



ROME, SEPTEMBER 12-14 2021  
UNIVERSITÀ URBANIANA

11<sup>TH</sup>

PROBIOTICS, PREBIOTICS  
& NEW FOODS, NUTRACEUTICALS AND BOTANICALS  
for NUTRITION & HUMAN and MICROBIOTA HEALTH

2<sup>ND</sup>

SCIENCE & BUSINESS SYMPOSIUM



## **SCIENTIFIC ORGANISERS**

L. Capurso (Italy)  
A. Gasbarrini (Italy)  
A. Guarino (Italy)  
L. Morelli (Italy)

## **INTERNATIONAL SCIENTIFIC COMMITTEE**

G. Barbara (Italy)  
R. Berni Canani (Italy)  
S. Binda (France)  
P. Brigidi (Italy)  
G. Clarke (Ireland)  
W. M. de Vos (Netherlands)  
D. Del Rio (Italy)  
V. Fogliano (Netherlands)  
F. Guarner (Spain)  
H. Kiyono (Japan)  
M. Koch (Italy)  
P. Lavermicocca (Italy)  
P. Malard (Switzerland - China)  
L. Monheit (USA)  
A. Ouwehand (Finland)  
G. Paraskevakos (Canada)  
R. Pecere (Belgium)  
L. Putignani (Italy)  
K. M. Tuohy (Italy)

## **PEDIATRIC DAY**

A. Guarino (Italy)  
R. Berni Canani (Italy)

## **GYNO SESSION**

F. Vicariotto (Italy)



# UNDER THE PATRONAGE



Consiglio Nazionale delle Ricerche



Società Italiana di Gastroenterologia  
ed Endoscopia Digestiva



Japan Bifidus Foundation



Fondazione Aldo Torsoli

**SINut**

Società Italiana di Nutraceutica

# UNDER THE PATRONAGE



MTCC, Mediterranean Task Force for Cancer Control



**AGUI**  
Associazione Ginecologi  
Universitari Italiani



# WITH THE SCIENTIFIC AGREEMENT OF



**Sunday, September 12**

|                       |       |
|-----------------------|-------|
| Aula Magna            | p. 1  |
| Aula Élie Metchnikoff | p. 5  |
| Aula Newman           | p. 10 |

**Monday, September 13**

|                       |       |
|-----------------------|-------|
| Aula Magna            | p. 12 |
| Aula Élie Metchnikoff | p. 17 |
| Aula Newman           | p. 22 |

**Tuesday, September 14**

|                       |       |
|-----------------------|-------|
| Aula Magna            | p. 24 |
| Aula Élie Metchnikoff | p. 26 |

|  |       |
|--|-------|
| Oral Communications                              | p. 28 |
| Posters  | p. 50 |
| Faculty  | p. 80 |
| Index of Oral Communications and Posters Authors | p. 86 |

|                            |        |
|----------------------------|--------|
| Abstract Authors Countries | p. 93  |
| General Information        | p. 94  |
| Scientific Information     | p. 100 |
| Exhibition Area            | p. 104 |

ROME, SEPTEMBER 2023

12<sup>TH</sup>

PROBIOTICS, PREBIOTICS  
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3<sup>RD</sup>

SCIENCE & BUSINESS SYMPOSIUM



# SCIENTIFIC PROGRAMME

**08.30 - 11.00****OPENING LECTURES**

*Chairs: M. Koch (Italy), F. Scalfaferrri (Italy)*

08.30 - 09.00

Microbiota studies to identify target pre, pro, and symbiotic intervention for patients' personalized treatment and primary prevention  
*A. Fasano (USA)*

09.00 - 09.20

The role of the gut microbiome in personalized nutrition  
*N. Segata (Italy)*

09.20 - 09.40

EBM and probiotics. AGA Guidelines: a critique  
*M. Koch (Italy)*

09.40 - 10.00

Post biotics: prospects for metabolic axes "intestines-another body parts"  
*V. M. Lakhtin (Russia)*

10.00 - 10.20

A novel 3D in vitro model of the human gut microbiota  
*E. Ghelardi (Italy)*

10.20 - 10.40

Inflamm-aging microRNAs may integrate signals from food and gut microbiota  
*L. Teodori (Italy)*

**GENERAL DISCUSSION****10.40 - 11.30****AGGEI (Associazione Giovani Gastroenterologi ed Endoscopisti Italiani)**

*Discussants: R. de Sire (Italy), F. Facciotti (Italy), L. R. Lopetuso (Italy), I. Marafini (Italy), D. Novicello (Italy), V. Petito (Italy), L. F. Pisani (Italy), F. Strati (Italy)*

**11.30 - 12.50****MICROBIOME ANALYTICS AND CLINICAL APPLICATIONS**

*Chairs & Introduction: L. Putignani (Italy), C. F. Perno (Italy)*

11.30 - 11.50

Microbiomics: disease-related profiling and clinical metagenomics  
*F. Del Chierico (Italy)*

11.50 - 12.10

Metaproteomics: challenges and bioinformatics issues microbiome profiling  
*S. Levi Mortera (Italy)*

12.10 - 12.30

Proteomics of microbial infectome  
*V. Marzano (Italy)*

12.30 - 12.50

Microbiomics: metabolomics profiling  
*P. Vernocchi (Italy)*

**13.00 - 14.00****Lunch**

**14.00 - 15.30****OPENING CEREMONY***Chair: L. Capurso (Italy)***WELCOME ADDRESSES**

WGO activities and its involvement in nutrition

*N. Lahbabi-Amrani (Morocco)**President WGO (World Gastroenterology Organization)**A. Benedetti (Italy)**President FISMA (Federazione Italiana Società Malattie Apparato Digerente)**A. Ghiselli (Italy)**President SISA (Società Italiana Scienza Alimentazione)**P. Brigidi (Italy)**National Committee for Biosafety, Biotechnology and Life Sciences of the Presidency of Council of Ministers (CNBBSV) and its "Microbiome initiative"**R. Pecere (Belgium)**IPA Europe (International Probiotic Association)*

Food cultures and gut health: defining the frame for consistent evidence

*F. Bourdichon (France)**International Dairy Federation***15.30 - 16.30****NEXT GENERATION MICROBIAL THERAPEUTICS***Chair: W. M. de Vos (Netherlands)*

15.30 - 15.50

Reconstruction of host-microbes symbiosis by full ecosystem microbiotherapy

*J. Doré (France)*

15.50 - 16.10

*Akkermansia**A. Brochot (Belgium)*

16.10 - 16.30

The potential use of the commensal bacterium *Faecalibacterium prausnitzii* to tackle IBD and other human diseases*P. Langella (France)*

**16.30 - 17.30****NUTRACEUTICALS, BOTANICALS AND MICROBIOTA: BIOACTIVATION AND MODULATION**

*Chairs: D. Del Rio (Italy), P. Lavermicocca (Italy)*

16.30 - 16.50

Benefits: tradition of use, experimental models and human studies to support health claims of botanicals

*P. Restani (Italy)*

16.50 - 17.10

Polyphenols as prebiotics and interaction with carbohydrate digestion

*M. G. Ferruzzi (USA)*

17.10 - 17.30

Bioactivation of polyphenols by the gut microbiome

*D. Del Rio (Italy)*

**17.30 - 19.10****ANIMAL NUTRITION & HEALTH**

*Chair: R. Marabelli (Italy)*

17.30 - 17.50

Animal health as fundamental part of "One Health" concept

*M. Eloit (France)*

17.50 - 18.10

The role of gut microbial communities to control pathogens colonization in poultry

*A. Ricci (Italy)*

18.10 - 18.30

Use of probiotics and prebiotics in animal farming: evidence of *Campylobacter jejuni* reduction in broilers with synbiotic administration

*G. Garofolo (Italy)*

18.30 - 18.50

The role of the industry and "Multi-omics characterization of milk resistome in a one-health perspective"

*L. Bonizzi (Italy), A. Soggiu (Italy)*

18.50 - 19.10

"One Health" synergies at the human/animal health interface

*P. Lecchini (Italy)*

19.10 - 19.45

**ORAL COMMUNICATIONS***Chair: A. Castellazzi (Italy)***OC.1 - IMPACT OF FERMENTED HEMPSEED BRAN ON THE HUMAN DISTAL COLON MICROBIOTA WITH MICODE IN VITRO MODEL**Lorenzo Nissen <sup>(1)</sup>, Flavia Casciano <sup>(2)</sup>, Andrea Gianotti <sup>(3)</sup><sup>(1)</sup> *Alma Mater Studiorum - University of Bologna, DiSTAL-Department of Agricultural and Food Sciences, Bologna, Italy*<sup>(2)</sup> *Alma Mater Studiorum - University of Bologna, DiSTAL-Department of Agricultural and Food Sciences, Cesena, Italy*<sup>(3)</sup> *Alma Mater Studiorum - University of Bologna, DiSTAL-Department of Agricultural and Food Sciences, Bologna, Italy***OC.2 - CANOLA MEAL FERMENTATION WITH PROBIOTIC *LACTOBACILLI*: IMPACT OF PHENOLIC ACIDS ON ANTIMICROBIAL ACTIVITY**Vi Pham <sup>(1)</sup>, Michael Gänzle <sup>(1)</sup><sup>(1)</sup> *University of Alberta, Agriculture, Food and Nutritional Science, Edmonton, Canada***OC.4 - ANALYSIS OF THE IMPACT ON HUMAN GUT MICROBIOTA AND OF COLONIZATION ABILITY OF PROBIOTIC MICROBES FROM FERMENTED FOODS THROUGH A SYSTEMATIC APPROACH**Chiara Devirgiliis <sup>(1)</sup>, Marianna Roselli <sup>(1)</sup>, Fausta Natella <sup>(1)</sup>, Paola Zinno <sup>(1)</sup>, Barbara Guantario <sup>(1)</sup>, Raffaella Canali <sup>(1)</sup>, Emily Schifano <sup>(1)</sup>, Giuditta Perozzi <sup>(1)</sup><sup>(1)</sup> *CREA (Council for Agricultural Research and Economics), Research Centre for Food and Nutrition, Rome, Italy***OC.5 - TEXTURED SOY PROTEIN MODULATES GUT MICROBIOTA AND SHORT-CHAIN FATTY ACIDS METABOLISM**Catarina Teixeira-Guedes <sup>(1)</sup>, Teresa Sánchez-Moya <sup>(2)</sup>, Gaspar Ros-Berruezo <sup>(2)</sup>, Cristina Pereira-Wilson <sup>(1)</sup>, Rubén López-Nicolás <sup>(2)</sup><sup>(1)</sup> *Centre for the Research and Technology of Agro-Environmental and Biological Sciences, Department of Biology, University of Minho, Braga, Portugal*<sup>(2)</sup> *Human Nutrition and Food Science, Faculty of Veterinary Sciences, University of Murcia, Murcia, Spain*

**09.30 - 10.10****FUNCTIONAL GASTROINTESTINAL DISORDERS**

*Chairs: E. S. Corazziari (Italy), G. Barbara (Italy)*

09.30 - 09.50

Worldwide prevalence and burden of functional gastrointestinal disorders, results of Rome Foundation Global Study

*E. S. Corazziari (Italy)*

09.50 - 10.10

FGID, gut microbiota and probiotics

*G. Barbara (Italy)*

10.10 - 10.40

LECTURE

Gut-Brain Axis: a promising paradigm for improving the development of novel therapeutics for brain disorders

*E. Merlo Pich (Italy)*

**10.40 - 11.10****ORAL COMMUNICATIONS****OC.6 - INADEQUATE SAFETY REPORTING IN RCTS IN IRRITABLE BOWEL SYNDROME. A SYSTEMATIC REVIEW: PHARMACEUTICAL INTERVENTIONS VS PROBIOTIC INTERVENTIONS**

Anne van der Geest <sup>(1)</sup>, Linda van de Burgwal <sup>(1)</sup>

*<sup>(1)</sup>Vrije Universiteit, Athena Institute, Amsterdam, Netherlands*

**OC.52 - IN VITRO INFLUENCE OF TILIA TOMENTOSA MOENCH ON SMALL INTESTINE NEUROMUSCULAR FUNCTION**

Silvia Cerantola <sup>(1)</sup>, Sofia Faggin <sup>(1)</sup>, Gabriela Annaloro <sup>(1)</sup>, Federica Mainente <sup>(2)</sup>, Anna Piovan <sup>(1)</sup>, Edoardo Savarino <sup>(3)</sup>, Gianni Zoccatelli <sup>(2)</sup>, Maria Cecilia Giron <sup>(1)</sup>

*<sup>(1)</sup>University of Padova, Department of Pharmaceutical and Pharmacological Sciences, Padova, Italy*

*<sup>(2)</sup>University of Verona, Department of Biotechnology, Verona, Italy*

*<sup>(3)</sup>University of Padova, Department of Surgery, Oncological and Gastrointestinal Science, Padova, Italy*

**OC. 53 - PRONEUROGENIC AND NEUROPROTECTIVE EFFECT OF A MULTI STRAIN PROBIOTIC MIXTURE IN A MOUSE MODEL OF ACUTE INFLAMMATION: INVOLVEMENT OF THE GUT-BRAIN AXIS**

Carla Petrella <sup>(1)</sup>, Georgios Strimpakos <sup>(1)</sup>, Alessio Torcinaro <sup>(1)</sup>, Silvia Middei <sup>(1)</sup>, Valentina Ricci <sup>(1)</sup>, Giorgio Gargari <sup>(2)</sup>, Diego Mora <sup>(2)</sup>, Francesca De Santa <sup>(1)</sup>, Stefano Farioli Vecchioli <sup>(1)</sup>

*<sup>(1)</sup>Institute of Biochemistry and Cell Biology - IBBC-CNR, Biomedical Sciences, Rome, Italy*

*<sup>(2)</sup>Department of Food Environmental and Nutritional Sciences DeFENS, University of Milan, Milan, Italy*



11.10 - 13.00

**ORAL COMMUNICATIONS***Chair: D. Del Rio (Italy)***OC.40 - TRANSCRIPTOME AND MITOCHONDRIAL ANALYSIS OF ASD CHILDREN COMPARED TO HEALTHY CONTROLS**Mark Cannon <sup>(1)</sup>, Sri Ganeshan <sup>(2)</sup>, Guru Banavar <sup>(3)</sup>, Momo Vuysich <sup>(4)</sup><sup>(1)</sup> Northwestern University, Otolaryngology, Chicago, United States<sup>(2)</sup> MitoSwab, Research, Plymouth, United States<sup>(3)</sup> VIOME, research, Los Alamos, United States<sup>(4)</sup> VIOME, Research, Los Alamos, United States**OC.8 - BIOCHEMICAL AND ANTIBIOTIC INFLUENCE OF GASTROINTESTINAL TRACTS MICROFLORA IN NEONATAL**Awatif Al-Judaibi <sup>(1)</sup>, Effat AlJudaibi <sup>(2)</sup>, Sahar AlShareef <sup>(2)</sup><sup>(1)</sup> Jeddah University, Biological Science, Microbiology Section, Jeddah, Saudi Arabia<sup>(2)</sup> Jeddah University, Biological Science, Jeddah, Saudi Arabia**OC.9 - A NOVEL SMALL INTESTINAL MICROBIOME ASPIRATION (SIMBA) CAPSULE DEVICE TO DETECT AND SAMPLE PROBIOTICS RELEASE IN THE HUMAN SMALL INTESTINE**Gang Wang <sup>(1)</sup>, Christopher Andrews <sup>(2)</sup>, Lynn Wilsack <sup>(2)</sup>, Renata Rehak <sup>(2)</sup>, Lawrence Lou <sup>(3)</sup>, Jeremie Auger <sup>(4)</sup>, Olivier Matieu <sup>(4)</sup>, Sabina Bruehlmann <sup>(1)</sup>, Sharanya Menon <sup>(1)</sup>, Qiang Tang <sup>(5)</sup>, Ge Jin <sup>(5)</sup>, Bangmao Wang <sup>(5)</sup><sup>(1)</sup> NIMBLE SCIENCE LTD, NIMBLE SCIENCE LTD, CALGARY, Canada<sup>(2)</sup> the University of Calgary, Cumming School of Medicine, CALGARY, Canada<sup>(3)</sup> EFW Radiology, EFW Radiology, CALGARY, Canada<sup>(4)</sup> Lallemand Health Solutions, Rosell Institute for Microbiome and Probiotics, Montreal, Canada<sup>(5)</sup> the Tianjin Medical University, Department of Gastroenterology, Tianjin, China**OC.10 - GENOME-GUIDED CREATION OF NEXT-GENERATION PROBIOTICS**Thomas Hitch <sup>(1)</sup>, Neeraj Kumar <sup>(1)</sup>, Thomas Clavel <sup>(1)</sup><sup>(1)</sup> Functional Microbiome Research Group, University Hospital RWTH Aachen, Aachen, Germany**OC.12 - ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF CELL FREE SUPERNATANT PRODUCED BY *LACTOBACILLUS REUTERI* DSM 17938**Irene Vitale <sup>(1)</sup>, Valentina Puca <sup>(1)</sup>, Simone Carradori <sup>(1)</sup>, Antonella Di Sotto <sup>(2)</sup>, Rossella Grande <sup>(1)</sup><sup>(1)</sup> University of Chieti-Pescara "G. d'Annunzio", Pharmacy, Chieti, Italy<sup>(2)</sup> Sapienza University of Rome, Physiology and Pharmacology "V. Erspamer", Rome, Italy



**OC.13 - INVESTIGATING THE SUSCEPTIBILITY OF THE NEXT GENERATION PROBIOTIC *FAECALIBACTERIUM PRAUSNITZII* UNDER STRESS CONDITIONS**

Daniela Machado <sup>(1)</sup>, Melany Domingos <sup>(1)</sup>, Diana Almeida <sup>(1)</sup>, Joana Cristina Barbosa <sup>(1)</sup>, José Carlos Andrade <sup>(2)</sup>, Ana Cristina Freitas <sup>(1)</sup>, Ana Maria Gomes <sup>(1)</sup>

<sup>(1)</sup> *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*

<sup>(2)</sup> *CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Paredes, Portugal*

**OC.14 - *AKKERMANSIA MUCINIPHILA* ANTIMICROBIAL SUSCEPTIBILITY PROFILE**

Joana Cristina Barbosa <sup>(1)</sup>, Daniela Machado <sup>(1)</sup>, Diana Almeida <sup>(1)</sup>, José Carlos Andrade <sup>(2)</sup>, Ana Cristina Freitas <sup>(1)</sup>, Ana Maria Gomes <sup>(1)</sup>

<sup>(1)</sup> *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*

<sup>(2)</sup> *Cespu, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Paredes, Portugal*

**OC.16 - LOCAL APPLICATION OF PROBIOTICS PROMOTES EXCISIONAL WOUND HEALING IN RATS**

Moysis Moysidis <sup>(1)</sup>, George Stavrou <sup>(1)</sup>, Vasiliki Birba <sup>(1)</sup>, Julia K. Tsetis <sup>(2)</sup>, Aristidis Ioannidis <sup>(1)</sup>, Georgia Tsaousi <sup>(1)</sup>, Katerina Kotzampassi <sup>(1)</sup>

<sup>(1)</sup> *Aristotle University of Thessaloniki, AHEPA University Hospital, Department of Surgery, Thessaloniki, Greece*

<sup>(2)</sup> *Uni-Pharma S.A., Pharmacist, Athens, Greece*

**OC.18 - COMPREHENSIVE PAN-GENOME ANALYSIS OF *LACTIPLANTIBACILLUS PLANTARUM* COMPLETE GENOMES**

Francesco M. Carpi <sup>(1)</sup>, Maria Magdalena Coman <sup>(1)</sup>, Stefania Silvi <sup>(2)</sup>, Matteo Picciolini <sup>(3)</sup>, Maria Cristina Verdenelli <sup>(1)</sup>, Valerio Napolioni <sup>(2)</sup>

<sup>(1)</sup> *Synbiotec Srl, Synbiotec Srl, Camerino, Italy*

<sup>(2)</sup> *University of Camerino, School of Biosciences and Veterinary Medicine, Camerino, Italy*

<sup>(3)</sup> *Independent Researcher, Independent Researcher, Gubbio, Italy*





**OC.20 - INNOVATIVE PERSPECTIVES ON THE DETOXYFING EFFECTS OF  
*LACTOBACILLUS* PROBIOTIC STRAINS**

Silvia Rapacioli<sup>(1)</sup>, Simone Stasi<sup>(1)</sup>, Annalisa Visciglia<sup>(2)</sup>, Angela Amoroso<sup>(2)</sup>, Marco Pane<sup>(2)</sup>

<sup>(1)</sup> *Bict Srl, Innovation Development, Lodi, Italy*

<sup>(2)</sup> *Probiotical Research Srl, R&D, Novara, Italy*

**OC.21- MULTISPECIES PROBIOTICS PROMOTE PERCEIVED HUMAN HEALTH  
AND WELLBEING: INSIGHTS INTO THE VALUE OF RETROSPECTIVE STUDIES  
ON USER EXPERIENCES**

Linda van de Brugwal<sup>(1)</sup>, Anne van der Geest<sup>(1)</sup>

<sup>(1)</sup> *Vrije Universiteit, Athena Institute, Amsterdam, Netherlands*

**OC.22 - VARIABILITY OF ANTIMICROBIAL AND ANTIFUNGAL EFFECT OF  
*LACTOBACILLUS PLANTARUM* AND *LACTOBACILLUS ACIDOPHILUS***

Oksana Rybalchenko<sup>(1)</sup>, Olga Orlova<sup>(2)</sup>, Valentina Kapustina<sup>(2)</sup>

<sup>(1)</sup> *Saint Petersburg State University, Medical Department, Saint-Petersburg, Russian Federation*

<sup>(2)</sup> *Saint Petersburg State University, Medical Department, Saint-Petersburg, Российская Федерация*

**13.00 - 14.00**

**Lunch**

**15.30 - 16.30**

**COELIAC DISEASE AND PROBIOTICS**

*Chair: R. Troncone (Italy)*

15.30 - 15.50

Celiac and gut microbiota

*R. Shamir (Israel)*

15.50 - 16.10

Antibodies in NCGS

*R. De Giorgio (Italy)*

16.10 - 16.30

Prevention strategies and probiotics in CD

*R. Auricchio (Italy)*

**16.30 - 18.10****NUTRITION FOUNDATION OF ITALY ROUND TABLE. FOOD SUPPLEMENTS AND FUNCTIONAL FOODS IN THE CONTROL OF PLASMA CHOLESTEROL LEVELS***Chair & Introduction: A. Poli (Italy)*

16.30 - 16.50

Food supplements and cholesterol reduction: when, why and for whom  
*A. F. G. Cicero (Italy)*

16.50 - 17.10

Supplements active on cholesterol absorption (phytosterols, beta-glucan,...)  
*F. Visioli (Italy)*

17.10 - 17.30

Supplements active on cholesterol synthesis or catabolism (fermented red rice, berberine)  
*N. Ferri (Italy)*

17.30 - 17.50

Increasing dietary fiber: effects on cholesterol, microbiota and more  
*L. S. A. Augustin (Italy)*

17.50 - 18.10

Lipidome and microbiota: gut microbiota and its host collaborate to regulate lipid metabolism  
*C. Ferreri (Italy), A. Castagnetti (Italy)*

18.10 - 18.40

LECTURE

Enhanced Omega-3 dietary supplements from sustainably sourced fish oil  
*M. Pagliaro (Italy)*

**18.40 - 19.30****INTERACTIONS OF NUTRACEUTICALS, BOTANICALS AND MICROBIOTA***Chairs: D. Del Rio (Italy), P. Lavermicocca (Italy)*

18.40 - 19.10

Sarcopenia and gut microbiota  
*S. Migliaccio (Italy)*

19.10 - 19.30

Mediterranean diet affects human metabolism and gut microbiome in overweight and obese subjects: perspectives on personalized nutrition approaches  
*P. Vitaglione (Italy)*

## THE PEDIATRIC DAY

# PROBIOTICS, PREBIOTICS, SYMBIOTICS TO TARGET MICROBIOME IN CHILDREN: A TRANSLATIONAL SCIENCE APPROACH

**08.00 - 08.30**

### INTRODUCTION

*A. Guarino (Italy)*

**08.30 - 09.00**

### LECTURE

High quality and low quality RCT and their impact on metanalysis  
*H. Szajewska (Poland)*

**09.00 - 10.30**

### COVID-19 IN CHILDREN

*Chair: H. Szajewska (Poland)*

09.00 - 09.30

Gastrointestinal involvement in Pediatric COVID  
*A. Lo Vecchio (Italy)*

09.30 - 10.00

Pathophysiology of COVID-19 associated diarrhea  
*M. Poeta (Italy)*

10.00 - 10.30

Targeting microbiome in COVID-19 in children  
*R. Berni Canani (Italy)*

**10.30 - 11.00**

### LECTURE

Indications and limits to target microbiome in vulnerable children  
*H. van Goudoever (Netherlands)*

**11.00 - 12.30**

### FUNCTIONAL GASTROINTESTINAL DISORDERS IN THE PEDIATRIC AGE

*Chair: R. Berni Canani (Italy)*

11.00 - 11.30

Potential indications and outcome parameters  
*R. Francavilla (Italy)*

11.30 - 12.00

The plausibility of the mechanisms of action  
*E. Scarpato (Italy)*

12.00 - 12.30

Clinical evidence and guidelines  
*F. Indrio (Italy)*

**12.30 - 13.00**

### LECTURE

Functional foods for functional disorders: probiotic for colic infants  
*F. Savino (Italy)*

**13.00 - 14.00****Lunch****15.30 - 17.00****OBESITY AND OBESITY-RELATED DISORDERS***Chair: E. Isolauri (Finland)*

15.30 - 16.00

Potential indications and outcome parameters

*T. Alterio (Italy)*

16.00 - 16.30

The plausibility of the mechanisms of action

*E. Isolauri (Finland)*

16.30 - 17.00

Clinical evidence and guidelines

*E. Verduci (Italy)***17.00 - 18.30****FOOD ALLERGY***Chair: R. Berni Canani (Italy)*

17.00 - 17.30

Potential indications and outcome parameters

*Y. Vandenplas (Belgium)*

17.30 - 18.00

The plausibility of the mechanisms of action

*L. Carucci (Italy)*

18.00 - 18.30

Clinical evidence and guidelines

*A. Fiocchi (Italy)***18.30****CLOSING REMARKS**

**08.30 - 10.00****TRIANGULATION OF MICROBIOME SCIENCE, INNOVATION AND REGULATION****TALKS + Q&A***Chair: Y. Sanz (Spain)*

08.30 - 08.45

Implications of the microbiome in regulatory science  
*M. Hugas (Italy)*

08.45 - 09.00

The regulatory path for probiotics *versus* live biotherapeutics  
*M. Cordaillat-Simmons (France)*

09.00 - 09.15

Human microbiome and chemical contaminants  
*M. I. Bahl (Denmark)*

09.15 - 09.30

Genome analysis for identification and safety assessment of the microorganisms used in the food chain  
*B. Mayo (Spain)*

09.30 - 09.45

The need of standards in the microbiome field  
*J. Doré (France)*

**10.00 - 11.00****GUT MICROBIOTA AND DIET***Chair: F. Guarner (Spain)*

10.00 - 10.20

Mediterranean diet for reverting microbial dysbiosis and immunosenescence  
*P. Brigidi (Italy)*

10.20 - 10.40

High fiber diets: microbiota benefits versus tolerance  
*F. Azpiroz (Spain)*

10.40 - 11.00

Impact of low FODMAP diet on gut microbiota  
*F. Guarner (Spain)*

**11.00 - 12.00****GUT MICROBIOTA, PROBIOTICS AND VITAMIN D IN IBD***Chair: M. Vecchi (Italy)*

11.00 - 11.20

The Microbiota-Gut-Brain Axis in IBD  
*S. M. Collins (Canada)*

11.20 - 11.40

Modulation of intestinal immune cell responses by eubiotic or dysbiotic microbiota  
*F. Facciotti (Italy)*

11.40 - 12.00

Vitamin D in IBD  
*F. Cominelli (USA)*

12.00 - 12.30

## ORAL COMMUNICATIONS

**OC. 23 - INTESTINAL EPITHELIAL PROTEASES CONTROL MUCOSAL BIOFILMS FOR BETTER, FOR WORSE, IN THICKNESS OR HEALTH**Nathalie Vergnolle <sup>(1)</sup>*<sup>(1)</sup>Inserm, U1220, Toulouse, France***OC.24 - EFFECTS OF A NOVEL PROBIOTIC COMBINATION ON CROHN'S DISEASE-LIKE ILEITIS MOUSE MODEL**Luca Di Martino <sup>(1)</sup>, Abdullah Osme <sup>(2)</sup>, Theresa Pizarro <sup>(2)</sup>, Mahmoud Ghannoum <sup>(3)</sup>, Fabio Cominelli <sup>(1)</sup>*<sup>(1)</sup>Case Western Reserve University, Digestive Health Research Institute, CLEVELAND, United States**<sup>(2)</sup>Case Western Reserve University, Pathology, CLEVELAND, United States**<sup>(3)</sup>Case Western Reserve University, Dermatology, CLEVELAND, United States***OC.26 - *LACTOBACILLUS RHAMNOSUS GG* THERAPEUTIC SUPPLEMENT IN MILD-MODERATELY ACTIVE ULCERATIVE COLITIS PATIENTS: RESULTS FROM A DOUBLE BLIND RANDOMIZED CLINICAL TRIAL**Cristiano Pagnini <sup>(1)</sup>, Maria Carla Di Paolo <sup>(1)</sup>, Fernando De Angelis <sup>(2)</sup>, Marco Mattana <sup>(2)</sup>, Riccardo Urgesi <sup>(1)</sup>, Lorella Pallotta <sup>(1)</sup>, Maria Giovanna Graziani <sup>(1)</sup>, Gianfranco Delle Fave <sup>(2)</sup>*<sup>(1)</sup>AO S. Giovanni Addolorata, Gastroenterologia ed Endoscopia Digestiva, Roma, Italy**<sup>(2)</sup>Università "Sapienza", Gastroenterologia, Roma, Italy*

**12.30 - 13.00****LECTURE***Chair: L. Capurso (Italy)*

Good practices in assessing the beneficial effect of foods: the case history of a fermented cereal ingredient

*L. Morelli (Italy)***13.00 - 14.00****Lunch****14.00 - 16.00****GUT MICROBIOTA, VIRUS, VACCINES AND FOOD COMPONENTS***Chair: G. Ippolito (Italy)*

14.00 - 14.20

COVID-19 and gut microbiota

*M. Clementi (Italy)*

14.20 - 14.40

Recombinant bacteria from the human microbiota as vaccine vectors

*G. Pozzi (Italy)*

14.40 - 15.00

Influence of intestinal microbiota on the vaccine induced immunity

*H. Kiyono (Japan)*

15.00 - 15.20

Vit D and COVID-19

*M. Miraglia del Giudice (Italy)*

**16.10 - 16.50****LECTURES**

*Chair: P. Lavermicocca (Italy)*

16.10 - 16.30

New insights on mechanisms of action and clinical evidence of a multistrain probiotic  
*L. Laterza (Italy)*

16.30 - 16.50

Efficacy of an orally administered combination of *Lactobacillus paracasei* LC11, cranberry extract and D-mannosio for the prevention of recurrent urinary tract infections in postmenopausal women: a randomized controlled prospective study  
*F. Vicariotto (Italy)*

**16.50 - 17.50****BILE ACIDS - MICROBIAL SIGNALS ACROSS THE DOMAINS OF LIFE**

*Chairs: K. M. Tuohy (Italy), M. Koch (Italy)*

16.50 - 17.05

Circulating bile acid profiles - biomarkers of health and disease risk  
*F. Gianfagna (Italy)*

17.05 - 17.20

Bile acid cell signaling - microbiome: host communication  
*S. A. Joyce (Ireland)*

17.20 - 17.35

Probiotics, prebiotics and polyphenols for modulating circulating bile acid profiles and human disease risk-the CABALA diet&health dietary intervention  
*J. A. Lovegrove (UK)*

17.35 - 17.50

Metabolomics of bile acids: from lab bench to clinical tool  
*M. Ulaszewska-Tarantino (Italy)*



**17.50 - 18.50****GUT-LIVER AXIS**

*Chairs: D. Alvaro (Italy), A. Benedetti (Italy)*

17.50 - 18.10

Cholangiocytes and gut microbiota

*D. Alvaro (Italy)*

18.10 - 18.30

Gut-Liver Axis and inflammasome activation in cholangiocyte pathophysiology

*L. Maroni (Italy)*

18.30 - 18.50

Basic knowledge and clinical consequences of the biliary involvement in the Gut-Liver Axis

*V. Cardinale (Italy)*

**19.00 - 19.20****ORAL COMMUNICATIONS**

**OC.28 - SPECIALIZED FOOD PRODUCTS MAY ENHANCE THE EFFICACY OF ISOCALORIC DIET IN THE TREATMENT OF NON-ALCOHOLIC STEATOHEPATITIS**

Sergey Morozov <sup>(1)</sup>, Armida Sasunova <sup>(1)</sup>, Irina Vorobyova <sup>(2)</sup>, Valentina Vorobiova <sup>(2)</sup>, Varuzhan Sarkisyan <sup>(2)</sup>, Alla Kochetkova <sup>(2)</sup>, Vasily Isakov <sup>(1)</sup>

<sup>(1)</sup> *Federal Research Center of Nutrition and Biotechnology, Dpt Gastroenterology, Hepatology and Nutrition, Moscow, Russian Federation*

<sup>(2)</sup> *Federal Research Center of Nutrition and Biotechnology, Lab Biotechnology and Specialized Food Products, Moscow, Russian Federation*

**OC.29 - PREBIOTIC LACTULOSE AS EFFICACIOUS MICROBIOTA AND METABOLITE MODULATOR IN CIRRHOSIS ENVIRONMENT**

Andrea Mancini <sup>(1)</sup>, Stefano Larsen <sup>(2)</sup>, Francesca Campagna <sup>(3)</sup>, Pietro Franceschi <sup>(2)</sup>, Piero Amodio <sup>(3)</sup>, Cecilia Pravadelli <sup>(4)</sup>, Massimo Pindo <sup>(5)</sup>, Kieran Tuohy <sup>(1)</sup>

<sup>(1)</sup> *Fondazione Edmund Mach, Food Quality and Nutrition, San Michele all'Adige, Italy*

<sup>(2)</sup> *Fondazione Edmund Mach, Computational Biology Unit, San Michele all'Adige, Italy*

<sup>(3)</sup> *University of Padova, Department of Medicine-DIMED, Padova, Italy*

<sup>(4)</sup> *Santa Chiara Hospital, Gastroenterology Unit, Trento, Italy*

<sup>(5)</sup> *Fondazione Edmund Mach, Computational biology unit, San Michele all'Adige, Italy*

