ROME, SEPTEMBER 8-10 2019
UNIVERSITÀ URBANIANA

10 TH

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

1 ST 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)

V. Fogliano (Netherlands)

F. Guarner (Spain)

H. Kiyono (Japan)

M. Koch (Italy)

P. Lavermicocca (Italy)

R. Pecere (Belgium)

M. Rescigno (Italy)

K. M. Tuohy (Italy)

PEDIATRIC DAY

A. Guarino (Italy)

R. Berni Canani (Italy)

UNDER THE PATRONAGE

Consiglio Nazionale delle Ricerche

Società Italiana di Gastroenterologia
ed Endoscopia Digestiva

AGGEI
Associazione Giovani
Gastroenterologi ed
Endoscopisti Italiani

SINut
Società Italiana di Nutraceutica

Nfi
NUTRITION FOUNDATION
OF ITALY

JAPAN BIFIDUS FOUNDATION

ipa
EUROPE
INTERNATIONAL PROBIOTICS ASSOCIATION

federSalus

UNDER THE PATRONAGE

Fondazione Aldo Torsoli

AGUI
Associazione Ginecologi Universitari Italiani

MTCC, Mediterranean Task Force for Cancer Control

FEDERCHIMICA
AISPEC MIAF
GRUPPO MATERIE PRIME PER INTEGRATORI ALIMENTARI E ALIMENTI FUNZIONALI

INDONESIAN SCIENTIFIC SOCIETY FOR PROBIOTICS AND PREBIOTICS
ISSPP

INTEGRATORI ITALIA

PROBIOSTUDIO
ITALIAN ACADEMY FOR THE STUDY OF HUMAN MICROBIOTA

INDEX

SCIENTIFIC PROGRAMME

08.30 - 10.00

OPENING LECTURE

Chair: L. Orfeo (Italy)

The role of nutrition, microbiome composition and function, and antigen trafficking in the pathogenesis of chronic inflammatory diseases: Academia-Industry partnership to exploit targets to reverse inflammation
A. Fasano (USA)

Q&A in collaboration with young gastroenterologists (AGGEI)
chaired by F. Scaldaferri (Italy)

LECTURE

Chair: L. Capurso (Italy)

Evidence based medicine and probiotics. To all involved: we have a problem
M. Koch (Italy)

10.00 - 11.15

NEW FOODS

Chair: V. Fogliano (Netherlands)

Healthy ellagitannins from nuts
F. A. Tomás-Barberán (Spain)

Processing or ultraprocessing: does it make a difference for health?
T. van Boekel (Netherlands)

Another *break* in the (cell) wall - Plant structure and nutrients utilization
E. Capuano (Netherlands)

Innovations in dairy through product-process interactions: the food matrix effect
T. Huppertz (Netherlands)

Polyphenol based new foods
I. Ferreira (Portugual)

OPEN COFFEE

11.15 - 11.30

ORAL COMMUNICATIONS

Chair: K. M. Tuohy (Italy)

OC.1 - GUT MICROBIOTA COMPOSITION AND IMMUNITY OF AGEING MICE SUPPLEMENTED WITH COW MILK CONTAINING A DIFFERENT CASEIN PROFILE

Barbara Guantario [(1)], Paola Zinno [(1)], Alberto Finamore [(1)], Vincenzo Motta [(2)], Paolo Trevisi [(2)], Marzia Giribaldi [(3)], Laura Cavallarin [(4)], Marianna Roselli [(1)], Chiara Devirgiliis [(1)]

[(1)] CREA, Centro Ricerca Alimenti e Nutrizione, Roma, Italy
[(2)] Università di Bologna, Dipartimento di Scienze e Tecnologie Agro-alimentari, Bologna, Italy
[(3)] CREA, Centro ricerca Ingegneria e Trasformazioni Agroalimentari, Torino, Italy
[(4)] CNR, Istituto di Scienze delle Produzioni Alimentari, Torino, Italy

OC.2 - HEALTHY PROTECTION OF BERGAMOT IS LINKED TO THE MODULATION OF MICROBIOTA

Giovanna Petrangolini [(1)], Valeria Longo [(1)], Davide Berlanda [(1)], Pietro Allegrini [(1)], Giulia Masetti [(2)], Sara Botti [(2)], Antonella Riva [(1)]

[(1)] R&D, Indena SpA
[(2)] PTP Science Park

OC.3 - PUTATIVE PROBIOTIC LACTIC ACID BACTERIA ISOLATED FROM GREEK PROTECTED DESIGNATION OF ORIGIN CHEESES USING TRADITIONAL AND METAGENOMIC MICROBIOLOGICAL ANALYSIS

Jonathan Rhoades [(1)], Sofia Michailidou [(2)], Iro Anastasiou [(1)], Anagnostis Argiriou [(2)], Eleni Likotrafiti [(1)]

[(1)] International Hellenic University, Food Science And Technology, Thessaloniki, Greece
[(2)] Center For Research And Technology Hellas, Institute Of Applied Biosciences, Thessaloniki, Greece

11.30 - 13.00

GUT MICROBIOTA AND IBD

Chairs: A. Armuzzi (Italy), R. Caprilli (Italy)

Bacterial microbiota and IBD
F. Scaldaferri (Italy)

Fungal microbiota and IBD
L. Putignani (Italy)

Viral microbiota and IBD
F. Ungaro (Italy)

ORAL COMMUNICATIONS

OC.4 - NOD2 PROMOTES HOST DEFENSE AND RECOVERY FROM *CANDIDA TROPICALIS* INFECTION DURING EXPERIMENTAL COLITIS
Kristine Ann Buela [(1)], Theresa Pizarro [(1)]
[(1)] Case Western Reserve University, Department of Pathology, Cleveland, United States

