oeni* (X2L and ST), native to the NOA region, were cultured at 30°C in modified MRS medium (5 g/L fructose and 4 g/L DL-malic acid) with and without GSH or cysteine and glutamate (5 mM). Cells were harvested at the end of the exponential phase (OD 0.9) at 600nm, washed with phosphate buffer, and treated with three types of cryoprotectants: sucrose (10%), sodium glutamate (2.5%), and glutamic acid (2.5%), suspended in phosphate buffer. Vials were previously frozen overnight to freeze-drying. Rehydration was carried out using the same cryoprotectants. Cell counts were performed before and after freeze-drying as well as at 7 days, 1, 4, and 8 months, to determine if cells remained viable over time. When sodium glutamate and glutamic acid at 2.5% were used as cryoprotectants, the initial cell concentration was 10^9 CFU/ml, and cell viability was maintained throughout the 8 months evaluated. However, when cells were freeze-dried with 10% sucrose, cell survival dropped by two logarithmic units after freeze-drying. Later, cells were inoculated into wine to assess cell viability and malolactic activity. In synthetic wine, both strains of *O. oeni* showed higher viability and malic acid consumption when glutamic acid was used as the cryoprotectant and GSH as the protective agent against stress wine. The results of this study confirmed glutathione has a positive effect on growth of *O. oeni* contributing to biomass preparation of MLF starter cultures and in combination with glutamic acid more resistant freeze-dried cells were obtained. Therefore, the combination GSH and glutamic acid could be used to optimize biomass production and enhance the quality of *O. oeni* starter cultures used for MLF in winemaking.

AB13 SENSORY ANALYSIS OF THE FINAL PROTOTYPE OF A SOY FOOD FERMENTED WITH *Lacticaseibacillus paracasei* subsp. *paracasei* CRL207

B.L. Nacchio[1], G.N. Avila Hael[1], O.A. Garro[2], R.B. Medina[1,3], **M.S. Garro**[1].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucuman, Argentina. [2]INIPTA-CONICET-UNCAUS. Cte Fernandez 755. 3700 Pcia R S Peña, Chaco, Argentina. [3]FAZyV, Universidad Nacional de Tucumán, Avda. Pte. N. Kirchner 1900. 4000 Tucumán, Argentina. E-mail: mgarro@cerela.org.ar

Soy foods have a low acceptance among consumers; this could be due to the organoleptic characteristics of soybean, especially the strange flavors and odors ("off flavors"). The use of fermentation with lactic acid bacteria confers to the matrix various properties, from the nutritional, functional and organoleptic point of view. In previous works it was determined that the *Lacticaseibacillus paracasei* subsp. *paracasei* CRL207 strain conferred favorable characteristics to soybean products and the aroma was studied sensorially, this study allowed obtaining descriptors to carry out a more detailed sensorial study of the product in its final phase. Sensory analysis involves an important phase in the final stage of the prototyping of a new food. The aim of the present work is to determine if the inclusion of flavorings improves the organoleptic profile of a food prototype fermented with lactic acid bacteria in a soybean matrix. A semi-trained panel selected the flavorings that were added to the food prototypes (strawberry and orange). The selected additives were then moved on to a further prototyping stage where a more detailed analysis was carried out with a panel of 50 untrained consumers who were given three coded samples, two of which were added with flavorings and as a control a sample without additives was given. Acceptability and association were evaluated in the survey using a "check all that apply" (CATA) type survey. The results showed that the samples with the addition of strawberry and without flavoring were the ones that the participants liked the most, obtaining a score greater than 4 considering the attributes of aroma, flavor and overall appreciation, while the sample added with flavoring was the one that obtained a lower score (4). Regarding the association: the participants described the sample without flavorings by selecting both positive and negative terms (cereals, nuts and unpleasant), the sample with added strawberry flavor was associated with descriptors with positive connotations such as sweet, yogurt and caramel; finally, the sample with orange flavor was described as sour, vinegar, soy and citrus, in this last case the selected terms also have a mixed connotation. In conclusion, we can say that based on the results obtained, the strawberry flavored sample is the one that obtained a better performance since it obtained a good acceptability score and was described with positive terms for consumers.

AB14 PRODUCTION OF MIXED FRUIT JUICE ENRICHED WITH PROBIOTIC LACTIC ACID BACTERIA

[1,4] **F. Escobar**, [1,2] D.A. Barrionuevo, [1] S.A. Isas,[4] B.J. Pérez, [1,3] C.P. Van Nieuwenhove.
[1] Centro de Referencia para Lactobacilos (CERELA)-CONICET, Laboratorio de Ecofisiología Tecnológica, Chacabuco 145, San Miguel de Tucumán, 4000, Tucumán, Argentina. [2] Facultad de Bioquímica Química y Farmacia-UNT, Batalla de Ayacucho 471, San Miguel de Tucumán, Argentina. [3] Fundación Miguel Lillo, Miguel Lillo 251, San Miguel de Tucumán, 4000, Tucumán, Argentina. [4] Facultad de Ciencias Naturales e IML, Miguel Lillo 205, San Miguel de Tucumán, 4000, Tucumán, Argentina. Email: fescobar@cerela.org.ar