**OC.30 - MANIPULATION OF GUT MICROBIOME BY PREBIOTIC TO COMBAT ALCOHOLIC LIVER DISEASES**

Ryan Yuki Huang <sup>(1)</sup>, Deron Raymond Herr <sup>(2)</sup>

<sup>(1)</sup> *San Diego State University and Canyon Crest Academy, Biology, San Diego, United States*

<sup>(2)</sup> *San Diego State University, Biology, San Diego, United States*

**08.30 - 10.20****MICROBIOME COMPUTATION: BIOINFORMATICS, DATA SCIENCE AND ARTIFICIAL INTELLIGENCE***Chair: L. Putignani (Italy)*

08.30 - 08.40

Microbiome, A.I. - driven diseases prediction

*M. Bianco Prevot (Italy)*

08.40 - 09.00

Network-based approaches for the identification of complex disease: signature from omics data

*F. Conte (Italy)*

09.00 - 09.20

The microbiota analysis: comparison between targeted and shotgun metagenomics

*G. Macari (Italy)*

09.20 - 09.40

Microbiota and Artificial Intelligence

*V. Guarrasi (Italy)*

09.40 - 10.00

Network approach to the study of microbiota

*G. Caldarelli (Italy)*

10.00 - 10.20

Multilayer structure of microbiota and relationship with human diseases

*T. Gili (Italy)***10.20 - 12.00****NEW FOODS***Chair: V. Fogliano (Netherlands)*

10.20 - 10.40

Functional bakery products

*N. Pellegrini (Italy)*

10.40 - 11.00

Microbiota and Mediterranean Diet

*D. Ercolini (Italy)*

11.00 - 11.20

How to design healthy ultra processed foods

*V. Fogliano (Netherlands)*

11.20 - 11.40

Discussion

**12.00 - 13.00****EARLY MICROBIOME COLONIZATION  
"BIOSTIME INSTITUTE NUTRITION & CARE - BINC"***Chair: P. Langella (France)*

12.00 - 12.20

Prebiotics, probiotics, symbiotic interactions in early life  
*J. Lane (Ireland)*

12.20 - 12.40

Cesarean-section microbiota alterations induce gut barrier dysfunction and impact host physiology at short- and long-term  
*P. Langella (France)*

12.40 - 13.00

Gut-brain interactions  
*V. Felice (Ireland)*

**13.00 - 14.00****Lunch****14.00 - 16.00****WOMEN MICROBIOME: A DIFFERENT WAY TO FEEL HEALTHY***Chair: F. Vicariotto (Italy)*

14.00 - 14.20

Microbiome think different: welcome to microgenderome  
*F. De Seta (Italy)*

14.20 - 14.40

The microbiome in pregnancy and pregnancy complications  
*O. Koren (Israel)*

14.40 - 15.00

Cervicovaginal microbiome and natural history of HPV  
*F. Murina (Italy)*

15.00 - 15.20

Microbiome of vaginal dysbiosis  
*L. Petricevic (Austria)*

15.20 - 15.40

Characteristics and changes of gut and vaginal microbiome in menopause  
*P. Villa (Italy)*

15.40 - 16.00

Microbiota: gut-vagina crosstalk  
*F. Franceschi (Italy)*



16.00 - 16.15

**ORAL COMMUNICATIONS**

*Chairs: F. Vicariotto (Italy), F. De Seta (Italy)*

**OC.31 - SACCHAROMYCES CEREVISIAE BASED PROBIOTICS OUTPERFORM LACTOBACILLI IN INHIBITION OF VAGINAL CANDIDIASIS**

Liesbeth Demuyser <sup>(1)</sup>, Mart Sillen <sup>(1)</sup>, Silke Baldewijns <sup>(1)</sup>, Ilse Palmans <sup>(1)</sup>, Paul Vandecruys <sup>(1)</sup>, Patrick Van Dijck <sup>(1)</sup>

<sup>(1)</sup> VIB-KU Leuven Center for Microbiology, Flanders Institute for Biotechnology, Biology, Leuven, Belgium

**OC.32 - EVALUATION OF NOMADIC AND NICHE-SPECIALIST LACTOBACILLI AS POTENTIAL VAGINAL PROBIOTICS**

Claudia Cappello <sup>(1)</sup>, Marta Acin-Albiac <sup>(1)</sup>, Daniela Pinto <sup>(2)</sup>, Fabio Rinaldi <sup>(2)</sup>, Emanuele Zannini <sup>(3)</sup>

<sup>(1)</sup> University of Bolzano-Bozen, Food Engineering and Biotechnology, Bolzano, Italy

<sup>(2)</sup> Human Microbiome Advanced Project, HMAP, Research & Development, Milano, Italy

<sup>(3)</sup> University College Cork, School of Food and Nutritional Sciences, Cork, Ireland

16.15 - 16.35

**LECTURE**

*Chair: F. Franceschi (Italy)*

Mutual interactions among exercise, sport supplements and microbiota  
*S. Donati Zeppa (Italy)*

16.35 - 17.35

**MEDITERRANEAN TASK FORCE FOR CANCER CONTROL (MTCC)  
*In memory of Massimo Crespi and Alberto Montori***

*Chair: P. G. Natali (Italy)*

16.35 - 16.50

Probiotics and liver diseases  
*A. Ascione (Italy)*

16.50 - 17.05

Mediterranean Diet and Cancer  
*A. Saggioro (Italy)*

17.05 - 17.20

Functional foods in the management of prostate benign hypertrophy  
*P. G. Natali (Italy)*

17.20 - 17.35

The protective role of tomato & olive micronutrients in the defense against Persistent Organic Pollutants induced-toxicities  
*E. Rubini (Italy)*

**17.35 - 18.55****LECTURES**

Chair: E. S. Corazziari (Italy)

17.35 - 17.55

Probiotics and Prostatitis

T. Cai (Italy)

17.55 - 18.15

The eye microbiome

P. Bonci (Italy), D. Borroni (Italy)

18.15 - 18.35

Microbiota in oral diseases

P. Simeone (Italy)

18.35 - 18.55

Dermobiotic: microbiome in the Gut-Skin Axis

M. Pignatti (Italy)

**18.55 - 19.30****ORAL COMMUNICATIONS**

**OC.33 - POTENTIAL ROLE OF THE INFLAMMATORY MOLECULES IN MODULATING SKIN MICROBIOME AND DYSBIOSIS IN ACNE VULGARIS**

Ilaria Cavallo <sup>(1)</sup>, Bruno Capitanio <sup>(2)</sup>, Francesca Sivori <sup>(1)</sup>, Fabrizio Ensoli <sup>(1)</sup>, Enea Gino Di Domenico <sup>(1)</sup>

<sup>(1)</sup> San Gallicano Dermatological Institute, IRCCS, Microbiology and Virology, Rome, Italy

<sup>(2)</sup> San Gallicano Dermatological Institute, IRCCS, Dermatology, Rome, Italy

**OC.34 - INVESTIGATIONS OF THE POTENTIAL MECHANISM OF ACTION OF A MULTI-STRAIN PROBIOTIC COMPOSITION AGAINST URO-GENITAL PATHOGENS BY EX-VIVO STUDIES**

Marisa Meloni <sup>(1)</sup>, Patrizia Malfa <sup>(2)</sup>, Demetrio Piro <sup>(2)</sup>, Laura Brambilla <sup>(3)</sup>, Silvana Giardina <sup>(4)</sup>, Silvia Lincetti <sup>(5)</sup>, Martina Masciarelli <sup>(6)</sup>, Federica Carlomagno <sup>(2)</sup>

<sup>(1)</sup> Vitroscreen, CEO, Milano, Italy

<sup>(2)</sup> Roelmi HPC, R&D, Origgio, Italy

<sup>(3)</sup> Vitroscreen, R&D, Milano, Italy

<sup>(4)</sup> Complife Group, R&D, Pavia, Italy

<sup>(5)</sup> Complife Group, R&D, Garbagnate Milanese, Italy

<sup>(6)</sup> Complife Group, R&D, Barcellona, Spain

**OC.35 - THE PROTECTIVE ROLE OF TOMATO AND OLIVE MICRONUTRIENTS IN THE DEFENSE AGAINST PERSISTENT ORGANIC POLLUTANTS-INDUCED TOXICITY**

Elisabetta Rubini <sup>(1)</sup>, Marco Minacori <sup>(1)</sup>, Fabio Altieri <sup>(1)</sup>, Giuliano Paglia <sup>(1)</sup>, Margherita Eufemi <sup>(1)</sup>, Pier Giorgio Natali <sup>(2)</sup>

<sup>(1)</sup> Sapienza University of Rome, Department of Biochemical Sciences "A.Rossi-Fanelli", Rome, Italy

<sup>(2)</sup> G. D'Annunzio University, Department of Medicine and Aging Sciences, Center for Advanced Studies and Technology (CAST), Chieti, Italy



**OC.36 - MODULATION OF SKIN MICROBIOTA AIMED TO ACNE MANAGEMENT THROUGH A IN&OUT TREATMENT**

Marco Pignatti <sup>(1)</sup>, Chiara Pesciaroli <sup>(2)</sup>, Federica Carlomagno <sup>(2)</sup>

<sup>(1)</sup> *Università di Modena, Clinica Dermatologica, Modena, Italy*

<sup>(2)</sup> *Roelmi HPC, R&D, Origgio, Italy*

**OC.37 - PROBIOTICS AND ACNE: IN VITRO TESTING OF NEW PROBIOTIC STRAINS TO COUNTERACT ACNE**

Maria Magdalena Coman <sup>(1)</sup>, Giulia Nannini <sup>(2)</sup>, Amedeo Amedei <sup>(2)</sup>, Stefania Silvi <sup>(3)</sup>,  
Maria Cristina Verdenelli <sup>(1)</sup>

<sup>(1)</sup> *Synbiotec Srl, R&D, Camerino, Italy*

<sup>(2)</sup> *University of Florence, Department of Experimental and Clinical Medicine, Florence, Italy*

<sup>(3)</sup> *University of Camerino, School of Biosciences and Veterinary Medicine, Camerino, Italy*



IPA IN COLLABORATION WITH IPA EUROPE



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## ROME CONGRESS, URBANIANA UNIVERSITY AULA NEWMAN - SEPTEMBER 13, 2021

RE: IPA Track Day Program

**Morning Session (10 am to 1:30 pm)****Welcome 10:00 am****Opening:** George Paraskevacos, Executive Director, IPA, Canada

Introduction from our Head Sponsor Deerland Probiotics &amp; Enzymes

**Regulatory Overview from a global perspective*****IPA Global - Government Collaborations***

- Probiotics as Natural Health Products: Overview, Updates, Prospects by Michael Steller, Supervisor at Health Canada, Canada.
- Argentina - Regulatory Framework: Probiotics in Foods. Harmonized Probiotic Guidelines for use in Foods and Food Supplements CCNFSDU current status by Andrea V. Moser, Head of the Food Evaluation and Registration Service, Establishments and Materials in Contact with Food, National Food Institute (INAL), Argentina.
- Current regulatory affairs for probiotics in Australia by Lucy Lang, Head of Regulatory Affairs, CMA, Australia.
- FDA Regulation of Probiotics in Dietary Supplements by Gregory Noonan, Acting Deputy Director, ODSP-FDA, USA.

***IPA EU -The Future of Probiotics in Europe: a Pathway for a sustainable growth***

- The EC "Farm to Fork strategy" food system and sustainability. by Esben Laulund, Vice President of Technology Search Coll. acting as IPA Eu President, Chr Hansen, Denmark.
- A flexible approach for Probiotics in Europe to promote responsible practices - IPA EU by Rosanna Pecere, IPA Europe, Executive Director, Belgium.
- Probiotics? Achieving a better regulatory fit: the legal interpretation by Katia Merten-Lentz, Partner, Keller & Heckman, Belgium.
- Presentation of the Italian approach by Valeria Di Giorgi PhD, Head of Office for the Directorate General for Food Hygiene, Food Safety and Nutrition, Italian Ministry of Health, Italy.



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### ***IPA Task Force Work at CODEX - Wrapping it all up***

- Regulation of Probiotics & Prebiotics in Europe: Domino effect of favourable opinions? by Dr. Luis Gosálbez, Managing Director and Co-Founder, Sandwalk BioVentures, Spain.
- The current regulatory state of play for probiotics internationally by David Pineda, LLM, Managing Director DPE International Consulting, Belgium.
- The importance of Codex standards in times of food systems transformation by Tom Heilandt, Secretary of the FAO/WHO Codex Alimentarius Commission, Italy.

### **Afternoon Session (2.30 to 6:00 pm)**

#### **Education and Science**

##### ***IPA Outreach and Communication***

- A Collaboration Between Industry and Academia: Preparing Tomorrow's Evidence-based Leaders by Dr. Gena E. Kadar, DC, MS. MedL, CNS, Dean, School of Nutrition, Southwest College of Naturopathic Medicine & Health Sciences, USA.
- Dietary Microbes: Can We Afford Not to Think About Them More Deeply? by Bruno Pot, Science Director Europe, Yakult Europe, Netherlands.
- 20 Years After the FAO/WHO Guidelines: What's Next? by Professor Lorenzo Morelli, Chair Professor of Food Microbiology and Director at the Institute of Microbiology UCSC, Italy.

##### ***IPA Research on Probiotics***

- Work on Resilience of the Gut Microbiota by Sylvie Binda, R&D Director for Microbiome Program and Bioprocess & Galenic platforms, Lallemand, France.

##### ***Closing Discussion***

- Science, Industry and Outreach come together.

##### ***Final Words***

- Closing: George Paraskevakos, Executive Director, IPA, Canada.

### **IPA Cocktail - 6.00 pm to 7:30 pm**

Sponsored by





**08.30 - 09.30****DRUGS AND BUGS**

*Chair: A. Gasbarrini (Italy)*

08.30 - 08.50

Drugs and Bugs: Pharmacomicrobiomics

*G. Clarke (Ireland)*

08.50 - 09.10

Psychobiotic

*B. Gonzalez Cautela (Canada)*

09.10 - 09.30

NSAIDS and gut microbiota

*F. Shanahan (Ireland)*

**09.30 - 09.50****LECTURE**

*Chair: F. Vicariotto (Italy)*

Specific strains of probiotic bacteria promote female urogenital health

*F. Vicariotto (Italy), G. Leyer (USA)*

**09.50 - 11.10****FECAL MICROBIOME TRANSPLANT**

*Chairs: G. Gasbarrini (Italy), A. Gasbarrini (Italy)*

09.50 - 10.10

FMT in the clinical practice

*G. Cammarota (Italy)*

10.10 - 10.30

FMT in oncology

*G. Ianaro (Italy)*

10.30 - 10.50

Could autologous Fecal Microbiota Transplantation attenuate weight regain? The DIRECT PLUS trial

*I. Shai (Israel)*

10.50 - 11.10

Microbiome clinics in FMT

*L. Gargiullo (Italy)*

**11.10 - 12.30****GUT MICROBIOTE AND CANCER**

*Chairs: P. Nisticò (Italy), G. Capurso (Italy)*

11.10 - 11.30

Ketogenic diet and immune response against cancer

*L. Derosa (Italy)*

11.30 - 11.50

Oleic acid for prevention of gut inflammation and cancer through the influence on gut microbiota

*A. Moschetta (Italy)*

11.50 - 12.10

Microbiota and pancreatic cancer

*G. Capurso (Italy)*

12.10 - 12.30

Microbiota modulation in colorectal cancer

*M. Libra (Italy)*

**12.30 - 12.45****ORAL COMMUNICATIONS**

**OC.38 - TUNING GUT MICROBIOTA THROUGH A PROBIOTIC BLEND IN GEMCITABINE TREATED PANCREATIC CANCER XENOGRAFTED MICE**

Concetta Panebianco <sup>(1)</sup>, Federica Pisati <sup>(2)</sup>, Maria Ulaszewska <sup>(3)</sup>, Annapaola Andolfo <sup>(3)</sup>, Annacandida Villani <sup>(1)</sup>, Federica Federici <sup>(4)</sup>, Laura Manna <sup>(4)</sup>, Eleonora Rizzi <sup>(4)</sup>, Adele Potenza <sup>(5)</sup>, Tiziana Pia Latiano <sup>(6)</sup>, Francesco Perri <sup>(1)</sup>, Claudio Tripodo <sup>(7)</sup>, Valerio Paziienza <sup>(1)</sup>

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<sup>(3)</sup>IRCCS San Raffaele Scientific Institute, Proteomics and Metabolomics Facility (ProMeFa), Milan, Italy

<sup>(4)</sup>Sintal Dietetics s.r.l, Sintal Dietetics s.r.l, Castelnuovo Vomano, Italy

<sup>(5)</sup>Fondazione IRCCS "Casa Sollievo della Sofferenza" Hospital, Dietetic and Clinical Nutrition Unit, San Giovanni Rotondo, Italy

<sup>(6)</sup>Fondazione IRCCS "Casa Sollievo della Sofferenza" Hospital, Oncology Unit, San Giovanni Rotondo, Italy

<sup>(7)</sup>University of Palermo, Tumor Immunology Unit, Department of Health Sciences, Palermo, Italy

**OC.39 - ANTICANCER EFFECTS OF LACTOBACILLUS RHAMNOSUS GG (LGG) SUPERNATANT**

Silvia Vivarelli <sup>(1)</sup>, Rossella Salemi <sup>(1)</sup>, Luca Falzone <sup>(2)</sup>, Maria Santagati <sup>(1)</sup>, Massimo Libra <sup>(1)</sup>

<sup>(1)</sup>University of Catania, Biomedical and Biotechnological Sciences, Catania, Italy

<sup>(2)</sup>IRCCS Istituto Nazionale Tumori "Fondazione G. Pascale", Epidemiology and Biostatistics Unit, Naples, Italy

**12.45****CLOSING**



10.00 - 11.10

**ORAL COMMUNICATIONS***Chairs: M. Marignani (Italy), M. Guarino (Italy)***GUT MICROBIOTA AND METABOLISM****OC.44 - SUPPLEMENTS ACTIVE ON CHOLESTEROL ABSORPTION**Francesco Visioli <sup>(1)</sup>*<sup>(1)</sup> University of Padova, Molecular Medicine, Padova, Italy***OC.41 - IMPROVED LIPID METABOLISM IN A MOUSE MODEL OF ALZHEIMER'S DISEASE UPON STRATEGIC MODULATION OF GUT MICROBIOTA**Chunmei Gong <sup>(1)</sup>, Laura Bonfilli <sup>(1)</sup>, Massimiliano Cuccioloni <sup>(1)</sup>, Valentina Cekarini <sup>(1)</sup>, Mauro Angeletti <sup>(1)</sup>, Anna Maria Eleuteri <sup>(1)</sup>*<sup>(1)</sup> School of Biosciences and Veterinary Medicine, University of Camerino, Via Gentile III da Varano, 62032 Camerino (MC), Italy, School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy***OC.45 - HAFNIA ALVEI HA4597 IMPROVES WEIGHT LOSS IN OVERWEIGHT SUBJECTS UNDER HYPOCALORIC DIET: A DOUBLE-BLIND, RANDOMIZED PLACEBO-CONTROLLED STUDY**Dechelotte Pierre <sup>(1)</sup>, Breton Jonathan <sup>(1)</sup>, Trotin Picolo Clementine <sup>(2)</sup>, Grube Barbara <sup>(3)</sup>, Erlenbeck Constantin <sup>(4)</sup>, Bothe Gordana <sup>(4)</sup>, Fetissov Serguei <sup>(5)</sup>, Gregory Lambert <sup>(2)</sup>*<sup>(1)</sup> Rouen University, Inserm UMR 1073, Rouen, France**<sup>(2)</sup> TargEDys, R&D, Longjumeau, France**<sup>(3)</sup> Practice, General medecine, Berlin, Germany**<sup>(4)</sup> Analyse & Realize GmbH, Clinical, Berlin, Germany**<sup>(5)</sup> Rouen University, Inserm UMR 1239, Rouen, France***OC.46 - MODULATION OF GUT MICROBIOTA AFTER SUPPLEMENTATION WITH CITRUS FRUIT EXTRACT USING THE TIM-2 IN VITRO MODEL OF THE HUMAN COLON**Sanne Ahles <sup>(1)</sup>, Mônica Maurer Sost <sup>(2)</sup>, Jessica Verhoeven <sup>(2)</sup>, Sanne Verbruggen <sup>(2)</sup>, Yala Stevens <sup>(3)</sup>, Koen Venema <sup>(2)</sup>*<sup>(1)</sup> Maastricht University, Department of Nutrition and Movement Sciences, Maastricht, Netherlands**<sup>(2)</sup> Maastricht University, Campus Venlo, Centre for Healthy Eating and Food Innovation, Venlo, Netherlands**<sup>(3)</sup> Maastricht University, Department of Internal Medicine, Maastricht, Netherlands*



**PROBIOTICS, MILK, FIBERS, YOGURT**

**OC.47 - EFFECTS OF A PREBIOTIC INTERVENTION WITH A HIGHLY PURIFIED EXTRACT OF BLACK ELDERBERRY - RESULTS FROM THE ELDERGUT TRIAL**

Simon Reider <sup>(1)</sup>, Julia Längle <sup>(1)</sup>, Nicole Przysiecki <sup>(2)</sup>, Alexandra Pfister <sup>(2)</sup>, Andreas Zollner <sup>(2)</sup>, Sonja Sturm <sup>(3)</sup>, Stephan Plattner <sup>(4)</sup>, Herbert Tilg <sup>(2)</sup>, Alexander Moschen <sup>(1)</sup>

<sup>(1)</sup> Johannes Kepler University Linz, Christian Doppler Laboratory for Mucosal Immunology, Linz, Austria

<sup>(2)</sup> Medical University Innsbruck, Department for Internal Medicine 1, Innsbruck, Austria

<sup>(3)</sup> Leopold-Franzens University Innsbruck, Department of Pharmacognosy, Innsbruck, Austria

<sup>(4)</sup> IPRONA AG/SPA, -, Lana (BZ), Italy

**OC.48 - PRODUCTION OF NATURALLY GAMMA-AMINOBUTIRIC ACID ENRICHED CHEESE FROM PASTEURIZED MILK, USING THE DAIRY STRAIN *LEVILACTOBACILLUS BREVIS* DSM 32386**

Elena Franciosi <sup>(1)</sup>, Andrea Mancini <sup>(1)</sup>, Maria Cid Rodriguez <sup>(1)</sup>, Tiziana Nardin <sup>(2)</sup>, Roberto Larcher <sup>(2)</sup>, Nicola Cologna <sup>(3)</sup>, Andrea Goss <sup>(3)</sup>, Kieran Tuohy <sup>(1)</sup>, Andrea Merz <sup>(4)</sup>

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<sup>(2)</sup> Edmund Mach Foundation, Technology Transfer Center, San Michele/adige, Italy

<sup>(3)</sup> Trentingrana Consorzio dei Caseifici Sociali Trentini s.c.a., Analysis Laboratory, Trento, Italy

<sup>(4)</sup> Trentingrana Consorzio dei Caseifici Sociali Trentini s.c.a., Management office, Trento, Italy

**OC.49 - YOGURT ENRICHED WITH FIBERS AND PROBIOTIC BACTERIA INCREASED THE ABUNDANCE OF *BIFIDOBACTERIUM ANIMALIS* subsp. *LACTIS BB-12* IN HUMAN GUT MICROBIOME**

Ene Viiard <sup>(1)</sup>, Madis Jaagura <sup>(1)</sup>, Natalja Part <sup>(1)</sup>, Jekaterina Kazantseva <sup>(1)</sup>, Kaarel Adamberg <sup>(1)</sup>

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**OC.50 - EFFECT OF FIBER TYPE ON THE METABOLISM OF *EX VIVO* HUMAN GUT MICROBIOTA**

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# ORAL COMMUNICATIONS

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

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### Objective:

Increasing intake of polyphenols could be a useful strategy to support and enhance gut health. Citrus flavonoids such as hesperidin and naringin have previously shown beneficial effects on barrier function and intestinal inflammation. The aim of this study was to assess effects of a natural citrus fruit extract (CFE) rich in hesperidin on gut microbiota composition and activity using the *in vitro* TIM-2 model of the human colon.

### Methods:

The TIM-2 units were inoculated with human fecal samples and supplemented with 250 mg CFE, 350 mg CFE, or control for 72 hours. Samples were collected at baseline, after 24h, 48h, and 72h. Gut microbiota composition and activity, and short-chain fatty acid (SCFA) production were determined using 16s rRNA gene sequencing and chromatography, respectively.

### Results:

On the genus level, a dose-dependent significantly increased abundance of *Roseburia* ( $q=0.134$ ) was observed after CFE supplementation. A similar trend was observed for *B.eggerthii* ( $q=0.184$ ) and *E.ramulus* ( $q=0.134$ ), although not significant. Moreover, the relative abundances of *Enterococcus* ( $q=0.134$ ) and *L.mucosae* ( $q=0.198$ ) were significantly increased after CFE supplementation. Cumulative production of total SCFAs was higher after CFE supplementation compared to control, which was reflected by increased production of acetate.

### Conclusions:

In conclusion, CFE supplementation increased abundance of microbes and SCFAs known for anti-inflammatory and anti-obesity effects. These results highlight the potential for supplementation with CFE as an enhancer for gut health.

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

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### Objective:

Irritable bowel syndrome (IBS) is characterized by abdominal pain, bloating and bowel disturbances. IBS therapy is primarily symptomatic, including treatment with herbal remedies. Flower extract of *Tilia tomentosa* Moench (TtM) is occasionally used as an anti-spasmodic in popular medicine. Since its effect on intestinal response is unknown, we evaluated TtM influence on small intestine contractility.

### Methods:

Ileal preparations from adult C57BL/6J mice were mounted in organ baths to assess changes in muscle tension, following addition of TtM extract (0.5–36 mcg/mL) or vehicle (ethanol). Ileal segments were pretreated with 12 mcg/mL TtM or vehicle and subjected to: cumulative addition of carbachol (0.001–100 microM); electrical field stimulation (EFS, 0–40 Hz); 10-Hz-EFS with 1 microM atropine + 1 microM guanethidine (non-adrenergic-non-cholinergic-conditions) with/without 0.1 mM L-NAME (pan-NOS inhibitor). The integrity of myenteric plexus was analyzed by immunofluorescence distribution of neuronal markers HuC/D and nNOS and of glial marker S100beta.

### Results:

Increasing addition of TtM induced a marked relaxation in ileal specimens compared to vehicle ( $p<0.001$ ). Pretreatment with TtM caused a significant reduction of CCh- and EFS-induced contraction compared to related control segments ( $p<0.001$ ). Following incubation with TtM, a significant reduction in 10 Hz-EFS-mediated relaxation ( $p<0.001$ ) sensitive to L-NAME was found. *In vitro* 1-hour-incubation of intestinal specimens with TtM did not affect myenteric plexus neuroglial network.

### Conclusions:

Our findings show that TtM-induced relaxation on small intestine neuromuscular contraction is mediated by nitric oxide pathways, providing a pharmacological basis for the use of TtM in IBS.

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

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### Background:

The intestinal microbiome is a major contributor to human health and disease. Influencing the microbiome potentially improves gastrointestinal symptoms. Prebiotics are one way to influence the microbiome and pre-existing microbiome configuration is influencing their effectivity. Better characterization of determinants for efficacy of prebiotics is needed. We aimed to characterize the interaction of a highly purified black elderberry extract rich in polyphenols with the intestinal microbiome and host physiology.

### Methods:

The ELDERGUT Trial was a longitudinal cohort trial in 30 healthy participants with 3 periods of 3 weeks. Prior to the intervention period patients were characterized for 3 weeks, and the intervention period was followed by a 3 week wash-out phase. Patients completed weekly symptoms questionnaires and provided a weekly biosample set. 16S amplicon sequencing was applied to fecal DNA and metabolomics data were generated from urine samples by nuclear magnetic resonance spectroscopy (NMR).

### Results:

While no effects on clinical symptoms were observed, microbiome analysis revealed a sharp increase in  $\alpha$ -diversity both at the beginning and after the end of the prebiotic intervention. A similar pattern was observed in an analysis of  $\beta$ -diversity (unweighted unifrac index), indicating prebiotic-induced changes of intestinal microbiome composition. On the genus level, changes in multiple taxa including *Lactobacillus* and *Akkermansia* could be observed.

### Conclusion:

The ELDERGUT trial reveals a rapid effect of a prebiotic intervention with black elderberry extract. After initial perturbation of community structures, counterregulatory responses seem to establish a new stable equilibrium accompanied by various changes in the taxonomic composition and metabolite output of the microbiome.

## SUPPLEMENTS ACTIVE ON CHOLESTEROL ABSORPTION

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### Objective:

Cholesterol control is mainly achieved via pharmacological treatment and some new compounds are being developed to further lower LDL concentrations and cardiovascular risk in high-risk patients. However, due to the rather recent availability of ad-hoc formulations, patients often independently self-administer supplements and functional foods without medical input, either inappropriately or in situations in which no significant advantage can be gained.

### Methods:

This lecture will outline the effects of the most frequently occurring cholesterol-lowering substances in functional foods or in supplements, with particular attention being paid to the inhibitors of cholesterol absorption.

### Results:

There are two major classes of molecules most active in inhibiting cholesterol absorption. Phytosterols (plant sterols and stanols) inhibit the intestinal absorption of cholesterol, which is partly derived from foods (300–500 mg/day), and largely from the bile (1000 mg/day), in a dose-dependent way, contingent upon their total intake with food or supplements. In order to obtain a significant cholesterol-lowering effect, at least 1.5 g of phytosterols must be consumed per day after the main meal.

Both dietary and supplementary intakes of fibre is also effective in the control of plasma LDL cholesterol levels, likely attributable to the increase of faecal excretion of cholesterol, bile acids or other dietary fats. Examples include beta glucan, glucomannan, psyllium, and chitosan. A positive side effect of such fibers is their prebiotic action.

### Conclusions:

Despite being freely available for purchase, these products should be used following shared agreement between the caring physician and the patient. Moreover, patients should consider the practicality of sustaining treatment costs over time, considering that such a treatment is often lengthy and in theory life-long. Yet, well-characterized and titrated formulations containing inhibitors of cholesterol absorption should be critically considered in the framework of a moderate risk cardiovascular patient in need of lowering his/her cholesterol concentrations.

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

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#### Objective:

The cheese-derived strain *Levilactobacillus brevis* DSM 32386 has been reported as able to produce high concentrations of GABA in experimental cheese from raw milk. In this study, we investigated the activity of this strain during the production of experimental cheeses from pasteurized milk, to test its ability to produce GABA before industrial scale-up.

#### Methods:

*Levilactobacillus brevis* DSM 32386 was tested alone (Lbr) or in combination with commercial proteolytic strains able to increase the amount of free glutamate in cheese, *Lactobacillus helveticus* LH4R and *Lactobacillus delbrueckii* subsp. *bulgaricus* LB1 and was compared with a cheese inoculated only with the starter culture (CTRL) or added with Lbr and glutamate. The GABA concentration was measured by means of UHPLC-HQOMS analysis on milk and cheese samples after 7, and 30 days ripening.

#### Results:

During the ripening, pH of all cheeses remained below 5.5, which is the value required for GABA production. GABA concentration increased during ripening, with the highest concentration in cheeses after 30 days of ripening where *L. brevis* DSM 32386 was added with glutamate (168.92 mg/kg), or with the commercial *L. delbrueckii* subsp. *bulgaricus* (150.89 mg/kg). In CTRL cheeses the GABA concentration was under 100 mg/Kg.

#### Conclusions:

The obtained data supported the hypothesis that *L. brevis* DSM 32386 can be exploited as probiotic culture, improving the *in situ* bio-synthesis of GABA not only in raw milk cheeses but also in pasteurized milk cheeses where the proteolytic activity is led by other lactic acid bacterial strains such as *L. delbrueckii* subsp. *bulgaricus*.

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

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#### Objective:

To evaluate the effect of a probiotic- and fiber-enriched symbiotic yogurt on human gut microbiota, digestive comfort, and selected blood markers. In addition to starter bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*), the yogurt contained probiotic bacteria *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus acidophilus* LA-5, and a mixture of soluble and insoluble fibers (resistant starch, polydextrose, high polymerization inulin, and xylooligosaccharides) in total 4.9 g / 100 g.

#### Methods:

The test subjects (N=81, double-blind controlled study) consumed 200 g of enriched yogurt for 14 days. Fecal and blood samples were collected before and after the intervention. The subjects filled in a 3-day food frequency questionnaire describing the consumption of main food groups. Standardized blood analyses were performed and metagenetic sequencing of 16S rRNA gene amplicons was used to determine the composition of fecal bacterial communities.

#### Results:

Both control and test yogurts reduced fasting blood lipid and glucose levels, while the fiber-enriched yogurt induced a remarkable increase of *B. animalis* BB-12 levels in the fecal samples. Increased fiber intake did not cause major digestive discomfort and helped maintain regular bowel movement during the study period.

#### Conclusions:

Enrichment of yogurt with a mixture of fibers significantly promoted the growth of *B. animalis* BB-12 in the human gut during two weeks of daily consumption and helped maintain a healthy digestive pattern. Regular consumption of symbiotic foods can be an effective strategy to modulate the human gut microbiota, improve the plasma lipids' profile and glucose levels.



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

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Bioactive metabolites produced by the gut microbiota are important for human health. Short-chain fatty acids (SCFAs) produced by carbohydrate fermentation and indole derivatives from tryptophan catabolism are well recognized for their impact on host physiology and can also have effects beyond the gut by entering the circulation. Here, we used an *ex vivo* experimental framework to investigate the effect of fiber type on the production of short-chain fatty acids (SCFAs) and indole derivatives. Pectin and inulin were chosen as examples of plant fibers to ferment with bacteria from the proximal colon (PC) and distal colon (DC) compartments of Simulator of Human Intestinal Microbial Ecosystem (SHIME®). Fermenter samples were collected to measure effects on human microbiota composition and diversity as well as metabolite production. Pectin and inulin fermentation resulted in distinct metabolic profiles in the PC and DC. Inulin yielded higher concentrations of SCFAs than the pectin after 24 h fermentation. The relative concentration of indole derivatives varied depending on the fiber used, with higher concentrations of indole-3-acetic acid and indole-3-aldehyde for pectin, and a higher concentration of indole-3-propionic acid for inulin. When PC and DC were supplied with the same amount and type of fibers, microbiota in the DC produced more SCFAs than the PC. Indole derivatives were largely produced in the DC. Fermentation with pectin or inulin suppressed microbiota catabolism of tryptophan in the PC. Taken together, our results suggest that the type of fiber must be considered in the formulation of functional foods for intestinal health benefits.

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

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#### Objective:

Cancer represents the second leading cause of death worldwide. Gut microbiota plays a dual role in cancer, as certain pathogens elicit tumorigenesis, whereas a number of beneficial probiotics can ameliorate immune defense against tumors. *Lactobacillus rhamnosus GG* (LGG) administration mitigates therapy-related intestinal damage and elicits the host's immune system. Additionally, LGG might help to directly arrest cancer growth, although the mechanism underneath remains yet elusive. The objective of this study was to assess the effect of LGG culture supernatant (LGG-SN) on the growth of a selected panel of cancer cells.