OC.5 - EFFECTS OF SYNBIO® PROBIOTIC FORMULATION ON PATHOGENS ISOLATED FROM CHRONIC ULCERATIVE LESIONS: IN VITRO STUDY
Lucia Mazzotti [(1)], Maria Magdalena Coman [(1)], Carla Orpianesi [(1)], Alberto Cresci [(1)], Stefania Silvi [(2)], Alessandro Scalise [(3)], Maria Cristina Verdenelli [(1)]
[(1)] Synbiotec Srl, Spin-off of University of Camerino, Camerino, Italy
[(2)] School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy
[(3)] Department of Plastic and Reconstructive Surgery, Marche Polytechnic University, Ancona, Italy

13.00 - 14.00 **Lunch**

14.00 - 16.00 **WELCOME ADDRESS**
L. Capurso (Italy)

ROUND TABLE IPA EUROPE
Chair: C. Hill (Ireland)

Outlook on probiotics food: the regulatory context in the EU, the economic perspective and how to move forward
R. Pecere (Belgium)

Introduction to EFSA remit and role
S. Valtueña Martínez (Spain)

The criteria approach for the use of the term probiotic in food to provide science-based information to the consumers. Presentation of the ISAPP-IPA EU paper
S. Binda (France), C. Hill (Ireland)

Panel discussion: representatives from National and European authorities. Scientists and nutrition experts will exchange on the requirements to define the category and the role of the national authorities in nutrition policies
C. Hill (Ireland), L. Morelli (Italy), B. Scarpa (Italy)

OPEN COFFEE

16.00 - 17.30 **LECTURES**
Chair: P. Aureli (Italy)

Physiopathology and dynamics of human microbiome
M. Clementi (Italy)

Gut microbiota type 2 diabetes and obesity
M. Federici (Italy)

Gut microbiota and infectious diseases
G. Ippolito (Italy)

Genomics approaches for today's and tomorrow's functional products
W. M. De Vos (Netherlands)

Low abundance, low relevance? Let's forget it. The importance of the so called "minor" species
L. Morelli (Italy)

17.30 - 18.30 **MICROBIAL METABOLITES AND POSTBIOTICS**
Chair: M. Rescigno (Italy)

Postbiotics: new players in health promotion
M. Rescigno (Italy)

Microbial metabolitis in the regulation of host immunity
H. Kiyono (Japan)

Person-specific gut mucosal colonization resistance to probiotics and their inhibitory effect on post-antibiotics microbiome recovery
J. Suez (Israel)

18.30 - 19.00 **LECTURE**
Chair: A. Castellazzi (Italy)

Impact of probiotic fermentation of food on their prebiotic activity and digestiblity
L. Morelli (Italy)

19.00 - 20.00 **CLINICAL MICROBIOLOGY**
Chair: L. Putignani (Italy)

Clinical microbiology of gut microbiota
L. Putignani (Italy)

Exclusion diets and gut microbiota modulation in pathological conditions
F. Del Chierico (Italy)

The concept of clouding microbiota profiles and associated diseases
A. Quagliariello (Italy)

20.00 **WELCOME COCKTAIL**

OPEN COFFEE

09.00 - 10.15

MICROBIOME & FOODS

Chair: K. M. Tuohy (Italy)

Exploitation of food chain microbiomes for a more sustainable food production
M. Candela (Italy)

Microbiome applications for sustainable food systems through technologies and enterprise
P. Cotter (Ireland)

The health effects associated to microbial biodiversity in raw milk and cheeses
E. Franciosi (Italy)

ORAL COMMUNICATION

OC.6 - THE HIGH POLYMERIZATION DEGREE INFLUENCES THE INULIN BIOACCESSIBILITY IN DURUM WHEAT SPAGHETTI

Angela Cardinali [1], Antonella Garbetta [1], Isabella D'Antuono [1], Maria Grazia Melilli [2], Vito Linsalata [1], Salvatore Scandurra [2]

[1] National Research Council, Institute of Science of Foods Production (ISPA), Bari, Italy
[2] National Research Council, Institute for Agricultural and Forest Systems in the Mediterranean (ISAFOM), Catania, Italy

10.15 - 11.00

LECTURES

Chair: M. Del Piano (Italy)

The lung microbiome
M. Miraglia del Giudice (Italy)

Efficacy of probiotic treatment in pediatric patients with allergic asthma and recurrent wheezing: a randomized, double-blind study
L. Mogna (Italy)

OPEN COFFEE

11.00 - 13.00

FIBERS AND POLYPHENOLS

Chairs: G. Riccardi (Italy), E. S. Corazziari (Italy)

Fibre, gut and cardiometabolic risk
G. Riccardi (Italy)

Role of dietary intervention in the prevention of age-related diseases
S. Vasto (Italy)

Impact of dietary fibers on metabolic cross talk between the splanchnic area and the muscle/adipose in mini pigs
I. Savary-Auzeloux (France)

Polyphenol rich diet and cardiovascular diseases
R. Giacco (Italy)

Polyphenols and microbiota
F. A. Tomás-Barberán (Spain)

Low-FODMAP diet and gut microbiota
E. S. Corazziari (Italy)

Adverse effects to plant food supplements
P. Restani (Italy)

Designing healthy sourdough bread
M. Agnolucci (Italy)

ORAL COMMUNICATIONS

OC.7 - EFFECTS OF B-FRUCTANS FIBER ON BOWEL FUNCTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

Le Bourgot Cindy [1], Respondek Frédérique [1], Calame Wim [2], de Vries Jan [3]

[1] Tereos, R&D department, Moussy-Le-Vieux, France
[2] StatistiCal BV, StatistiCal BV, Wassenaar, Netherlands
[3] De Vries Nutrition Solutions Inc., De Vries Nutrition Solutions Inc., Gorssel, Netherlands

OC. 8 - EFFECTS OF RESVERATROL IN A MOUSE MODEL OF ALCOHOL ADDICTION

Marco Fiore [1], Carla Petrella [1], Fausta Natella [2], Mauro Ceccanti [3]