The consumption of functional foods has acquired great interest over the years, as they provide health benefits beyond the basic nutritional characteristics of fresh foods. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host. Among functional foods, probiotic foods and drinks are in great demand due to their close relationship with the prevention of multiple diseases. Fermented fruit-based juices are one of the most active functional food categories. They provide several bioactive compounds and could be used for probiotic delivery as an alternative to dairy foods, useful for those consumers who are vegan or intolerant to dairy products. The aim of this study was to evaluate a mixed fruit juice as a vehicle for three commercial probiotic strains: *Lactiplantibacillus plantarum* CRL 972 (ATCC 14917), *Lacticaseibacillus casei* CRL 1110 (ATCC 393) and *Lacticaseibacillus rhamnosus* CRL 1227 (ATCC 11443). The juice was obtained by mixing pomegranate (20% v/v), blueberries (10% v/v), blackberries (10% v/v) juices, and water (60% v/v), 6º Bx, pH 4.4 adjusted with sodium carbonate. Juices were pasteurized at 65°C during 15 min, then immediately cooled. Probiotic strains were individually inoculated at 2% (v/v) (10^7 CFU/mL), then incubated at 37°C for 48 h, further refrigerated at 4°C for 28 days. Viable cell count, pH, total phenolic content (TPC) and antioxidant capacity by three different methods (FRAP, DPPH and TEAC) were measured in the formulated juices during fermentation and cold storage. *L. plantarum* CRL 972 grew after 48 h of fermentation (up to 10^8 UFC/mL) and maintained at a high viable cell count of 10^7 UFC/mL after 28 days at 4ºC. The viability of *L. casei* CRL 1110 was around 10^7 CFU/mL during fermentation and shelf life; whereas *L. rhamnosus* CRL 1227 decreased near one log unit after 28 days of cold storage (from 10^7 CFU/mL to 10^6 CFU/mL). The TPC significantly increased after shelf life in fermented juice by *L. casei* CRL 1110 ($p< 0.05$). Antioxidant activities were similar in all juices (with or without probiotic strains) and times assayed. No yeast, mold or *Enterobacteriaceae* were found in probiotic juices. Results show that the mixed fruit juice could be used as an alternative functional drink, with high antioxidant capacity and preserved polyphenol content, for delivering probiotic strains.

AB15 IMPAIRMENT OF *Listeria monocytogenes* DEVELOPED ON INDUSTRIAL SURFACES BY *Latilactobacillus curvatus* CRL1579 BACTERIOCIN

C. Melián[1], D. Ploper[2], R. Chehín[2], G. Vignolo[1], P. Castellano[1]
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucuman, Argentina. [2] IMMCA-CONICET-UNT-Ministerio de Salud Pública, Gobierno de Tucumán. Pje. Dorrego 1080. 4000 Tucuman, Argentina. E mail: cmelian@cerela.org.ar

Under environmental stress, *Listeria monocytogenes* can form biofilms by attaching to a variety of surfaces like stainless steel (SS), polytetrafluoroethylene (PTFE) or glass. Biofilm protected pathogens from sanitization procedures, allowing them to survive and persist as a chronic source of contamination, increasing their resistance to antimicrobials and sanitizers. The use of eco-friendly approaches involving microorganisms and their metabolites has recently emerged as alternative anti-biofilm strategies. Lactic acid bacteria (LAB) are known for their ability to produce bacteriocins, which are "friendly" antimicrobial agents. Particularly, the bacteriocin lactocin AL705 produced by *Latilactobacillus (Lat.) curvatus* CRL1579 showed high effectiveness at inhibiting Listeria in different meat systems. In addition, at sub-inhibitory concentrations was able to control *L. monocytogenes* FBUNT biofilm formation by disruption of quorum sensing through a signal molecule inactivation. On this basis, the aim of this study was to evaluate the ability of *Lat. curvatus* CRL1579 and its bacteriocin lactocin AL705 to displace *L. monocytogenes* FBUNT biofilm formed SS and PTFE industrial surfaces at 10 °C under static and continuous flow conditions. The bacteriocin was purified from an overnight culture of *Lat. Curvatus* CRL1579 in MRS by fractional precipitation with ammonium sulfate, solid phase chromatography and subsequent evaporation of solvents by rotary evaporator. The minimal inhibition concentration (MIC) was determined. *L. monocytogenes* FBUNT biofilms were formed on SS and PTFE coupons during 3 and 6 days at 10 °C under static and continuous flow conditions using LB as a culture medium. The inhibitory effect of *Lat. curvatus* and lactocin AL705 was evaluated by displacement assay and analyzed by confocal laser scanning microscopy (CLSM). Partially purified bacteriocin (800 UA/mL) effectively inhibited *L. monocytogenes* preformed biofilm through displacement strategy, reducing the pathogen by 5.54 ± 0.26 and 4.74 ± 0.05 log cycles at 3 and 6 days, respectively. The bacteriocin-producer decreased the pathogen biofilm by 2.84 log cycles. CLSM allowed to visualize the ability of lactocin AL705 to reduce *L. monocytogenes* FBUNT biofilm under static and hydrodynamic flow conditions. A greater effect of the bacteriocin was found at 3 days independently of the surface matrix and pathogen growth conditions at 10 °C. As a more realistic approach, biofilm displacement strategy under continuous flow conditions showed a significant loss of biomass, mean thickness and substratum coverage of pathogen biofilm. These findings highlight the antibiofilm capacity of lactocin AL705 and their potential application in food industries.