#### Methods:

Cell-free LGG-SN was obtained by growing LGG at steady conditions in a culture medium compatible with human cells. LGG-SN was administered to four cancer cell lines (both local colorectal cancer and distal melanoma). To assess the effects of LGG-SN on cancer growth, several complementary approaches were used including: MTT metabolic assay, Trypan blue cell death count, BrdU proliferation assay, Propidium iodide cell cycle analysis and cleaved-Caspase-3 apoptotic readout.

#### Results:

LGG-SN significantly reduces the viability of all the cancer cell lines in a concentration-dependent manner. Moreover, LGG-SN when administered in combination with cytotoxic drugs show a synergic effect. Importantly, LGG-SN inhibits cancer cell proliferation and specifically induces a cell cycle G2/M arrest, without promoting apoptosis.

#### Conclusions:

Overall, these results suggest the potential use of LGG-SN as adjuvant of anti-cancer therapies. Future studies are needed to identify the active molecule(s) contained in LGG-SN and to validate these findings in translational models (i.e., patient-derived tumor organoids).

## PREBIOTIC LACTULOSE AS EFFICACIOUS MICROBIOTA AND METABOLITE MODULATOR IN CIRRHOSIS ENVIRONMENT

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### Objective:

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

### Methods:

Here we investigated the modulation of gut microbiota, in terms of microbial composition and metabolism, upon fermentation of lactulose, rifaximin and VSL#3, using in vitro-24hours anaerobic pH-controlled batch cultures inoculated with faecal microbiota of cirrhotic patients.

### Results:

Over time, cirrhotic microbiota responded dynamically to the treatments. In particular, significant differences (PERMANOVA Weighted/Unweighted/Bray-Curtis estimators) were observed after 24h. The main taxa associated with decompensated cirrhosis, were reduced with lactulose. At the same time, taxa associated with healthy conditions, such as Lachnospiraceae, Ruminococcaceae, *Blautia* and *Bifidobacterium*, were promoted as confirmed by the Indicator Species Analysis. Lactulose alone or in combination with the probiotic VSL#3 led to an increase production of SCFA and decrease in ammonia production. These shifts in metabolite production are indicative of carbohydrate fermentation and are consistent with improved gut health and reduced risk of HE.

### Conclusions:

We demonstrated that lactulose is able to significantly increase the relative abundance and absolute numbers of bifidobacteria, which was associated with an increased production of SCFA and a reduction in ammonia content. Future investigations should assess the molecular pathways that are involved in the modulation of gut microbiota and its metabolic reprogramming while translational studies should examine the clinical potential of this *in vitro* observations.

## BIOCHEMICAL AND ANTIBIOTIC INFLUENCE OF GASTROINTESTINAL TRACTS MICROFLORA IN NEONATAL

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### Objective:

Colonisation of the neonatal intestinal tract by microbiota may occur as early as the foetal stage, and this colonisation preforms the intestinal microbiota, which is reflected in the intestinal activity and neonate vitality. This study aimed to isolate and identify common bacteria in 19 preterm neonates spending their first weeks of life in the neonatal intensive care unit.

### Methods:

First, stool samples were collected, and bacteria were isolated and purified from those samples.

Common bacterial species were investigated regarding their susceptibility or resistance to antibiotics. From 19 stool samples, 15 contained three species in common: *Enterobacter cloacae*, *Enterococcus faecalis* and *Klebsiella pneumoniae*.

### Results:

Differences in the microbial formation and density were correlated with the type of delivery and feeding as well as the administration or no administration of antibiotics to the preterm neonate. Antibiotic susceptibility testing was undertaken, and minimum inhibitory concentrations were determined. The results showed that the minimum level of isolates was affected by several of the most commonly used antibiotics from the following families: aminoglycosides, carbapenems, fluoroquinolones, glycolcyclines and polymyxins.

### Conclusions:

In the present study, we identified the most common bacterial species present in the intestinal microflora of premature infants during their first days after birth. *Enterobacter cloacae* and *Klebsiella pneumoniae* were the most common gram-negative bacteria, while *Enterococcus faecalis* was the most prevalent gram-positive bacterium. Our microbial susceptibility testing showed that these isolates were sensitive to several of the most commonly used antibiotics from the following families: aminoglycosides, carbapenems, fluoroquinolones, glycolcyclines and polymyxins. The analysis revealed a significant level of sensitive towards most tested antibiotics among certain strains isolated from neonates, which raises concern.

### INVESTIGATIONS OF THE POTENTIAL MECHANISM OF ACTION OF A MULTI-STRAIN PROBIOTIC COMPOSITION AGAINST UROGENITAL PATHOGENS BY EX-VIVO STUDIES

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#### Objective:

The urogenital microbiota is dominated by lactobacilli able to counteract pathogens growth. Vaginal infections occur when the urogenital microbiota is unbalanced. The aim of the study was to evaluate the efficacy of SynBalance® Femme, a product containing *L. plantarum* PBS067, *B. animalis* subsp. *lactis* BL050 and *L. rhamnosus* LRH020, to inhibit the adhesion and the growth of pathogens involved in uro-vaginal infections.

#### Methods:

The antimicrobial and preventive effects of the three probiotic strains and their combination SynBalance® Femme, have been evaluated on a reconstructed bladder epithelium (HBE), infected with *E. coli* and on a reconstructed vaginal human epithelium (VHE, A431 modified) infected with *C. glabrata* and *C. albicans*, *G. vaginalis*, *N. gonorrhoeae* and *T. vaginalis*, respectively. In addition, the effects on the viability and the integrity of reconstructed tissues after TEER treatment were also assessed.

#### Results:

A strong antimicrobial activity was observed for *B. lactis* BL050, *L. plantarum* PBS067 and *L. rhamnosus* LRH020, on HBE previously colonized by *E. coli*. For *L. rhamnosus* LRH020 a preventive activity was also observed by SEM analysis. TEER results showed that none of the strains have negatively influenced the integrity of HBE.

On the vaginal epithelium, SynBalance® Femme and its corresponding strains showed a full inhibition of all tested pathogens, together with a strong reduction of their adhesiveness. The prevention model demonstrated a very strong effect as well.

#### Conclusions:

These results underling the potential mechanism of action of SynBalance® Femme and their single strains in the prevention and treatment of several urogenital infections.

### TRANSCRIPTOME AND MITOCHONDRIAL ANALYSIS OF ASD CHILDREN COMPARED TO HEALTHY CONTROLS

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#### Objective:

Autism Spectrum Disorder (ASD), like many modern society pathologic conditions, is an epigenetically initiated disease. The purpose of this study was to determine the differences in the oral microbiome and mitochondrial health between children from a "blue zone" in Colombia, healthy and A.S.D. children in the U.S.A.

#### Methods:

The A.S.D. section included 30 children and young adults, ages 6 to 21, who were sampled at three different intervals. The sampling consisted of buccal swabs for Mitoswab testing, and saliva for full transcriptomics, in order to determine the entire virus, bacteria, archaea, and fungus range of species. Dietary and health information was obtained, as were consents and assents per I.R.B. requirements. The Colombia component included 30 children, ages 6-16, who were healthy and within normal behavior standards. Buccal swabbing and salivary sampling was performed only once with this group, as with the typical healthy USA controls (30). The USA Healthy Control group consisted of children 6-16 who had no history of any medications.

#### Results:

Significant differences between each subject and intervention group were demonstrated by the Richness and Shannon Diversity plots. The USA healthy children group had a greater Richness than the other two groups but the Shannon diversity was not significantly different.

#### Conclusions:

The microbiomes of individuals diagnosed with A.S.D. is significantly different from healthy children in a developing country and healthy children from the USA. Microbiome shifts may have strong epigenetic consequences that may be involved in ASD development.

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

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### Objective:

Previous studies demonstrated that probiotics counteract Alzheimer's disease (AD) progression and restore glucose homeostasis in 3xTg-AD mice [Bonfilli et al 2017, 2020]. Considering the debated role of deregulated lipid homeostasis in AD [McGuinness B et al 2010] and in light of the promising effects of probiotics on energy metabolism, this study aims at elucidating the mechanisms through which gut microbiota manipulation ameliorates impaired lipid metabolism in AD.

### Methods:

8 week-old 3xTg-AD mice and their wild-type counterpart were chronically administered with a multi-strain probiotic formulation and changes in the plasma lipid profile (using lipidomic analyses and enzymatic colorimetric assays), along with the cerebral and hepatic expression levels of key regulators of cholesterol metabolism (through Western blotting and ELISA), were evaluated.

### Results:

Upon probiotics administration, cholesterol biosynthesis was inhibited in AD mice, with the involvement of sterol regulatory element binding protein 1c and liver X receptors mediated pathways. Decreased plasma and brain concentration of 27-hydroxycholesterol and increased brain expression of cholesterol 24S-hydroxylase indicated that alternative pathways of bile acid synthesis are influenced. These data, together with the hypocholesterolemic effects and the ameliorated fatty acids profile, demonstrated that microbiota modulation through probiotics can positively change lipid composition in AD mice, with arachidonic acid representing a hub metabolite in the interactions among probiotic-induced lipid profile changes, insulin sensitivity, and inflammation.

### Conclusions:

These data provide important contribution in filling the knowledge gap in the neuroprotective microbiota-lipid-glucose crosstalk in AD and may pave the way for the identification of new therapeutic targets and effective treatments.

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

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Just like dysbiosis and decreased microbiota diversity have been associated with intestinal pathologies, changes in proteolytic homeostasis at mucosal surfaces have been observed in gut pathological situations such as infection, chronic inflammation, functional disorders or even cancer. Studies have demonstrated in the gut that luminal proteases are for the most part from host origin. We have postulated that the intestinal epithelium is a major source of proteases and protease inhibitors, which together exert controls on mucosal microbiota biofilms. Our objectives were to i) investigate which epithelial proteases are controlled by the presence of microbiota, ii) investigate the effects of identified proteases on human intestinal biofilms in health and disease, iii) determine the contribution of such proteases to microbial biofilm homeostasis or dysbiosis.

Using activity-based probes, we have identified several proteases that are produced and secreted in an active form, by human intestinal epithelial cells. Among such proteases, thrombin, elastase-2 and trypsin-1 and -2 were released in large amounts on the luminal side of the epithelium. Germ-free mice lack the expression of such proteases in the epithelium, but their expression was up-regulated upon microbiota transfer. This indicated that the expression of these epithelial proteases is controlled by the presence of microbiota.

Further, we have investigated the effects of protease exposition to human intestinal biofilms (from healthy donors and from inflammatory bowel disease: IBD patients). Results showed that thrombin exposure reduced bacterial survival and decreased biofilm biomass of healthy individual biofilms, while biofilms from Crohn's disease patients seemed to be resistant to the effects of thrombin. Thrombin exposure also increased the virulence (translocation) of bacteria detached from healthy biofilms. Elastase-2 exposure increased healthy biofilm bacterial survival and conferred a pro-invasive phenotype to biofilm detached bacteria. However, in Crohn's disease patient biofilms, Elastase-2 increased biofilm bacteria survival. Finally, Elastase exposure of human biofilms from healthy or Crohn's disease individuals seemed to increase firmicute and proteobacteria abundance, while the same dose of Elastase in biofilms from Ulcerative Colitis disease patients decreased proteobacteria and increased firmicute abundance.

Taken together these results demonstrate that epithelial proteases are controlled by the presence of microbiota and conversely can control microbial biofilms in terms of their composition, their growth and their virulence. Such interactions seemed to be very complex and will require further investigation, but our results clearly point to proteolytic homeostasis as a major component of microbiota behavior in the gut.

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

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#### Background:

Persistent Organic Pollutants (POPs) such as lindane, hexachlorobenzene, DDT, dioxin etc. belong to a large class of diffused organic compounds well-known for their toxic effects on human health. Among POPs, particular attention has been focused on the  $\beta$ -hexachlorocyclohexane ( $\beta$ -HCH), the more stable and persistent isomer of the hexachlorocyclohexanes family. In fact, despite its worldwide environmental distribution,  $\beta$ -HCH effects on human health have not been largely investigated. Previous cellular and molecular studies performed on prostate cancer cells demonstrated that  $\beta$ -HCH activates a wide range of signaling pathways and acts as an endocrine disruptor, promoting cellular processes related to carcinogenesis, tumor progression and chemoresistance. In spite of its small size,  $\beta$ -HCH has a relevant impact on cellular homeostasis, making it mandatory to explore defense strategies against its multifaceted biological effects.

#### Methods:

For this purpose, a screening of natural substances was carried out on the above enlisted cell targets to test their capability to counteract  $\beta$ -HCH actions by performing many different biochemical and cellular assays.

#### Results:

Among a wide array of selected compounds, extracts containing micronutrients from tomato and olive show a dose-dependent significant chemoprotective activity in the considered cell lines by contrasting  $\beta$ -HCH-induced intracellular responses such as anti-apoptotic and prometastasing events, increase in ROS production and DNA damage.

#### Conclusions:

These experimental outcomes identified the chemoprotective effects of tomato and olive-derived micronutrients, recommending the development and testing of tailored enriched formulations for exposed individuals. Investigations along this line are ongoing.

This study was supported by *Fondazione Federico Calabresi*.

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

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#### Objective:

Recommendations on diet modification for patients with non-alcoholic fatty liver disease (NASH) are predominantly restrictive. We aimed to assess the efficacy of the approach that enriched isocaloric diet (ID) with active ingredients provided in the form of newly developed specialized food product (SFP) for patients with NASH.

#### Methods:

Formula of SFP was developed according to literature data on the efficacy of nutrients for NASH. Product (code name "SPP1") contained (%RDA): proteins-8%; fats-7% (including  $\omega$ -3 PUFA-40%); soluble dietary fiber-160%; phospholipids-25%; alpha-lipoic acid-33%; betaine-10%; minerals-13%-44%; vitamins (A, E, D3, K1, C, B1, B2, B6, B12, PP, Folic acid, Pantothenic acid, Biotin) 24%-140%. Patients enrolled to the study (NCT04308980) had confirmed NASH (per EASL), gave informed consent. They were randomized into the group received ID with 2 portions of SFP daily 2 weeks, or ID only. Repeated (baseline and on 15th day) clinical evaluations, body composition (InBody, Korea), blood chemistry measurement were performed.

#### Results:

Twelve subjects were enrolled to ID+SFP and 8 in ID group. Groups didn't differ by age, sex, and BMI. The product was well tolerated. In contrast to ID group, those received SFP demonstrated significant decrease of weight and loss of body fat (table 1). In both groups, we found a trend for ALT and AST decrease; however, it was significant in ID+SFP group only.

#### Conclusions:

New foods in combination with isocaloric diet may be beneficial in the treatment of NASH. Our study showed greater body fat loss and improvement of laboratory markers when new food product was used

## GENOME-GUIDED CREATION OF NEXT-GENERATION PROBIOTICS

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Metagenomic analysis has provided detailed taxonomic and functional insights into host-associated microbiota. Using this wealth of information, consortia of microbes that confer health benefits to their hosts can be developed, termed 'next-generation probiotics'.

Host-associated microbiota consist of hundreds of species, each containing thousands of protein families (Pfams), making them functionally complex. While a few species dominate most microbiota profiles, species occurring at lower abundances are known to contribute important functions. Due to this, consortia based on dominant species in an environment are likely to miss key or essential functions. To solve this, we developed an automated system for the creation of representative synthetic communities, such as next-generation probiotics, called MiMiC (Minimal Microbial Consortia), based on the functional repertoire of an input microbiome.

MiMiC predicts functionally representative minimal consortia using an iterative scoring system based on maximal match-to-mismatch ratios of Pfams between a database of genomes (generic or isolate specific) and input metagenomes. Reduction of metagenomes, and genomes to the presence and absence of Pfams was confirmed to retain resolution and allow metagenomic profiles between six environmental and host-derived microbial communities to be distinguished. Furthermore, when looking at microbiome from pigs or humans from different countries, significant differences were observed within their predicted consortia. MiMiC represents a step forward in the automated development of synthetic communities and can be applied to generate next-generation probiotics for both generic use (replacement of FMT or neonatal probiotics), or personalized treatments (supplementation of missing functions to improve gut health).

## LOCAL APPLICATION OF PROBIOTICS PROMOTES EXCISIONAL WOUND HEALING IN RATS

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### Objective:

Excisional wounds are one of the most used wound-healing models and are considered to resemble acute clinical wounds requiring healing by second intention. In such wounds, skin barrier function must be promptly restored for further damage or infection to be prevented and an aesthetically acceptable scar to be achieved. Nowadays, there is ongoing knowledge that bacteria residing on the skin are involved in the complex process of defense against pathogens and tissue healing. Hence, the objective of this preliminary study on a rat model of excisional wounds was to evaluate the influence of a locally applied probiotics compound on wound healing rates.

### Methods:

Thirty-two Wistar rats were randomly allocated into probiotics [*L. rhamnosus*, *B. longum*,  $10^{11}$  cfu/gr] and control groups. Six excisional full-thickness wounds were created on each dorsum by using a sterile, 8-mm circular punch [registration 227933(934)]. Probiotics or saline were applied, and wounds covered with sterile adhesive dressing; the same treatment applied every 4 days. On days 0, 4, 8, 16 wound healing area [mm<sup>2</sup>] was assessed, by means of photography [Cannon EOS-50D, EF-100mmf/2.8L Macro lens] and digital planimetry [Image J, Bethesda, MD] on each time-point. ANOVA analysis was then applied.

### Results:

Probiotics-treated rats experienced a rapid decline of the wounded area [mm<sup>2</sup>] in relation to controls,  $p=0.0001$ , at every time point; D4:  $42.05 \pm 12.65$  vs  $49.37 \pm 10.63$ ; D8:  $14.71 \pm 4.51$  vs  $26.33 \pm 3.86$ ; D16:  $2.43 \pm 1.00$  vs  $11.06 \pm 2.92$ .

### Conclusions:

These preliminary results clearly demonstrate that the local application of probiotics significantly promotes the wound healing process. Further studies are in process.

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

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#### Objective:

Food safety is a major concern for Health Agencies and consumers worldwide. Poor quality or contaminated food can constitute a significant risk factors for Human health. The objective of this study was to evaluate whether strains of probiotics of the genus *Lactobacillus* are capable of degrading or sequestering chemical contaminants such as heavy metals, glyphosate and biogenic amines.

#### Methods:

Five *Lactobacillus* strains were evaluated. At this purpose, the microorganisms were grown *in vitro* and exposed to different concentrations of contaminants. Through validated analytical methods and statistical analysis, the concentration of contaminants in the culture broth was evaluated and their level of sequestration or degradation by probiotics was quantified.

#### Results:

Data analysis showed that a strain of *L. plantarum* was able to degrade glyphosate in an amount of 12% in respect to the initial quantity added. A degree of uptake of several heavy metals was also observed: in particular cadmium is sequestered up to 72% and chromium up to 20%. Three strains showed degradative activity towards amines by degrading up to 50% of Tryptamine, Spermidine and Spermine.

#### Conclusions:

In this investigation it has been demonstrated how some strains belonging to the genus *Lactobacillus* are able to sequester or degrade food contaminants. The population of the Human gut with these bacteria could lead to a decreased risk of absorption of these contaminants, thus introducing a novel perspective of the beneficial effects of probiotics on Human health.

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

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#### Objective:

We identified beneficial probiotic strains including *S. boulardii*, *L. acidophilus*, *L. rhamnosus* and *B. breve* that antagonize elevated bacterial pathogens in the inflamed gut. Our aim is to characterize the effect of the probiotic supplement in the murine model of ileitis SAMP1/YitFc (SAMP).

#### Methods:

Two groups of SAMP mice have been used for this experiment. The experimental group was administered with one dose of the probiotic nutritional supplement diluted in sterile PBS (0.25 mg/100uL of PBS) everyday for 60 days through gavage technique. The control group was administered with sterile PBS. At the end of the treatment, ilea and stool samples were collected for histology and 16S rRNA analysis.

#### Results:

The histology score shows that probiotic treated-mice had a significant decrease of ileitis compared to the control group (unpaired t test:  $7.2 \pm 0.5$  vs.  $13.4 \pm 2.5$ ; \*\*  $P = 0.0069$ ). Principal component analysis showed that for the bacteriome, mice before the treatment clustered together. In contrast, probiotic treated-samples were widely scattered compared to the limited scattering observed in the control group. 16S rRNA analysis showed that abundance of species belonging to genus *Lactobacillus* was significantly decreased compared to controls ( $P < 0.05$ ). Levels of *Rikenellaceae* were significantly increased in probiotic-treated mice compared to controls ( $P < 0.02$ ).

#### Conclusions:

The changes followed probiotic use show that the microbiome was positively impacted. In fact, previous studies found that Non-Alcohol Fatty Liver disease patients have significantly higher levels of *Lactobacillus* and lower levels of *Rikenellaceae* compared to healthy subjects.

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

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### Background:

Therapeutic administration of probiotic bacteria in ulcerative colitis (UC) patients appears rational and attracting, but consistent dishomogeneity exists in published studies. We have previously demonstrated that *Lactobacillus rhamnosus* GG (LGG) has favourable properties such as adhesion to the colonic mucosa and anti-inflammatory and immunomodulatory effect.

Aim of the study: To investigate the potential clinical application of therapeutic supplement of LGG (ATCC 53103) in mild-moderately active UC patients, evaluating efficacy and safety.

### Materials and methods:

UC patients with mild-moderately active disease (Clinical Mayo score  $\geq 2$ ) despite oral treatment with oral mesalamine, after a wash-out period of 2 weeks from mesalamine, were randomized to assume a regular ( $1.2 \times 10^{10}$  CFU/day) or a double ( $2.4 \times 10^{10}$  CFU/day) dose of LGG for 1 month. Clinical activity before and after treatment were compared and clinical response was defined as a reduction of Clinical Mayo score  $\geq 1$  point. Primary end-points were the improvement of clinical symptoms and the safety evaluation, and secondary end-points was comparison between the two dosages of LGG. Patients who had a disease flare stopped LGG supplement and went back to regular therapy. Intention-to-treat (ITT) and per protocol (PP) analysis were performed. The trial has been registered to ClinicalTrials.gov (NCT04102852).

### Results:

40 patients were preliminarily included in the study (M/F=21/19), and 31(78%) completed the treatment period. In the ITT analysis: 19/40 (48%) patients showed clinical response, 12/40 (32%) remained stable, and 8/40 (20%) had a disease flare. In the PP analysis, 20/32 (63%) had a clinical response, and 12/32 (37%) remained stable. The mean reduction of Clinical Mayo score was 0.6 points ( $p=0.004$ ). No serious adverse event was recorded. No significant difference in efficacy and safety was observed between the two different doses of LGG.

### Conclusion:

In the present interim analysis of a double-blind randomized clinical trial, LGG administration was effective and safe in UC patients with mild-moderate clinical activity.

## MANIPULATION OF GUT MICROBIOME BY PREBIOTIC TO COMBAT ALCOHOLIC LIVER DISEASES

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### Objective:

Pathogens in the gut microbiome are associated with detrimental effects to the liver by producing alcohol. We asked whether prebiotics can induce these pathogenic bacteria such as virulent *Klebsiella pneumoniae* K1 (*K. pneumoniae*; *Kpn*) strain to produce beneficial short-chain fatty acids (SCFAs) instead of alcohol to prevent liver damages.

### Methods and Results:

1. No auto-antibodies to *Kpn* strains in blood were produced. Commensal *Kpn* strains expressed higher pyruvate dehydrogenase than virulent *Kpn* strains.
2. The sequence elements with 32mer-T or 32mer-G nucleotides in the promoter region of aldose/keto reductase, an enzyme converting toxic aldehyde to inactive alcohol, vary between virulent (K1, K2, W14 and TH1) and commensal *Kpn* strains.
3. PCR validated that 32mer-T can differentiate virulent (K1 and K2) *Kpn* strains from commensal *Kpn* strains.
4. Incubation of virulent *Kpn* K1 strains or commensal *Kpn* C3 strains with 2% glucose produced both alcohol and SCFAs.
5. Replacing glucose with linolenic acid significantly lowered the alcohol amounts yet increased the production of SCFAs including propionic acid in *Kpn* K1, suggesting that linolenic acid can serve as a prebiotic to manipulate the *Kpn*'s role in the gut microbiome for prevention or treatment of alcohol-related liver diseases.
6. Administration of propionic acid diminished the alcohol-induced 4-hydroxynonenal formation in mouse livers.

### Conclusions:

The virulence of *Kpn* bacteria was lowered by giving linolenic acid as prebiotics. Propionic acid and its derivatives as postbiotics hold promise for treatment of liver diseases.



## POTENTIAL ROLE OF THE INFLAMMATORY MOLECULES IN MODULATING SKIN MICROBIOME AND DYSBIOSIS IN ACNE VULGARIS

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### Objective:

Acne vulgaris is a common inflammatory disorder affecting more than 80% of young adolescents. *Cutibacterium acnes* plays a role in the pathogenesis of acne lesions, although the mechanisms are poorly understood. The study aimed at measuring the prevalence of *C. acnes* in skin lesions, from comedogenesis to the progression towards inflamed acne lesions; analyze biofilm production of *C. acnes* isolates; assess the level of different inflammatory molecules in the skin of acne patients and their putative role in promoting bacterial growth and persistence.

### Methods:

Samples for microbiological analysis were collected from the unaffected skin, the comedones, and the papulo-pustular lesions of 15 acne patients and the skin of 10 healthy subjects.

### Results:

Microbiota analysis showed a significantly higher relative abundance of *C. acnes* ( $P < 0.05$ ) in inflammatory compared with non-inflammatory acne lesions, unaffected skin, and the skin of healthy subjects. All the strains analyzed were able to produce biofilm. The level of cutaneous interleukin (IL)-alpha and vascular endothelial growth factor (VEGF) was significantly higher ( $P < 0.05$ ) in the skin of acne patients as compared to control subjects. Additionally, IL-1alpha and VEGF were capable of promoting a concentration-dependent increase of *C. acnes* growth in-vitro.

### Conclusions:

*C. acnes* proliferates in the inflammatory lesions of acne patients. Biofilm production by *C. acnes* may contribute to sustaining bacterial adhesion and chronic persistence in acne. Besides, the inflammatory milieu and the increased IL-1alpha and VEGF levels observed in acne patients' skin may play a role in promoting the growth of *C. acnes*.

## MODULATION OF SKIN MICROBIOTA AIMED TO ACNE MANAGEMENT THROUGH A IN&OUT TREATMENT

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### Objective:

Skin manifestations are often correlated to gastrointestinal disorders. Acne is a multifactorial inflammatory skin disease characterized by sebum overproduction, follicular hyperkeratinisation, colonization by *Corinebacterium acnes* and inflammation. Recently, acne definition has been shifted from infectious disease to skin dysbiosis controlled by the intestinal microbiota. The aim of the research was to assess the efficacy of a combined treatment, a cosmetic cream containing ÆCTive® (ectoine from fermentation) and a probiotic supplement SynBalance® ProBeautyShield (*L. plantarum* PBS067, *L. reuteri* PBS 072 and *L. rhamnosus* LRH020), in improving acneic skin appearance through a positive modulation of gut and skin microbiota.

### Methods:

A RDBPC trial was carried-out on eighty subjects with acneic skin; volunteers were randomly assigned to 4 groups: ÆCTive® cream + nutraceutical placebo, ÆCTive® cream + SynBalance® ProBeautyShield, cosmetic benchmark + nutraceutical placebo, cosmetic benchmark + SynBalance® ProBeautyShield. Skin moisturization, pH and sebum content were observed together with clinical evaluations of acne lesions and comedones, skin complexion evenness and inflammatory status of the acneic area at the beginning, after 28 days (T28) and at the end of the treatment (T56).

### Results:

The combined active treatment resulted effective in ameliorating the instrumental parameters, with significant results on skin hydration (3.9%) and sebum reduction (-22%). The dermatological assessment of skin complexion evenness and inflammatory status reported significant amelioration compared to control (75% vs 40% respectively).

### Conclusions:

These results confirm the efficacy of the In&out treatment, targeting gut and skin microbiota, as an excellent support for acneic subjects, representing a sustainable alternative to more invasive medical treatments.

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

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### Objective:

Acne is a highly prevalent inflammatory skin condition involving the interaction between skin microbes and host immunity, which lead with the changes in microbial composition and activity disturbing the microbiota balance. Several probiotic strains have been tested for their anti-pathogenic activity against the main pathogens responsible for acne, capacity to fight pathogenic adhesion to HaCaT cells and the *in vitro* down-regulation of innate immunity.

### Methods:

To investigate whether probiotics have direct effects on the growth of *Cutibacterium acne*, *Staphylococcus aureus* and *Streptococcus pyogenes*, the antimicrobial activity of live LAB was analysed by a modified cross-streak method. It was also investigated the *in vitro* blockage of pathogens adherence by six probiotic strains to HaCaT cells, under three possible mechanisms: exclusion by adhered probiotics, displacement of adhered pathogens and competition for receptor sites (exclusion test). The inflammatory response was monitored by immunohistochemistry and ELISA assays, targeting a selection of innate immune markers (IL-4, IL-6, IL-8, IL-10, IL-17, TGF- $\beta$  and IFN- $\gamma$ ).

### Results:

All strains shown anti-pathogenic activity against the tested pathogens. The inhibition results on HaCaT cells highlights a significant ( $P < 0.05$ ) competition of all probiotics against the three pathogens. Every pathogenic strain alone has been shown to lead to up-regulation of innate immune markers, while restoration of the microbiome diversity by probiotics presence suppressed inflammation via down-regulation of innate immunity.

### Conclusions:

The results suggest that the probiotics used in the present study could prevent colonization of the skin by relevant pathogens through barrier and interference mechanisms (mainly exclusion and competition), suggesting a successfully use in future conventional therapies of skin disorders (acne).

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

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### Objective:

Scientific results describing the microbial flow connecting food and gut microbiomes are still fragmented. The aim of the analysis was to provide a state-of-the-art knowledge-base about the scientific literature addressing the connection between foodborne and gut microbiomes, focusing on probiotics added to fermented foods, their possible impact on human gut microbiota composition and their ability to colonize the gut environment. An additional aim was also to highlight experimental approaches and study designs which could be better standardized to improve comparative analysis of published datasets.

### Methods:

A systematic literature search for peer-reviewed research articles was carried out on two databases to identify intervention and observational studies analyzing the impact on human gut microbiota composition and colonization ability of probiotic bacteria from fermented foods. Forty-two papers were finally selected.

### Results:

Overall, recurrent gut microbial groups mainly affected by probiotic-added fermented food consumption resulted to be Lactobacilli and Bifidobacteria, whose levels increased following supplementation. Most probiotics were recovered in faecal samples, suggesting colonization ability, although only few studies provided direct evidence of the presence of viable bacterial cells. Moreover, the overall results indicate that colonization was a transient condition, lasting only during the supplementation period.

### Conclusions:

Further research employing standardized and trans-disciplinary approaches aimed at understanding how probiotic- added fermented foods can be tailored to positively influence human gut microbiota and, in turn, host health, are therefore of pivotal importance.

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

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#### Objective:

The aim of this work was to refine the taxonomy and the functional characterization of publicly available *Lactiplantibacillus plantarum* complete genomes through a pan-genome analysis. Particular attention was paid in depicting the probiotic potential of each strain.

#### Methods:

127 complete genome sequence of *L. plantarum* strains, without detected anomalies, was downloaded from NCBI.

#### Results:

Roary analysis of *L. plantarum* pan-genome identified 1,436 core, 414 soft core, 1,858 shell and 13,203 cloud genes, highlighting the "open" nature of *L. plantarum* pan-genome.

Identification and characterization of plasmid content, mobile genetic elements, adaptative immune system and probiotic marker genes (PMGs) revealed unique features across all the *L. plantarum* strains included in the present study.

Considering our updated list of PMGs, we determined that approximately 70% of the PMGs belongs to the core/soft-core genome.

#### Conclusions:

The comparative genomic analysis conducted in this study provide new insights into the genomic content and variability of *L. plantarum*. This study provides a comprehensive pan-genome analysis of *L. plantarum*, including the largest number (N=127) of complete *L. plantarum* genomes retrieved from publicly-available repositories. Our effort aimed to determine a solid reference panel for the future characterization of newly sequenced *L. plantarum* strains useful as probiotic supplements.

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

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#### Objectives:

Feed fermentations in animal production deliver high cell counts of probiotic lactobacilli as well as bioactive compounds. Antimicrobial phenolic compounds are abundant in canola meal but can be converted to derivatives with different antimicrobial activity during lactic fermentation. This study aimed to quantify phenolic acids in canola meal fermented with probiotic lactobacilli, and to investigate the antimicrobial activity of fermented and unfermented canola meal against intestinal bacteria in chickens *in-vitro*.

#### Methods:

Canola meal was fermented for 24 h with *Lactiplantibacillus plantarum* TWM1.460, *Furfurilactobacillus rossiae* FUA3583 or its mutant FUA3583 *deltapar1deltapar2*, or *Limosilactobacillus reuteri* FUA3536. Unfermented and chemically acidified samples were incubated in the same condition. Phenolic acids were quantified via high-performance liquid chromatography. Antimicrobial activity against *Fructilactobacillus sanfranciscensis* FUA3024, *Limosilactobacillus reuteri* FUA3613, and *Salmonella* FUA10060 was assessed using a critical dilution assay.

#### Results:

Sinapic acid was the major phenolic acid in canola meal with a concentration of 1498 ±3.1 mg/kg. After fermentation with *Lp. plantarum* TMW1.460, *Ff. rossiae* FUA3583 and FUA3583 *deltapar1deltapar2*, *Lm. reuteri* FUA3536, sinapic acid concentrations were reduced to 193±8.1mg/kg, 178±18mg/kg, 494±197mg/kg, 604±114mg/kg, respectively. Unfermented canola meal was most inhibitory to *Fl. sanfranciscensis*; *Lm. reuteri* and *Salmonella* were more resistant. The inhibitory activity of unfermented and fermented canola meal was generally similar but fermentation with *Lp. plantarum* decreased the antimicrobial activity.

#### Conclusions:

Lactic metabolism decreased the antimicrobial activity of phytochemicals in canola meal but this effect was not related to metabolism of sinapic acid. This result could provide insight on improved canola fermentations for enhanced gut health.

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

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### Objective:

The SIMBA capsule is a novel ingestible device aiming to sample luminal fluid in the small intestine. We aim to test performance characteristics of SIMBA in healthy volunteers and demonstrate SIMBA's safety and efficacy in tracking the microbiome profile's change in small intestine during oral probiotics ingestion in real time.