[1] Institute of Cell Biology and Neurobiology, IBCN-CNR, Rome, Italy
[2] CREA-AN, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Food and Nutrition Research Centre, Rome, Italy
[3] Centro Alcologico della Regione Lazio (CRARL), Department of Clinical Medicine, Sapienza University of Rome, Italy

OPEN COFFEE

13.00 - 14.00 **Lunch**

14.00 - 16.00 **FOOD SUPPLEMENTS, PROBIOTICS & WOMEN MICROBIOME: THE KEY ROLE OF GYNECOLOGISTS**

Chairs: N. Colacurci (Italy), F. Vicariotto (Italy)

The importance of a woman maintaining a healthy microbiome
F. Vicariotto (Italy)

Role of the genital tract microbiome in female sexual health
F. Murina (Italy)

Therapy of vaginal dysbiosis with oral probiotics
L. Petricevic (Austria)

Role of microbiome in preterm deliveries
I. Cetin (Italy)

HPV and microbioma
F. De Seta (Italy)

16.00 - 17.25 **WOMEN AND THEIR MICROBES**

Chair: F. Vicariotto (Italy)

The importance of the maternal microbiome
F. Vicariotto (Italy)

The female microbiome journey
N. Giovannini (Italy)

LECTURE

Maternal antibiotic prophylaxis affects bifidobacterium spp. counts in the human milk, during the first week after delivery
S. M. Isay Saad (Brazil)

17.25 - 19.15

HUMAN INTESTINAL MICROBIOME IN THE FIRST 1000 DAYS AND ITS CONSEQUENCES

Chairs: D. Caserta (Italy), N. Colacurci (Italy)
Opening remarks: P. Maniglio (Italy)

Microbiome in reproductive health
R. Marci (Italy)

Microbiome and nutrition in pregnancy
F. Torcia (Italy)

Microbiome and cesarean section
I. Ruscito (Italy)

Microbiome and implantation failure
M. Schimberni (Italy)

Microbiome in natural delivery
C. Assorgi (Italy)

Discussants: L. Di Benedetto (Italy), E. Matteucci (Italy)

11.30 - 13.00 **ALIMENTI PROBIOTICI: NUTRIRE L'INNOVAZIONE**
100 ANNI DI DANONE

11.30 - 11.35 Benvenuto e introduzione ai lavori
Modera: F. Mereta (Italia)

11.35 - 11.45 Le aspettative crescenti sul ruolo del microbiota e l'importanza del probiotico
L. Morelli (Italia)

11.45 - 12.15 Alimenti funzionali arricchiti con probiotici: la continua innovazione dei *latti fermentati*

La ricerca alla base dello sviluppo tecnologico
L. Morelli (Italia), A. Senizza (Italia)

Il ruolo di alimenti funzionali arricchiti con probiotici all'interno della dieta
G. Ianiro (Italia)

Dal laboratorio alla divulgazione scientifica
S. Di Maio (Italia)

12.15 - 12.50 Talk session «come sta cambiando l'approccio al probiotico?»
C. Bastetti (Italia), L. Morelli (Italia), G. Ianiro (Italia), V. Monda (Italia), S. Ferretti (Italia)

12.50 - 13.00 100 anni dal primo yogurt Danone: prospettive future
A. Salvia (Italia)

SESSIONE NON ACCREDITATA E.C.M.

MEET.
GREET.
DON'T MISS
A BEAT.

B2B/2GO MEETINGS DURING THE FOLLOWING DAYS AND TIMES

SEPTEMBER 8

10.00 - 11.00 and 16.00 - 18.00

SEPTEMBER 9

10.00 - 12.30 and 16.00 - 18.00

SESSIONE NON ACCREDITATA E.C.M.

08.30 - 9.30

SUPPORTING RESEARCH: 25 YEARS OF *FONDAZIONE INVERNIZZI*

Chair: L. Capurso (Italy)

Invernizzi Foundation and gut microbiota: looking for ARCHAEA
L. Morelli (Italy)

Invernizzi Foundation and the pediatric age
C. Bandi (Italy)

Invernizzi Foundation and the market for functional foods
V. Fiorillo (Italy)

09.30 - 11.00

RECENT ADVANCES ON NEXT GEN PROBIOTICS AND BACTERIAL THERAPEUTICS

Chair & Introduction: W. M. De Vos (Netherlands)

Functionality of next generation probiotics in humans; a gut brain perspective
R. J. M. Brummer (Sweden)

Akkermansia muciniphila: from mice to man
P. Cani (Belgium)

Growth and impact of *Faecalibacterium spp*
P. Langella (France)

OPEN COFFEE

11.00 - 12.00

LECTURES

Chair: M. Koch (Italy)

Probiotics and heart health. The emerging role of gut microbes in cardiovascular health
G. Leyer (USA)

Role of antibiotics in medicine. Always *anti?* The role of eubiosis
C. Scarpignato (Italy)

ORAL COMMUNICATIONS

OC.9 - PROBIOTICS IN METABOLIC SYNDROME

Emre Avci [1]

[1] *Hitit University, Molecular Biology And Genetics, Corum, Turkey*

OC.10 - A RANDOMIZED CONTROLLED TRIAL ON THE EFFECTS OF A SYNBIOTIC COMPOUND ON MARKERS OF INFLAMMATION IN OBESE ADOLESCENTS

Roya Kelishadi [1]

[1] *Child Growth and Development Research Center,Research Institute for Primordial Prevention of Non Communicable Disease, Isfahan University of Medical S, Pediatrics, Isfahan, Iran (Islamic Republic of)*

OC.11 - OBESITY MANAGEMENT WITH PROBIOTICS: MODULATION OF GUT MICROBIOTA COMPOSITION AND BODY WEIGHT CONTROL

Maria Magdalena Coman [1], Cinzia Cecchini [1], Maria Vittoria Micioni di Bonaventura [2], Stefania Silvi [3], Carla Orpianesi [1], Alberto Cresci [1], Carlo Cifani [2], Maria Cristina Verdenelli [1]