AB16 STABILITY OF PREBIOTIC GALACTOOLIGOSACCHARIDES DURING YOGHURT MANUFACTURE AND STORAGE

A. Fara[1], J. Palacios[1]; A. Montilla[2], G. Zárate[1,3].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. [2]Instituto de Investigación en Ciencias de la Alimentación (CIAL-CSIC), Madrid, España. [3]Universidad de San Pablo Tucumán, Argentina. E-mail: gzarate@cerela.org.ar

Prebiotics, are substrates selectively utilized by host microorganisms conferring a health benefit. Among the targets for prebiotics, the intestinal microbiota is the most relevant due to its crucial role in physiology and well-being. Since diet greatly influences the composition and metabolism of the intestinal microbiota, positive microbiome modulation by functional food ingredients, such as prebiotics, has become a strategy for reducing disease risk and fortifying homeostasis. At present, most widespread prebiotics are carbohydrates, particularly oligosaccharides such as fructans (inulin and fructo-oligosaccharides), galacto-oligosaccharides (GOS), and lactulose. In particular, GOS have attracted the interest from researchers and food industry because of the presence of galactose-based units with relative structural similarity to human milk oligosaccharides. GOS derived from lactose, lactulose (OsLu) and lactitol (LOS) are synthetized by transgalactosylation catalyzed by microbial β-galactosidases (β-gal). In previous studies we reported the synthesis and characterization of GOS by the β-gal from *Acidipropionibacterium acidipropionici* LET 120 (PAB-GOS) and *Lactobacillus bulgaricus* CRL 450 (LAB-GOS). GOS produced by both strains were scarcely digested by the gastrointestinal tract and demonstrated prebiotic effects on recognized probiotic microorganisms (CRL 431 and LGG). In the present study we manufactured a yoghurt supplemented with 2% PAB-GOS or LAB-GOS before and after fermentation of free-lactose milk with *S. thermophilus* CRL 1598 and *L. bulgaricus* CRL 450. The growth of the starter cultures (by plate count method), acidification of milk (by pH measurement) and consumption of GOS and monosaccharides (by HPLC) were determined at intervals during 8 h of fermentation and 28 days of storage at 4 °C. PAB-GOS remained unaltered throughout fermentation, while the viability of starter culture was similar to that observed in the control yoghurt. When LAB-GOS were added to milk prior fermentation, CRL 1598 grew like the control while CRL 450 reached 0.5 log CFU/mL more than the control and reached a lower pH (4.81 vs 4.93). Growth during fermentation was mainly due to monosaccharides consumption whereas less than 10% of LAB-GOS were consumed. During cold storage, GOS were not significantly hydrolyzed whereas pH decreased in a similar manner in both control and GOS supplemented yoghurts and starters remain higher than 10^7 CFU/mL. Our results show the feasibility of obtaining a reduced-lactose yogurt enriched in stable prebiotic GOS. The manufacture of a prebiotic yoghurt with probiotic starters would further increase the functional properties of the product.

AB17 EVALUATION OF THE PROTEOLYTIC PROFILE AND ANTIOXIDANTS OF LEGUME FLOURS FERMENTED WITH SELECTED LACTIC CULTURES

A. P. Pezuk[1], L. R. M. Juarez[1], G. Zárate[1,2], M. S. Garro[1].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. [2]Universidad de San Pablo-Tucumán. Gral. José de San Martín 435. 4000 Tucumán, Argentina. E-mail: ppezuk@cerela.org.ar