### Methods:

20 healthy volunteers ingested 2 SIMBA capsules after fasting and underwent abdominal X-rays every 30 mins out to 210 min to assess capsules' location and deployment. Capsules were independently collected and returned with a stool sample. One week later, 2 further SIMBA capsules were ingested simultaneously with a dual strain probiotic and collected when passed. Endpoints: sampling location at baseline, capsule sample and stool microbiota analysis using 16S sequencing, qPCR probiotic strain detection, safety, and subject usability assessment for both capsule sets (total 80 capsules).

### Results:

78/80 SIMBA capsules were successfully retrieved for analysis. 65/66 selected SIMBA capsules had sufficient DNA for 16s sequencing, which showed clearly different microbiota composition between SIMBA samples and stools, and between baseline and intervention SIMBA samples. Absolute quantification using probiotic strain-specific qPCR results showed SIMBA capsules detect an increase of the probiotics concentration in the small intestine after oral ingestion of the probiotics. The rest 12 capsules were sent for metabolomic analysis and results will be published in future.

### Conclusion:

The SIMBA capsule appears safe and reliable for collection of SI content which can be used for tracking spatial and temporal microbiome change in small intestine without the need for deep endoscopy.

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

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### Objective:

*Faecalibacterium prausnitzii* is a multi-skilled intestinal bacterium proposed as a next generation probiotic. However, detailed information addressing the safety of this novel probiotic (in terms of antimicrobial susceptibility) and its technological fitness is still lacking. These data are important when developing probiotic products. This work aimed to evaluate *F. prausnitzii* DSM17677 susceptibility when exposed to selected antimicrobials, oxygen, acidic pH and bile.

### Methods:

Antimicrobial susceptibility of *F. prausnitzii* DSM17677 to ampicillin, vancomycin, gentamicin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline and chloramphenicol was assessed following European Food Safety Authority guideline. *Faecalibacterium prausnitzii* DSM17677 cultures were exposed to: 1) ambient air up to 5-minutes; 2) acidic pH (3 and 5) during 2-hours; 3) bile concentrations (0.1, 0.25 and 0.5 %) up to 3-hours. Viability was determined by colony-forming units plating (CFU) at defined time-points.

### Results:

*Faecalibacterium prausnitzii* DSM17677 was susceptible to vancomycin, clindamycin, tetracycline and chloramphenicol. Moreover, this strain exhibited high viability reductions (>4 log CFU/ml) after 1-minute of aerobic exposure of inoculated plates, and after 1-hour exposure to pH 3 and in all bile concentrations tested. However, this strain tolerated well the exposure to pH 5 for 2-hours.

### Conclusions:

Given high *F. prausnitzii* DSM17677 sensitivity to aerobic atmosphere, pH 3 and bile, our data revealed the need to develop delivery systems able to promote the viability and stability of this bacterium when subject to such environmental stresses, envisaging its future application as a probiotic strain. Furthermore, this work contributes to the establishment of *F. prausnitzii* DSM17677 antimicrobial susceptibility profile.

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

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##### Objective:

*Lactobacillus reuteri* colonizes the human gastrointestinal tract, where it can stimulate the host immune system, modulate the microbiota composition, and prevent pathogen colonization thanks to the release of several antimicrobial compounds. The aim of this work was the assessment of the antimicrobial and antibiofilm activity of the Cell Free Supernatant (CFS) produced by *L. reuteri* DSM 17938 versus *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus mutans*.

##### Methods:

The CFS was obtained through the centrifugation with centrifugal filter devices of 10K of *L. reuteri* broth cultures. The Minimum Inhibitory Concentration was determined through the broth microdilution method, the viability assay and Colony Forming Units (CFU) counts, while the Minimum Bactericidal Concentration through CFU counts. The antibiofilm activity was evaluated by assessing the Minimal Biofilm Inhibitory Concentration and the Minimal Biofilm Eradication Concentration through CFU counts, Cristal Violet and a metabolic assay. In addition, the cytotoxicity of CFS was tested on human cell lines.

##### Results:

The CFS showed a satisfactory antibacterial activity toward all the microorganisms tested. Regarding the antibiofilm efficacy the CFS showed MBEC corresponding to 1×MIC versus *P. aeruginosa* and *S. aureus* and corresponding to 2-3×MIC versus *E. coli*. No effect has been detected toward *S. mutans*. The safety profile toward human cell lines showed promising sureness.

##### Conclusions:

CFS can be useful for developing alternative therapeutic strategies against bacterial infections associated with biofilm-producer microorganisms. Further studies should be performed to detect the CFS components responsible of the antimicrobial and antibiofilm activities.

#### INADEQUATE SAFETY REPORTING IN RCTS IN IRRITABLE BOWEL SYNDROME. A SYSTEMATIC REVIEW: PHARMACEUTICAL INTERVENTIONS VS PROBIOTIC INTERVENTIONS

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Randomised clinical trials (RCTs) offer a unique opportunity to obtain controlled efficacy and safety data to support clinical decisions. However, most RCTs have a stronger focus on efficacy rather than safety. In connection to this paper, a meta-analysis was conducted and published to evaluate the efficacy of probiotics compared to that of pharmaceuticals in Irritable Bowel Syndrome (IBS). To compare the burden to benefit ratio between probiotic as well as pharmaceutical interventions, we aimed to identify the safety profile of both intervention types. RCTs including participants (>16 years old) with IBS comparing probiotic or pharmaceutical interventions with placebo were identified by systematic searching of MEDLINE (January2015 – November2020). Although inclusion criteria were similar for both intervention types, substantial differences between safety profiles for both pharmaceutical and probiotic *control* groups were identified. Several inconsistencies in safety reporting were identified between and within pharmaceutical and probiotic studies, that could be categorized by: didn't report on safety; only reported Adverse Reactions (ARs) or Adverse Events (AEs) with a certain severity; didn't report the total number of AEs; didn't split in the control- or experimental arm; didn't specify AEs; and used different thresholds for 'common' AEs. Hence, it is difficult to compare safety data from pharmaceutical and probiotic RCTs across and between different studies. In conclusion, based on the current approaches to safety reporting we could not establish an unambiguous safety profile for probiotic and pharmaceutical interventions in IBS. Therefore, a critical comparison of the benefit to burden ratio was not possible.

### VARIABILITY OF ANTIMICROBIAL AND ANTIFUNGAL EFFECT OF *LACTOBACILLUS PLANTARUM* AND *LACTOBACILLUS ACIDOPHILUS*

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An essential point in the prevention and complex bacteriotherapy of dysbiosis is the level and spectrum of antagonistic activity of probiotic bacteria.

#### Aim:

To reveal the nature of the relationship between industrial strains of lactobacilli (LB) and opportunistic microorganisms (UPM) at the ultrastructural level.

#### Materials and methods:

Industrial strains *Lactobacillus plantarum* 8RA-3 and *L. acidophilus* D75. Clinical isolates: 8 strains of *S. aureus* producing  $\alpha$ -hemolysin, 20 strains of *E. coli* Hly+, 12 strains of *C. albicans* were detected using transmission electron microscopy on a JEM-100C (JEOL, Japan).

#### Results:

With the manifestation of antagonistic activity of LB in relation to UPB of various types, significant changes were found in all interacting cells. Thus, extensive invaginations of intracytoplasmic membrane structures appeared in LB cells, the formation of which indicated the activation of metabolic processes in them.

Electron microscopic examination of co-grown cultures of LB with UPB and *C. albicans* in places of close cell contact revealed significant destructive changes in the cells of LB themselves. The main differences were in the nature of the destruction of cell walls by the type of desquamation of small layer-by-layer fragments of peptidoglycan layers. Along with destructive changes in the cell wall, a specific change in the ultrafine structure of the protein-ribosomal complex of the cytoplasm of lactobacilli was noted.

#### Conclusion:

Ultrastructural changes revealed during the joint cultivation of LB with *S. aureus*, *E. coli* Hly+, and *C. albicans* testified to the strict specificity of the interaction of these microorganisms.

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

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

**AKKERMANSIA MUCINIPHILA ANTIMICROBIAL SUSCEPTIBILITY PROFILE**

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**Objective:**

This study aims to characterize the antimicrobial susceptibility profile of *Akkermansia muciniphila* DSM 22959, a human commensal and next-generation probiotic candidate, using phenotypic and *in silico* analyses.

**Methods:**

**Phenotypic antibiotic susceptibility assessment:** *A. muciniphila* DSM 22959 was grown in PYGM medium and subcultured at least twice before use. Minimum inhibitory concentration was determined for 8 clinically relevant antimicrobials, as recommended by EFSA-FEEDAP, using broth microdilution and E-test<sup>®</sup> methods. Both assays were performed at least with three independent replicates and with technical duplicates.

**In silico analysis:** Antimicrobial resistance genes (ARG), virulence factors (VF), genomic islands (GI) and mobile genetic elements (MGE) were predicted in *A. muciniphila* DSM 22959 whole genome (accession number: NZ\_CP042830.1) using several available databases and bioinformatics tools.

**Results:**

Phenotypically, *A. muciniphila* DSM 22959 shows susceptibility to ampicillin, tetracycline, colistin and fosfomicin and is resistant to gentamycin, kanamycin, streptomycin (aminoglycosides) and ciprofloxacin. *Akkermansia muciniphila* contains 26 annotated ARG that support the observed resistance profile. Other ARG might not be expressed under the tested conditions. Most ARG and VF are not embedded within GI or MGE. No plasmids were reported for this strain.

**Conclusions:**

The same susceptibility categorization was obtained in both phenotypic methods. The phenotypic resistance profile is supported by the genomic context. However, there is no evidence of horizontal acquisition or potential transferability of the identified ARG and VF. Thus, the antimicrobial susceptibility profile of the probiotic candidate *A. muciniphila* DSM 22959 meets the safety criteria required to be considered for human consumption.

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

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**Objective:**

After *Hafnia alvei* HA4597 proved able to produce Caseinolytic peptidase B (ClpB), a peptide mimicking the satiety hormone alpha-MSH and showed promising results on the reduction of food intake and body weight gain in mice models of obesity (Lucas et al., 2020, Legrand et al., 2020), the objective was to investigate the probiotic strain's efficacy in weight reduction and metabolic health parameters in overweight adults.

**Methods:**

236 overweight adults were included in this 12-week prospective study. They received standardized counselling for a -20% hypocaloric diet. Subjects of the HA group received 2 capsules per day providing 100 billion bacteria, while the placebo group received 2 capsules of placebo. The primary outcome was the proportion of responders, defined as subjects who lost at least 3% of baseline body weight at 12 weeks.

**Results:**

The proportion of responders was significantly superior in the HA group (57,7%) compared to the placebo group (41,7%). In addition, the reduction in Body Mass Index (BMI), hip circumference and fasting glycemia were significantly greater in the probiotic group. The feeling of fullness and the global satisfaction were also greater in the HA group.

**Conclusions:**

A 12 week supplementation with HA4597 significantly improves weight loss, feeling of fullness and glycemia levels in overweight subjects under hypocaloric diet. These data support the use of *Hafnia alvei* HA4597 in the global management of excess weight.

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

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### Objective:

The use of hemp seed bran (HB) in industrial food application has not been tackled yet, and hemp bran has traditionally been discarded during hemp seed powder processing. Knowledge on the functional capabilities of HB is very limited. For example, it is not known the impact of HB on intestinal microbiota, in particular on that of large intestine, where the vegetable fibers are fermented and degraded.

### Methods:

In this work, we investigated in depth the prebiotic potential of HB and transformed HB in comparison to fructooligosaccharides (FOS) underwent human distal colonic fermentation using the *in vitro* colon model MICODE (multi-unit *in vitro* colon gut model). During the 24 h of fermentation at different time points, volatilome analysis (SPME GC/MS), and microbiota analyses (MiSeq and qPCR) were performed.

### Results:

The results indicated that HB transformed samples in an healthy ecological condition of the human colon are able to: i) preserve microbial eubiosis; ii) increase the abundance of beneficial bacterial groups, such as *Bifidobacterium* and *Akkermansia*; iii) produce bioactive low organic fatty acids; iv) reduce detrimental compounds, such as p-cresol; v) generate a striking value of prebiotic index; vi) limit opportunistic and proteolytic bacteria (*Collinsella* and *Desulfovibrio*).

### Conclusions:

Our study evidenced the prebiotic role of transformed HB through a critical evaluation of its functionalities on the gut microbiota, thereby valorizing the use of hemp seed byproduct, as a food supplement

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

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### Objective:

Texturized soy protein has been widely used as a meat analogs and garnering attention due to its nutritional advantages when compared to conventional animal proteins. The present work aims to compare the impact of textured soy with traditional food containing pasta and meat, on pH shift, intestinal microbiota, and metabolism of short-chain fatty acids (SCFA) using a batch of human fecal culture fermentation.

### Methods:

Prior to the fermentation, samples were *in vitro* digested, passing through mouth, gastric, and small intestine simulation phases, and then *in vitro* fermented for 6, 24, and 48 h. The shift of pH, gas, and short-chain fatty acids (SCFAs) production, as well as changes in gut microbiota, were evaluated along the fermentation time.

### Results:

A significant decrease was observed in pH over time in media with fermentable sources when compared with negative control. SCFA concentration increased over time and it was significantly higher for both textured soy and potato:meat when compared with inulin (positive control). For potato:meat and inulin, acetate was the major SCFAs produced during fermentation time whereas for textured soy was butyric acid. Butyric acid production was 10-fold higher in medium containing textured soy when compared with potato:meat and inulin. Textured soy showed a significant increase in *Bifidobacterium* and *Lactobacillus* when compared to the remaining fermentable sources.

### Conclusions:

Textured soy presented a strong prebiotic effect and significant increase butyric acid production that plays an important role in the prevention of colorectal cancer. These results suggest that consumption of textured soy used alone or as an ingredient of novel functional foods, may contribute to improving intestinal health and therefore human health promotion.



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

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#### Objective:

Pancreatic cancer (PC) is an aggressive and chemotherapy-resistant cancer. Gemcitabine treatment shifts the intestinal microbiota of PC mice towards an inflammatory profile which may worsen side effects. We investigated whether a specific probiotic blend, by rebalancing microbiota, could reduce gemcitabine-induced inflammation and side effects.

#### Methods:

Probiotics were administered to PC xenografted mice. Histopathological stainings were performed on cancer sections to evaluate morphology, proliferation, DNA damage, collagen deposition and epithelial-mesenchymal transition. Intestinal sections were stained with HE, Ki67 and Alcian Blue/PAS. Fecal DNA underwent 16S rRNA sequencing to analyze microbiota, blood samples were collected to assess blood cell count, biochemical parameters and to perform serum metabolomics. Cell-free supernatants (CFSs) prepared from single probiotic strains were assayed on BxPC-3 cells and their composition was analyzed by metabolomics approach.

#### Results:

Mice receiving probiotics displayed a retardation trend in tumor growth, with tumor showing a decreased stromatogenesis and mesenchymal phenotype. A milder intestinal damage, an improved blood count, an increase in fecal species richness and in short chain fatty acids-producing bacteria were also observed. Serum levels of amino acids, choline and pyruvic acid significantly dropped upon probiotics consumption. CFSs-derived from *Bifidobacterium bifidum* and *Bifidobacterium breve* were the most effective in inhibiting cell migration, affecting cell cycle and inducing apoptosis. Amino acids, nitrogenous bases, vitamins, pyruvate and butyrate metabolism resulted among the most represented pathways in CFSs.

#### Conclusions:

These results suggest that specific probiotics administration could help relieving some adverse effects of gemcitabine in the setting of PC treatment by restoring a favorable microbiota.

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

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#### Objective:

Neuroinflammation can severely affect brain homeostasis and adult hippocampal neurogenesis with detrimental effects on cognitive processes. Brain and gut are connected via the "gut-brain axis", a bidirectional communication system, whose modulation through probiotics could represent an intriguing approach for the prevention or even the cure of several diseases. In the present study we evaluated the putative neuroprotective effect of prolonged consumption of a multi-strain probiotic formulation (named OttaBac) based on food-associated strains and human gut bacteria in a mouse model of acute inflammation.

#### Methods:

Mice were gavaged with OttaBac (10<sup>9</sup> CFU/mouse/day) for 15 days before a single intraperitoneal injection of LPS (0.1mg/kg). We sacrificed the animal after 2 and 24 hours from LPS treatment and we evaluated physiological and behavioral changes, proliferation and differentiation of the new neurons within the hippocampal dentate gyrus, the neuroinflammatory response, the modifications of intestinal permeability and of the inflammatory state in OttaBac versus vehicle-administered mice

#### Results:

The results indicate that the administration of OttaBac not only prevents the LPS-dependent increase of pro-inflammatory cytokines in specific regions of the brain (hippocampus and cortex) and in the gastrointestinal district, but also triggers a potent proneurogenic response capable of enhancing hippocampal neurogenesis. This effect is accompanied by a potentiation of intestinal barrier, as documented by the increased epithelial junction expression in the colon.

#### Conclusions:

Our hypothesis is that pre-treatment with the multi-strain probiotic formulation helps to create a systemic protection able to counteract or alleviate the effects of LPS dependent acute pro-inflammatory responses.

## SACCHAROMYCES CEREVISIAE BASED PROBIOTICS OUTPERFORM LACTOBACILLI IN INHIBITION OF VAGINAL CANDIDIASIS

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### Objectives:

Although progress has been made to equalize the rights of men and women, female-specific issues, such as vulvovaginal candidiasis (VVC), are still studied less compared to males. This is striking, as VVC affects 75% of all women at least once in their life. In case of recurrent (R)VVC, women experience at least four episodes of infection every year, further increasing the emotional and economic burden.

Especially in case of RVVC, treatment is insufficient, as misdiagnosis, recolonization and resistance impair clearance. Here, we aim to develop a probiotic therapy to target *Candida* infections in the vaginal niche.

### Methods:

Most of the research on VVC has been performed in non-optimal conditions, using systemic *Candida* isolates and non-representative medium. We used an optimized *in vitro* platform to select promising probiotics against VVC.

### Results:

1) In this study, we screened a large set of vaginal *Candida* isolates for virulence properties in the vaginal niche. The isolate representing the highest pathogenicity was used to identify promising probiotic organisms. 2) The use of lactobacilli as probiotic therapy against VVC is debated. We find that certain bacterial strains increase rather than inhibit pathogenicity of *Candida* and point out the role of lactic acid in this process. We also show the potential of *Saccharomyces cerevisiae* strains in inhibiting virulence by *Candida* species. We identified a role for specific fatty acid metabolites in this process.

### Conclusions:

By using an appropriate platform, we validated the potential of *S. cerevisiae* in inhibition of *Candida* virulence in the vagina.

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

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### Objective:

The aim of this study is the development of a multi strain probiotic gel to promote lactobacilli-dominated vaginal microbiota in pregnant women and to establish a proper eubiosis on the new-born. Mainly nomadic lactobacilli, isolated from food sources, were screened for functional characteristics and the capability to inhibit *Streptococcus agalactiae*, *Staphylococcus aureus* and *Candida albicans*, which may lead to adverse pregnancy-related outcomes.

### Methods:

One hundred fourteen strains were screened for hydrophobicity, auto-aggregation, peptide hydrolysis, hydrogen peroxide production, and lactic acid isomers quantification. Cell-free supernatants (CFSs) of the candidate strains were co-inoculated with vaginal pathogens for high-throughput inhibition screening. Aiming to evaluate the reduction of the expression of genes involved in the inflammatory cascade the best performing strains were investigated *in vitro* alone and in combination.

### Results:

Fifteen *Lactiplantibacillus plantarum* strains showed outstanding hydrophobicity traits. The auto-aggregation capacity was specie-independent, while the peptide concentration distribution was quite similar among lactobacilli. The production of hydrogen peroxide was strain dependent, with the highest concentrations found for *Lactocaseibacillus paracasei*. *Lb. plantarum* produced both isomers of lactic acid, while *Lb. paracasei* produced only L-isomer. *S. aureus* and *S. agalactiae* were strongly inhibited by a wide range of CFS in different modes of action, whereas *C. albicans* inhibition was less frequent.

### Conclusions:

Overall, *L. plantarum* had the highest pathogen inhibition score and the best functional traits. Two of the best performing strains showed a reduction on the expression of genes involved in the inflammatory cascade in human keratinocytes.

# POSTERS

## EUBIOTICS, A NEW CATEGORY OF MICROBIOTA REGULATORS FOR IRRITABLE BOWEL SYNDROME (IBS): RESULTS FROM A RANDOMIZED DOUBLE BLIND PLACEBO-CONTROLLED TRIAL.

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### Objective:

Essential oils (EO) are volatile compounds that include several aromatic and aliphatic constituents generally belonging to the chemical families of terpenes and terpenoids. EOs have shown to have multitarget positive effects on the intestinal microbiota, different from those obtained with the use of probiotics, prebiotics and postbiotics. The primary objective of the study was to evaluate changes in the microbiota after 4 weeks of administration a food supplement based on Palmrose Essential Oil (*Cymbopogon martinii*) titrated in geraniol (>80%) absorbed on ginger rhizome fiber in patients with a confirmed diagnosis of IBS (Roma III criteria). The secondary objective of the study was to evaluate the impact of the food supplement on clinical symptoms (abdominal pain, bloating, intestinal habits,) of IBS patients, registered through the validated IBS-Visual Analogue Scale questionnaire.

### Methods:

The study was multicenter, prospective, double-blind, placebo controlled. 56 patients were enrolled, in the study and were randomized 1:1 to receive a food supplement (50 mg/Kg<sup>(-1)</sup> die) consisting of Palmrose Essential Oil adsorbed on ginger root powder or placebo (corn starch) for four weeks. The study consisted of two visits, on each visit a clinical examination was performed, fecal and blood samples were collected, and patients filled the IBS-VAS questionnaire. The intestinal microbiota was analyzed starting from fecal samples using the 16sRNA analysis (NGS).

### Results:

Microbiota analysis showed a significant rise ( $P < 0.05$ ) in alpha diversity in the active group which was not detectable in the placebo group. A rise of SCFAs producers *Faecalibacterium* (near to statistical significance,  $p = 0.1$ ) and *Blautia* ( $p = 0.047$ ), a non-significant decrease of *Collinsella* and a rise of *Prevotella* were observed at V2 in the active group. A significant reduction in the IBS-VAS Score was registered in the active group, with the average value decreasing from  $226.60 \pm 52.55$  to  $162.60 \pm 70.74$  ( $p = 0.0007$ ) after 4 weeks of treatment. No significant decrease was detected in the placebo arm after 4 weeks of treatment (from  $238.54 \pm 63.51$  to  $229.79 \pm 64.98$ ;  $p = 0.6393$ ).

### Conclusions:

Our study showed that oral administration Palmrose EO titrated in geraniol absorbed on ginger root fiber is able to counteract intestinal dysbiosis and ameliorate clinical symptoms in IBS patients.

## EFFECT OF LACTOFLORENE PANCIA PIATTA® IN WOMEN WITH SYMPTOMS RELATED TO IRRITABLE BOWEL SYNDROME

Alessia Farioli<sup>(1)</sup>

Montefarmaco, marketing, Milan, Italy<sup>(1)</sup>

### Objective:

#### Background

Irritable bowel syndrome (IBS) is a non-organic gastrointestinal disorder that adversely affects the quality of life. The etiology of the disease is not fully understood, and appropriate treatment is still a matter of debate. The prevalence of IBS in the global population is estimated at 11% and the prevalence in women is about twice as high as in men. Half of patients report their first symptoms before the age of 35. In patients with IBS there are qualitative and quantitative changes in the composition of the gut microbiota, which has significant therapeutic implications. Disturbed motor activity of the gastrointestinal tract and visceral hypersensitivity are typical but not completely specific features of IBS. The diagnosis of IBS should be based on the Rome IV criteria. They consisted in recurrent abdominal pain on average at least 1 day/week in the last 3 months, associated with 2 or more of the following:

1. Related to defecation and/or
  2. Associated with a change in frequency of stool and/or
  3. Associated with a change in form (appearance) of stool
- Rome IV recognizes that patients may also report symptoms of abdominal bloating (subjective) and/or distension (objective/visible increase in abdominal girth).

The Research Hypothesis for the present study is to assess the effects of a food supplement containing probiotic live lactic acid bacteria, with enzymes, Melissa, Passionflower and Chamomile in toward symptoms in women with IBS

### Methods:

#### Design:

Interventional, controlled prospective trial on one cohort of patients

#### Inclusion Criteria

- Women between 18-70 years with symptoms of IBS who meet the Rome IV criteria for the diagnosis of IBS
- Read and signed informed consent.

#### Number of patients and sample size

30 enrolled women

#### Trial design

The study was a 20-days interventional trial. During the intervention, participants will take Lactoflorene Pancia Piatta® sachets, once a day for 20 days. Throughout the study, the subjects will not allow to consume any medications that could influence gut motor or microbiota, including laxatives, anti-diarrheal agents, antibiotics, and probiotics. They will fill out the weekly questionnaire GSRS. After 4 weeks a comprehensive evaluation of data will be performed for each patient.

### Results:

The outcomes of the trial confirm the assumption of a beneficial effect of a food supplement containing probiotic live lactic acid bacteria, with enzymes, Melissa, Passionflower and Chamomile as an adjuvant in women suffering from abdominal symptoms, especially characterized by bloating

### Conclusions:

Treatment with Lactoflorene Pancia Piatta®, a food supplement containing probiotic live lactic acid bacteria, with enzymes, Melissa, Passionflower and Chamomile proved to be effective and safe as an adjuvant in the management of women with abdominal symptoms related to irritable bowel syndrome

### ONE HEALTH CONCEPT: ESBLs AND PLASMID AMPC IN RESISTANT *ESCHERICHIA COLI* FROM PIGLETS FECES

Olga Cardoso<sup>(1)</sup> - Sofia Osório<sup>(2)</sup> - Fernando Ramos<sup>(3)</sup> - Maria Manuel Donato<sup>(4)</sup>  
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#### Objective:

Global emergence of antimicrobial resistance can be disseminated from animals to humans and vice-versa leading to the One Health concept. This concept is particularly relevant in food safety and combatting antibiotic resistance. Transmission of multidrug-resistant strains to humans can occur through direct contact or consumption of contaminated food, water or surfaces. The aim of this study was to determine susceptibility profiles of *Escherichia coli* in piglets intestinal microbiota. The occurrence of multiple antibiotic resistance and genotypic detection of ESBLs and pAmpC were done.

#### Methods:

Feces from piglets (n=75) of a dense livestock industry, were obtained in slaughter house, and 340 *E. coli*, bacteria biomarker, were identified. Determination of susceptibility, by agar disk diffusion, to amikacin, ciprofloxacin, cotrimoxazole, aztreonam, ceftazidime, cefepime, amoxicillin+clavulanic acid, ceftiofur and piperacillin. Detection of ESBL-genes (*bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX-M</sub>) and AmpC-genes (*bla*<sub>CMY-2</sub>, *bla*<sub>ACC</sub>), using Real-Time PCR.

#### Results:

Twelve isolates were classified as extensively drug-resistant, and one as pandrug-resistant and presented different enzyme profiles. The most observed resistant determinant was *bla*<sub>CTX-M</sub> (n=9), alone (n=3) and associated with other determinants (n=6). Associations of *bla* genes were detected in 7 isolates: two with 3 genes; two with 2 ESBLs genes, and three with 1 ESBL and 1 pAmpC.

#### Conclusions:

*E. coli* isolates showed high resistance to antimicrobials. High presence of ESBL and AmpC genes (alone or associated) was found. According to the One Health, monitoring multidrug-resistant bacteria in different environmental matrices is essential not only from the perspective of environmental safety but also to prevent the spread of antimicrobial resistance.

### ONE HEALTH CONCEPT: RESISTANT ENTEROCOCCI FROM PIGLETS FECES

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 University of Coimbra, CIMAGO, FMUC, Coimbra, Portugal<sup>(1)</sup> - Augusto Virgílio Sousa & Filho, Lda, Augusto Virgílio Sousa & Filho, Lda, Coimbra, Portugal<sup>(2)</sup> - University of Coimbra, University of Oporto, FFUC, REQUIMTE/LAQV, Coimbra, Portugal<sup>(3)</sup> - University of Coimbra, CIEQPF, FFUC, Coimbra, Portugal<sup>(4)</sup>

#### Objective:

In One Health concepts, antimicrobial resistance is usually ranked amongst the top concerns on which veterinary and public health need to collaborate.

Frequent occurrences of antimicrobial agents-resistant enterococci have been observed among food animals and it has been suggested that animals may be a reservoir of resistant genes capable of transferring to humans through food chain. The aim was to determine the resistance profile of enterococci isolated from piglets feces in dense livestock industry of Portugal.

#### Methods:

Feces from piglets (n=75) were obtained in abattoir, and 132 enterococci, bacteria biomarker, were identified. Susceptibility determination, by agar disk diffusion, to vancomycin (VAN), ciprofloxacin (CIP), linezolid (LNZ), tigecycline (TIG), ampicillin (AMP) and imipenem (IP). *Enterococcus* species were confirmed by Real-Time PCR.

#### Results:

*Enterococcus faecalis* (N=40), *Enterococcus faecium* (N=47) and other *Enterococcus* sp. (N=45) were identified. *E. faecium* showed 68% of resistance, *E. faecalis* 60% and *Enterococcus* sp. 58%. Resistance to one antibiotic was verified in more than 50% of the enterococci. 2 *Enterococcus* sp, 2 *E. faecium* and 1 *E. faecalis* were considered multidrug-resistant (resistance to three antibiotics). Enterococci showed high resistance to CIP (35.6%) and IP (31.8%). Resistance to IP was essentially observed in *E. faecium* (61.7%). The major resistance to CIP was detected in *Enterococcus* sp (46.7%) and *E. faecalis* (45%).

#### Conclusions:

The resistance observed is worrisome as these antibiotics are used in human medicine. According to One Health, this study contributes to development of policies in order to reduce these bacteria in livestock and their spread to humans and throughout the environment.

#### DEVELOPMENT OF FIBER-ENRICHED YOGURT USING ISOTHERMAL MICROCALORIMETRY FOR THE ASSESSMENT OF FIBERS' STABILITY DURING FERMENTATION

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AS TFTAK, Food Department, Tallinn, Estonia <sup>(1)</sup> - AS TFTAK, Analytics Department, Tallinn, Estonia <sup>(2)</sup>

##### Objective:

To develop a fiber-enriched yogurt containing at least 6 g of fibers per 100 g of the product and assess the stability of fibers during shelf-life. The mixture of the fibers added to the yogurt should consist of both water soluble and insoluble fibers and contain 3-4 different fibers to support the growth of beneficial bacteria in the human gut.

##### Methods:

The growth of bacteria in fiber-enriched yogurt was studied using a multi-channel isothermal microcalorimeter. Starter culture used for fermentation contained *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Probiotic bacteria *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus acidophilus* LA-5 were added for additional health benefits. Microcalorimetric approach allowed evaluation of the stability of added fibers during the fermentation process and assure that the fibers are not consumed by bacteria. The total dietary fiber content was measured with AOAC 2017.16 method.

##### Results:

Yogurt was enriched with a mixture of four different soluble and insoluble fibers: resistant starch, polydextrose, high polymerization inulin, and xylo-oligosaccharides in total 6.35 g / 100 g. Calorimetric results confirmed intactness of the added fibers both at the end of the fermentation process and shelf-life.

##### Conclusions:

Developed yogurt was enriched with a mixture of four fibers supporting the growth of beneficial gut microbiota. Microcalorimetric method gave a comprehensive overview of the peculiarities of bacterial growth in yogurt, and moreover provided information about the metabolic processes that occurred in the media, such as the possible degradation of fibers.

#### CONSUMPTION OF MULTI-FIBER ENRICHED YOGURT INCREASED THE LEVELS OF CATENIBACTERIUM MITSUOKAI AND PARABACTEROIDES DISTASONIS.

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##### Objective:

Previous human trials involving synbiotics have mainly concentrated on identifying the positive effects of prebiotics on the viability and growth of supplemented probiotic strains. Only recent studies incorporating 16S rRNA amplicon sequencing have been able to detect the effect of synbiotic products on the other members of the gut consortia. Here, we aimed to evaluate the effect of synbiotic yogurt on human gut microbiota, stool form and frequency.

##### Methods:

In the 2-week parallel controlled intervention, 81 subjects consumed either a 200 g of yogurt (control) or the same yogurt supplemented with 9.8 g of dietary fibers (test): resistant starch, polydextrose, high polymerization inulin, and xylo-oligosaccharides. Fecal samples were collected before and after the intervention, and 16S sequenced. At both timepoints, participants provided information about Bristol Stool Scores along with weekly fecal frequency.

##### Results:

Both control and test yogurts increased the prevalence of butyrate-producing bacteria and reduced the prevalence of *Escherichia coli*. Intake of fiber-enriched yogurt specifically increased the levels of *Catenibacterium mitsuokai* and *Parabacteroides distasonis* in the fecal microbiota. *Escherichia* was correlated negatively with *Bifidobacterium animalis* in the test group. Stool frequency was reduced only in the control group.

##### Conclusions:

Intake of fiber-enriched yogurt introduced significant changes to the gut microbial community and supported the maintenance of gastrointestinal activity compared to regular yogurt. However, the effect on prevalence of gut bacteria was similar in both groups. We have made an important step towards analysing the full effect of a synbiotic products on gut communities.

### UNDERSTANDING THE CONTRIBUTION OF DIFFERENT FOOD GROUPS TO GUT MICROBIOME IN ELDERS: AN EXPLORATORY APPROACH

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#### Objective:

This contribution aimed to gain insight about the relationship between dietary habits, and other relevant lifestyle factors, on the relative abundance of colonic species in the microbiota of Spanish elders participating in the study.

#### Methods:

A cohort of 20 volunteers  $\geq 65$  years-old were asked to answer a questionnaire of 35 items related to the intake frequency of different food groups and other relevant lifestyle habits. Fecal microbiome of participants was characterized by amplicon-based sequencing of the region V3-V4 16S rRNA by Illumina MiSeq sequencer using the automated cluster generation and paired-end sequencing with dual indexes reads (2  $\times$  300 bp). A Principal Component Analysis (PCA) was also applied to establish the significance and relationship among the studied variables.

#### Results:

PCA separated two different groups of Spanish elders according to their profiles of microbiota. On one hand, PCA found relationship between an increased relative abundance of phyla Firmicutes, Verrucomicrobia and Actinobacteria (56, 1.3 and 0.2%, respectively), genera *Ruminococcus*, *Roseburia* and *Bifidobacterium* (3.8, 2.2 and 0.04%, respectively) and a higher weekly consumption of meat, legumes and desserts. On the other hand, phyla Proteobacteria and Bacteroidetes (2.1 and 32.2%) and the genus *Faecalibacterium* (9.25%) were more abundant in those individuals who presented a higher intake of dairy products, fish and fruits, and with a higher intestinal transit.