[1] *Synbiotec Srl, Spin-off of University of Camerino, Camerino, Italy*
[2] *School of Pharmacy, University of Camerino, Camerino, Italy*
[3] *School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy*

OPEN COFFEE

PEDIATRIC DAY

12.00 - 13.30 **PROBIOTICS IN CHILDHOOD WITH GASTROENTERITIS**
Chairs: S. Guandalini (USA), A. Guarino (Italy)
Introduction: A. Guarino (Italy)

The impact of gastroenteritis in childhood
A. Guarino (Italy)

The standard management of gastroenteritis
A. Lo Vecchio (Italy)

Active treatment of gastroenteritis with probiotics: pros & cons
S. Freedman (Canada), H. Szajewska (Poland)

13.30 - 14.30 **LUNCH WITH THE EXPERT (SECOND FLOOR)**

Effect of probiotics in cancer
R. Angioli (Italy)

Probiotics for the prevention of recurrent uti, synergistic effect of a combination of *Lactobacillus Paracasei LC11,* cranberry expract and D-Mannose in humans
F. Vicariotto (Italy)

To count or not to count? Pros and Cons of probiotic enumeration methods
M. Pane (Italy)

13.30 - 14.30 **Lunch**

14.30 - 16.30 **NUTRACEUTICALS AND BOTANICALS FOR A FUNCTIONAL DIET**
Chairs & Introduction: P. Lavermicocca (Italy), M. Ferruzzi (USA)

Nutraceuticals and biomarkers
M. Renis (Italy)

Food matrix effects on bioavailability and metabolism of (poly) phenols from botanicals
M. Ferruzzi (USA)

Dietary flavan-3-ols, gut microbes, and health: how does it work?
D. Del Rio (Italy)

Systems metagenomics of human fungal populations: passengers, colonizers and invaders
C. De Filippo (Italy)

Botanicals, new foods and supplements: what is the European normative?
L. Scotti (Italy)

OPEN COFFEE

ORAL COMMUNICATIONS

OC.12 - PRODUCTION OF FRUIT BASED DRINKS AS CARRIERS OF PROBIOTIC BACTERIA *LACTOBACILLUS RHAMNOSUS SP*

Perihan Kubra Akman [1], Fatih Törnük [1], Hasan Yetim [2]
[1] *Yildiz Technical University, Food Engineering Department, Istanbul, Turkey*
[2] *Sabahattin Zaim University, Food Engineering Department, Istanbul, Turkey*

OC.13 - FORTUNELLA MARGARITA: THE CITRUS FRUIT OF THE MOMENT? INVESTIGATION OF BIOACTIVE COMPOUNDS: VITAMINS, MACROELEMENTS AND POLYPHENOLS

Clarice Silva e Souza [1], Pamella Cristine Anunciação [2], Ceres Mattos Della Lucia [2], Rosana Gonçalves Rodrigues das Dôres [3], Regina Célia Rodrigues de Miranda Milagres [2], Helena Maria Pinheiro-Sant'Ana [2]
[1] *Institution, Szent István University, Gödöllő, Hungary*
[2] *Institution, Universidade Federal de Viçosa, Viçosa, Brazil*
[3] *Institution, Universidade Federal de Ouro Preto, Ouro Preto, Brazil*

OC.14 - PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM FERMENTED CEREAL FOODS

Ilkin Yucel Sengun [1], Kivanc Atlama [1], Gulden Kilic [1]
[1] *Ege University, Engineering Faculty, Food Engineering, Izmir, Turkey*

16.30 - 17.45

LECTURES

Chair: C. Cricelli (Italy)

Body microbiomes and their dysbiosis: new approaches for health & personal care
F. Carlomagno (Italy)

Multistrain and singlestrain probiotics
A. Ouwehand (Finland)

Cytofluorimetry in the 21st century: between compelling scientific evidence and new regulatory horizons
M. Pane (Italy)

Gut barrier and diarrheal diseases
M. Aloi (Italy)

Selected probiotics for support of iron status and bone health when needed most
N. Larsson (Sweden)

17.45 - 19.30

GUT LIVER AXIS

Chairs: D. Alvaro (Italy), M. Koch (Italy)

Gut barrier role in gut - liver axis
P. Brescia (Italy)

Gut microbiota, biliary salts and FXR
D. Alvaro (Italy)

Gut liver axis in cholagiopathies
A. Benedetti (Italy)

LECTURE

Gut dysbiosis and liver disorders
H. Tilg (Austria)

OPEN COFFEE

PEDIATRIC DAY
HUMAN INTESTINAL MICROBIOME IN THE FIRST 1000 DAYS AND ITS CONSEQUENCES IN LATER HEALTH

08.30 - 09.30 **AGE RELATED STRUCTURE OF INTESTINAL MICROBIOME**
Chair: R. Berni Canani (Italy)

The development of intestinal microbiome: from pregnancy to 2 years
F. Indrio (Italy)

The development of gut microbiome-immune system axis
L. Stronati (Italy)

The development of gut microbiome brain axis
Y. Vandenplas (Belgium)

09.30 - 10.30 **MICROBIOME AND NUTRITION**
Chair: H. Szajewska (Poland)

Breast milk: pro-pre and post-biotics features
E. Isolauri (Finland)

Plausible role of diet and microbiota in the pathogenesis of intestinal inflammation
P. Lionetti (Italy)

Solid foods, junk foods, mediterranean diet and intestinal microbiota
D. Ercolini (Italy)

10.30 - 11.45 **NUTRITION AND MICROBES: AN APPROACH TO PREVENTION IN AT RISK CHILDREN**
Chair: E. Isolauri (Finland)