Currently, the production of legumes represents an important economic support for the Northwest region of Argentina, particularly soybeans, chickpeas and beans, due to their high nutritional value and vegetable protein content. The fermentation with lactic acid bacteria is an attractive strategy to improve the nutritional, sensory and techno-functional characteristics of legume flours with applications in novel foods. The objective of this work was to evaluate the effect of lactic fermentation on the proteolysis, antioxidant activity and the in vitro digestibility of legume flours. The microorganisms used were: *Limosilactobacillus fermentum* CRL 251, *Lacticaseibacillus paracasei* subsp. *paracasei* CRL 207 and *Lactiplantibacillus plantarum* CRL 2211. Pastes were made with the individual legume flours: soybean paste (PS), bean paste (PP), kabuli chickpea paste (PG) and a mixture (1:1:1 ratio) of the three flours (PSGP). Pastes containing 65% of moisture, were inoculated at 1% rate (~10^6 CFU/g) with each strain and incubated at 37°C for 24 h, uninoculated pastes were used as control. Bacterial growth during fermentation was followed by the plate dilution count method (log CFU/g of sample) and pH. The concentration of soluble amino acids in the pastes was measured by the o-phthaldialdehyde method (OPA), antioxidant activity by the DPPH method and the quantification of in vitro protein digestibility (IVPD) was carried out through a multienzyme complex. The assayed strains showed a significant microbial growth and increase of acidification in all pastes, compared to the controls. All the fermentation increased the soluble amino acids contents particularly 40% in PS and PP, 30% in PSGP and 20% in PG, compared to their controls. The antiradical activity showed the greatest increase in PSGP (30% in the three strains compared to the control) in contrast to the individual pastes (20% compared to the control). The strains CRL 251 (in PS) and CRL 207 (in PG) did not show significant variations with respect to the control. Regarding the IVPD%, values above 20% IVPD were observed in all treatments compared to their controls, except in PG fermented with CRL 251 which was significantly lower compared to the other treatments. In this work it was demonstrates that the three strains grew and acidified the vegetable matrices at 37°C for 24 h, observing an improvement in the antioxidant, proteolytic activity and digestibility of the flours. Our results demonstrate the potential of selected lactic acid bacteria to improve the properties of legume flours by increasing their antioxidant activity, the availability of peptides and amino acids for the consumer, promoting their use as starters for the formulation of products derived from legumes.

AB18 TECHNOLOGICAL STRATEGY TO IMPROVE THE ORGANOLEPTIC CHARACTERISTICS OF SOY FOOD PRODUCTS: FERMENTATION WITH SELECTED LACTIC ACID BACTERIA

G.N. Avila Hael[1], B.L. Nacchio[1], O.A. Garro[2], R.B. Medina[1], **M.S. Garro**[1].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. [2]INIPTA-CONICET-UNCAUS. Cte Fernandez 755. 3700 Pcia R S Peña, Chaco, Argentina. E-mail: mgarro@cerela.org.ar

The production of plant-based foods is currently expanding. Soybean is a widely cultivated, legume rich in vegetable protein (35–40%), lipids (19–22%) and other important nutrients (isoflavones, minerals and dietary fiber). However, its consumption is limited by the beany flavors (off flavors). The use of selected lactic acid bacteria (LAB) emerges as a technological strategy to modify the soy matrix, increasing its nutritional value, and improving the organoleptic characteristics. LAB that metabolize citrate produce compounds such as acetate, diacetyl, acetaldehyde and acetoin, which determine the flavor of many fermented foods. The objective of this work was to evaluate the production of organic acids, diacetyl (DA) and acetoin (AC) in fermented soybean products added with different sources of citric acid. Aqueous soy extract (EAS) and soy pastes (PS) were used with the addition of strawberry, orange peel or pulp, and/or sodium citrate. In total, 6 PS matrices and 5 EAS matrices were evaluated without and with additions of the different citrate sources. Each matrix was individually inoculated at 2% with *Lacticaseibacillus paracasei* subsp. *paracasei* CRL 207 (strain selected for its ability to utilize citrate) and incubated at 37°C for 8 h in EAS and 16 h in PS. PS and EAS without and with aggregates uninoculated were used as controls. Organic acids were determined by HPLC, and volatile compounds by GC. The main acids detected in all samples were lactic, citric and acetic. It was shown that the soybean matrix contains citric acid (EAS and PS without aggregates: 1.24 and 6.03 mg acid/g of sample, respectively). The addition of orange or strawberry to EAS produced a slight increase in the initial citric acid concentration, being higher with strawberry (EAS+strawberry: 1.79 mg citric acid/g of sample). After 8 h of incubation was production of lactic acid was observed in all EAS sample. In EAS + commercial citrate presented the highest concentration of lactic acid (12.63 mg lactic acid/g of sample). In all PS citrate consumption was observed, detecting high consumed in PS + strawberry (95%). In this sample, the highest production of lactic acid (9.64 mg acid/g of sample) was obtained. DA and AC (DA-AC) production was detected in all fermented PS (138.4 mM to 219.29 mM), while in EAS these compounds were detected at very low concentrations (4.68 mM to 17.85 mM). In soy matrices added orange peel, was observed the highest values of the DA-AC. In conclusion, a strategy to improve the organoleptic characteristics of soy products consisting in ferment soybean paste added of oranges or sodium citrate inoculated with *L. paracasei* subsp. *paracasei* CRL 207 for 16 h at 37 °C.