#### Conclusions:

Protein dietary source (legumes, fish, meat and dairy products) together with the intestinal transit frequency seem to be responsible of the differences found in the microbiome of the Spanish elders volunteers. Even though all subjects presented, abundance of phyla and genera commonly associated to a good health status; the specific differences, mainly in *Bifidobacterium*, suggest that fruits, fish and dairy consumption increase its abundance.

### MICROBIAL DIVERSITY OF FERMENTED GREEK OLIVES AS REVEALED BY METAGENOMIC ANALYSIS AND PROBIOTIC POTENTIAL OF ISOLATES

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#### Objective:

To evaluate the bacterial and yeast diversity of fermented olives of different varieties from different regions - green olives, cv. Halkidiki, and black olives, cv. Konservolia, - by conventional microbiological methods and NGS. Furthermore to evaluate the probiotic potential of isolated lactic acid bacteria (LAB) and yeasts.

#### Methods:

Total Viable Counts, Lactic acid bacteria (LAB), yeast and molds, and *Enterobacteriaceae* were enumerated. Microbial genomic DNA subjected to NGS for the identification of bacteria and yeasts. The probiotic potential of LAB and yeasts was evaluated by in vitro tests according to Argyri et al. 2013 including survival under conditions simulating the human GI tract, antimicrobial activity, haemolytic activity, antibiotic resistance etc.

#### Results:

NGS analysis showed no difference in LAB diversity and dominance of *Lactobacillus*. *Wickerhamomyces* was the most abundant yeast genus in Conservolea olives from Magnesia region, while *Pichia* sp. and *Pichia membranifaciens* dominated in Conservolea olives from Fthiotida and *Pichia manshurica* in Chalkidiki olives from both regions. A significant number of isolates showed probiotic potential.

#### Conclusions:

The results will contribute to the microbial identity of table olive varieties in relation to their origin. In addition probiotic candidates can be used as probiotic starters for the improvement of the traditional fermentation process and the production of novel foods.

This research has been financed by Greek national funds through the Public Investments Program (PIP) of GSRT, under the Emblematic Action "The Olive Road" (2018ΣΕ01300000). Partial contribution of the project STER1-0026295 funded under the Operational Program "CENTRAL GREECE" 2014 - 2020 is acknowledged.

**EFFECT OF ORGANIC HERBS ON GROWTH OF SALMONELLA ENTERITIDIS IN MEAT BROTH**

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**Objective:**

This study aimed to evaluate the effect of organic herbs on the growth of *Salmonella* Enteritidis in meat broths.

**Methods:**

*Salmonella* was inoculated in meat broths containing organic oregano, thymus, summer savory and crithmum as well as mixtures of them. Two growth media were used i.e the laboratory medium meat extract (ME) broth and chicken meat broth (B) which was prepared by cooked meat to better simulate the food matrix. The effect of these herbs, on *Salmonella* Enteritidis growth parameters was monitored by microplate reader for 24 hours at 37°C.

**Results:**

According to the obtained results the organic herbs influenced the growth of the pathogen. This influence was related with the herb species, the added quantity and the growth medium used. More specific the lag phase of *Salmonella* was increased in most of the cases, when *Salmonella* was grown on chicken meat broth with herbs, where notable differences in lag phase were also observed in meat extract broth.

**Conclusions:**

The observed differences between the effect of the same herb on the growth of *Salmonella* in different growth media, highlights the importance of the choice of the most appropriate medium to simulate the food matrices. This observation could be fundamental for understanding the possible actions to be taken for controlling the pathogens on food, food chain and food processing environment.

This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH-CREATE-INNOVATE (project code: T2EDK-00056)

**PROBIOTIC'S FERMENTED SMOOTHIES AND GUT MICROBIOTA: POTENTIALITY OF DELIVERING PHYTOCHEMICAL IN THE LARGE INTESTINE**

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**Objective:**

Understand modifications of phytochemicals of blueberry smoothies during fermentation carried out by probiotics microorganisms and after digestion during the passage through large intestine.

**Methods:**

Fresh blueberries were purchased from local market and milled with the addition of 10% mineral water. After milling, samples of blueberries smoothies were inoculated with 10<sup>6</sup> log CFU/mL of a mix of 2 probiotics microorganisms, one *Lactiplantibacillus plantarum* (Lp 32) and one *Levilactibacillus brevis* (Lb 19) belonging to the University of Wageningen, Food Quality and Design group. Smoothies were incubated for 4 days at 30°C to ferment (Komatsuzaki et al., 2005)NFRI 7415, which was isolated from a Japanese traditional fermented fish (funa-sushi. After fermentation, samples were digested following INFOGEST protocol (Brodkorb et al., 2019) and this impedes the meaningful comparison of results. The standardized protocol presented here is based on an international consensus developed by the COST INFOGEST network. The method is designed to be used with standard laboratory equipment and requires limited experience to encourage a wide range of researchers to adopt it. It is a static digestion method that uses constant ratios of meal to digestive fluids and a constant pH for each step of digestion. This makes the method simple to use but not suitable for simulating digestion kinetics. Using this method, food samples are subjected to sequential oral, gastric and intestinal digestion while parameters such as electrolytes, enzymes, bile, dilution, pH and time of digestion are based on available physiological data. This amended and improved digestion method (INFOGEST 2.0 and ultra-centrifuged. Precipitate was used as supplementation for fecal batch cultures. In a SHIME apparatus, microorganisms mimicking ascending, transversal and descending colon microbiotas were isolated. With these bacteria a fecal batch culture was setted up, inoculating also fermented blueberries smoothie as substrates. Fecal batch cultures were carried out for 48 hours and samples for GC/MS and LC/MS were taken every 12 hours.

**Results:**

Probiotics resulted to be able to ferment the matrix maintaining the viability. Thus the product represents a candidate able to deliver probiotics microorganisms. Both fermentation of the smoothies and fecal batch cultures induced changes on matrix' polyphenols and phytochemicals.

**Conclusions:**

Blueberries smoothies resulted to be a suitable substrate for fermentation by probiotics microorganisms, that induce modification in the chemical structure of phytochemicals. Furthermore, fecal batch cultures with different microbiotas induced further modifications with a possible positive effect



### IMPACT AND COMPARISON OF PLANT MATERIALS ENHANCEMENT ON THE PRODUCTION OF FERMENTED BEVERAGES USING *MEDUSOMYCES GISEVII* SYMBIOTIC CULTURE AND BIRCH SAP

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#### Objective:

Fermented beverages enhancement of different plant materials increases the bioactive composition and sensory acceptance disparate. The present study aimed to evaluate biochemical and physicochemical properties in fermented beverages enhanced with different morphological part of plant materials.

#### Methods:

Fermentation was carried out at 28°C for 7 days aerobic and 3 days anaerobic conditions. Therefore, after 7 days, fermented solutions were enhanced with hemp leaves (*Cannabis sativa* L.), honeysuckles (*Lonicera japonica* T.) and chokeberries (*Aronia melanocarpa* L.). The total phenolic content (TPC) of samples was determined using Folin-Ciocalteu method after both fermentations. In addition, antioxidant activity was measured using DPPH<sup>+</sup> and FRAP.

#### Results:

The results showed that after additional enhancement TPC and antioxidant activities increased, including mesophilic lactic acid bacteria variety in the range of  $1.4 \times 10^4$  –  $9.2 \times 10^7$  (CFU/ml). The higher content for *M. gisevii* and birch sap was with chokeberries, where TPC (GAE/100g) increases  $21.09 \pm 1.56$ ,  $66.20 \pm 3.20$  and  $13.10 \pm 0.50$ ,  $43.1 \pm 2.53$ , respectively. For further determination, DPPH<sup>+</sup> ( $\mu\text{M TE}/100\text{g}$ ) and FRAP ( $\mu\text{M TE}/100\text{g}$ ) for birch sap before and after anaerobic fermentation were  $0.45 \pm 0.11$ ,  $2.11 \pm 0.15$  and  $0.84 \pm 0.20$ ,  $2.60 \pm 0.23$ , respectively, and for *M. Gisevii* samples were  $2.46 \pm 5.25$ ,  $2.11 \pm 0.15$  and  $4.15 \pm 0.10$ ,  $11.46 \pm 0.23$ , respectively. Moreover, the color coordinates of the products differed statistically significantly depending on the type and amount of raw materials used for the secondary fermentation.

#### Conclusions:

The novel approach of acceptable flavor probiotic beverages was investigated. The results showed that TPC and antioxidant activity significantly increased after additional enhancement. However, samples with hemp leaves had lower phenolic content and antioxidant activity than samples containing berries material.

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### SMALL INTESTINAL BACTERIAL OVERGROWTH AND PROGNOSIS IN CIRRHOSIS

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#### Objective:

Small intestinal bacterial overgrowth (SIBO) is associated with various complications of cirrhosis. The aim of the study is to determine whether the presence of SIBO affects the prognosis in cirrhosis.

#### Methods:

Fifty patients with cirrhosis were enrolled in a prospective case-control study. All participants underwent lactulose hydrogen breath test for SIBO. The follow-up period was 4 years.

#### Results:

SIBO was detected in 26 (52.0%) patients: in 10 (52.6%) patients with compensated cirrhosis and in 16 (51.6%) ones with decompensated cirrhosis. Twelve (46.2%) patients with SIBO and four (16.7%) patients without SIBO died within 4 years ( $p=0.009$ ). Among patients with decompensated cirrhosis, 8 (50.0%) patients with SIBO and 3 (20.0%) patients without SIBO died ( $p=0.027$ ). Among patients with compensated cirrhosis, 4 (40.0%) patients with SIBO and 1 (11.1%) patients without SIBO died ( $p=0.045$ ). Among patients with SIBO, there was no difference in mortality between patients with compensated and decompensated cirrhosis ( $p=0.209$ ). It was the same for patients without SIBO ( $p=0.215$ ). SIBO affects the prognosis only in the first year of follow-up in decompensated cirrhosis, and only in subsequent years with compensated cirrhosis. Presence of SIBO ( $p=0.028$ ; HR=4.2[1.2-14.9]) and blood albumin level ( $p=0.027$ ) were significant independent risk factors for death in cirrhosis.

#### Conclusions:

SIBO is associated with poor prognosis in cirrhosis.

### EFFECT OF LACTOFERRIN AS A PROTECTIVE FACTOR AGAINST INTESTINAL INFLAMMATION INDUCED BY ANTIBIOTICS

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The alteration of intestinal microbiota, or gut dysbiosis, is associated with functional changes in the microbial transcriptome, proteome or metabolome, and has been linked to many diseases. Intestinal dysbiosis can be caused by alterations in the relative abundance of bacteria, due to changes in diet, immunodeficiency, inflammation, infection or exposure to antibiotics or toxins. In this context, the search for microbiota modulators that prevent the effects of antibiotics is interesting. Bovine lactoferrin (LF) is an iron-binding glycoprotein with numerous activities: anti-inflammatory, antimicrobial and immunomodulatory. Therefore, the aim of this study was to investigate the ability of native (nLF) and saturated (sLF) lactoferrin to reverse the effects of clindamycin (Clin) on the murine gut microbiota. For this study, male C57BL/6 mice were used and divided into six groups: control, Clin, nLF, nLF/Clin, sLF and sLF/Clin. At the end of the treatments, the ileum was collected, from which the RNA was extracted and, subsequently, the cDNA. These samples were analyzed by qPCR to determine the expression of different receptors involved in the inflammation cascade, such as NOD1, NOD2, TNF-alpha, IL6, IL10, IL12p35 and IL12p40. Additionally, the histopathology of the collected tissues was studied to evaluate inflammation signs. The results showed that antibiotic treatment generally increases the expression of inflammatory receptors, whereas the addition of LF caused a decrease in the expression of receptors such as TNF-alpha or IL6. With these results, we conclude that LF could be used as a prebiotic to protect the microbiota and reduce intestinal dysbiosis when administering antibiotics.

### GUT MICROBIOTA INVESTIGATION IN EARLY RHEUMATOID ARTHRITIS (ERA) PATIENTS AFTER THREE MONTHS OF METHOTREXATE TREATMENT.

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#### Objective:

Rheumatoid Arthritis (RA) is a chronic systemic inflammatory disease, impacting small and large joints, that can also comprise other systems and organs. RA treatment is based on immunosuppressants, such as methotrexate (MTX), even if the response to treatment is not always positive. Gut involvement in RA pathogenesis seems related to the influence of colonizing bacteria on resident immune cells. Nevertheless, the impact of intestinal microbiota on therapies outcome is still unclear, as well as the therapy impact on microbiota. Our aim was to evaluate faecal microbiota composition in Early RA (ERA) patients, before (T0) and after (T1) three months of MTX treatment.

#### Methods:

We collected faecal samples from 19 ERA patients and 20 controls and characterized the microbiota. From total DNA samples extracted, the V3-V4 region of 16S rRNA gene was sequenced by Illumina MiSeq platform.

#### Results:

Reads were analyzed for biodiversity, in term of alpha and beta-diversity. Co-occurrence networks at species level were computed. Regardless of MTX treatment, for the first time an association between *Ruminococcus gnavus* and ERA patients was showed. By networks analysis *R. gnavus* seems to have a central role in microbial ecosystem balance.

#### Conclusions:

Results showed that MTX treatment exerts a positive pressure on both gut microbiota structure and its biodiversity. ERA (T1) microbiota was no longer distinct from that of controls, indicating the important role played by the microbiota in this disease. This suggests that an improvement in MTX therapy could be achieved by dispensing a therapy aimed at restoring the microbiota.

### EFFECT OF DIET MICROBIOTA-DERIVED SHORT CHAIN FATTY ACIDS IN COLORECTAL CANCER CELLS

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Colorectal cancer (CRC) is the main cause of cancer deaths worldwide. Diet modulates the colon microbiota interfering with the production of short-chain fatty acids (SCFA) (acetate, butyrate, propionate). CRC is associated with gut dysbiosis and a decrease in SCFA concentrations. SCFA interfere with several biological processes. We demonstrated that acetate presents effects in CRC cells, such as inhibition of proliferation, induction of apoptosis and lysosomal membrane permeabilization and energetic metabolism modulation. CRC cells are exposed to SCFA in combination, but their combined effect is poorly understood. We aim to understand the individual and combined effects of SCFA, unveiling their impact on CRC cells hallmarks.

We applied for the first time the concentration addiction model to predict the effects of SCFA combinations in CRC cells growth and compared it with the results obtained by sulforhodamine B. We evaluated SCFA effects in survival (by colony formation assay and annexin V/PI), energetic metabolism (glucose consumption, lactate production and expression of metabolic proteins).

SCFA, alone and combined, affect cells viability in a dose-dependent manner, being more selective to CRC cells. SCFA are more potent in combination than individually, affecting different CRC hallmarks.

We showed for the first time that the effect of SCFA on CRC cells are potentiated when combined. SCFA play a key role in the regulation of biological processes of CRC cells. These promising results indicate that increased levels of SCFA might be important for new therapeutic strategies for CRC.

### IDENTIFICATION OF THE SOLID PHASE ENZYMEBIOTIC SYSTEMS AND THEIR PROSPECTS AGAINST MICROBIAL PATHOGENS

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#### Objective:

The aim was, based on own results, to evaluate prospects of the investigated by us some solid phase enzymebiotics (EB) – enzymes with actions against pathogens.

#### Methods:

Own preparations of the probiotic lectins (PL) and L from the drug plants were used. Preliminary separated protein fractions were further identified and characterized using gel electrophoreses followed by electroblotting on membrane sandwich. L, EB, peptides, biosurfactants (BS) and polysaccharides (PS) were stained with SYPRO or glycoconjugates-biotin ([www.lectinity.com](http://www.lectinity.com)). Luminescence was analyzed in regime of live bioimagination using BioChemi System (UVP). Strains from the G.N.Gabrichevsky Research Institute collection of microorganisms were used. Activities were analyzed by standard methods.

#### Results:

(prospects- in brackets): \*purification and separation of EB on conventionally treated hydrophobic membrane (increase of antipathogenic potential of components and their complexes); \*identification of active blot zones of "EB+L" (the direct realization of antipathogenic activities of EB); \*identification of 4 protease systems of *Acilact* and its strains (the use for constructing protective consortia); \*establishment of serial active assembled oxydoreductases of *Acilact* (pl 5-5,6) and drug plants (pl 3,3-3,8) (constructing antipathogenic consortia synergistic with PL and phytolectins); \*visualization of microbial EB associated with PS or activity of L (constructing multifunctional EB, modulation/switching antipathogenic activities); \*identification of bifidobacterial probiotic system "Depolymerase+PS" (screening of depolymerases against pathogens); \*associates of bifidobacterial and lactobacillar BS with cationic endogenic PL or peptides (delivery of "EB+L" in areas of wishible degradation and/or lysis of massifs/biofilms of pathogens).

#### Conclusions:

1. EB as L or in associates with L are perspective against target sets within massifs/ biofilms of pathogens. 2. Applications of systems "EB+L" open prospects for constructing pro/pre/syn/ postbiotic systems against mixed pathogens in mucosal biotopes.

### IMMUNOMODULATORY PROPERTIES OF A PROBIOTIC COMBINATION: EFFECTS ON IMMUNE CELL POPULATIONS AND MACROPHAGE POLARIZATION IN MICE

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#### Objective:

To assess the immunomodulatory properties of a probiotic combination on innate and adaptive immune responses in mice.

#### Methods:

C57BL/6 mice were orally treated with the combination of *L. acidophilus* LA201 and *L. paracasei* LA802 (Lactibiane® Immuno, PiLeJe Laboratoire; 10<sup>9</sup> CFU/mouse) or phosphate-buffered saline (n=10/group) once daily for 14 days and then *intraperitoneally injected* or not with LPS (0.5mg/kg). Twelve hours after LPS injection, modulation of immune cell populations and activation of macrophages were assessed by flow cytometry and RT-qPCR, respectively. *Ex-vivo* microbicidal functions of macrophages (phagocytosis and killing) were evaluated against *Streptococcus pneumoniae*.

#### Results:

Interestingly, the probiotic combination decreased inflammatory response to LPS and induced changes in peritoneal immune cell populations by increasing the percentages of neutrophils, dendritic cells, natural killer cells, CD4+ and CD8+-activated T cells, Th1 cells and decreasing those of eosinophils and Th2 cells. Interestingly, peritoneal macrophage subpopulations were also modified. Although SPM were decreased in favor of LPM under physiological conditions, they were strongly induced by LPS treatment in line with the decrease of precursor blood monocytes.

Moreover, probiotics reduced the expression of pro-inflammatory genes (*Tnf-α*, *Il-1β*, *Il-6*) by peritoneal macrophages, thus promoting a less inflammatory phenotype without any change of macrophages' response to LPS. Phagocytosis and bactericidal activity against *S. pneumoniae* was increased in macrophages isolated from mice supplemented with the probiotic combination *versus* phosphate-buffered saline.

#### Conclusions:

Our study promotes the probiotic combination tested as a good candidate to stimulate immune responses involved in defense mechanisms against invading pathogens.

### AGGREGATION PROPERTIES OF PROBIOTIC STRAINS UNDER AEROBIC AND ANAEROBIC CONDITIONS

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#### Objective:

Aggregation between microorganisms from the same species (auto-aggregation) and from different species (co-aggregation) is considered a desirable property of probiotics, since it has been related with successful gut colonisation by probiotic strains and clearance of intestinal pathogens, respectively. Our study aimed to evaluate the auto- and co-aggregation, with the pathogens Methicillin-resistant *Staphylococcus aureus* [MRSA] and *Escherichia coli* O157:H7, of a novel probiotic candidate *Akkermansia muciniphila* DSM 22959 and the commercial probiotics *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus rhamnosus* GG, under two atmospheric conditions (aerobiosis and anaerobiosis).

#### Methods:

Auto- and co-aggregation abilities of *A. muciniphila* DSM 22959, *B. animalis* subsp. *lactis* BB-12 and *L. rhamnosus* GG were determined at different time-points (2, 4, 20 and 24-h) under aerobic and anaerobic conditions, via spectrophotometric method, according to the protocols of Collado et al. (DOI: 10.1007/s00217-007-0632-x) and Jena et al. (DOI: 10.1111/1348-0421.12054).

#### Results:

All tested probiotic strains were able to auto- and co-aggregate with pathogens at all time-points and under both atmospheres and, in general, these aggregation properties increased with increasing incubation period.

#### Conclusions:

This work provides novel insights regarding aggregation properties of novel probiotic candidate *A. muciniphila* DSM 22959 and commercial probiotics (*B. animalis* subsp. *lactis* BB-12 and *L. rhamnosus* GG) under two atmospheric conditions. Furthermore, the proven aggregation properties of *A. muciniphila* DSM 22959 support its use as probiotic.

### HIGH MOLECULAR MASS POSTBIOTICS AGAINST PATHOLOGIES: METABOLIC AXES “INTESTINE—OTHER BODY PARTS”

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#### Objective:

Postbiotics (P) - products of live bacteria. High molecular mass P (HMMP) include adhesins and probiotic lectins (PL), oxydoreductases and proteinases, exopolysaccharides (EPS), peptidoglycans and biosurfactants (BS), other exopolymers with not enough studied (co)functioning. The aim is, based on own data, to evaluate potential of the probiotic HMMP against pathologies.

#### Methods:

Standard as well as adapted and developed by us procedures in study of the purified and separated HMMP using electrophoresis and blotting followed by bioluminescent assays in *BioChemi System* (UVP) in live imagination were used.

#### Results:

1.HMMP improve organism status in cases of pathologies of mucosal tracts, liver, fat tissue, skin, lung, nerve tissue and blood in metabolic axes “Intestine—Other body parts”. They reveal immune-modulating, anti-inflammatory, anti-tumor and cytokines-modulation activities. 2.Cultural fractions of human intestinal bifidobacteria and lactobacilli include perspective synergistic antibiotic-like systems (products of Trp catabolism and HMMP) against Gram positive bacteria and microfungi. Degradation/lysis of massifs/biofilms of *S.aureus* или *C.albicans* takes place in the presence of synergistic PL conversing into sets of P retaining interaction with glycoconjugates. Antimicrobial HMMP of the *Acilact* involve action of peroxydoreductases, caseinases (decrease of allergenicity), and sets of BS. *Acilact* strain K<sub>3</sub>III<sub>24</sub> contributes into bacteriocins-like system. Bifidobacterial strains complete probiotic protective system with BS and EPS (modified with depolymerases).

#### Conclusions:

The data indicate a network of simultaneous action of HMMP against groups of pathologies or diseases. The action of HMMP is implemented through functionally coupled branches “Intestine—Other body parts”. HMMP participate directly in forming specific sets from the cultural original P.

### <sup>1</sup>H NMR-BASED METABOLOMICS TO INVESTIGATE THE EFFECT OF PROBIOTICS ON HUMAN PHENOTYPE

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#### Objective:

The human gut hosts microorganisms that play a crucial role in health promotion, implementing host's metabolism. In this perspective, probiotics are used, with the aim of manipulating the composition of the gut microbiota and improving balanced microbial communities. Using a Nuclear Magnetic Resonance (NMR)-based metabolomic approach, we highlighted the molecular effects obtained by microorganisms' modulation through probiotics treatment on human urine and serum metabolome.

#### Methods:

21 healthy volunteers were enrolled in the study and administered with probiotics. 20 urine samples per subject and 1 serum sample per subject were collected before and during the probiotic assumption. Univariate (Wilcoxon-test) and multivariate statistical analyses (PCA-CA and MPLS-DA) were used to evaluate the <sup>1</sup>H-NMR urine and serum spectra, and to characterize the effects of the treatment.

#### Results:

Probiotics influence the urinary and serum metabolic profiles of the volunteers (overall accuracy of ≈70%), without altering their subject-specific phenotypes. In urinary metabolome, modifications in metabolite levels, especially in glucose, isoleucine, valine, 3-hydroxyisobutyric acid, 4-hydroxyphenylacetate, and acetoacetic acid levels, were monitored. We observed, also, that probiotics influence the serum metabolic profiles inducing fluctuations in acetone, ascorbate, and citrate levels.

#### Conclusions:

We investigated the urinary and serum metabolic profiles of 21 healthy volunteers before and during the supplementation of probiotics, using an NMR-based approach that turns out to be a powerful tool to estimate the host-microbiome interactions and the response to probiotics assumption. After treatment, probiotics influence the metabolic profiles of the volunteers, without alter the phenotypes, making changes in terms of concentrations of specific metabolites.

### ANTIMICROBIAL AND ANTIPROLIFERATIVE ACTIVITIES OF *L. FERMENTUM* SOLUBLE PEPTIDOGLYCAN FRAGMENTS

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#### Objective:

Our previous studies showed that a strain of *Limosilactobacillus fermentum* (LAC92) exhibited good amensalistic properties. In particular, it showed high resistance to bile salts and low pH, slightly production of hydrogen peroxide, strong biofilm production, and interestingly, its cell-free supernatant (CFS) exhibited strong antibacterial activity. The present study describes the isolation steps of CFS in order to partially purify the active components.

#### Methods:

Postbiotics were collected after growth in MRS medium broth and, CFS was treated for the isolation of soluble peptidoglycan fragments (SPF). Moreover, antimicrobial activity by both microdilution and agar well diffusion assay was assessed. MRS medium, crude CFS and the strain killed by tyndallization were used as internal controls. Finally, a proliferation study on human cell line HTC116 was performed by xCELLigence analysis.

#### Results:

SPF showed antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. Crude CFS was active against all bacterial strains tested. MRS medium and LAC92 tyndallized not showed antibacterial activity. Preliminary results of the SPF by xCELLigence analysis showed substantial reduction of cell index (CI) at 13% v/v, demonstrating cytostatic activity. MRS medium showed no cytotoxic activity.

#### Conclusions:

Our results showed good antibacterial and antiproliferative activity due to SPF. Moreover, the results obtained by xCELLigence showed a reduction of CI depending on the concentration. Although, the characterization of all molecules present in CFS has not yet occurred, chemical analysis are in progress in order to thoroughly investigate and fully characterize those are involved in antibacterial and cytostatic activity.

### INVESTIGATION OF THE ROLE OF A MULTI-STRAIN PROBIOTIC ON BABY BLUES AND BREASTFEEDING IN WOMEN AFTER DELIVERY

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#### Objective:

After pregnancy, mothers can suffer from a common non-pathologic condition called "baby-blues", leading them to feel anxious, sad and irritable. Factors like infant colic and mastitis further concur to the onset. The aim of the study is to evaluate the efficacy of a multi-strain probiotic composition (*L. reuteri* PBS072, *B. breve* BB077), to support new-mothers' mood and reduce baby cries and mastitis incidence.

#### Methods:

A RDBC clinical trial is on-going, involving 200 postpartum healthy mothers: one group were allocated to use the active treatment (*L. reuteri* PBS072, *B. breve* BB077 and vitamins) while the other used a multivitamin product as positive control. The study starts within 3 days after delivery and has a duration of 90 days with three checkpoints: before the delivery, after 45 and 90 days. At T45 and T90, the gynecologist evaluates mother depression-related symptoms with the Edinburgh Postnatal Depression Scale (EPDS), the quality of breastfeeding and the baby crying duration.

#### Results:

The expected results are linked to a general improvement of mother's mood in terms of anxiety and stress in the weeks after delivery. Also, we expect the reduction of baby cries and mastitis incidence thanks to the modulation of gut and local microbiota of both mothers and new borns.

#### Conclusions:

The final results (first half of 2022), will underline the primary importance of probiotic supplements for stressed subjects facing acute non-pathological conditions like post-partum. This clinical trial will lay the groundwork for innovative approaches, taking care of the last pregnancy quarter and the first days of baby life.

### MODULATION OF THE ENDOCANNABINOID SYSTEM BY PROBIOTIC LACTIPLANTIBACILLUS PLANTARUM IMC513 IN ZEBRAFISH EXPOSED TO ENDOCRINE DISRUPTORS

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#### Objective:

Environmental pollutants, such as endocrine disruptors (EDs), have become harmful for their impact on human health and, among them, phthalates used as plasticizers can lead to a dysregulation of the endocannabinoid system (ECS). The present study is aimed at evaluating a potential neuroprotective effect of *Lpb. plantarum* IMC513, with a focus on the ECS of zebrafish exposed to di-n-hexyl phthalate (DnHP).

#### Methods:

The probiotic was daily delivered for six months either alone or as a combined treatment. Morphometric data were collected after zebrafish necroscopy. Gene expression analysis of the ECS elements in the brain of zebrafish was performed by means of qRT-PCR analysis. The levels of two endocannabinoids, 2-arachidononyl-glycerol (2-AG) and anandamide (AEA), and the endocannabinoid-like mediator palmitoylethanolamide (PEA) were detected through LC/MS-MS. The study was approved by the Italian Ministry of Health (n: 529/2018-PR).

#### Results:

No fish died in all the treated groups. DnHP and probiotic did not affect body weight and total fish length. mRNA levels of ECS key genes revealed a promising protective role of probiotic through the downregulation of the ECS mirroring in a restoration of gene expression to the control level. In addition, the levels of 2-AG, AEA and PEA were modulated by the daily intake of *Lpb. plantarum* IMC513.

#### Conclusions:

These findings highlight the potential ability of probiotics to modulate at the level of the central nervous system some ECS components in zebrafish, suggesting the use of probiotics as innovative dietary strategy to counteract the emerging health risk of EDs, which is worth to be further investigated.

### PROBIOTICS AND GUT-BRAIN AXIS: INSIGHTS ON LOCAL AND SYSTEMIC MECHANISMS OF ACTION

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Probiotal Research srl, Research, Novara, Italy<sup>(1)</sup> - University of Piemonte Orientale, Department of Health Sciences, Novara, Italy<sup>(2)</sup>

#### Objective:

There are increasing evidences showing reciprocal interaction between chronic intestinal inflammation and psychiatric disorders that involves multiple pathways highly debated. Herein, we have investigated a probiotic mixture (*Limosilactobacillus fermentum* LF16 DSM26956, *Lactocaseibacillus rhamnosus* LR06 DSM21981, *Lactiplantibacillus plantarum* LP01 LMGP21021, *Bifidobacterium longum* BL04 DSM23233) in order to understand its potential biological and mechanistic effects on *in vitro* cellular models.

#### Methods:

It has been tested the probiotic influence on cytokines release in the PBMCs and their capacity to inhibit some pathogens (*K.pneumoniae* and *E.coli*). Then, cellular models of intestinal-endothelial and intestinal-neuronal cross-talking have been developed with the aim to investigate both the ability of probiotics to recover eukaryotic cells after the occurrence of inflammatory damage (exerted by IL1beta and TNFalpha) and their capacity to prevent negative effects of the same damage by performing different assays (MTT, TEER method, Adhesion test, ROS detection). Subsequently, it has been investigated if probiotics could affect phosphorylation of STAT3, a transcription factor able to increase tight junctions and viability-related genes expression and it has been tested if they could increase tight junctions.

#### Results:

Data demonstrated that Caco2 cells treated with probiotics were able to crosstalk with endothelial and neuronal cells. Results show that all probiotics are immune-modulators able to inhibit the growth of the analyzed pathogens and positively affect membrane integrity by increasing pSTAT3 and tight junctions.

#### Conclusions:

In conclusion, our results suggest that selected probiotics could be a promising strategy for the mental disorder treatments by means of systemic mechanisms of actions.

### A GALACTO-OLIGOSACCHARIDE EXERTS AN ANTI-INFLAMMATORY EFFECT IN AN IN VITRO MODEL OF INFLAMED INTESTINAL CELLS MIMICKING ULCERATIVE COLITIS

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#### Objective:

Considering the capacity of prebiotics in reducing gut inflammation, the aim of this study was to investigate the anti-inflammatory activity of a galacto-oligosaccharide (Bimuno GOS) in an in vitro model of ulcerative colitis (UC)-like inflamed intestinal cells.

#### Methods:

Differentiated Caco-2 cells were exposed to 2% dextran-sulphate-sodium salt (DSS) to induce inflammation, or treated with different concentrations of Bimuno GOS, either alone or simultaneously with DSS. Cell monolayer permeability, tight- and adherent junction protein distribution, pro-inflammatory cytokines secretion and NF- $\kappa$ B cascade were assessed.

#### Results:

Bimuno GOS, at different concentrations, was able to counteract UC-like intestinal inflammatory responses and damages induced by DSS. Cell monolayer permeability was not affected by Bimuno GOS. Bimuno GOS was able to counteract the detrimental effects of DSS on cell permeability, determined by transepithelial electrical resistance, phenol red apparent permeability, and tight- and adherent junction protein distribution. Furthermore, Bimuno GOS inhibited DSS-induced NF- $\kappa$ B nuclear translocation and proinflammatory cytokines secretion. Further analyses showed that Bimuno GOS was able to revert the expression levels of most of the proteins involved in NF- $\kappa$ B cascade to control levels.

#### Conclusions:

The prebiotic Bimuno GOS can be a safe and effective way to modulate the gut inflammatory state and could possibly further improve efficacy in inducing remission of UC. This study indicates that its potential mechanisms of action include NF- $\kappa$ B pathway modulation.

### CHARACTERIZATION OF THE GLUCAN-BRANCHING ENZYME GENE GLGB FROM SWINE INTESTINAL BACTERIA AND POTENTIAL ROLE IN LOW DIGESTIBLE OLIGOSACCHARIDES PRODUCTION

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#### Objective:

To biochemically characterize the glucan-branching enzyme *glgB* gene from the swine intestine and to investigate its role formation of alpha(1→4,6)-branched oligosaccharides from starch

#### Methods:

Two *glgB* gene variants, B342 and B344, encoding alpha(1→4)-glucan branching enzymes were identified in a swine metagenomic dataset, cloned in *Escherichia coli* and purified by affinity chromatography. Branching activity of purified *glgB* was determined with six different starches by quantification of the amylose and by oligosaccharide analysis after hydrolysis of products by alpha- and betaamylases. The *in vitro* digestibility of the enzymatically synthesized products was quantified after treatment with pancreatic and brush border enzyme.

#### Results:

The enzyme activity of *glgB*, reduced the amylose content of all six starches by more than 85%. The highest reduction of the amylose content by 97.5%, 97.6% and 99.2% was observed in starches with higher amylose content including fava bean, pea and potato starch, respectively. The oligosaccharide profile after hydrolysis with amylases showed an increased concentration of higher molecular weight oligosaccharides with DP > 10 in samples treated with the branching enzyme and a reduced *in vitro* digestibility when compared to untreated starch.

#### Conclusions:

The glucan-branching enzyme *glgB* gene cloned from swine intestinal bacteria showed branching activity which forms branched oligosaccharides that are not hydrolyzed by intestinal enzymes. The results improve our understanding of colonic starch fermentation and may conversion of starch to products with reduced digestibility.



### PROBIOTICS: A POTENTIAL THERAPEUTIC STRATEGY IN RESPIRATORY INFECTIONS

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#### Objective:

Respiratory diseases have a significant impact on morbidity and mortality worldwide and could be caused by pathogens, such as viruses and bacteria. The most frequent viruses include coronaviruses, influenza viruses, parainfluenza viruses, and rhinoviruses. In this regard, currently, there is a raised interest in the Coronaviridae family due to severe acute respiratory syndrome 2 (SARS-CoV-2) global pandemic. The aim of this project is to investigate how and if probiotics can prevent the spread of viral respiratory infections.