The guidelines for prevention of NEC in preterm infants
J. B. H. van Goudoever (Netherlands)

A global approach to prevent atopy
R. Berni Canani (Italy)

Respiratory infections: what are we trying to prevent
I. Hojsak (Croatia)

12.00 - 13.30 **AULA MAGNA**
PROBIOTICS IN CHILDHOOD WITH GASTROENTERITIS

11.45 - 13.30

THE GLOBAL VOICE OF PROBIOTICS IPA/IPA EUROPE SESSION
How to define a comprehensive approach for the definition of the probiotic category (nutrition relevance and scientific requirements)

Welcome of the IPA Europe
President: E. Laulund (Denmark)

Nutritional relevance of the daily ingestion of live bacteria (including probiotics)
S. Lortal (France)

Could "probiotics" deserve a category definition such as "prebiotics"?
B. Pot (Belgium)

Learn more about probiotic and prebiotic
Y. Vandenplas (Belgium)

Introduction to the Codex on probiotics: the IPA initiative and future perspectives
G. Paraskevakos (Canada)

Probiotic definition and elements of the proposal on harmonised Codex approach
F. Bourdichon (France)

Panel discussion and conclusions: "How to build a category approach for probiotics"
Chair: Y. Vandenplas (Belgium)

13.30 - 14.30 **Lunch**

14.30 - 16.00

MICROBIOME AND PEDIATRIC DISEASES
Chair: A. Guarino (Italy)

The microbiome in cystic fibrosis - prognostic aspects
M. Wilschanski (Israel)

Microbiome and neuropsychiatric conditions
S. Cucchiara (Italy)

Hepato-metabolic syndrome and probiotics
A. Alisi (Italy)

Probiotics and functional GI disorders
Closing remarks
S. Guandalini (USA)

16.00 - 17.30

GUT MICROBIOTA AND COELIAC DISEASE

Chair: R. Troncone (Italy)

Microbiota and coeliac disease: studies at a risk cohorts
Y. Sanz (Spain)

Intestinal handling of gliadin peptides: modulation by gut microbiota
M. V. Barone (Italy)

Role of gut microbiota in animal models of coeliac disease
A. Caminero (Canada)

Risk factors for coeliac disease: role of infections
R. Auricchio (Italy)

Is there a rationale for the use of probiotics in coeliac disease?
M. Silano (Italy), V. Madiai (Italy)

17.30 - 19.00

PROBIOTICS: MARKET, TECHNICAL, SCIENTIFIC AND QUALITY TOPICS IN ITALY AND IN EUROPE - FEDERSALUS

Chair: M. Fiorani (Italy)

Probiotics market trend and perspective in Italy and in Europe
I. Cecchini (Italy)

Probiotics quality control: the ESLP (European Scientific League for Probiotics) experience
J.P. Warzée (Belgium)

The analytical best practice of microorganisms in Italy: FederSalus' point of view
M. Elli (Italy)

Bifidobacterium adolescentis: a diverse species with probiotic potential
T. Leser (Denmark)

Formulation and production of food supplement containing probiotics: a case history
M. Mori (Italy)

Discussion, Q&A

NEW TOOLS FOR NEW FOODS: INNOVAZIONE E SCIENZA INCONTRANO L'INDUSTRIA

Prima edizione

Questo laboratorio di idee è strutturato come un "gioco di ruolo" compartecipato, costituito da due gruppi di lavoro paralleli animati da tecnici delle aziende aderenti al gruppo Miaf, coordinati da un motivatore esperto in comunicazione e formazione. I partecipanti avranno a disposizione informazioni di base con spunti pratici per elaborare e sviluppare nuove formulazioni di integratori, partendo dalla materia prima per arrivare al concetto del prodotto finito innovativo. Obiettivo principale: avvicinare gli studenti universitari al mondo dell'Industria, stimolando le loro potenzialità su argomenti attinenti il percorso di studio, ma con una visione ampliata all'ambito applicativo e lavorativo (Think out the box) e creare legami di reciproca conoscenza e collaborazione.

Relatori: F. Dal Bello (Italy), G. Faravelli (Italy), L. Mogna (Italy), F. Stratta (Italy)

10.30 - 13.30	Formazione teorica e working group
13.30 - 14.30	Lunch
14.30 - 16.00	Sessione conclusiva

SESSIONE NON ACCREDITATA E.C.M.

08.30 - 10.30

LECTURES

Chair: G. Gasbarrini (Italy)

Probiotics in *Helicobacter Pylori* infection and in gut microbiota disequilibrium
G. Gasbarrini (Italy), C. Mosoni (Italy), F. Bonvicini (Italy)

How to obtain nowadays reliable high quality multistrain probiotic for clinical practice: the case of VSL#3
D. Mora (Italy)

Gut microbiota as an emerging target to attain longevity
P. Brigidi (Italy)

Clinical applications of Probio'Stick® in the brain-gut axis: recent clinical findings
B. V. Gonzalez Cautela (Canada)

Nasal probiotics
M. Lehtinen (Finland)

10.30 - 11.30

FECAL MICROBIOME TRANSPLANT

Chair: A. Gasbarrini (Italy)

Presentation of second consensus conference on FMT. The first global summit
A. Gasbarrini (Italy)

Europe consensus data on FMT
G. Ianiro (Italy)

European consensus conference on stool banking dor FMT
J. Keller (Netherlands)

11.30 - 12.10

LECTURES ON GUT MICROBIOTE AND CANCER

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

MAIN LECTURE
Pre - and probiotics against cancer
L. Zitvogel (France)

LECTURE
Cancer of the colon and microbial signature
A. Tett (Italy)

12.10 - 13.20

LECTURES & ORAL COMMUNICATIONS

Chair: M. Guarino (Italy)

Pharmacomicrobiomics: the microbiome cloud as double-edged shield against drugs and nutrients
R. K. Aziz (Egypt)