乳酸菌

MOLECULAR BIOLOGY AND GENOMICS OF LACTIC ACID BACTERIA

健康

食料

MG1 GENOMIC CHARACTERIZATION OF THE HONEYBEE-PROBIOTIC STRAIN *Ligilactobacillus salivarius* subsp. *salivarius* A3iob

M. Elean[1], A. Arroyo Guerra[2], L. Albarracín[1], C. Audisio[2], J. Villena[1].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucuman, Argentina. [2]INIQUI-CONICET-UNSA, Salta, Argentina. E-mail: carina.audisio@exa.unsa.edu.ar, jcvillena@cerela.org.ar

Previous studies demonstrated the beneficial effects of the probiotic strain *Ligilactobacillus salivarius* A3iob on honeybee (*Apis mellifera*) colonies. This bacterium is able to enhance protection against bee pathogens. Furthermore, hives from honeybees treated with the A3iob strain displayed significant increases in honey production. The present study aimed to assess the genomic characteristics of the probiotic *L. salivarius* A3iob to understand its ability to improve bee´s health. The comparative genomic analysis was performed with the A3iob genome and the genomes of probiotic *L. salivarius* strains from human, porcine and chicken origin as well as bacteria isolated from the bee´s gut including *Bombella intestine*, *Bombella apis*, *Bifidobacterium lactis*, *Apilactobacillus kunkeei*, *Enterococcus durans*, *Apilactobacillus micheneri*, *Apilacobacillus timberlakei*, *Apilactobacillus quenuiae*, *Apilactobacillus apinorum* and *Apilactobacillus waqarii*. The analysis included the examination of metabolic and functional genes related to adhesion, production of bioactive compounds, modulation of the host's immune system, and antimicrobial substances. The total size of the A3iob genome is 2.05 Mb with a 34.6% GC. *In silico* studies revealed that *L. salivarius* A3iob possesses genes for glycosyltransferases (GT) from the families GT2, GT4 and GT similar to *B. apis* and *B. intestinalis* and glycosylhydrolases (GH) from the families GH1, GH2, GH13, GH36, GH65 and GH177 similar to *A. kunkeei*, *E. durans* and bifidobacteria isolated from bee intestine. It was also observed that the A3iob strain has a unique genetic profile with a high number of secretion system genes and adhesion genes including the ones coding for the SecA2/Y2 system, the mucus biding proteins MucBP1, MucBP2 and MucBP3 and a pilli cluster (*pilA, SpaA, SpaB, sortaseA*) that was described only in five strains of the *L. salivarius* species and in the intestinal bee-derived strain *E. durans* EDD2, indicating its potential for successful colonization in the bee gastrointestinal tract. Additionally, the A3iob strain showed the presence of exopolysaccharide biosynthesis clusters (EPS1 and EPS2) described in the probiotic *L. salivarius* UCC118 (LSL_0977-LSL_0997 and LSL_1547-LSL_1574, respectively), which have been associated to adhesive and immunomodulatory effects. Genes related to oxidative stress response (thioredoxin and NrdH-redoxin system) were detected, indicating that the A3iob strain may help bees to maintain a healthy intestinal redox balance. In addition, the bacteriocin genes *abp118A* and *abp118B* were found in the A3iob genome. The genomic characterization of the probiotic strain *L. salivarius* A3iob performed in this work provides some clues about the genetic mechanisms underlying the probiotic properties of the strain, paving the way for future research aimed at improving bee´s health and productivity in the face of environmental challenges.

MG2 FUNCTIONAL AND GENOMIC CHARACTERIZATION OF *Pediococcus pentosaceus* TUCO-3 ISOLATED FROM CAT MILK: A POTENTIAL PROBIOTIC STRAIN TO COMBAT INFECTIONS

S. Quilodrán-Vega[1], M. Elean[2], L. Albarracín[2], C. Muñoz[1], J. Toledo[1], J. Villena[2].
[1]University of Concepción, Concepción, Chile. [2]CERELA-CONICET, Tucuman, Argentina. E-mail: squilodran@udec.cl

Diarrheal pathologies in cats are the most common cause of consultation in veterinary clinics. The most common bacteria that affect pets are Gram negative pathogens. Usually, there is no investigation of the diarrhea causative agent, so the most common treatment consists of the administration of broad-spectrum antibiotics, which leads to the selection of antibiotic-resistant microorganisms and intestinal dysbiosis in cats. Therefore, it is necessary to find alternatives to prevent or reduce diarrhea in cats. In this work, lactic acid bacteria were isolated from breast milk samples of cats and potential feline probiotic strains were selected based on their ability to inhibit pathogenic bacteria including *Escherichia coli, Salmonella enterica*, and *Klebsiella pneumoniae*. Among the studied strains, *Pediococcus pentosaceus* TUCO-3 stood out because of its remarkable capacity to inhibit the growth of *E. coli* ATCC25922, *S. enterica* ATCC13076, and *K. pneumoniae* ATCC13883, as well as enterohemorrhagic *E. coli* isolates. *In vitro* studies showed that *P. pentosaceus* TUCO-3 has resistance to pH=3, pancreatine and bile salts, indicating its potential to survive gastrointestinal conditions. The TUCO-3 strain is susceptible to most antibiotics assessed and revealed no hemolysin activity. In addition, the complete genome of the TUCO-3 strain was sequenced using the Illumina Miseq platform. *P. pentosaceus* TUCO-3 identity was determined via average nucleotide identity (ANI), obtaining an identity value of 98,96% compared with strains of the *P. pentosaceus* species. The annotation of the genome was performed with the NCBI Prokaryotic Genome Annotation Pipeline. The total size of the TUCO-3 genome is 1,9 Mb with a 37% GC content and a total of 1,996 coding sequences. Several genes consistent with features of probiotic microorganisms are detected in the TUCO-3 genome, including a *MucBP* gene associated with adhesion and colonization (NVZ01419.1), and two putative genes coding for class IIa bacteriocins: pediocin A and a bacteriocin with 69.7% identity to hiracin JM79 (NVZ00664.1). The production and secretion of these bacteriocins by the TUCO-3 strain could contribute to maintaining intestinal health in cats by inhibiting the growth of pathogenic bacteria such as *Clostridium perfringens*, a major cause of gastrointestinal infections. In addition, a vitamin B2 biosynthetic cluster was found; however, the *ribDG* gene has a premature termination codon, suggesting that the B2 vitamin *de novo* synthesis pathway is likely incomplete. Additional phenotypic tests are necessary to evaluate vitamin B2 production. Screening for the virulence factor genes in the genome of TUCO-3 showed no hits using the Virulence Factor Database (VFDB), confirming the safety of the tested strain. *P. pentosaceus* TUCO-3 is potential probiotic candidate for improving intestinal health and resistance to infections in cats.