#### Methods:

In order to characterize the effect of *alive probiotic strains, their supernatants and the cellular extracts* from 14 *Lactobacillus* strains and 12 *Bifidobacterium* strains on the inhibition of the binding between human receptor angiotensin-converting enzyme 2 (ACE2) and the receptor binding domain (RBD) of the spike protein from SARS-CoV-2, "SARS-CoV-2 Inhibitor Screening Assay" by AdipoGen Life Sciences (AG-48B-0001-KI01), a colorimetric sandwich E.L.I.S.A, was used.

#### Results:

Data demonstrated that the most of alive probiotic strains and their supernatants from *Lactobacillus* strains exhibit, with an interesting strain-specific activity, a capacity over 50% to contrast the binding RBD-ACE2. Instead, the activity of the cell extract of the probiotic strains had an inhibitory effect never more than 30% and the best results were from *Bifidobacterium* strains.

#### Conclusions:

These experiments demonstrated the effect of probiotic strains, through the release of some metabolic products, on the inhibition of the binding of the receptor binding domain (RBD) of the spike proteins of SARS-CoV-2 to human ACE2 receptor, particularly relevant for the entry of the virus into the host cell.

### LACTIC ACID BACTERIA ISOLATED FROM THE ARTISANAL CULTURES AS POSTBIOTICS PROMOTERS BY FERMENTATION OF UNCONVENTIONAL SUBSTRATES

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#### Objective:

Study the artisanal cultures such as milk kefir grains (MKG), water kefir grains (WKG) and kombucha (KB) as sources for obtaining lactic acid bacteria (LAB) strains as the postbiotic promoters by fermentation of unconventional substrates.

#### Methods:

The isolation of the newly LAB strains (coded MIUG) from the artisanal cultures microbiota was assessed by specific isolation techniques. Additionally, the fermentation on the unconventional substrate, based on 5% red lentil flour, 4% sweet potato peel flour and 3% okara, was performed. 2% of LAB cultures was used as inoculum, followed by incubation at 37°C for 72 h. The total titratable acidity (TTA), antioxidant (DPPH method) and antimicrobial activity (well diffusion method) of the fermented products (FPs) were analysed.

#### Results:

Newly 16 LAB strains were isolated and selected based on the functional characteristics. The FPs obtained with the strains codified MIUG BL79 (from WKG) and MIUG BL80 (from WKG) showed higher values of TTA (15.72 – 16.01 mL NaOH 0.1N). Regarding the antibacterial activity, for FPs obtained with MIUG BL78 (from WKG) and MIUG BL91 (from MKG) strains values for inhibition zone between 4.83 – 4.00 mm were measured against spore forming bacteria. The radical scavenging activity (RSA) for all FPs were ranging from 53.18% - 88-65%.

#### Conclusions:

Through the unconventional substrate and newly LAB isolates from artisanal starter microbiota driven by fermentation-enabled processes enhanced the bioavailability and effects of postbiotics.

#### Acknowledgements:

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### ENHANCEMENT OF THE POSTBIOTICS PRODUCTION BY LACTIC ACID BACTERIA ON AGRI-FOOD RESIDUAL SUBSTRATES

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#### Objective:

The optimisation of the postbiotics production by lactic fermentation through valorising the agri-food residues, such as okara and sweet potato peel (SPP), by statistical analysis was targeted.

#### Methods:

In order to improve the bioactive compounds by fermentation with selected *Lactobacillus* spp. strains coded MIUG BL13 and MIUG BL4, Response Surface Methodology by using Minitab 17 Statistical Software (USA) was employed. Concentration of okara (1-5%), sweet potato peel (2-6%) and MIUG BL13 inoculum (0-4%) were varied while red lentil flour (5%), inoculum of MIUG BL4 (4%), temperature (37°C) and fermentation time (72 h) were maintained constant. The analysed responses were total titratable acidity (TTA), antioxidant activity (DPPH method), and antimicrobial activity (diffusion method).

#### Results:

The results showed that the parameters that influenced the responses were as follows: concentration of okara and SPP for TTA, SPP concentration for antibacterial and antioxidant activity, SPP concentration and inoculum of MIUG BL13 for antifungal activity. The optimised fermentation solution comprise 5% okara, 5.5% SPP and 5% inoculum of MIUG BL13, which will lead to an antioxidant activity of 2.58 mM TE/mL, values of 42.27% for antifungal activity, 4.31 mm for antibacterial activity and 18.79 mL NaOH 0.1N, for TTA.

#### Conclusions:

By valorisation of agri-food residues through lactic fermentation, functional bioactive compounds (postbiotics) with technological and functional impacts can be obtained.

#### Acknowledgments:

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI – UEFISCDI, project number PCE 159/2021, within PNCDI III.

### POSTBIOTIC COMPOSITION OF THE BOVINE COLOSTRUM IMPROVED BY LACTIC ACID FERMENTATION IN OPTIMIZED CONDITIONS

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#### Objective:

The concentration of colostrum (% w/v), the volume of lactobacilli cultures used as inoculum (% v/v) and the time of fermentation (h) with a co-culture of selected lactobacilli strains *Lactobacillus* spp. MIUG BL13 and *Lactobacillus* spp. MIUG BL37 were analysed by the design of the experiments and Response Surface Methodology (RSM) in order to increase the functional properties of the fermented product.

#### Methods:

The colostrum concentration, inoculum and fermentation time (h) as independent variables were varied according to the matrix designed. Titratable acidity (°Th), antioxidant activity (DPPH method, mM TE/mL) and antimicrobial activity (agar diffusion method for the antibacterial activity against the *Bacillus subtilis* MIUG B1 and growth inhibition ratio for antifungal activity against *Aspergillus niger* MIUG M5), were considered as responses associated to the fermented product (FP) characteristics

#### Results:

The results of the mathematical modelling and statistical analysis lead to an optimized variant of the fermentation parameters. Thus, the FP showed titratable acidity between 50-150°Th, antioxidant potential between 0.15-0.50 mM TE/mL, an antibacterial activity of 2.5-6 mm against *B. subtilis* and growth inhibition ration of 20-36 % against *A. niger*.

#### Conclusions:

The results demonstrated that a colostrum concentration of 9%, an inoculum volume of 5% MIUG BL37 and a fermentation time of 88 h assured the improving of the bovine colostrum postbiotic composition with positive impact due to their functional characteristics.

#### Acknowledgments:

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### CHARACTERIZATION OF CELL-FREE SUPERNATANTS OF LACTIC ACID BACTERIA FROM DIFFERENT MICROENVIRONMENTS

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#### Objective:

The study aims to: compare the antimicrobial efficiency of cell-free supernatants (CFS) of lactobacilli from humans and bees gut, obtained with different incubation time and liquid broths; design a broad inhibitory spectrum CFS-mixture (CFSM); characterize the major bioactive components.

#### Methods:

Nine strains of lactobacilli were cultivated in MRS-original broth or glucose-supplemented (MRS-G) for 24 and 48 hours. All CFSs were obtained by centrifugation and filtration. The antimicrobial efficiency of single CFSs and CFSM were investigated through agar-well diffusion method. Using pH, heat, enzymes treatments and analysis by gas chromatography coupled with flame ionization detection, the major antimicrobial compounds of CFSM and its short-chain fatty acids composition were characterized.

#### Results:

MRS-G enhanced the bioactivity against *Pseudomonas aeruginosa*, *Salmonella enterica* as well as *Proteus mirabilis*, whereas for other pathogens higher inhibitory ability was observed in CFSs-derived from MRS. The 48h-incubation time increased the anti-*Escherichia coli*, anti-*Ps. aeruginosa*, anti-*Listeria* capacities, but reduced the inhibitory spectrum. The inhibition on *Staphylococcus aureus*, *P. mirabilis* and *Enterococcus faecium* was only found in certain CFSs at both times. The designed CFSM was effective against all target strains and acetic acid was the major detected active compound.

#### Conclusions:

The nutrients and the incubation time affect the production and bioactivity of lactobacilli metabolites. The mixtures of different CFS showed broad antimicrobial spectrum, demonstrating natural food preservation potential.

### EXPLORING SOLID STATE FERMENTATION OF LENTIL GRAINS AND FLOUR AS STRATEGY TO IMPROVE PROTEIN CONTENT AND OTHER FUNCTIONAL COMPOUNDS

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#### Objective:

In the last years, postbiotic foods have gained much popularity in food technology area. The objective of this study was to evaluate the benefits resulting from solid-state fermentation (SSF) on functional compounds in Pardina lentils (*Lens culinaris var. variabilis*).

#### Methods:

Solid-state fermentation with *Pleurotus ostreatus*, an edible fungus, was conducted on lentil whole grains and flour during 14 days at 28°C. Biomass production, phenolic compounds, phytic acid and antioxidant activity (ABTS, DPPH and FRAP) were assessed at different fermentation times: 2, 4, 6, 8, 10, 12 and 14 days for whole grains, and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 14 days for flour. Results obtained from fermented samples were compared to the control samples (without inoculum).

#### Results:

Along this study of SSF, the maximum production of fungus biomass was reached after 14 days of fermentation, concretely 294 mg/g in lentil flour and 281 mg/g in lentil grains. Phenolic compounds in fermented flour and grains slightly decreased over the time. In general, fermented grains (0.90 mg gallic acid/g) showed higher phenolic concentration compared to the flour (0.79 mg gallic acid/g). Therefore, antioxidant activity was not enhanced by fermentation process. Finally, SSF slightly decreased phytic acid concentration in lentil flour (5%) and in lentil grains (10%).

#### Conclusions:

Despite the advantages reported in other vegetal substrates, SSF in Pardina lentils reported neither a significant increase of antioxidant properties nor a decrease in measured antinutrients. Therefore, SSF in lentils can be considered of interest if the aim focuses on digestible protein content resulting from the great increase of unicellular protein coming from biomass production.

### SELECTION OF LACTOBACILL STRAINS WITH PROBIOTIC POTENTIAL FROM ALGERIAN GOAT'S MILK: ANTILISTERIAL ACTIVITY AND MODULATION OF TNF INDUCED CHEMOKINE SECRETION

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#### Objective:

In Algeria consumption of fresh milk and traditional dairy products from raw milk rich in autochthonous microflora with beneficial effects on health is decreasing. The objective of this investigation is the selection of lactobacilli strains with probiotic potential from goat's milk.

#### Methods:

Lactobacilli isolates were identified at species levels by using an API 50 CHL system, sequencing of 16S rRNA gene succeeded by phylogenetic tree construction. The probiotic potential of lactobacilli is evaluated were assessed *in vitro* by accessing their a) growth capacity in acid broth adjusted at different pH levels, and bile tolerance at 0.5 and 1%, b) antilisterial activity against *Listeria monocytogenes* CECT 932 and c) modulation of TNF-induced chemokine secretion of human colon tumorigenic cell. The IP-10 and IL-8 concentrations were determined in cell culture supernatants using appropriate ELISA-Kits.

#### Results:

Lactobacilli strains were identified as *Lactiplantibacillus plantarum* (41), *Limosilactobacillus fermentum* (7), *Lacticaseibacillus rhamnosus* (3). The cell free supernatant of 10% strains had antibacterial activity against *Listeria monocytogenes* CECT 932<sup>T</sup>. Ability to grow at pH 3, 3.5 and 4 were shown for 9.80, 15.68 and 70 % respectively. All strains exhibited bile tolerance. A good decrease in IP10 interferon secretion by HT-29 stimulated with TNF- $\alpha$  was observed after incubation with three Lactobacilli strains. Whereas, IL-8 interleukin secretion by HT-29 cells was not reduced.

#### Conclusions:

The use of lactobacilli selected strains having a probiotic potential in the industrial sector as an auxiliary culture remains one of the solutions to partially preserve the benefits of these indigenous microorganisms on health.

### ANTIPROLIFERATIVE EFFECT OF A PROBIOTIC MICROORGANISM IN HUMAN BREAST ADENOCARCINOMA CELL LINES: PRELIMINARY DATA

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#### Objective:

Epidemiological studies have shown a lower risk of breast cancer in women who consume probiotics. These observations suggested that probiotics may exert chemoprotective activities, directly or indirectly. The aim of this study was to assess the direct effect of a probiotic microorganism in two human breast adenocarcinoma cell lines. In particular, the antiproliferative effect of heat-inactivated bacteria and the supernatant as such, heat-treated and neutralized was evaluated in cancer cells.

#### Methods:

The antiproliferative effect was determined via MTT assay. SK-BR-3 and MDA-MB-231 cells were treated with the heat-inactivated cells of *Streptococcus thermophilus* 11-137, and with the supernatant from the bacterial suspension, used as such, neutralized to neutral pH and heat-treated; then the MTT assay was performed and results were recorded after 72 and 96 hours.

#### Results:

Incubation of the MDA-MB-231 cells with heat-treated supernatant diluted at 30% led to the highest reduction in cell vitality: 25% and 36% after 72 and 96 h of incubation, respectively. In SK-BR-3 cells, incubation with the supernatants as such and boiled, both diluted at 30%, caused a vitality reduction of 67% and 69% after 96 h, respectively.

#### Conclusions:

The heat-treated supernatant of the *S. thermophilus* 11-137 exerted a dose- and time-dependent antiproliferative effect more clearly in one of the two tested cancer cell lines. One or more soluble compounds produced by this strain, including organic acids, could be responsible for this effect.

### CHARACTERIZATION OF NEW CANDIDATE PROBIOTIC STRAINS FOR IBS TREATMENT

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#### Objective:

Characterize new probiotic strains for IBS treatment using large-scale *in vitro* and *in vivo* approaches.

#### Methods:

A collection of 63 strains belonging to *Bifidobacterium* and *Lactobacillus* species were characterized by their ability to modulate IL-8 level in HT-29/TNF-alpha cell model, IL-10/IL-12 ratio in peripheral blood mononuclear cell (PBMC), and intestinal permeability in Caco-2 cells. Six selected strains were evaluated in one low-grade inflammation model induced by DiNitroBenzene Sulfonic acid (DNBS), and two of them in one neonatal maternal separation (NMS) model.

#### Results:

Thirty-seven strains were able to decrease IL-8 production up to 60% in HT-29/TNF-alpha model. Twenty-seven strains displayed IL-10 immunomodulation in PBMC and only 22 strains improved the barrier integrity in Caco-2 cells. Six-selected strains improved the inflammatory markers in mice caused by the low doses of DNBS, as observed by weight gain, macroscopic scores and reduction of myeloperoxidase, lipocaline-2 and fecal protease activities. Two strains were able to reduce the pro-inflammatory cytokines at systemic level and to protect from intestinal hyper-permeability (FITC-dextran) by enhancing *Cingulin* and *Tight Junction Protein 1* (TJP1) expression. Additionally, these strains significantly decreased the intestinal hyper-permeability in NMS-mice model accompanied by Lipocaline-2 and fecal protease reduction, but only one improved the visceromotor response (VMR).

#### Conclusions:

Together, these results demonstrated the strong probiotic activities of two strains in cellular and preclinical models of IBS

### IDENTIFICATION OF NOVEL PROBIOTIC STRAINS WITH BENEFICIAL POTENTIAL IN GASTROINTESTINAL DISORDERS

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#### Objective:

The goal is to identify promising bacterial strains against gastrointestinal disorders that will next be tested *in vivo*. We have used an *in vitro* approach to compare the anti-inflammatory properties of 41 bacterial strains, including commercialized probiotics as benchmark controls.

#### Methods:

Growth kinetics were determined by optical density and colony forming unit counts. All strains were recovered and cryoconserved at the beginning of their stationary phase. Bacterial supernatants were analyzed for short chain fatty acid and Arylhydrocarbon Receptor (AhR) agonists production, using gas chromatography and HepG2 Lucia reporter line, respectively. Strains were then tested *in vitro* with equal multiplicity of infection: dosing of cytokines produced after co-incubation on challenged HT-29 cells and non-stimulated peripheral blood mononuclear cells (PBMC), and measures of TransEpithelial Electrical Resistance of TNF-alpha challenged Caco-2 cells.

#### Results:

7 strains demonstrated acetate production and 10 demonstrated AhR agonists production. Co-incubation on HT29 resulted in a 20% decrease of IL8 production for 6 strains; and co-incubation on PBMC revealed a change in the TH1/TH2 profile through modulation of IL10 / IL12 production for 22 strains. 15 strains were able to restore the paracellular permeability of Caco2.

#### Conclusions:

41 strains were screened for intestinal disorders parameters: production of anti-inflammatory metabolites, immuno-modulation and capacity to restore the intestinal barrier function. Combining these data allowed a ranking of strains and selection of 4 promising ones that will be further tested in *in vivo* murine gastrointestinal disorders models.

### PROBIOTICS SUPPLEMENTS REDUCE ER STRESS AND GUT INFLAMMATION ASSOCIATED WITH GLIADIN INTAKE IN A MOUSE MODEL OF GLUTEN SENSIVITY

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#### Objectives:

Celiac Disease (CD) is an autoimmune disorder resulting in intestinal chronic inflammation and tissue damages induced by gluten and gluten-related proteins ingestion, with individual genetic susceptibility playing a key role. Currently, no pharmacological treatment is available to CD patients, with a strict, life-long gluten-free diet is the only therapy, although its efficacy is limited. Here we evaluated whether probiotics are able to mitigate gut inflammation induced by gliadin exposure, by using *in vivo* and *in vitro* models.

#### Methods:

Gluten-sensitive Balb-c mice were exposed to gliadin alone or in combination with two probiotic formulations, and gut dysfunction was evaluated. To this aim, we evaluated: the expression of the key CD marker TG2, by both western blotting and qPCR analysis; intestinal permeability, by qPCR analysis of Tight Junction proteins level, and FITC-dextran permeability; ER stress, by measuring the expression of the key UPR markers ATF4, ATF6, and XBP1s; inflammation, by measuring the production of pro-inflammatory cytokines (IFN $\gamma$ , IL15, and IL17A). This analysis was also carried out in 2D cell culture conditions, by using Caco-2 cells as a model.

#### Results:

Our results clearly show that probiotics formulations efficiently reduced the gliadin-dependent gut inflammation and tissue dysfunction. Indeed, probiotics consistently reduced TG2 levels, ER stress, and pro-inflammatory cytokine production, restoring physiological permeability and intestinal morphology.

#### Conclusions:

Our data show that probiotics administration might potentially represent a new valuable strategy to treat gluten-sensitive patients, as those affected by CD.

### EFFECT OF A MULTISTRAIN PROBIOTIC ON LEAKY GUT IN PATIENTS WITH DIARRHEA-PREDOMINANT IRRITABLE BOWEL SYNDROME

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#### Objective:

To evaluate the effect of a multistrain probiotic on leaky gut associated with diarrhea-predominant IBS (IBS-D).

#### Methods:

In this pilot, open-label, single-center, proof-of-concept study, 30 IBS-D patients with increased intestinal permeability assessed by (51)Cr-EDTA or (99m)Tc-DTPA received 2 capsules of a multistrain probiotic a day for 30 days (10 billion CFU/capsule of *Bifidobacterium lactis* LA303, *Lactobacillus acidophilus* LA201, *Lb plantarum* LA301, *Lb salivarius* LA302, and *B lactis* LA304). On D30, patients repeated intestinal permeability assessment were measured and patients completed the Bristol Stools, VAS-IBS and IBS-QOL.

#### Results:

Of the 30 included patients (mean age: 42.1 [SD: 13.1] years; female: 60%), 27 completed the study. On D30, 10 patients (37%) had normal intestinal permeability. As compared with baseline, mean intestinal permeability had significantly decreased [ $\Delta$ : -3.4 (95%CI, 1.65, 5.18)], 97% of patients claimed that their IBS symptoms have been satisfactory alleviated by the treatment. A significant improvement in stool consistency, abdominal pain, diarrhea, impact of gastrointestinal problems in daily life and for the Total score of the IBS-QOL was reported. Supplementation was well tolerated.

#### Conclusions:

This open label study suggests that the multistrain probiotic tested improve intestinal permeability in IBS-D. Moreover, almost all patients significantly ameliorated IBS symptoms. A placebo controlled study however remains needed.

### THE KNOWLEDGE OF PROBIOTICS, PREBIOTICS AND SYNBIOTICS AMONG SOCIAL NETWORK USERS IN SLOVENIA

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#### Objective:

Probiotics, prebiotics and synbiotics are becoming well-known among consumers due to their many beneficial effects, proven in several well-designed randomized controlled clinical studies. The aim of our study was to examine the awareness and knowledge of social network users of these supplements.

#### Methods:

We conducted the survey questionnaire using 1KA online survey application and distributed it via Facebook. The obtained data were statistically analyzed (IBM SPSS Statistic 25.0). We used the Cramér correlation coefficient, T-test, and Hi-square test.

#### Results:

We found that the awareness and use of probiotics, prebiotics and synbiotics among web users is very good. Education level and age did not affect the use of these alone. A statistically significant difference existed in age and usage of supplements ( $p=0.002$ ). The older respondents were less familiar with probiotics, prebiotics and synbiotics, as previous research also revealed.

#### Conclusions:

Research shows that respondents would like to use probiotics when they are presented with their beneficial effect on health. Proper knowledge of health workers is therefore crucial to know how to give patients the correct and evidence-based information.

#### Keywords:

probiotics, prebiotics, synbiotics, social networking, general population

### SELECTION OF PROBIOTIC MICROORGANISMS FOR THEIR POTENTIAL PSYCHOBOTIC ACTIVITY

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#### Objective:

The study aims to select probiotic strains with potential effect on mental health outcomes, and to develop a probiotic formulation that may provide an effective therapeutic option for boosting cognitive performance, alleviate anxiety symptoms and manage psychological reaction to stress in healthy people.

#### Methods:

Probiotic microorganisms belong to the culture collection of the Progefarm. The screening activity to assess the ability of the strains to produce neurotransmitters has been carried out in the University of Naples Federico II inoculating the strains in growth media containing the precursors of the neurotransmitters and the supernatant analyzed with LC-MS/MS. The strains that showed the best performance were selected for the growth test on industrial level and used for the formulation of a dietary supplement. *In vitro* batch fermentation, has been performed in Wageningen University to mimic the effect of the dietary supplement on the microbiota of the distal colon.

#### Results:

*L. brevis* P30021 produced the highest amount of GABA, 77,26 mM. Low but considerable activity was observed in *L. plantarum* strains. The acetylcholine content was detected in *L. plantarum* P30025, reaching quantity of 0,36mM. The *in vitro* batch fermentation also showed higher amount of GABA than the amount detected in the control (only fermented stools).

#### Conclusions:

The probiotic strains have demonstrated *in vitro* potential psychobiotic activity. The data obtained in this study encourage to investigate the activity of the formulation in a clinical trial as potential microbiota-targeted interventions that can positively modify mental health in healthy volunteers with moderate level of stress.

### FATE OF LISTERIA MONOCYTOGENES DURING PRODUCTION AND STORAGE OF FETA CHEESE USING MULTI-FUNCTIONAL STRAINS WITH PROBIOTIC POTENTIAL

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#### Objective:

The aim of the current study was to monitor the survival of *Listeria monocytogenes* during ripening and storage of Greek Feta-cheese with or without the addition of 2 multi-functional lactic acid bacteria (LAB) strains.

#### Methods:

Feta-cheese was manufactured using commercial starter-culture (control) and with the addition (co-culture) of 2 multi-functional strains (*Leuconostoc mesenteroides*-FMX3, *Lactococcus lactis*-SMX2) with technological and probiotic properties *in vitro*. Then, *L. monocytogenes* (4-strains) was added (6 log CFU/g) and ripening followed. Microbiological, pH, and sensory (non-inoculated samples) analyses were performed during ripening and cold-storage, while, the presence of the strains in the product was determined using RAPD-PCR.

#### Results:

Results showed that LAB population exceeded 8.0 and 7.0 log CFU/g during ripening, 7.8 and 7.1 log CFU/g during storage at probiotic and control case, respectively. The population of *L. monocytogenes* was found lower by 0.5-1.0 log CFU/g in the probiotic case compared to the control during early-ripening, while it was found below the detection limit after 45d and 60d of ripening at the probiotic and control case, respectively. During storage, pathogen was not detected (after enrichment) at both cases. RAPD-PCR showed that the probiotic strains were maintained in high percentages during storage, whereas probiotic samples exhibited better sensory characteristics.

**Conclusions:** The results of the study are promising for the production of Feta cheese with enhanced safety and functional properties.

#### Acknowledgment:

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### POTENTIAL THERAPEUTIC BIOMOLECULES OF LACTOBACILLUS STRAINS ISOLATED FROM HUMAN MILK MICROBIOTA

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#### Objective:

This study aimed to select the *Lactobacillus* strains, from human milk microbiota, with specific properties of synthesis of exopolysaccharides (EPS), S-proteins and bacteriocins with the potential to be characterised as therapeutic biomolecules.

#### Methods:

Detection of the „ropy“ phenotype was used for screening of EPS producers, while bacteriocinogenic activity was analysed by the determination of antagonistic activities *in vitro*. S-proteins of *Lactobacillus brevis* strains MB1, MB2, MB13 and MB20, were detected by SDS-PAGE analysis of GHI extracted surfaceome and further analysed by LC-MS/MS using database search by Mascot. For the identification of the genes of interest, responsible for the expression of targeted biomolecules, PCR with specific primers was applied.

#### Results:

<sup>1</sup>H-NMR analysis confirmed that *Lactobacillus fermentum* MC1 strain produces EPS. PCR method with *eps* primers confirmed the genes of the *eps* cluster. Dendrograms of SDS-PAGE electrophoretic profiles, obtained using GelCompar II, showed characteristic bands of approximately 45 kDa in *L. brevis* strains identified by mass spectrometry. The spectrum of *Lactobacillus* strains showed bacteriocinogenic activity, however, the presence of *plnJ*, *plnA* and *plnEF* genes involved in the bacteriocin biosynthetic pathway was confirmed in the genome of six *Lactobacillus plantarum* strains. The comparative genomic analysis provided insights into the clusters related to the expression of potential biomolecules.

#### Conclusions:

The analysis of breast milk microbiota revealed the presence of *Lactobacillus* strains with the potential to express specific biomolecules. Further investigations will be directed towards deciphering mechanisms of probiotic actions triggered by these biomolecules in order to develop next-generation probiotics.



### PROBIOTICS DECREASE LPS INDUCED INFLAMMATORY RESPONSE IN HUMAN BRONCHIAL CELLS – A POTENTIAL THERAPEUTICS IN CHRONIC INFLAMMATORY LUNG DISEASES

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#### Objective:

The alteration of lung microbiota in healthy and diseased lungs implicates that its composition can modulate inflammatory response of the lungs. We suppose that health-benefit of probiotics could be effective in treatment of chronic inflammatory lung conditions. However, the effects of lactic-acid bacteria (LAB), that feature the microbiota of healthy human lungs, on bronchial cells has been insufficiently investigated.

The main objective of this research is to explore the immunomodulatory abilities of natural LAB isolates in human bronchial cells as potential therapeutics for chronic inflammatory lung diseases.

#### Methods:

The ability of 21 LAB isolates to reduce the expression and secretion of pro-inflammatory mediators IL-1, IL-6, IL-8, TNF and MCP-1 is tested in LPS induced BEAS-2B cells using RT-PCR and ELISA approaches, respectively. The cytotoxicity is measured by LDH assay.

#### Results:

Five LAB strains showed a decrease of the expression of at least one pro-inflammatory cytokine and did not increase the secretion of measured cytokines. The strains *Lactobacillus brevis* BGZLS10-17, *Lb. plantarum* PKM22 and GO7-29, significantly decreased LPS induced IL8, MCP and TNF ( $p < 0,01$ ) while *Lb. rhamnosus* BGHi22 and *Streptococcus thermophilus* BGKMJ1-36 significantly attenuated the induction of IL6 and TNF ( $p < 0,05$ ). The strains with anti-inflammatory abilities showed no cytotoxicity.

#### Conclusions:

Available anti-inflammatory pharmacological treatments, are not effective in suppression of lung inflammation and there is an urgent need for the development of novel approaches. LAB isolates with anti-inflammatory abilities, identified in our research, may be employed in manipulation of the lung microbiota that can alleviate the symptoms in chronic inflammatory lung diseases.

### DARK CHOCOLATE AS A PROMISING CARRIER FOR PROBIOTIC STRAINS

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#### Objective:

Chocolate is one of the most attractive food products among the majority of population. Furthermore, probiotic-containing chocolates have been proposed as new functional food candidates. Our study aimed to evaluate flavanol-rich dark chocolate as a carrier for probiotic bacteria (*Bifidobacterium animalis* subsp. *lactis* BB-12 and *Akkermansia muciniphila* DSM 22959), through measurement of cell viability during aerobic storage at 20°C.

#### Methods:

Upon cultivation, pellets from probiotic strains (*B. animalis* subsp. *lactis* BB-12 and *A. muciniphila* DSM 22959) were incorporated, at 10% (w/w) inoculum, in melted 70% cocoa dark chocolate at 37°C, followed by tempering at 34°C during 10 minutes. Next, chocolates were molded and cooled at 11°C, for 2h. Lastly, produced chocolates were stored aerobically at 20°C. Viability of probiotic strains was assessed after 6 and 12 days by plating colony-forming units in appropriate media.

#### Results:

Incorporation of *B. animalis* subsp. *lactis* BB-12 into dark chocolate showed a high viability level (ranging between 8 to 9 log CFU/g), even after 12 days of aerobic storage at 20°C. In contrast, *A. muciniphila* DSM 22959 incorporated into dark chocolate displayed a high viability reduction at similar storage conditions.

#### Conclusions:

Our findings indicate that dark chocolate constitutes a promising carrier for delivery of probiotic *B. animalis* subsp. *lactis* BB-12. However, incorporation of *A. muciniphila* DSM 22959 in dark chocolate still requires further improvements.

**LIVE BIOTHERAPEUTIC PRODUCTS FOR HUMAN USE AND THEIR REGULATORY PATHWAYS IN ITALY**

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**Objective:**

Live biotherapeutic products (LBPs) are biological medicinal products containing as active ingredient live microorganisms with an intended therapeutic or preventive effect in humans, regardless the route of administration. Here, we give an overview of the regulatory pathway for the marketing authorization of this kind of medicines in Italy.

**Methods**

An analysis of the requirements needed for the marketing authorization of LBPs in Italy has been conducted.

**Results**

Most of the LBPs currently available in Italy have been registered in the '70/'80 following national regulation. According to the Italian legislation, LBPs marketing approval need to be supported by sound quality, safety and efficacy data for the intended clinical use. Furthermore, the Common Technical Document (CTD) needs to be regularly updated, in particular regarding safety aspects. LBPs are manufactured according to Good Manufacturing Practices for Medicines and since 2019 have to comply with the quality requirements, legally binding, of the European Pharmacopeia monograph 3053 on LBPs for human use. Once approved, LBPs may be sold in Italian pharmacies as over the counter medicines.

**Conclusions:**

Bacteria/yeasts present in LBPs might be the same probiotic microorganisms used as food supplements. Because of their historical use in food, they are considered safe. However, a certain strain, even with a long history of safe use in healthy population cannot be automatically considered safe in debilitated/diseased/immunocompromised subjects. Important differences exist between LBPs and probiotic supplements regulations as different is the target population and the health claims, but from a practical point of view, as healthy and diseased individuals have free access to them, a harmonization of the two regulatory framework should be evaluated.

**PROBIOTICS DETOXIFICATION CAPACITY – HUMAN AND FOOD SAFETY CONCERNS**

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**Objective:**

Analyze the recent literature regarding the probiotics (*Lactobacillus*, *Bifidobacteria*, and *Saccharomyces*) ability to protect the human body and the food products from different contaminants (mycotoxins, aflatoxins, acrylamide, benzopyrene, and heavy metals). In addition, probiotics consumption and possible risks are also evaluated.

**Methods:**

We proposed evaluating the last five years' scientific literature studies on probiotics' capacity to reduce contaminants level or annihilate their impact on human health.

**Results:**

The literature review reveals that, despite the wide variety of food contaminants (physical, chemical and microbiological) and the narrow likelihood to appraise all the pollutants, probiotics may be safe. Besides this, all the literature results showed that this pathway is economically feasible and maybe a versatile tool in biodetoxification. Therefore, we evaluated the biodetoxification process in food products. This fact raised issues as influences on taste, structure, nutritional value, and probiotics viability.

**Conclusions:**

A careful and precise choice of the probiotic strain, applied in certain foods, frequently incriminated by the presence of toxins, and consumed in substantial quantities, may significantly influence food safety.

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### THE EFFECTS OF PROBIOTIC SUPPLEMENTATION ON GUT MICROBIOTA, OBESITY AND TYPE 2 DIABETES: META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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#### Objective:

This study discusses the role of probiotic supplementation on gut microbiota, obesity and type 2 diabetes.

#### Methods:

The research strategy was applied to the PubMed and Springer-link. Only randomized controlled studies were included. The study was independently evaluated for eligibility, extracted data and assessed the risk of bias of the included studies. Data were pooled using the randomized effect model and expressed as a Standard Mean Difference (SMD) with a 95% Confidence Interval (CI). Heterogeneity was assessed and quantified (I<sup>2</sup>).

#### Results:

From a total of 1082 scientific papers, 21 studies were included, involving 1445 subjects in the control group and 1557 in the intervention group. The meta-analysis shows that BMI (SMD= - 0.06 kg/m<sup>2</sup>, 95% CI [-0.39, 0.28], p= 0.0004, (I<sup>2</sup>= 74%, p=0.0004)). HDL (SMD= 0.09 mmol/l, 95% CI [-0.08, 0.27], p= 0.30, (I<sup>2</sup>= 1%, p=0.42)). LDL (SMD= - 0.23 mmol/l, 95% CI [-0.58, 0.12], p= 0.20, (I<sup>2</sup>= 65%, p< 0.009)). HOMA-IR (SMD= - 0.48, 95% CI [-0.18, 0.22], p= 0.18, (I<sup>2</sup>= 86%, p< 0.00001)). Fasting glucose (SMD= - 0.09 mmol/l, 95% CI [-0.28, 0.48], p= 0.64, (I<sup>2</sup>= 64%, p= 0.02)). For insulin (SMD= - 0.15 uU/ml, 95% CI [0.78, 0.22], p= 0.64, (I<sup>2</sup>= 79%, p= 0.002)), and HbA1c (SMD= 0.00, 95% CI [- 0.18, 0.18], p= 0.99, (I<sup>2</sup>= 0%, p= 0.89)). The Egger regression indicated no significant publication bias.

#### Conclusions:

Dietary agents for modulation the gut microbiota have insignificant effects on fasting glucose and HDL in type 2 diabetes and obesity.