OPEN COFFEE

Gut microbiota & metabolism: prebiotic effect on T2DM, microbiome profile
I. S. Surono (Indonesia)

ORAL COMMUNICATIONS

OC.15 - PERIODONTAL DISEASES & SYSTEMIC ORGANS INTERACTION

Piero Simeone [1]
[1] *Active member of Italian Academy of Prosthetic Dentistry*

OC.16 - MICROBIOTA CHANGES IN CHILDREN WITH VIRAL GASTROENTERITIS

Konstantin Ermolenko [1], Natalia Gonchar [1], Yurii Lobzin [1]
[1] *Pediatric Research and Clinical Center for Infectious Diseases, Intestinal infection, Saint-Petersburg, Russian Federation*

OC.17 - VITAMIN D RECEPTOR CONTRIBUTES TO THE HEALTH BENEFITS OF PROBIOTIC CONSUMPTION

Carolina Battistini [1], Yong-guo Zhang [2], Ishita Chatterjee [2], Rong Lu [2], Jilei Zhang [2], Susana M. I. Saad [1], Jun Sun [2]
[1] *University of São Paulo, School of Pharmaceutical Sciences / Department of Pharmaceutical and Biochemical Technology / FoRC - Food Research Center, São Paulo, Brazil*
[2] *University of Illinois at Chicago, Department of Medicine, Chicago, United States*

OC.18 - DETECTION OF NEW PROBIOTICS IN THE PRESENCE OF VIRAL GASTROENTERITIS

Gulcin Alp Avci [1]
[1] *Hitit University, Molecular Biology And Genetics, Corum, Turkey*

OC.19 - ANTI-CAMPYLOBACTER EFFECTS OF PROBIOTICS IN VITRO AND IN VIVO

Elena Ermolenko [1], Konstantin Ermolenko [2], Elvira Martens [3], Sergey Sidorenko [3], Marina Kotyleva [1], Alexander Suvorov [1], Guo Danyang [4]
[1] *Institute of Experimental Medicine, Department of experimental microbiology, Saint-Petersburg, Russian Federation*
[2] *Pediatric Research and Clinical Center for Infectious Diseases, Department of intestinal infections, Saint-Petersburg, Russian Federation*
[3] *Pediatric Research and Clinical Center for Infectious Diseases, Laboratory of medical microbiology, Saint-Petersburg, Russian Federation*
[4] *Agro-food Science and Technology, SAAS, Shandong, China*

OC.20 - EFFECT OF GLUTEN FREE DIET ON INTESTINAL MICROBIOTA IN PEDIATRIC CELIAC PATIENTS

Yasemin Ertaş Öztürk [1], Efsun Karabudak [1], Ödül Eğritaş Gürkan [2], Buket Dalgıç [2]
[1] *Gazi University, Department of Nutrition and Dietetics, Ankara, Turkey*
[2] *Gazi University, Division of Pediatric Gastroenterology, Ankara, Turkey*

08.30 - 09.50

MICROBIOTA AND FUNCTIONAL BOWEL DISORDERS

Chair: G. Barbara (Italy)

Psychobiotics and the microbiome-gut-brain axis: moving the goalposts in psiychiatry?
G. Clarke (Ireland)

Fungal dysbiosis in FBD
R. van den Wijngaard (Netherlands)

Evidence based management of abdominal symptoms with probiotics
P. Fracasso (Italy)

Advanced in vitro models: studying organ and tissue cross-talk at the microscale
C. Magliaro (Italy)

ORAL COMMUNICATION

OC.21 - COMPLIANCE TO PROBIOTIC THERAPY IN IRRITABLE BOWEL SYNDROME IN CLINICAL PRACTICE: A REAL-LIFE STUDY

Lucrezia Laterza [(1)], Marco Napoli [(1)], Valentina Petito [(1)], Franco Scaldaferri [(1)], Eleonora Gaetani [(1)], Antonio Gasbarrini [(1)]

[(1)] *Università Cattolica del Sacro Cuore, Digestive Disease Center, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy*

09.50 - 11.20

SKIN GUT MICROBIOME

Chair: M. Picardo (Italy)

Microbial biofilms and the human skin microbiome in the pathogenesis of dermatological diseases
E. G. Di Domenico (Italy)

Staphylococcus aureus and atopic dermatitis: the selective pressures acting on the skin microbiome
I. Cavallo (Italy)

Dermobiotic: microbiome in the gut-skin axis
M. Pignatti (Italy)

Probiotic formulations for healthy skin: from lab to slab
I. Pal Kaur (India)

ORAL COMMUNICATION

OC.22 - SKIN MICROBIOTA AND BACTERIAL BIOFILMS IN PATIENTS WITH ATOPIC DERMATITIS AND IN HEALTHY HUMAN HOSTS
Oksana Rybalchenko [1], Olga Orlova [1], Valentina Kapustina [1]
[1] Saint-Petersburg State University, Medical department, Saint-Petersburg, Russian Federation

11.20 - 13.00 **MEDITERRANEAN TASK FORCE FOR CANCER CONTROL**
Meeting in memory of Massimo Crespi
Chairs: P. G. Natali (Italy), A. Montori (Italy)

The Mediterranean Task force for Cancer Control: an evergreen commitment
P. G. Natali (Italy)

The complex connection between diet, microbiota, and colorectal cancer
A. Saggioro (Italy)

Functional foods in liver diseases
A. Ascione (Italy), F. Morisco (Italy)

Gut microbiota: role play in obesity
M. K. Shaker (Egypt)

PROCEEDINGS

DESIGNING HEALTHY SOURDOUGH BREAD

Monica Agnolucci [1,2*], Michela Palla [1], Manuela Giovannetti [1,2]
[1] *Department of Agriculture, Food and Environment - University of Pisa*
[2] *Interdepartmental Research Center "Nutraceuticals and Food for Health" – University of Pisa*