MG3 PROTEOMIC REGULATION AND GENE EXPRESSION OF SELENIZED CELLS OF *Fructobacillus tropaeoli* CRL2034 IN A FERMENTED FRUIT-BASED MEDIUM

L. Crespo[1], F. Mozzi[1], M. Pescuma[2].
[1]CERELA-CONICET. Chacabuco 145. 4000 Tucumán, Argentina. [2]CIEFAP-CONICET. Ruta Nacional 259 Km 16, Esquel, Chubut, Argentina. E-mail: lcrespo@cerela.org.ar

Selenium (Se) is an essential micronutrient for humans. Some lactic acid bacteria (LAB) can grow on fruit substrates and biotransform selenite into Se-nanoparticles and Se-amino acids. These LAB are considered an interesting alternative to formulate new fermented fruits-based beverages fortified with Se. This work aimed to compare the growth and the differential protein and gene expression of *Fructobacillus tropaeoli* CRL2034 grown in MRSf (fructose 20 g/L) with selenized (Se^+) and control (Se^-) cells grown in a fruit-like medium (SP). Se^+ and Se^- cells were grown in MRSf and SP for 6 h (exponential growth phase). The protein differential expression was analyzed by LC-MS/MS (CEQUIBIEM-CONICET), while the expression of those genes likely related to Se metabolism was analyzed by RT-qPCR. When Se^- cells were grown in SP, 146 proteins were differentially expressed; 118 were upregulated and 28 were downregulated, while the Se^+ cells differentially expressed 228 proteins; 152 upregulated, and 76 downregulated compared with cells grown in MRSf. Venn diagram showed that 125 proteins were deregulated independently of the Se growth condition, being 103 proteins deregulated in Se^+ cells. When *F. tropaeoli* CRL2034 was grown in SP, two important enzymes involved in the synthesis and incorporation of seleno-amino acids into proteins were differentially expressed. SerC showed a fold chance of 0.82 and 0.92 times, while methionyl $_t$RNA synthetase increased its expression 1.58 and 2.00 times in Se^- and Se^+ cells, respectively. Moreover, two glutathione reductases showed higher expression in the Se^+ cells (1.84 and 1.49 times) than in Se^- cells (1.00 and 1.24 times), while its gene expression increased in Se^+ cells between 0.22 and 0.38 units with respect to MRSf grown cells. Mannitol 2-dehydrogenase (MDH), an enzyme involved in fructose metabolism, showed a negative deregulation trend with a fold change of 0.89 and 0.76 times in the Se^+ and Se^- cells, respectively. Consistently, *mdh* expression decreased by 0.48 and 0.60 units in cells grown in SP with respect to MRSf grown cells. Malolactic enzyme showed a positive deregulation of 162.86 and 148.96 times for Se^+ and Se^- cells, respectively. Similar results were observed in its gene expression which increased by 1.23 and 1.46 units compared to the cells grown in MRSf. This fact was probably due to the presence of malic acid in the SP medium. Proteomic and gene expression analysis indicated that the fruit-like medium influenced the regulation of proteins involved in cellular processes. Moreover, an increase in the expression of proteins related to Se metabolism was observed in Se^+ cells with respect to Se^- cells grown in SP, compared with cells grown in MRSf.

MG4 GENOMIC AND PROTEOMIC CHARACTERIZATION OF *Lactiplantibacillus plantarum* CRL681 FOCUSING ON CELL SURFACE AND SECRETED PROTEINS

A. Baillo[1], L. Albarracín[1], J. Villena[1], S. Fadda[1]
[1]CERELA-CONICET. Chacabuco 145. Tucuman, Argentina. E-mail: sfadda@cerela.org.ar