#### Keywords:

intestinal microbiota, type 2 diabetes, obesity.

### 31 - DIET AS TERTIARY PROPHYLAXIS RELATED TO OBESITY AND METABOLIC SYNDROME: A CASE-STUDY ON PATIENTS WITH COMPLICATED DIABETES MELLITUS TYPE-2, TREATED INTO A COUNTY HOSPITAL FROM TIMISOARA, ROMANIA

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#### Objective:

The aim of the performed study was to investigate if there is a relation between diet as tertiary prophylaxis, obesity and Metabolic Syndrome (MS) in patients with complicated Diabetes Mellitus Type-2 (DMT2).

#### Methods:

The study was performed on a sample that consisted of 427 patients (53.2% females, 46.8% males, aged 35-91 years) treated at the Diabetes, Nutrition and Metabolic Diseases Clinique into a County Hospital from Timisoara, Romania, using an observational inquiry (case-study) method from 1<sup>st</sup> January to 31<sup>st</sup> December 2016. A statistical analysis (Correlations, Chi-square and Binomial logistic regression) was performed with the aid of a SPSS20 Program.

#### Results:

All recorded patients (427) with complicated DMT2 were overweight or obese. 53.9% patients with complicated DMT2 were diagnosed with Metabolic Syndrome (MS). A weak relation between MS and age (P=0.01) and no relations between MS and gender and MS and obesity were found. The prescribed diet for caloric regimen was regulated through variation of the carbohydrate regimen (r=0.89, P<0.001). The carbohydrate and caloric regimens were related to gender ( $\chi^2=12.33$ , P=0.002 and  $\chi^2=13.69$ , P=0.003), to age groups (P=0.01 and P=0.03) and to obesity (P=0.02 and  $\chi^2=24.76$ , P=0.003, respectively). An inverse relation between recommended diet with Sodium Chloride and MS (B=-1.38, OR=0.25, P=0.003) resulted.

#### Conclusions:

Diet as tertiary prophylaxis in patients with complicated DMT2 relates to gender, age groups and obesity for carbohydrates and caloric regimens, and to MS for Sodium Chloride regimen.

There is a weak relation between MS and age and no relations between MS and gender and obesity.

### EFFECTS OF PROBIOTIC SUPPLEMENTATION ON INFLAMMATORY STATUS OF OBESE WOMEN

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#### Objective:

Scientific evidences suggest that intestinal microbiota is a key player in development of chronic low-grade inflammation associated with obesity. Studies showed that circulating proinflammatory proteins (IL-6, TNF-alpha, leptin) are positively, while antiinflammatory protein adiponectin is negatively correlated with body fat accumulation. The aim of this study is to assess the effects of probiotic supplementation on inflammatory status of overweight and obese women.

#### Methods:

Twenty overweight (BMI=25.0-29.9 kg/m<sup>2</sup>) and obese (BMI≥30.0 kg/m<sup>2</sup>) women participated in double-blind randomized placebo-controlled trial (RCT). They were randomly assigned to receive one capsule daily of probiotics (7x10<sup>10</sup> CFU *Lactobacillus plantarum* 299v (DSM9843), 5x10<sup>9</sup> CFU *Saccharomyces cerevisiae* var. *boulardii* and 40mg octacosanol; N=12) or placebo (N=8) for 3 months. Plasma concentrations of IL-6, TNF-alpha, leptin and adiponectin were measured before the initiation of intervention (t0), at the end of intervention (t1) and three months after intervention period (t2), using RT-6100 Microplate Reader (Rayto, China).

#### Results:

Concentration of IL-6 was significantly decreased by the intervention with probiotics (p=0.05), specially at t2 compared to the t0 (p=0.003), without significant change in placebo group. In probiotic-supplemented group, significant increase of adiponectin concentration was observed (p=0.049), specially at t2 compared to the t0 (p=0.041). Although levels of TNF-alpha and leptin in probiotic group were slightly decreased, they didn't reach statistical significance.

#### Conclusions:

Probiotic supplementation modestly improved inflammatory status of obese women. Further studies are warranted to confirm these results, and such confirmation may lead to the introduction of probiotics as adjunctive therapy for obesity.

### THE KNOWLEDGE OF FERMENTATION AND HEALTH BENEFITS AMONG GENERAL POPULATION IN NORTH EASTERN SLOVENIA

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#### Objective:

Fermented foods are staples of the human diet. The preparation of fermented foods was performed in the past without knowledge of the role of microorganisms involved or beneficial effects on health. Nowadays, due to the proclaimed health benefits, fermented foods are becoming increasingly popular. The aim of our study was to examine the awareness and knowledge of people in Northeastern Slovenia with fermentation, fermented foods and beneficial impact on health.

#### Methods:

The cross-sectional study included 367 individuals (16-89 years of age). A questionnaire was designed to assess the participants' knowledge of fermentation, fermented foods, the consumption of fermented foods and awareness of the health benefits.

#### Results:

Compared with the youngest participants (<21 years) knowledge about fermentation was higher in older individuals (p<0,001). More than a half of participants knew the fermentation process is led by lactic acid bacteria and yeasts, however, only 17,4 % of participants were aware of the role of the molds. Only 24,5 % of the participants became acquainted with fermented foods at home and 61,3 % of them were aware of health benefits of fermented foods, but mostly on gut health and immune system.

#### Conclusions:

As people today live predominantly in urban areas and incline towards westernized foods, younger generations often lack the knowledge of fermentation and nutritional value of fermented foods. Steps should be taken to educate younger generations regarding the health benefits of fermented foods especially taking into account that most of them expressed their interest in learning more about this process.

#### Key words:

awareness, fermentation, general population, health benefits, knowledge.

#### EFFECT OF LACTOBACILLUS ACIDOPHILUS LA5 ON GUT MICROBIOTA USING AN IN VITRO GUT MICROBIOME MODEL

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##### Objective:

Verify the impact of probiotic *Lactobacillus acidophilus* - LA5 on the gut microbiota of adults (40-50 years old) and the production of metabolites using the The Simulator of the Human Intestinal Microbial Ecosystem (SHIME <sup>®</sup>).

##### Methods:

The experimental period in the SHIME<sup>®</sup> reactor lasted 6 weeks. After 7 and 14 days of colonic fermentation, samples of the colonic contents were evaluated microbiologically and chemically [production of short chain fat acids (SCFA) and ammonium ions]. The colonic fermentation was realized in triplicate. Statistical analyses were performed using two-way ANOVA, with Tukey's post-test ( $p < 0.05$ ).

##### Results:

The results showed significant increase in *Bifidobacterium* ssp, *Lactobacillus* ssp, total anaerobes and *Clostridium* ssp after 14 days La-5 treatment when compared with control period. SCFA production reduced after 7 days of treatment, but with a significant increase after 14 days of La-5 treatment. For ammonia ion production was observed a decrease ( $p < 0.05$ ) after 7 days of treatment when compared with control period.

##### Conclusions:

Finally, this study using gut microbiome model showed a positive and promising results of La-5 treatment in enhanced of gut homeostasis.

#### RIFAXIMIN FOR SMALL INTESTINAL BACTERIAL OVERGROWTH: A RETROSPECTIVE STUDY

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##### Objective:

Rifaximin seems to be effective and safe in treating gastrointestinal aspecific symptoms associated with small intestinal bacterial overgrowth (SIBO), however to date there is no consensus regarding the proper timing of the therapy.

The present study aims to provide preliminary data regarding the effects of rifaximin administered at the dosage of 600 mg/day for five days in patients with an established diagnosis of SIBO.

##### Patients/methods:

We retrospectively analysed clinical records and lactulose breath tests of 15 otherwise healthy patients (8 males and 7 females) aged between 30 and 60 years old, complaining of gastrointestinal symptoms consistent with SIBO. All the patients had a baseline lactulose breath test suggestive of SIBO. 7 subjects were treated with rifaximin at the daily dose of 600 mg (200 mg after each of the 3 meals), for 5 days per month (group 1). The other 8 subjects were treated with the same daily dose of Rifaximin, but for two monthly cycles of 5 days each (group 2). All the patients repeated the breath test after one month.

The results of the breath tests performed at the baseline and after a month were compared to determine whether the different dosage of the therapy had had different effects.

##### Results and conclusion:

When comparing breath test results between the two groups, we observed that Rifaximin may be able to improve intestinal dysbiosis and gastrointestinal symptoms due to SIBO, with results that seem to be more evident when rifaximin is administered in two monthly cycles rather than one.

### APPLICATION OF NATIVE LACTIC ACID BACTERIA WITH TECHNOLOGICAL AND PROBIOTIC PROPERTIES IN A PILOT SCALE FETA CHEESE PRODUCTION

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#### Objective:

The aim of the study was the incorporation of native lactic acid bacteria (LAB) with multi-functional properties in Feta cheese to develop a product with enhanced quality and distinctive organoleptic characteristics.

#### Methods:

9 LAB isolates (including *Lactococcus lactis*, *Lactobacillus brevis*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Leuconostoc mesenteroides* isolated from artisanal Greek cheeses) were applied as co-starters (mono and mix-cultures) in industrial-scale Feta cheese production. Feta was produced with the commercial starter-culture (control) and with the further addition of the LAB strains (12-cases). Microbiological, pH, HPLC (organic acids determination) and sensory analyses took place during ripening and storage at 4°C.

#### Results:

The results showed that after manufacture, LAB population was ~9.0 log CFU/g at all samples, whereas during storage their population declined (6.5-7.0 log CFU/g, depending on the case). pH was similar at all cases. Amongst the organic acids, citric and lactic were the most abundant acids, whereas during storage a notable reduction in the concentrations of all acids was observed. Furthermore, the addition of selected strains (*Lc. lactis*, *Ln. mesenteroides*, *Lb. brevis*, *Lb. plantarum* in mono- or co-cultures) led to desirable and distinctive organoleptic characteristics, compared to the control.

#### Conclusions:

The results of the present study are promising for the inclusion of selected indigenous LAB in the production of functional Feta cheese with distinctive organoleptic characteristics and traditional character.

#### Acknowledgment:

This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH-CREATE-INNOVATE (project code:T1EDK-00968)

### EFFICACY OF AN ORALLY ADMINISTERED COMBINATION OF LACTOBACILLUS PARACASEI LC11, CRANBERRY EXTRACT AND D-MANNOSIO FOR THE PREVENTION OF RECURRENT URINARY TRACT INFECTIONS IN POSTMENOPAUSAL WOMEN

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#### Objective:

##### Background

Approximately 40% of women experience during lifetime at least one urinary tract infection episode requiring antibiotic treatment and the implicated pathogen is *Escherichia coli* in 75–90% of cases. About one-third of these women develop recurrent infections, that is two or more episodes within 6 months or three or more episodes within 12 months documented by significant positive urine cultures. The risk of developing a recurrent episode is greater when the first infection was caused by *E. coli* than another pathogen. The period of a woman's life more targeted by the onset of recurrent cystitis certainly is postmenopausal age, when the fall of estrogen levels and its impact on urogenital mucosa predispose to bladder infections. In the management of these infections, the optimization of antibiotic strategies appears fundamental in view of the increasing bacterial resistance.

##### Trial rationale

The Research Hypothesis for the present study is to assess whether the oral therapy with compound containing *Lactobacillus paracasei* LC11, cranberry extract (Cranpure) and D-mannosio in the prevention of uncomplicated, recurrent urinary tract infections, eventually associated with local estrogen therapy, is effective in the prevention of recurrent urinary tract infections (UTIs) in postmenopausal women.

##### Primary outcome.

The primary outcome of the study is to evaluate the UTIs recurrence rate with a sub-categorization of the patients as follows:

- Responder = no episode of UTIs relapses (pain in the projection of the urinary bladder, frequent urination, a sensation of urethral discomfort and a positive urine culture) at final visit of follow up (day 150)
- Partial responder = no more than 1 episode of UTIs during the therapy or during the 60 days observation period
- Non responder = more than 1 recurrence of UTIs during the therapy or during the 60 days observation period

##### Secondary outcome.

The secondary endpoint is to evaluate the reduction of related symptoms and improvement in patients' quality of life at 5-months follow-up compared to baseline. The tool used for this purpose is The Pelvic Pain and Urgency/Frequency (PUF) patient symptom scale, a validated questionnaire used to evaluate patients with chronic pelvic pain (range 0–35, score >12 indicative of significant symptoms)

#### Methods:

##### Trial population

- Women between 40–70 years with physiological menopause
- No have taken hormonal (topical and/or systemic) treatment and SERM for menopausal symptoms in the past 3 months
- Women with mild-to-moderate urogenital atrophy, corresponding to a Vaginal Health Index (VHI) score between 10 and 15
- History of recurrent UTIs (two or more episodes within 6 months or three or more episodes within 12 months)
- Not taking antibiotics for their recurrent UTIs since at least one month
- Negative urine culture at baseline.
- Read and signed informed consent.

**Trial design**

The original intent-to treat includes 50 women randomized to receive one of the two treatment groups as follows:  
 -Group 1: local estrogen therapy only (0.005% estriol vaginal gel, daily for three weeks and then twice weekly up to 120 days  
 -Group 2: local estrogen therapy (0.005% estriol vaginal gel) with the same administration schedule as above + oral therapy with Lactoflorene Cist once a day for 60 days.

**Results:**

The outcomes of the trial confirm the assumption of a beneficial effect of a food supplement containing Lactobacillus Paracasei LC11, D-mannose and Cranberry Extract (Cranpure) in the prevention of recurrent urinary tract infection in post menopausal women.

**Conclusions:**

Prophylactic treatment with Lactoflorene Cist® proved to be effective and safe in the management of recurrent uncomplicated UTIs in menopausal women, thus confirming previous findings in the premenopausal female population

**BACILLUS LIPOPEPTIDE FENGYCIN ATTENUATES CLOSTRIDIAL VIRULENCE EXPRESSION VIA SIGNALLING INTERFERENCE**

Chuan Hao (Grant) TAN<sup>(1)</sup> - Noele Ng<sup>(1)</sup> - Tricia Lim<sup>(1)</sup> - Hari Chirakkal<sup>(1)</sup> - Hai Ming Tan<sup>(1)</sup>

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The accessory gene regulator (Agr)-based quorum sensing (QS) signalling is instrumental to *Clostridial* and *Staphylococcal* virulence expression for intestinal colonization and infection. Fengycin, a lipopeptide produced by many probiotic *Bacilli*, including *Bacillus subtilis*, was shown to inhibit the *Staphylococcal* Agr-QS, resulting in complete elimination of the pathogen from the hosts. The QS system in *Clostridium perfringens* (i.e., *agrBD* and *virSA*) shares a homology with that in *Staphylococcus aureus* (i.e., *agrBDCA*), but the impact of fengycin on *C. perfringens* remains unexplored. Using a farm strain 4.6 (toxintype G) as a *C. perfringens* model, we show that fengycin inhibited expression of perfringolysin O toxin, a hemolytic factor modulated by QS, in a concentration-dependent manner. Perfringolysin O expression was reduced significantly by fengycin at 1.5 ppm and inhibited completely at 15 ppm. Transcription of *agrD* and *netB*, encoding for the QS signalling peptide and a QS-regulated toxin key to avian necrotic enteritis, respectively, were downregulated by fengycin at concentrations as low as 0.1 ppm. While fengycin could suppress early biofilm formation (~ 3 h), such QS blocking effect was revoked at later stages (i.e., 6 h or more) presumably due to accumulation of endogenous signals in the culture that subdue the binding of fengycin to the QS receptors. Our findings demonstrate, for the first time, that fengycin can interfere with the *Clostridial* QS system and attenuate virulence expression at concentrations typically produced by *B. subtilis* culture, suggesting a novel mode of action for probiotic nutrition in governing animal health from pathogens.

### THE EFFECTIVENESS AND SAFETY OF MULTI-STRAIN PROBIOTIC PREPARATION IN PATIENTS WITH DIARRHEA - PREDOMINANT IRRITABLE BOWEL SYNDROME: A RANDOMIZED DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY

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It is supposed that the intestinal dysbiosis inducing disturbances in the functions of the gut-brain axis may be a main factor responsible for most of pathological conditions in irritable bowel syndrome (IBS). Several studies have reported significant alterations in the gut microbiota that may promote the development and persistence of IBS. Thus, modification of the gut microbiota composition by probiotics plays an important role in the treatment of IBS. The aim of the randomized double-blind placebo-controlled study was to evaluate the effectiveness and safety of multi-strain probiotic in adults with diarrhea-predominant IBS (IBS-D). The patients were randomized to receive a mixture of *Lactobacillus*, *Bifidobacterium* and *Streptococcus thermophilus* strains or placebo for eight weeks. Primary endpoints included changes in symptom severity and improvement assessed with the IBS Severity Scoring System (IBS-SSS) and Global Improvement Scale (IBS-GIS). The probiotic in comparison with placebo significantly improved the IBS symptom severity (the change of total IBS-SSS score from baseline  $-165.8 \pm 78.9$  in the probiotic group and  $-105.6 \pm 60.2$  in the placebo group,  $p=0.005$ ) and in the specific scores related to the severity of pain ( $p=0.015$ ) and the quality of life ( $p=0.016$ ) after eight weeks of intervention. The probiotic group indicated an improvement of symptoms with the use of the IBS-GIS compared with the placebo group after four ( $p=0.04$ ) and eight weeks ( $p=0.003$ ). The occurrence of adverse events did not differ between study groups. In conclusion, the multi-strain probiotic intervention resulted in a significant improvement in IBS symptoms evaluated with the use of both IBS-SSS and IBS-GIS scales. These results suggest that the administration of studied probiotic preparation is well tolerated and safe, and can offer a benefit for patients with IBS-D. [Registration number in Clinicaltrials.gov NCT 04662957].

### ISOLATION AND PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF THE POTENTIAL PROBIOTIC STRAINS OF *LACTOBACILLUS* FROM THE IRANIAN POPULATION

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#### Objective:

Among different causes of inflammatory bowel disease (IBD), the imbalance of the gut microbiome (dysbiosis) is one of the main reasons in the development of the disease. Probiotics are the live microorganisms that can maintain gut microbiota by different mechanisms. We aimed to isolate and characterize the potential probiotic strains of *Lactobacillus* from the Iranian population.

#### Methods:

This cross-sectional study was conducted during 2019 on fecal samples of 83 volunteer individuals living in Guilan Province, North Iran. The primary identification of *Lactobacillus* strains was performed by standard microbiological tests and confirmed by amplification of 16s rRNA specific primers. The acid and bile salt tolerance were assessed for all recovered strains. Also, the presence of 3 bacteriocins encoding genes was investigated by PCR method.

#### Results:

Totally, 42 samples were positive for *Lactobacillus* species. Acid and bile resistance assay showed that 67% and 33% of strains were resistant to acid and bile salt stress, respectively. We find out that 28% of our *Lactobacillus* strains have the ability for resistance to acid and bile conditions. PCR results revealed that the prevalence of gassericin A, plantaricin S, lactacin bacteriocin genes were 16.6%, 12%, and 9.5%, respectively. Meanwhile, 5 out of 12 *Lactobacillus* strains that were resistant to acid and bile conditions contained one of the gassericin or plantaricin bacteriocins.

#### Conclusions:

We isolated 42 potential probiotic strains of *Lactobacillus*. Of which, results of 5 strains were more promising and can be considered as potential probiotics sources for future functional products.



FACULTY

| <b>NAME</b>               | <b>COUNTRY</b> | <b>PAGE</b> |
|---------------------------|----------------|-------------|
| Alterio Tommaso           | <i>Italy</i>   | 11          |
| Alvaro Domenico           | <i>Italy</i>   | 16          |
| Ascione Antonio           | <i>Italy</i>   | 19          |
| Augustin Livia S.A        | <i>Italy</i>   | 9           |
| Auricchio Renata          | <i>Italy</i>   | 8           |
| Azpiroz Fernando          | <i>Spain</i>   | 12          |
| Bahl Martin Iain          | <i>Denmark</i> | 12          |
| Barbara Giovanni          | <i>Italy</i>   | 5           |
| Benedetti Antonio         | <i>Italy</i>   | 2, 16       |
| Berni Canani Roberto      | <i>Italy</i>   | 10, 11      |
| Bianco Prevot Michela     | <i>Italy</i>   | 17          |
| Binda Sylvie              | <i>France</i>  | 23          |
| Bonci Paola               | <i>Italy</i>   | 20          |
| Bonizzi Luigi             | <i>Italy</i>   | 3           |
| Borroni Davide            | <i>Italy</i>   | 20          |
| Bourdichon Francois       | <i>France</i>  | 2           |
| Brigidi Patrizia          | <i>Italy</i>   | 2, 12       |
| Brochot Amandine          | <i>Belgium</i> | 2           |
| Cai Tommaso               | <i>Italy</i>   | 20          |
| Caldarelli Guido          | <i>Italy</i>   | 17          |
| Cammarota Giovanni        | <i>Italy</i>   | 24          |
| Capurso Gabriele          | <i>Italy</i>   | 25          |
| Capurso Lucio             | <i>Italy</i>   | 2, 14       |
| Cardinale Vincenzo        | <i>Italy</i>   | 16          |
| Carucci Laura             | <i>Italy</i>   | 11          |
| Castagnetti Andrea        | <i>Italy</i>   | 9           |
| Castellazzi Annamaria     | <i>Italy</i>   | 4           |
| Cicero Arrigo F. G.       | <i>Italy</i>   | 9           |
| Clarke Gerard             | <i>Ireland</i> | 24          |
| Clementi Massimo          | <i>Italy</i>   | 14          |
| Collins Stephen M.        | <i>Canada</i>  | 12          |
| Cominelli Fabio           | <i>USA</i>     | 12          |
| Conte Federica            | <i>Italy</i>   | 17          |
| Corazziari Enrico Stefano | <i>Italy</i>   | 5, 20       |

| <b>NAME</b>                   | <b>COUNTRY</b>     | <b>PAGE</b> |
|-------------------------------|--------------------|-------------|
| Cordailat-Simmons Magali      | <i>France</i>      | 12          |
| De Giorgio Roberto            | <i>Italy</i>       | 8           |
| De Seta Francesco             | <i>Italy</i>       | 18, 19      |
| de Sire Roberto               | <i>Italy</i>       | 1           |
| de Vos Willem M.              | <i>Netherlands</i> | 2           |
| Del Chierico Federica         | <i>Italy</i>       | 1           |
| Del Rio Daniele               | <i>Italy</i>       | 3, 6, 9     |
| Derosa Lisa                   | <i>Italy</i>       | 25          |
| Di Giorgi Gerevini Valeria D. | <i>Italy</i>       | 22          |
| Donati Zeppa Sabrina          | <i>Italy</i>       | 19          |
| Doré Joel                     | <i>France</i>      | 2, 12       |
| Eloit Monique                 | <i>France</i>      | 3           |
| Ercolini Danilo               | <i>Italy</i>       | 17          |
| Facciotti Federica            | <i>Italy</i>       | 1, 12       |
| Fasano Alessio                | <i>USA</i>         | 1           |
| Felice Valeria                | <i>Ireland</i>     | 18          |
| Ferreri Carla                 | <i>Italy</i>       | 9           |
| Ferri Nicola                  | <i>Italy</i>       | 9           |
| Ferruzzi Mario G.             | <i>USA</i>         | 3           |
| Fiocchi Alessandro            | <i>Italy</i>       | 11          |
| Fogliano Vincenzo             | <i>Netherlands</i> | 17          |
| Francavilla Ruggiero          | <i>Italy</i>       | 10          |
| Franceschi Francesco          | <i>Italy</i>       | 18, 19      |
| Gargiullo Livia               | <i>Italy</i>       | 24          |
| Garofolo Giuliano             | <i>Italy</i>       | 3           |
| Gasbarrini Antonio            | <i>Italy</i>       | 24          |
| Gasbarrini Giovanni           | <i>Italy</i>       | 24, 76      |
| Ghelardi Emilia               | <i>Italy</i>       | 1           |
| Ghiselli Andrea               | <i>Italy</i>       | 2           |
| Gianfagna Francesco           | <i>Italy</i>       | 15          |
| Gili Tommaso                  | <i>Italy</i>       | 17          |
| Gonzalez Cautela Brunella     | <i>Canada</i>      | 24          |
| Gosálbez Luis                 | <i>Spain</i>       | 23          |
| Guarino Alfredo               | <i>Italy</i>       | 10          |

| <b>NAME</b>             | <b>COUNTRY</b>   | <b>PAGE</b> |
|-------------------------|------------------|-------------|
| Guarino Michele         | <i>Italy</i>     | 26          |
| Guarner Francisco       | <i>Spain</i>     | 12          |
| Guarrasi Valerio        | <i>Italy</i>     | 17          |
| Heilandt Tom            | <i>Italy</i>     | 23          |
| Hugas Marta             | <i>Italy</i>     | 12          |
| Ianiro Gianluca         | <i>Italy</i>     | 24          |
| Indrio Flavia           | <i>Italy</i>     | 10          |
| Ippolito Giuseppe       | <i>Italy</i>     | 14          |
| Isolauri Erika          | <i>Finland</i>   | 11          |
| Joyce Susan A.          | <i>Ireland</i>   | 15          |
| Kadar Gena E.           | <i>USA</i>       | 23          |
| Koch Maurizio           | <i>Italy</i>     | 1, 15       |
| Koren Omry              | <i>Israel</i>    | 18          |
| Kiyono Hiroshi          | <i>Japan</i>     | 14          |
| Lahbabi-Amrani Naima    | <i>Morocco</i>   | 2           |
| Lakhtin Vladimir        | <i>Russia</i>    | 1, 58, 60   |
| Lane Jonathan           | <i>Ireland</i>   | 18          |
| Lang Lucy               | <i>Australia</i> | 22          |
| Langella Philippe       | <i>France</i>    | 2, 18       |
| Laterza Lucrezia        | <i>Italy</i>     | 15          |
| Laulund Esben           | <i>Denmark</i>   | 22          |
| Lavermicocca Paola      | <i>Italy</i>     | 3, 9, 15    |
| Lecchini Pierdavide     | <i>Italy</i>     | 3           |
| Leyer Gregory           | <i>USA</i>       | 24          |
| Levi Mortera Stefano    | <i>Italy</i>     | 1           |
| Libra Massimo           | <i>Italy</i>     | 25          |
| Lo Vecchio Andrea       | <i>Italy</i>     | 10          |
| Lopetuso Loris Riccardo | <i>Italy</i>     | 1           |
| Lovegrove Julie A.      | <i>UK</i>        | 15          |
| Macari Gabriele         | <i>Italy</i>     | 17          |
| Marabelli Romano        | <i>Italy</i>     | 3           |
| Marafini Irene          | <i>Italy</i>     | 1           |
| Marignani Massimo       | <i>Italy</i>     | 26          |
| Maroni Luca             | <i>Italy</i>     | 16          |

| <b>NAME</b>                  | <b>COUNTRY</b>   | <b>PAGE</b> |
|------------------------------|------------------|-------------|
| Marzano Valeria              | <i>Italy</i>     | 1           |
| Mayo Baltasar                | <i>Spain</i>     | 12          |
| Merlo Pich Emilio            | <i>Italy</i>     | 5           |
| Merten-Lentz Katia           | <i>Belgium</i>   | 22          |
| Migliaccio Silvia            | <i>Italy</i>     | 9           |
| Miraglia del Giudice Michele | <i>Italy</i>     | 14          |
| Morelli Lorenzo              | <i>Italy</i>     | 14, 23      |
| Moschetta Antonio            | <i>Italy</i>     | 25          |
| Moser Andrea V.              | <i>Argentina</i> | 22          |
| Murina Filippo               | <i>Italy</i>     | 18          |
| Natali Pier Giorgio          | <i>Italy</i>     | 19          |
| Nisticò Paola                | <i>Italy</i>     | 25          |
| Noonan Gregory               | <i>USA</i>       | 22          |
| Noviello Daniele             | <i>Italy</i>     | 1           |
| Pagliaro Mario               | <i>Italy</i>     | 9           |
| Paraskevacos George          | <i>Canada</i>    | 22, 23      |
| Pecere Rosanna               | <i>Belgium</i>   | 2, 22       |
| Pellegrini Nicoletta         | <i>Italy</i>     | 17          |
| Perno Carlo Federico         | <i>Italy</i>     | 1           |
| Petito Valentina             | <i>Italy</i>     | 1           |
| Petricevic Ljubomir          | <i>Austria</i>   | 18          |
| Pignatti Marco               | <i>Italy</i>     | 20, 21      |
| Pineda David                 | <i>Belgium</i>   | 23          |
| Pisani Laura Francesca       | <i>Italy</i>     | 1           |
| Poeta Marco                  | <i>Italy</i>     | 10          |
| Poli Andrea                  | <i>Italy</i>     | 9           |
| Pot Bruno                    | <i>Belgium</i>   | 23          |
| Pozzi Gianni                 | <i>Italy</i>     | 14          |
| Putignani Lorenza            | <i>Italy</i>     | 17          |
| Restani Patrizia             | <i>Italy</i>     | 3           |
| Ricci Antonia                | <i>Italy</i>     | 3           |
| Rubini Elisabetta            | <i>Italy</i>     | 19, 20      |
| Saggioro Alfredo             | <i>Italy</i>     | 19          |
| Sanz Yolanda                 | <i>Spain</i>     | 12          |
| Savino Francesco             | <i>Italy</i>     | 10          |

| <b>NAME</b>                  | <b>COUNTRY</b>     | <b>PAGE</b>        |
|------------------------------|--------------------|--------------------|
| Scaldaferri Franco           | <i>Italy</i>       | 1                  |
| Scarpato Elena               | <i>Italy</i>       | 10                 |
| Segata Nicola                | <i>Italy</i>       | 1                  |
| Shai Iris                    | <i>Israel</i>      | 24                 |
| Shamir Raanan                | <i>Israel</i>      | 8                  |
| Shanahan Fergus              | <i>Ireland</i>     | 24                 |
| Simeone Piero                | <i>Italy</i>       | 20                 |
| Soggiu Alessio               | <i>Italy</i>       | 3                  |
| Steller Michael              | <i>Canada</i>      | 22                 |
| Strati Francesco             | <i>Italy</i>       | 1                  |
| Szajewska Hanja              | <i>Poland</i>      | 10                 |
| Teodori Laura                | <i>Italy</i>       | 1                  |
| Troncone Riccardo            | <i>Italy</i>       | 8                  |
| Tuohy Kieran M.              | <i>Italy</i>       | 15                 |
| Ulaszewska-Tarantino Marynka | <i>Italy</i>       | 15                 |
| van Goudoever Hans           | <i>Netherlands</i> | 10                 |
| Vandenplas Yvan              | <i>Belgium</i>     | 11                 |
| Vecchi Maurizio              | <i>Italy</i>       | 12                 |
| Verduci Elvira               | <i>Italy</i>       | 11                 |
| Vernocchi Pamela             | <i>Italy</i>       | 1                  |
| Vicariotto Franco            | <i>Italy</i>       | 15, 18, 19, 24, 77 |
| Villa Paola                  | <i>Italy</i>       | 18                 |
| Visioli Francesco            | <i>Italy</i>       | 9, 26              |
| Vitaglione Paola             | <i>Italy</i>       | 9                  |

**INDEX OF ORAL COMMUNICATIONS  
AND POSTERS AUTHORS**

### A

Abad Inés  
Acin-Albiac Marta  
Adamberg Kaarel  
Aguirre-Ramírez Diego  
Ahles Sanne  
Ahmed Marroki  
Ait-Abdellah Samira  
Alieva Aliya  
Al-Judaibi Awatif  
AlJudaibi Effat  
Almeida Diana  
AlShareef Sahar  
Altieri Fabio  
Amar Abid  
Amedei Amedeo  
Amodio Piero  
Amoruso Angela  
Andolfo Annapaola  
Andrade José Carlos  
Andrés Grau Ana  
Andrews Christopher  
Angeletti Mauro  
Annaloro Gabriela  
Argri Konstantina  
Argyri Anthoula  
Asadzadeh Aghdaei Hamid  
Asensio-Grau Andrea  
Ashorov Olga  
Ashrafian Fatemeh  
Auger Jeremie  
Authier Hélène  
Azzimonti Barbara

### B

Bahrim Gabriela Elena  
Baldewijns Silke  
Baltazar Fátima  
Banavar Guru  
Bangotra Ridhika  
Banić Martina  
Banovic Pavle  
Barbara Grube  
Barbosa Joana Cristina  
Barbut Frédéric  
Battista Natalia

Bellés Andrea  
Bellich Barbara  
Belovic Miona  
Ben Simon Shira  
Bermudez-Humaran Luis G.  
Bertrand Bénédicte  
Bierla Joanna Beata  
Bikouli Vasiliki  
Birba Vasiliki  
Bobinas Ceslovas  
Bona Gianni  
Bonfilli Laura  
Bottari Benedetta  
Brambilla Laura  
Bruehlmann Sabina  
Butorac Katarina

### C

Cakarevic Jelena  
Calcabrini Annarica  
Campagna Francesca  
Canali Raffaella  
Cannon Mark  
Capitano Bruno  
Cappello Claudia  
Capuano Edoardo  
Capuzzo Elena  
Cardoso Olga  
Carlomagno Federica  
Carpi Francesco M.  
Carradori Simone  
Carvalho Frédéric  
Casciano Flavia  
Casertano Melania  
Castellone Vincenzo  
Cavallo Ilaria  
Cecarini Valentina  
Cerantola Silvia  
Cescutti Paola  
Chain Florian  
Chirakkal Hari  
Chorianopoulos Nikos  
Cichoż-Lach Halina  
Cid Rodriguez Maria  
Clavel Thomas  
Clementine Trotin Picolo



Cologna Nicola  
 Colombi Stefano  
 Colone Marisa  
 Coman Maria Magdalena  
 Cominelli Fabio  
 Constantin Erlenbeck  
 Corazzari Marco  
 Cormio Luigi  
 Corsetti Aldo  
 Costa Francisca  
 Coste Agnès  
 Cotârleţ Mihaela  
 Crespo Yanguas Marta  
 Cristina Petrescu  
 Cuccioloni Massimiliano  
 Cukrowska Bożena  
 Čuljak Nina

## D

Daneshazari Roya  
 De Angelis Fernando  
 De Prisco Annachiara  
 De Santa Francesca  
 Deidda Francesca  
 Delannoy Johanne  
 Delle Fave Gianfranco  
 Demuyser Liesbeth  
 Devirgiliis Chiara  
 Di Cesare Francesca  
 Di Domenico Enea Gino  
 Di Franco Manuela  
 Di Martino Luca  
 Di Paolo Maria Carla  
 Di Sotto Antonella  
 Dickman Ram  
 Dionisi Tommaso  
 Distefano Alfio  
 Domingos Melany  
 Donato Maria Manuel  
 Đorđević Brižita  
 Doulgeraki Agapi  
 Dragačević Luka