Fermentation is one of the oldest method for preserving foods and it is currently used to produce more than 5000 different types of fermented foods and beverages all over the world. Among fermented foods, sourdough bread is one of the most important baked goods derived from cereal fermentation (Vogel *et al.*, 1999). Sourdough is a mixture of cereal flour and water, fermented by a complex microbiota, consisting of lactic acid bacteria (LAB) and yeasts, which interact among themselves, often establishing stable associations and contributing to the peculiar properties of sourdough breads (Gobbetti, 1998). In particular, such autochthonous microorganisms produce a large variety of compounds, originating diversified flavours, increasing shelf-life and enhancing nutritional and nutraceutical value of fermented goods. Each sourdough harbours different LAB and yeast communities, whose diversity depends on process technologies, types of flour and other ingredients traditionally associated with local culture and origin. The diverse LAB and yeast strains synthesize essential amino acids and vitamins, *i.e.* thiamine, vitamin E and folates, produce prebiotic exopolysaccharides (EPS) and bioactive compounds, such as peptides and amino acid derivatives, *i.e.* γ-amino butyric acid (GABA) (Gobbetti *et al.*, 2014). Moreover, they may degrade anti-nutritional factors, such as raffinose and phytate, produce anti-oxidant compounds and enzymes, such as protease, phytase or lipase, positively affecting human health (Gadaga *et al.*, 1999).

Recently, the increasing demand for healthy baked goods boosted studies on sourdough microbiota with beneficial metabolic traits, to be used as potential functional starters. Actually, as microbial functional traits are strictly related to strains, the characterization of sourdough microbial isolates is crucial for their biotechnological exploitation to obtain functional baked end-products.

The overall objective of this research project was the selection of yeast strains with interesting functional and nutritional traits to be used as functional starters for the production of healthy breads. A total of 139 yeasts isolated from cereal-based fermented food and drink, were characterized for their pro-technological, functional and molecular traits. Analyses were carried out in triplicate with three biological replicates. In particular, 78 yeast strains isolated from Tuscan sourdoughs (Palla *et al.*, 2019), 3 *Saccharomyces cerevisiae* strains isolated from PDO Tuscan bread sourdough (Palla *et al.,* 2017), 43 yeasts isolated from other Italian sourdoughs and 15 yeast strains isolated from Boza were evaluated *in vitro* for their functional traits *i.e.* phytase and antioxidant activities. Phytase activity was evaluated measuring the production of *halo zones* on the Phytate Screening Medium (PSMG). To eliminate false positive, plates were counterstained with cobalt chloride and molybdate ammonium (Bae *et al.*, 1999; Pepe *et al.,* 2004). The ability to solubilize phytate was detected in 77% of isolates, 40% of which showed a *halo zone* higher than 2 mm. The antioxidant activity was determined by the free radical scavenging capacity using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). Results showed a high variability among yeast isolates, ranging from 65.11 ± 14.43 to 0.34 ± 0.34 nmol TEAC/mL. In particular, 75% of yeasts exhibited the radical scavenging capability (mean 17.50 nmol TEAC/mL).

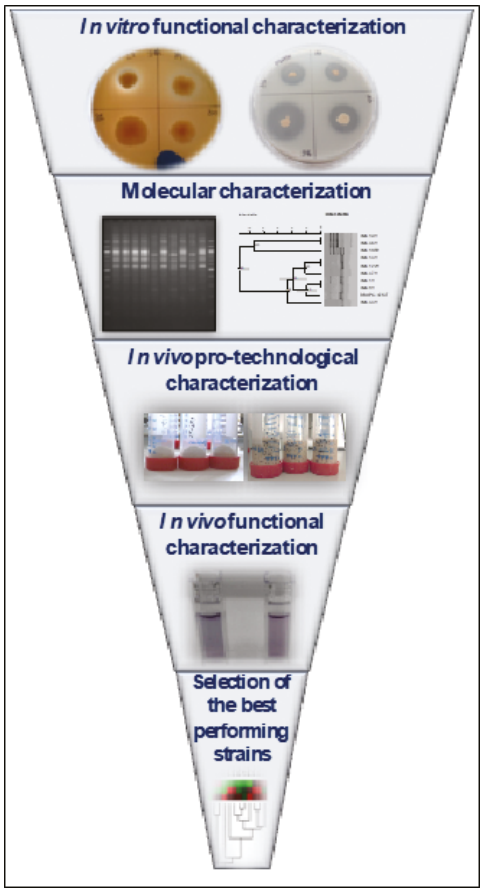


Figure 1. Flow chart showing the different operational steps for designing healthy sourdough bread. Selection of yeast strains to be used as functional starters for the production of healthy breads.

Based on results of the in vitro qualitative screening, the best 39 performing yeast isolates (34 *S. cerevisiae,* 2 *Pichia fermentans,* 1 *Kazachstania humilis,* 1 *Candida sake and* 1 *Torulaspora quercuum*) were selected and characterized at strain level by inter-delta regions analysis (Palla *et al.,* 2019). The dendrogram, obtained comparing the inter-delta profiles of the selected isolates and of two commercial baker's yeasts - *S. cerevisiae* Zeus IBA (ZEUS IBA srl) and *S. cerevisiae* Lievitalia (Lesaffre Italia spa) - showed a high intraspecific diversity, discriminating 20 biotypes out of 34. Moreover, none of our isolates shared the profiles with the two commercial baker's yeast strains.