Lactiplantibacillus plantarum CRL681 (Lp681) can inhibit the growth of pathogenic *Escherichia coli* both in food matrices and in the intestinal mucosa. In addition, this strain can modulate intestinal innate immune responses. Considering that the bacterial surface and secreted factors have been associated with the interaction of beneficial lactobacilli with the host as well as in their abilities to inhibit pathogens, in this work, we performed genomic and proteomic studies to characterize the cell surface and secreted proteins of Lp681. In a first set of studies an *in silico* analysis of cell surface and secreted factors was performed using the complete genome sequence of Lp681. The extracellular proteins in the genome were identified by bioinformatic analysis considering their subcellular location (secreted or expressed on the surface), their type of anchorage and the secretory mechanism (proteins with signal peptides). A total of 277 proteins were detected in Lp681 which were classified into eight groups: C-terminal anchored proteins (with and without cleavage sites) (10), N-terminal anchored proteins (with and without cleavage sites) (138), lipid-anchored proteins, proteins anchored to the wall by the LPxTG domain (45), secreted proteins with cleavage sites (55) and proteins secreted by minor pathways (7). Among the surface proteins, factors involved in adhesion were detected including MubB2, MucBP2, MucBP5, MucBP-MubB2-YGX, MucBP-MubB2-YGX, PgrA, and proteins of adhesion to collagen and fibronectin. Among the secreted proteins, genes coding for resistance to gastrointestinal conditions and adhesion (*cbh*, *treA*, *celB*, *eno2*) were detected. In a second set of experiments, proteins secreted by Lp681 in the presence and absence of enterohemorrhagic *E. coli* (EHEC) O157:H7 in a chemically defined medium were studied. The cell-free supernatant proteins were precipitated with trichloroacetic acid, while the surface cells proteins were obtained by scraping bacteria with trypsin (5 µg). The samples were analyzed by mass spectrometry and the identified proteins were analyzed by bioinformatics. A total of 113 and 533 proteins were identified in the exoproteome and the surface of Lp681, respectively, in the presence of EHEC. Interestingly, the most abundant proteins belonged to the categories of membrane and cell wall biogenesis (hydrolases), and translation (ribosomal proteins). In addition, proteins with moonlighting functions were found. The results show that Lp681 possesses several surface adhesion factors that would allow its interaction with the intestinal mucosa as well as to coaggregate with pathogenic *E. coli*. In addition, Lp681 secrete proteins that would exert an antimicrobial effect on EHEC through the generation of reactive oxygen species and cell lysis by membrane permeabilization.

Author index

L. Abán	HH24
M.C. Abeijón-Mukdsi	AH8
A. Abraham	HH9
G. Acciarri	S7A
G. Agüero	HH12
J. Aguilar	AH2
L. Albarracín	HH3 HH5 HH23 HH24 HH25 AH3 AH5 MG1 MG2 MG4
J.C. Alcocer	MP3
A. Aliano	S6A
S. Alvarez	HH12 HH19 HH21
N. Álvarez	MP3
E. Andrada	HH17 HH20 AH1 AH8
L. Arce	S3A
L. Arellano-Arriagada	HH12 HH23 AH3
J.N. Argañaraz Aybar	HH23 HH24
C. Aristimuño Ficoseco	S6B AH7
J. Arrieta	HH8
A.A. Arroyo Guerra	AH6 MG1
A. Aucouturier	AB10
M.C. Audisio	AH6 MP1 MP3 MG1
G.N. Avila Hael	AB13 AB18
J.D. Babot	AH1
A. Baillo	HH26 AB1 AB2 MG4
M.F. Balcells	HH14
D.A. Barrionuevo	AB3 AB14
C. Belloch	MP8
L. BermúdEz-Humarán	AB10
A. Bernal	HH9
E.V. Betencourt	AB12
V.S. Blancato	S7A
M. Bruzone	AB5
G.A. Bulacios	HH1 MP2

Author index

C. Burgos	HH16
J. Burini	AB5
A.Y. Bustos	MP4 MP5 MP6 AB6
C. Cancino	HH9
J. Carol	MP5 MP6
Y. Carpintero Polanco	HH9
N. Carrasco	AH7
P. Castellano	HH16 AB11 AB15
P.G. Cataldo	HH1 MP2
S.I. Cazorla	HH4 HH8 HH10 HH14
M.N. Cerasuolo	HH2 HH11 HH13 HH15
R. Chehín	AB15
L. Contreras	AB8
C. Coronel	MP3
P.D. Cotter	S4
L. Crespo	AB5 MG3
H. Cristóbal	MP3
A. de Moreno de LeBlanc	HH2 HH6 HH11 HH13 HH15 AH7 AB10
S. Dentice Maidana	HH3 HH25
M. Elean	S3A HH3 HH5 HH23 HH25 HH26 AH3 MG1 MG2
F. Escobar	AB3 AB14
S. Fadda	S6B HH26 AB1 AB2 AB9 MG4
F. Fanelli	S6B
A. Fara	AB16
S. Feliu	HH14
R. Fernández Brando	HH9
M. Flores	MP8
K. Fukuyama	HH12
V. Fusco	S6B
C. García	AB9
M.S. Garro	AB13 AB17 AB18
O.A. Garro	AB13 AB18