## E

Efremova Irina  
 Eleuteri Anna Maria

Ensoli Fabrizio  
 Ercolini Danilo  
 Esmaeili Hossein  
 Eufemi Margherita

## F

Faggin Sofia  
 Falzone Luca  
 Fanti Federico  
 Farioli Alessia  
 Farioli Vecchioli Stefano  
 Farmani Maryam  
 Favaro Wagner  
 Federici Federica  
 Ferrari Eleonora  
 Fijan Sabina  
 Finamore Alberto  
 Fiorini Dennis  
 Fogliano Vincenzo  
 Fonseca Mariana  
 Forte Silvia  
 Franceschi Pietro  
 Franciosa Giovanna  
 Franciosi Elena  
 Freitas Ana Cristina  
 Fuochi Virginia  
 Furneri Pio Maria

## G

Gabiaelli Rosita  
 Gagliardi Mara  
 Gal Caroline  
 Ganeshan Sri  
 Gänzle Michael  
 García Alonso María Luisa  
 Garcia-Gonzalez Natalia  
 García-Hernández Jorge  
 Gargari Giorgio  
 Gasbarrini Antonio  
 Gasbarrini Giovanni  
 Genaro Sandra  
 Ghannoum Mahmoud  
 Ghini Veronica  
 Gianotti Andrea  
 Giardina Silvana  
 Gingold Belfer Rachel

Gioia Chiara  
Giron Maria Cecilia  
Golić Nataša  
Gomes Ana Maria  
Gomes Sara  
Gomez Miguel Maria Jesus  
Gong Chunmei  
Gordana Bothe  
Goss Andrea  
Grande Rossella  
Grasa Laura  
Graziani Maria Giovanna  
Graziano Teresa  
Grimaldi Roberta  
Grounta Athena  
Guantario Barbara  
Guerrieri Francesca  
Guida Serena  
Gusman Vera  
Guzzo Maria Paola

### H

Habib Bisma  
Haddad Kashani Hamed  
Hamouda Dalal  
Hang Tianyu  
Harthorn Lucien  
Hasannejad-Bibalan Meysam  
Haslberger Alexander  
Hayat Fetati  
Heredia Ana  
Hernández Artur  
Hernandez Stéphanie  
Hernandez-Olivas Ever  
Herr Deron Raymond  
Hippe Berit  
Hitch Thomas  
Holowacz Sophie  
Hosseini-Abari Afrouzossadat  
Hu Ying  
Huang Ryan Yuki  
Huang Xiaohui  
Huang Zhan

### I

Iacobelli Stefano

Iannuccelli Cristina  
Ilić Vesna  
Imberti Luisa  
Imbesi Veronica  
Ioannidis Aristidis  
Isakov Vasily  
Ivanovic Nevena  
Ivashkin Vladimir

### J

Jaagura Madis  
Jacouton Elsa  
Jalilpour Jalil  
Jin Ge  
Jonathan Breton  
Jusius Gintaras

### K

Kamarinou Christina  
Kapustina Valentina  
Kawata Salgaço Mateus  
Kazantseva Jekaterina  
Kazazić Saša  
Kazazić Snježana  
Khoramjo Seyed Mobin  
Kiiker Liisa  
Kochetkova Alla  
Kombarova Svetlana  
Koren Omry  
Kos Blaženka  
Kotzampassi Katerina  
Kriščiunaite Tiina  
Kumar Neeraj

### L

Lakhtin Vladimir  
Lambert Gregory  
Langella Philippe  
Längle Julia  
Larcher Roberto  
Larsen Stefano  
Laterza Lucrezia  
Latiano Tiziana Pia  
Lazarević Slavica  
Leboš Pavunc Andreja  
Leila Bousmaha-Marroki

Lenoir Loïc  
 Li Volti Giovanni  
 Libra Massimo  
 Lim Tricia  
 Lincetti Silvia  
 Llaveró Carolina  
 Lopetuso Loris Riccardo  
 López-Nicolás Rubén  
 Lorenzo González Óscar  
 Lou Lawrence  
 Luchinat Claudio  
 Lumpuy Castillo Jairo

## M

Machado Daniela  
 Maillard Flore  
 Mainente Federica  
 Malfa Patrizia  
 Mancini Andrea  
 Manna Laura  
 Marazzato Massimiliano  
 Maroua Khedroughi  
 Martin Rosique Rebeca  
 Martinović Kristina  
 Maruszak Aleksandra  
 Masciarelli Martina  
 Maslennikov Roman  
 Matieu Olivier  
 Mattana Marco  
 Maurer Sost Mônica  
 Medic Deana  
 Méghit Boumédiène Khaled  
 Melikhova Alexandra  
 Meloni Marisa  
 Menon Sharanya  
 Merola Carmine  
 Merz Andrea  
 Mičetić Turk Dušanka  
 Michaličkova Danica  
 Middei Silvia  
 Mikov Momir  
 Milinkovic Neda  
 Minacori Marco  
 Mitrović Hristina  
 Monzani Romina  
 Mora Diego

Mora Vincenzina  
 Morazzoni Carlotta  
 Moreno Yolanda  
 Moreno-Mesonero Laura  
 Morozov Sergey  
 Moschen Alexander  
 Mosoni Carolina  
 Mostefa Naimi  
 Moysidis Moysis  
 Murina Filippo

## N

Nannini Giulia  
 Napoli Marco  
 Napolioni Valerio  
 Nardin Tiziana  
 Natella Fausta  
 Neroni Bruna  
 Ng Noele  
 Nikolic Natasa  
 Nikzad Hossein  
 Nissen Lorenzo  
 Novak Jasna

## O

Okuka Nina  
 Oliviero Teresa  
 Orlova Olga  
 Osme Abdullah  
 Osório Sofia

## P

Pacularu-Burada Bogdan  
 Paglia Giuliano  
 Pagnini Cristiano  
 Pallotta Lorella  
 Palmans Ilse  
 Pańczyszyn Elżbieta  
 Pane Marco  
 Panebianco Concetta  
 Pantanella Fabrizio  
 Pantić Višnja  
 Papadopoulou Olga  
 Part Natalja  
 Patić Aleksandra  
 Pavlović Nebojša

Pazienza Valerio  
Pereira-Wilson Cristina  
Perozzi Giuditta  
Perri Francesco  
Perugini Monia  
Pesciaroli Chiara  
Petrella Carla  
Pfister Alexandra  
Pham Vi  
Piantelli Mauro  
Picciolini Matteo  
Pierre Dechelotte  
Pignatti Marco  
Pihurov Marina  
Pindo Massimo  
Pinto Alves Mayer Marcia  
Pinto Daniela  
Piovan Anna  
Piro Demetrio  
Pisati Federica  
Pizarro Theresa  
Plattner Stephan  
Polovina Snezana  
Poluektova Elena  
Pop Oana Lelia  
Potenza Adele  
Pravadelli Cecilia  
Prete Roberta  
Preto Ana  
Prozorow-Król Beata  
Przysiecki Nicole  
Puca Valentina

### Q

Quiros-Roldan Eugenia

### R

Rabelo Socca Eduardo Augusto  
Radocchia Giulia  
Raia Valeria  
Ramos Fernando  
Rapacioli Silvia  
Rehak Renata  
Reider Simon  
Ribeiro de Souza Sasaki Bianca  
Ricci Chiara

Ricci Valentina  
Rinaldi Fabio  
Rizzello Fernando  
Rizzi Eleonora  
Rodríguez-Largo Ana  
Ros-Berruezo Gaspar  
Roselli Marianna  
Rossi Giacomo  
Rubert Josep  
Rubini Elisabetta  
Ruiz Tovar Jaime  
Rybalchenko Oksana

### S

Salemi Rossella  
Sanchez García Janaina  
Sánchez Lourdes  
Sánchez-Moya Teresa  
Santagati Maria  
Sarkisyan Varuzhan  
Sartoratto Adilson  
Sasunova Armida  
Savarino Edoardo  
Saverio Valentina  
Schifano Emily  
Schippa Serena  
Sedigh Ebrahim-Saraie Hadi  
Serguei Fetissof  
Settanni Carlo  
Shahrokh Shabnam  
Shao Yuqi  
Sharma Surbhi  
Shenavar Masouleh Alireza  
Šikić Pogačar Maja  
Sillen Mart  
Silva Elisabete  
Silva Mariana  
Silvi Stefania  
Sivieri Katia  
Sivori Francesca  
Skrzydło-Radomańska Barbara  
Sliupas Rokas  
Spampinato Mariarita  
Spigarelli Renato  
Spisni Enzo  
Spivak Mykola

Squarzanti Diletta  
 Stanimirov Bojan  
 Stanković Marija  
 Stasi Simone  
 Stavrou George  
 Stevens Yala  
 Streimikyte Paulina  
 Strimpakos Georgios  
 Stringaro Annarita  
 Stulova Irina  
 Sturm Sonja  
 Suharoschi Ramona  
 Sumarac-Dumanovic Mirjana  
 Šušković Jagoda

## T

Tan Chuan Hao (Grant)  
 Tan Hai Ming  
 Tang Qiang  
 Tassou Chrysoula  
 Teixeira-Guedes Catarina  
 Tenori Leonardo  
 Tilg Herbert  
 Torbica Aleksandra  
 Torcinaro Alessio  
 Torres-Maravilla Edgar  
 Tripodo Claudio  
 Trudic Anika  
 Tsaousi Georgia  
 Tsetis Julia K.  
 Tuohy Kieran M.  
 Turrone Silvia

## U

Udenigwe Chibuikwe  
 Ulaszewska Maria  
 Urbonaviciene Dalia  
 Urgesi Riccardo

## V

Valerii Maria Chiara  
 van de Burgwal Linda  
 van der Geest Anne  
 Van Dijck Patrick  
 Vandecruys Paul  
 Vasile Mihaela Aida

Velickovic Ksenija  
 Veljović Katarina  
 Venema Koen  
 Verbruggen Sanne  
 Verdenelli Maria Cristina  
 Vergnolle Nathalie  
 Verhoeven Jessica  
 Vicariotto Franco  
 Viiard Ene  
 Villani Annacandida  
 Villella Valeria Rachela  
 Visciglia Annalisa  
 Visioli Francesco  
 Viskelis Jonas  
 Viskelis Pranas  
 Vitale Irene  
 Vivarelli Silvia  
 Von Hunolstein Christina  
 Vorobiova Valentina  
 Vorobyova Irina  
 Vukmirović Saša  
 Vuysich Momo

## W

Waligora-Dupriet Anne-Judith  
 Wang Bangmao  
 Wang Gang  
 Wang Weilan  
 Wells Jerry  
 Wilsack Lynn

## Y

Yadegar Abbas

## Z

Zali MohammadReza  
 Zannini Emanuele  
 Zhilenkova Olga  
 Zineb Benmechernene  
 Zinno Paola  
 Živković Irena  
 Zoccatelli Gianni  
 Zollner Andreas



- |                    |             |                       |
|--------------------|-------------|-----------------------|
| Algeria            | Germany     | Republic of Singapore |
| Austria            | Greece      | Romania               |
| Belgium            | India       | Russian Federation    |
| Bosnia Herzegovina | Iran        | Saudi Arabia          |
| Brazil             | Israel      | Serbia                |
| Canada             | Italy       | Slovenia              |
| China              | Lithuania   | Spain                 |
| Croatia            | Netherlands | Switzerland           |
| Estonia            | Poland      | Ukraine               |
| France             | Portugal    | USA                   |

# GENERAL INFORMATION

## GENERAL INFORMATION

### **DATES & TIMES**

September 12 - 14, 2021.

The Meeting will commence at 08.30 (CET) Sunday 12 September and will conclude on Tuesday 14 September at 13.00 (CET).

Invitations to participate are personable and non-transferable.

### **CONFERENCE VENUE**

Università Urbaniana, Via Urbano VIII, 16 - 00165 Rome, Italy  
Phone +39 06 69889611- Fax +39 06 69881871  
www.urbaniana.edu

### **COVID-19**

Covid-19 procedures will be communicated and constantly updated.

### **LANGUAGE**

The Meeting will be held in English. Translation services will not be available.

### **DRESS CODE**

Smart casual.

### **CLIMATE**

September is one of the most beautiful months to visit Rome. Whilst approaching the end of summer, the weather in September is still warm and sunny. Temperatures range from a warm 25° degrees during the day and drop to a cool 15° degrees in the evening. A light weight jacket/cardigan/scarf is recommended for the evenings.

### **CLOAKROOM**

If required, luggage can be left in the cloakroom at the venue.

### **REGISTRATION & NAME BADGES AVAILABLE AT THE ORGANISING SECRETARIAT DESK**

On-site registration and issuance of badges is available daily from:

- Sunday 12 September: 08.00 - 19.00
- Monday 13 September: 08.00 - 18.00
- Tuesday 14 September: 08.00 - 14.00

Registration payment can be made by credit card or cash directly at the Organising Secretariat desk.

For security purposes participants will be requested to present an identification document.

Participants and exhibitors will be required to wear name badges for access to the venue and all the meeting rooms.



**REGISTRATION FEES (22% VAT included)**

|   |          |
|---|----------|
| Participants  | € 300,00 |
| Presenting Authors (abstract fee included)          | € 100,00 |
| Biologists/Pharmacists/Chemist                      | € 150,00 |
| Dieticians/Nutritionists                            | € 150,00 |
| Nurses  | € 100,00 |
| IPA/GPA Members                                     | € 200,00 |
| Members Mediterranean Task Force for Cancer Control | € 150,00 |
| Under 35*   | € 150,00 |
| Pediatric Day**                                     | € 200,00 |
| Under 35 Pediatric Day                              | € 100,00 |
| Daily Registration                                  | € 200,00 |

\* the applicant's registration form must be accompanied by a copy of an official document.

\*\* If you are not registered to the Meeting.

**Registration fee includes:**

- Admission to scientific sessions, technical exhibition
- Final programme
- Selected proceedings and abstract
- Access to B2B meeting platform
- Coffee corner and lunch
- Opening ceremony and welcome cocktail September 12
- Pasta Party September 13
- Certificate of attendance
- Italian CME certificate (to whom entitled)

**Cancellation Policy**

Cancellations must be sent via email to the Organising Secretariat.

In the case of cancellation by July 31, 2021, a 50 % refund of the total registration amount will be refunded (bank commissions/ expenses are excluded).

Please note that refunds will not be possible after this date. Refunds will be issued within 30 days of the Meeting conclusion.

**BANKING AND CURRENCY EXCHANGE**

The Italian monetary system is the Euro (€). Foreign currency can be exchanged at banks, currency exchange offices, hotels and at the airport upon presentation of an identification document. All major credit cards are accepted in most hotels, restaurants and shops.

### LIABILITY AND INSURANCE

The Organising Secretariat cannot accept liability for personal injuries or for loss of, or damage to, property belonging to Meeting participants (or accompanying persons) either during or as a result of the Conference. Please check the terms and conditions of your health insurance.

### CERTIFICATE OF ATTENDANCE

Certificates of attendance will be provided to all registered participants by the Organising Secretariat desk at the conclusion of the Conference.

### FOOD AND BEVERAGES

Sunday 12 September - A buffet lunch will be served at the Meeting venue  
Sunday 12 September - Welcome Cocktail at the Meeting venue  
Monday 13 September - A buffet lunch will be served at the Meeting venue  
Monday 13 September - Pasta party  
Coffee & snacks are available from the coffee corner areas.

### TRANSPORTATION

#### AIRPORT INFORMATION

Rome is served by two international airports:

Rome Leonardo da Vinci International Airport, located in Fiumicino, 34 km from Rome's city centre.

Rome-Ciampino International Airport, located 15 km from Rome's city centre.

#### ACCESS TO CONFERENCE VENUE FROM ROME LEONARDO DA VINCI INTERNATIONAL AIRPORT

- **Taxi:** The Meeting venue is located 30 km from the airport. Allow 35 mins by taxi, depending on traffic. The taxi fare costs € 48.00 (fixed fare).
- **Public transport:** Participants may take the Leonardo Express, a non-stop service which operates to/from Rome Termini railway station departing every 15 minutes. The train trip takes 30 minutes. The ticket costs € 14.00. <https://www.trenitalia.com/en.html>. From Rome Termini participants may take Bus no.64 to the Hospital S. Spirito (Lgt. Sassia) bus stop. The trip takes approximately 30 minutes and costs € 1.50. Bus tickets must be purchased at the station. The Università Urbaniana is a 6-minute walk from the Hospital S. Spirito bus stop.

#### ACCESS TO CONFERENCE VENUE FROM ROME-CIAMPINO INTERNATIONAL AIRPORT

- **Taxi:** The Meeting venue is located 20 km from the airport. Allow 40 minutes by taxi, depending on traffic. The taxi fare costs € 50.00 (fixed fare).
- **Public transport:** Service Provider Terravision Bus Company. [https://www.terravision.eu/airport\\_services.html?noredirect=en\\_US](https://www.terravision.eu/airport_services.html?noredirect=en_US). Participants make take the bus to Rome Termini railway station. Allow approximately 40 minutes. The ticket costs € 5.80 and can be purchased either online or at the airport. From Rome Termini participants may take Bus no. 64 to the Hospital S. Spirito (Lgt. Sassia) bus stop. The trip takes approximately 30 minutes and costs € 1.50. Bus tickets must be purchased at the station. The Università Urbaniana is a 6-minute walk from the Hospital S. Spirito bus stop.
- There is no train station at Rome-Ciampino International Airport.

**TAXI SERVICES**

We recommend using only licensed taxis located outside the airports and train stations. For taxi/shuttle services from the Meeting venue, please contact the Organising Secretariat desk.

For reputable taxi companies, the following phone numbers are provided:

+39 06 3570 Radio Taxi  
 +39 06 5551 Samarcanda  
 +39 06 4994 La Capitale

Upon calling, the operator will provide the taxi identification number and indicate the time it will take the taxi to reach the caller.

**UBER SERVICES**

Uber remains legal to use in Rome; however, Italy only allows Uber Black (and UberVans) as drivers must possess the car NCC license in order to operate. Due to the fact that there is no UberX or UberPOOL, Uber in Italy tends to be more expensive, on average, than taxis.

**PARKING AT CONFERENCE VENUE**

Parking is available at the Terminal Gianicolo, Via Urbano VIII, 16C, Rome, which is located adjacent to the Università Urbaniana.

Meeting participants will be given a discounted rate.

For more information, please see the staff at the Organising Secretariat desk.

**THE CITY OF ROME**

Rome is the capital city of Italy and of the Lazio region. It has a population of approximately 2.8 million residents. The metropolitan area has a population of about 4 million. Rome is located in the central-western portion of the Italian peninsula, where the Aniene river joins the Tiber river.

An enclave of Rome is the State of the Vatican City, the sovereign territory of the Holy See. It is the smallest nation in the world, and the capital of the only religion to have representation in the United Nations (as a non-member observer state).

Rome, referred to as Caput mundi ("capital of the world"), la Città Eterna ("the Eternal City"), Limen Apostolorum ("threshold of the Apostles"), la Città dei Sette Colli ("the city of the seven hills") or simply l'Urbe ("the City"), is modern and cosmopolitan. As one of the few major European cities that escaped World War II relatively unscathed, central Rome remains essentially Renaissance and Baroque in character. The historic centre of Rome is listed by UNESCO as a World Heritage Site.

**ORGANISING SECRETARIAT**

For additional information or queries, please address all correspondence to the Organising Secretariat:

**MEETING&CONSULTING**

Via Michele Mercati, 33 - 00197 Rome, Italy  
 Phone +39 06 80693320 - Fax +39 06 3231136  
 E-mail: [probiotics2021@emec-roma.com](mailto:probiotics2021@emec-roma.com)  
 Website: [www.probiotics-prebiotics-newfood.com](http://www.probiotics-prebiotics-newfood.com)  
[www.emec-roma.com](http://www.emec-roma.com)



# SCIENTIFIC INFORMATION

### ORGANISING SECRETARIAT DESK AT THE MEETING VENUE WILL BE OPEN AS FOLLOWS:

| <b>DAY</b> | <b>DATE</b>  | <b>FROM</b> | <b>TO</b> |
|------------|--------------|-------------|-----------|
| Sunday     | September 12 | 08.00       | 20.00     |
| Monday     | September 13 | 08.00       | 19.00     |
| Tuesday    | September 14 | 08.00       | 14.00     |

### ORAL COMMUNICATIONS

Oral communications sessions are scheduled as follows:

September 12 AULA MAGNA from 11.15 to 11.30 - from 12.50 to 13.00  
AULA METCHNIKOFF from 09.55 to 10.00 - from 12.50 to 13.00

September 13 AULA MAGNA from 11.45 to 12.00 - from 16.15 to 16.30

September 14 AULA MAGNA from 12.50 to 13.20  
AULA METCHNIKOFF from 09.20 to 09.30 - from 11.15 to 11.20

### POSTERS

The virtual poster area will be open from 10.30 on September 12 to 12.00 on September 14. The poster area is on the second floor.

The posters will be available on the meeting app. Further information about the app is available on the meeting website.

### SLIDE CENTERS

All speakers and authors must deliver their presentation (CD Rom, USB) to the slide centers 2 hours in advance or the day before their speech

**ITALIAN CME ACCREDITATION ECM (Italian CME Certificate)**

e meeting&consulting in qualità di Provider standard ha accreditato:

**“11<sup>th</sup> Probiotics, Prebiotics & New Foods, Nutraceuticals and Botanicals - for Nutrition & Human and Microbiota Health”** per le seguenti categorie:

Medico Chirurgo (discipline di riferimento): Allergologia ed Immunologia Clinica; Biochimica Clinica; Dermatologia e Venereologia; Endocrinologia; Farmacologia e Tossicologia Clinica; Gastroenterologia; Ginecologia e Ostetricia; Igiene degli Alimenti e della Nutrizione; Malattie dell'apparato Respiratorio; Malattie Infettive; Malattie Metaboliche e Diabetologia; Medicina Generale (Medici di Famiglia); Medicina Interna; Microbiologia e Virologia; Neonatologia; Oncologia; Patologia Clinica (Laboratorio di Analisi Chimico-Cliniche e Microbiologia); Pediatria; Pediatria (Pediatri di Libera Scelta); Reumatologia; Scienza dell'alimentazione e Dietetica

Biologo

Chimico (chimica analitica)

Dietista

Farmacista (ospedaliero - territoriale)

Infermiere

Infermiere pediatrico

Rif. n. 326559 - Crediti assegnati 6

Obiettivo formativo - Appropriatelyzza delle prestazioni sanitarie, sistemi di valutazione, verifica e miglioramento dell'efficienza ed efficacia. Livelli essenziali di assistenza (LEA)

Per avere diritto ai crediti E.C.M. è necessario frequentare il 90% delle ore di formazione

Si rammenta al partecipante che il limite massimo dei crediti formativi ricondotti al triennio di riferimento 2020-2022 acquisibili mediante invito da sponsor è di 1/3

THE MEETING WAS MADE POSSIBLE THANKS TO THE UNCONDITIONAL GRANT OF:

**PLATINUM**

AG PHARMA  
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FARMACEUTICI PROCEMSA  
IPA: INTERNATIONAL PROBIOTICS ASSOCIATION/DEERLAND  
MONTEFARMACO OTC  
PROBIOTICAL  
SANOFI

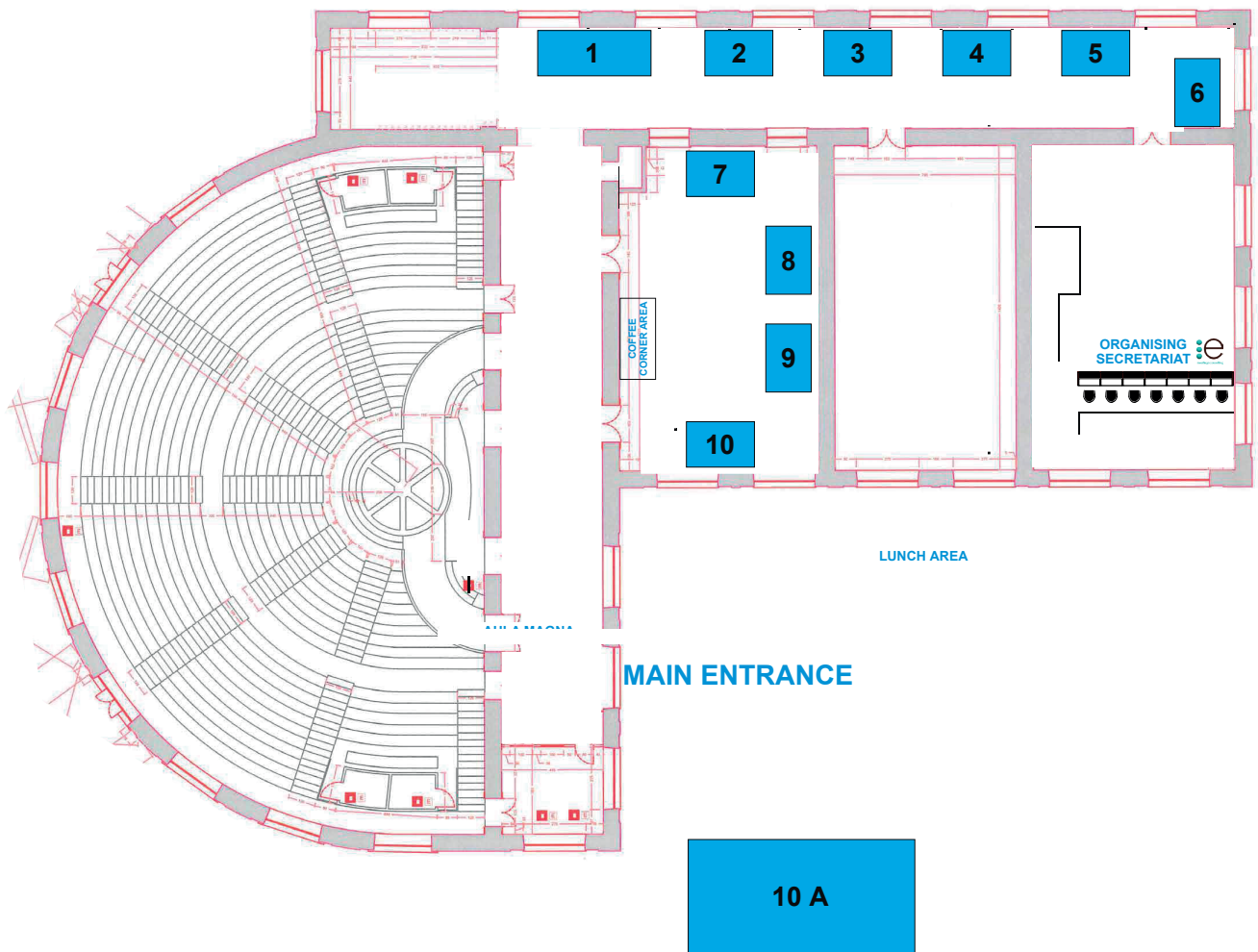
**&**

ABOCA  
BIOACTOR  
BIOSPHERA PHARMA  
BONOMELLI  
DOX – AL ITALIA  
GUNA  
IPSEN CONSUMER HEALTHCARE  
JANUS PHARMA  
LACTALIS ITALIA  
PHARMEXTRACTA  
ROELMI HPC  
SOCIETA' SPORTIVA CALCIO NAPOLI



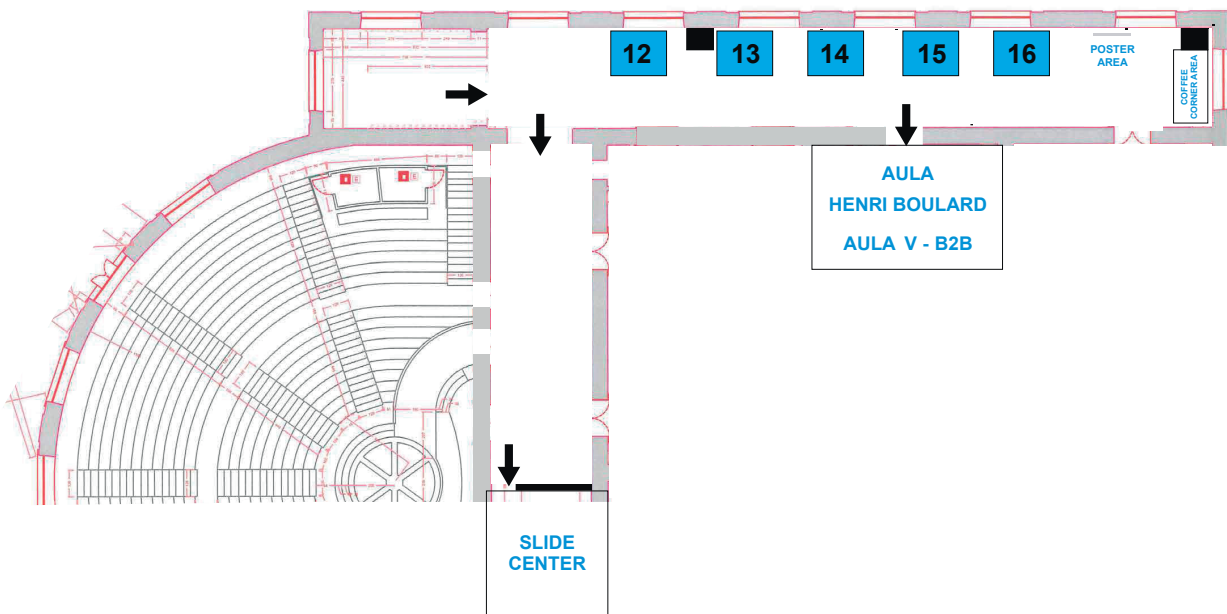
**EXHIBITION AREA**

FIRST FLOOR



- |          |  |            |   |
|----------|--|------------|---|
| <b>1</b> | IPA: INTERNATIONAL PROBIOTICS ASSOCIATION/DEERLAND | <b>8</b>   | FARMACEUTICI PROCEMSA                                     |
| <b>2</b> | CHR HANSEN   | <b>9</b>   | OFMOM   |
| <b>3</b> | IPSEN  | <b>10</b>  | BIOSTIME INSTITUTE OF NUTRITION AND CARE (BINC)/H&H GROUP |
| <b>4</b> | MONTEFARMACO OTC                                   | <b>10A</b> | AG PHARMA/DICOFARM  |
| <b>5</b> | PROBIOTICAL  |            |   |
| <b>6</b> | YAKULT ITALIA                                      |            |   |
| <b>7</b> | AG PHARMA/DICOFARM                                 |            |   |

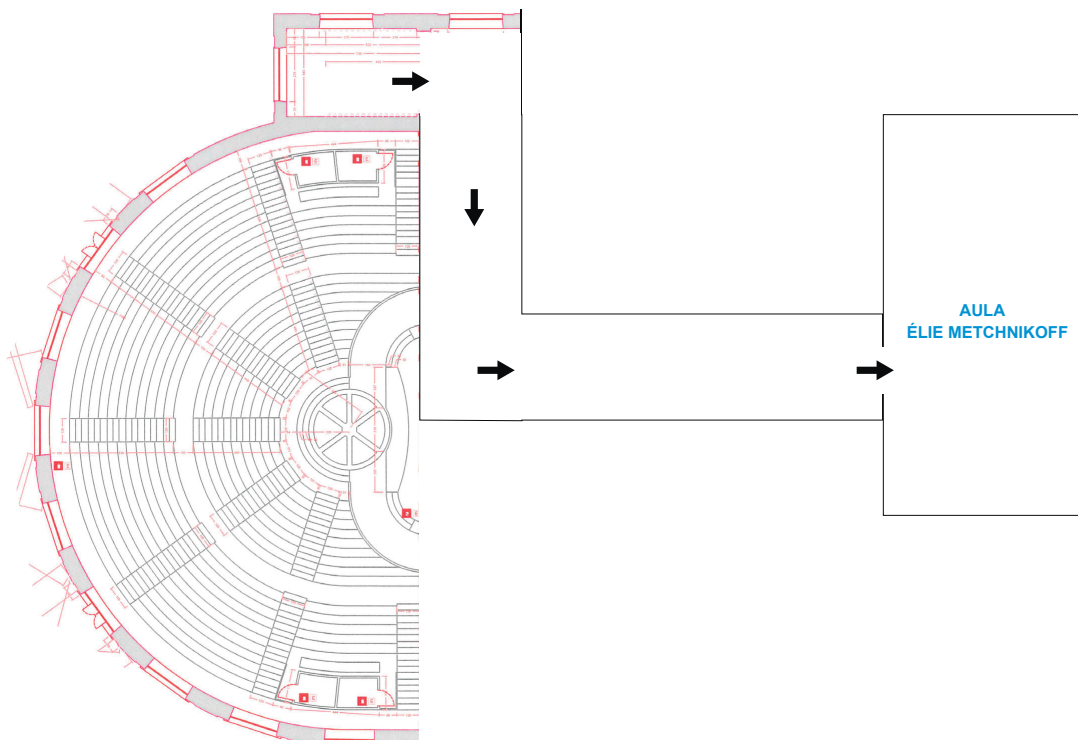
SECOND FLOOR



- 12 GUNA
- 13 PHARMEXTRACTA
- 14 BIOACTOR

- 15 BIOSPHAERA PHARMA
- 16 JANUS PHARMA

THIRD FLOOR



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# Enterogermina<sup>®</sup>

Bacillus clausii  
spores

# Dicoflor 60 Immuno

Integratore alimentare di Probiotici e Vitamina D<sub>3</sub>

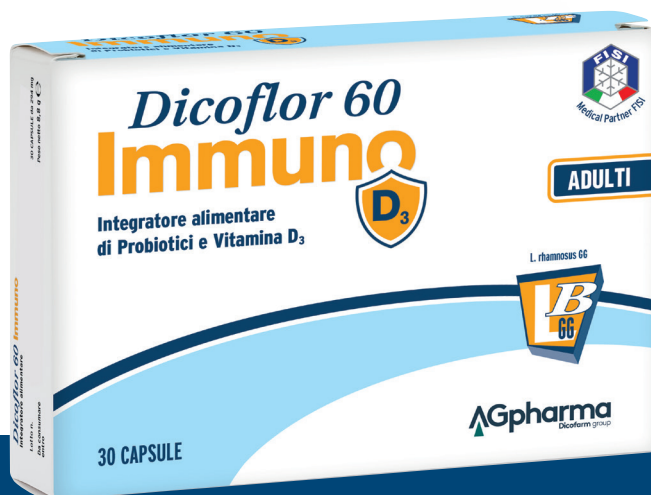


L. rhamnosus GG



**Synergistic  
anti-inflammatory action  
of *L. rhamnosus* GG  
and Vitamin D<sub>3</sub>**

*for the patient with  
Inflammatory Bowel Disease<sup>1-2</sup>*



**POSODOLOGY: 1 capsule per day**

**2000 IU of Vitamin D<sub>3</sub>,  
10 billion of *L. rhamnosus* GG**

21x29,7/lor60Imm0721-1

1.Pagnini et al. 2018  
2.White et al. 2018