Author index

G. Garrote	HH9
P. Gauffin-Cano	HH17 HH20
F. Gharzia	AB4
K. Gianni de Carvalho	AB4 AB7
F.O. Gizzi	S7A
N. Gobbato	HH23 HH24
J. Gomez	AB4
J. Gomez	AB6
N. Gómez	MP4
F. Gutiérrez	HH19 HH21
J. Gutierrez-Merino	S5B
E.M. Hebert	HH1 MP2
M. Hidalgo	S6A
V.P. Houde	HH1
C. Ibarguren	MP1 MP3
M. Ibarra	MP6
Y. Imamura	S3A
A.S. Isas	AB3 AB14
M.I. Isla	AB8
J. Islam	S3B
M. Ivir	HH19 HH21
L.R.M. Juarez	AB17
M.A. Jure	HH3
H. Kitazawa	S2A S3A HH5 HH12 HH18 HH23 HH24 HH25 AH3
J. Laiño	HH7
P. Langella	AB10
J.G. LeBlanc	HH2 HH6 HH11 HH13 HH15 AH1 AH7 AB10
A. Ledesma	MP4 MP5 MP6 AB6
R.M. Lenz	MP1 MP3
R. Levit	HH13 HH15 AH7 AB10
A. Llorente	AB9
S. López Pérez	AB11

Author index

G.L. Lorca	S5A
M.J. Lorenzo Pisarello	HH5
C. Magni	S7A
E. Maldonado	S5B
C. Maldonado Galdeano	HH4 HH8 HH10 HH14
C. Maluquer de Motes	S5B
A. Márquez	HH17 HH20
G. Marranzino	HH12
B. Martínez Caliari	AB7
M.J. Martínez Monteros	HH4
K. Matsumoto	HH12
R. Medina	S3C HH17 HH20 AH1 AH8 AB13 AB18
C. Melián	AB11 AB15
L.M. Mendoza	MP8 AB12
C. Mercado	AB9
C. Minahk	HH1
M.H. Miranda	AH4
F.R.E. Molina	MP7 AB3
A. Montilla	AB16
C. Moreno	MP4
H. Mouguelar	AH2
F. Mozzi	MP7 AB5 MG3
S. Mukhopadhyay	S5B
C. Muñoz	AH5 MG2
B.L. Nacchio	AB13 AB18
M.E.F. Nader	S6B HH1 AH2 AH4 AH7
Z. Nakamura	S3B
F. Namai	S2A S3A HH5 HH12 HH25 AH3
L. Nelegatti	AB2
K. Nishiyama	S2A S3A HH24
T. Nochi	S3B
M. Obregozo	AH1
O. Ordoñez	AB5

Author index

S. Orosco	HH23 HH24
S. Ortiz Mayor	HH24
R. Ortiz Moyano	HH25 HH26
N. Ohtani	S3B
J. Palacios	AB16
M. Palermo	HH9
J. Palma	AB9
R. Parada	AB7
E. Parente	S6A
M.S. Pellegrino	AH2
G. Perdigón	HH4 HH14
N. Pereyra	AH2
B.J. Pérez	AB14
L. Perret	HH24
M. Pescuma	AB5 MG3
A.P. Pezuk	AB17
R. Pietrafesa	S6A
D. Ploper	AB15
S. Quilodrán-Vega	AH5 MG2
V. Ramos	HH9
R.R. Raya	MP7 AB12
A. Ricciardi	S6A
L.G. Ruiz-Rodríguez	MP7
M. Russo	HH17 HH20
L. Saavedra	HH1 MP2
J. Sacur	HH18
G. Sáez	HH22
A. Salas Burgos	S7B
E. Salguero	AB8
S. Salva	HH12 HH16 HH19 HH21
I. Sandoval	AB9
B. Sede Lucena	AB5
R. Serda	HH3 HH5

Author index

Y. Shimizu	S3B
T. Shimosato	S2A
H. Shirakawa	S1
J.A. Silva	AH2
K. Sivieri	S2B
S. Solis	S5B
F. Sosa	HH9
F. Sosa	AB7
C. Soto	AB9
N. Suarez	AH8
Y. Suda	HH5 HH25
M.E. Taborra	S7A
H. Tanaka	S3B
M.P. Taranto	HH7 MP4 AB6
J. Toledo	AH5 MG2
C. Tomei	HH17 HH20
M. Torino	AB2
S. Torres	AB8
M.P. Urquiza	HH1 MP2
J.C. Valdéz	HH23 HH24
M. Vallejo	AB7
C.P. Van Nieuwenhove	AB3 AB14
B. Vasile	HH16 HH19 HH21
A. Vasqueto	AH2
E.M. Vélez	HH8 HH10 HH14
A. Venecia	HH24
M.D. Vera	HH18
A. Vestani	HH4 HH8 HH10
G. Vignolo	S6B AB15
J. Villena	S3A HH3 HH5 HH12 HH18 HH23 HH24 HH25 HH26 AH3 AH5 AH6 AB1 MG1 MG2 MG4
M.G. Vizoso Pinto	HH18
H. Yamamuro	S3A

Author index

J. Yamazaki	S3B
P.A. Yánez	HH6
G. Zárate	HH22 AB16 AB17
M.H. Zelaya	HH7 HH12