All the selected isolates were further analyzed for their pro-technological (leavening ability) and functional (phytase activity and content of polyphenols) features, by in vivo screening using three different wholegrain flours derived from an ordinary bread-making wheat variety (*T. aestivum* L. cv Aubusson), a wheat variety rich in anthocyanins (*T. aestivum* L. cv Skorpion) and hull-less barley (*Hordeum vulgare* L. var. nudum Hook) Rondo. In particular, three different batches of each flour were pooled and used to prepare 100 g of dough (dough yield, dough weight × 100/flour weight, 160) supplemented with chloramphenicol (0.1 g/L), and individually inoculated with ca. 6.0 Log cfu/g of cellular suspension of each yeast. Finally, doughs were fermented at 30°C for 24h, according to the common temperature used in sourdough preparation at artisanal and industrial levels (Minervini et al., 2012). Not inoculated doughs, prior (CT_0) and after (CT_{24}) the incubation and Zeus IBA and Lievitalia commercial baker's yeasts were used as controls.

The increase of volume was monitored every two hours and the leavening performance was calculated as the difference in dough volume during the time of fermentation (cm3/h) (Perricone *et al.,* 2014). All strains inoculated in wheat flours, except for *C. sake* IMA BL3, showed leavening ability (mean 1.89 and 1.63 cm^3/h for Aubusson and Skorpion, respectively), while only 33% of yeasts inoculated in Rondo flour showed such ability (≤ 0.51 ± 0 cm^3/h). Many *S. cerevisiae* strains showed higher leavening performance, compared to commonly used commercial baker's yeasts. The 10 best performing yeasts for each flour were selected and further analyzed for their functional traits.

The analysis of total phenols content (antioxidant activity) was carried out on methanolic extracts (ME) of fermented doughs following the method described by Slinkard and Singleton (1997), based on the reaction between phenols with Folin-Ciocalteu reagent. Results showed that almost all the strains led to increases of total phenols content, ranging from 1.1 ± 0.03 to 0.68 ± 0 mg GAE/g, without significant differences among them. It is interesting to note that three strains for Abusson, 5 for Skorpion and all the strains for Rondo doughs showed antioxidant activity higher than commercial baker's yeast *S. cerevisiae* Zeus IBA.

Phytase activity was determined on the water-salt soluble extract of fermented doughs, by monitoring the rate of hydrolysis of *p*-nitrophenyl phosphate (*p*-NPP) as described by Rizzello et al. (2010). Results showed a high variability of phytase activity, ranging from 26.9 ± 0.7 to 2.8 ± 1.4 U, among doughs started with the different strains. Moreover, almost all the strains inoculated in Aubusson and in Skorpion flours exhibited phytase activity values significantly higher compared to the commercial baker's yeast.

The combination of leavening ability, phytase activity and phenols content values by Permutation analysis (PermutMatrix) allowed the detection of 2 best performing strains for each flour, which were further analysed for their ability to produce organic acids, such as lactic, acetic, propionic and butyric acid. All fermented doughs showed a significantly higher content of the four acids tested than non inoculated doughs. Moreover, with the only exception of *S. cerevisiae* IMA L10Y, all the other strains produced significantly higher quantities of total organic acids than the commercial strain.

References

1. Bae H.D., Yanke L.J., Cheng K.J., Selinger L.B., 1999. A novel staining method for detecting phytase activity. *J. Microbiol. Methods 39,* 17–22.
2. Gadaga T.H., Mutukumira A.N., Narvhus J.A., Feresu S.B. 1999. A review of traditional fermented foods and beverages of Zimbawe. *Int. J. Food Microbiol. 53,* 1–11.
3. Gobbetti M., 1998. The sourdough microflora: interactions of lactic acid bacteria and yeasts. *Trends Food Sci. Technol. 9,* 267–274.
4. Gobbetti M., Rizzello C.G., Di Cagno R., De Angelis M., 2014. How the sourdough may affect the functional features of leavened baked goods. *Food microbiol. 37,* 30–40.
5. Minervini F., Di Cagno R., Lattanzi A., De Angelis M., Antonielli L., Cardinali G., Cappelle S., Gobbetti M., 2012. Lactic acid bacterium and yeast microbiotas of 19 sourdoughs used for traditional/typical Italian breads: interactions between ingredients and microbial species diversity. *Appl. Environ. Microbiol. 78,* 1251–1264.
6. Palla M., Cristani C., Giovannetti M., Agnolucci M., 2017. Identification and characterization of lactic acid bacteria and yeasts of PDO Tuscan bread sourdough by culture dependent and independent methods. *Int. J. Food Microbiol. 250,* 19-26.
7. Palla M., Agnolucci M., Calzone A., Giovannetti M., Di Cagno R., Gobbetti M., Rizzello C.G., Pontonio E., 2019. Exploitation of autochthonous Tuscan sourdough yeasts as potential starters. *Int. J. Food Microbiol. 302,* 59-68.
8. Pepe O., Blaiotta G., Anastasio M., Moschetti G., 2004. Technological and molecular diversity of Lactobacillus plantarum strains isolated from naturally fermented sourdoughs. *Syst. Appl. Microbiol. 27,* 443–453.
9. Perricone M., Bevilacqua A., Corbo M.R., Sinigaglia M., 2014. Technological characterization and probiotic traits of yeasts isolated from Altamura sourdough to select promising microorganisms as functional starter cultures for cereal-based products. *Food Microbiol. 38,* 26-35.
10. Rizzello C.G., Nionelli L., Coda R., De Angelis M., Gobbetti M., 2010. Effect of sourdough fermentation on stabilisation, and chemical and nutritional characteristics of wheat germ. *Food Chem. 119,* 1079–1089.
11. Slinkard K., Singleton V.L., 1997. Total phenol analysis: automation and comparison with manual methods. Am. J. Enol. Vitic. 28, 49-55.
12. Vogel R.F., Knorr R., Muller M.R.A., Steudel U., Gänzle M.G., Ehrmann M.A., 1999. Non-dairy lactic fermentations: the cereal world. *Lactic Acid Bacteria: Genetics, Metabolism and Applications.* Springer, Netherlands, 403-411.

HEPATO-METABOLIC SYNDROME AND PROBIOTICS

Anna Alisi[1], Antonella Mosca[2], Nadia Panera[1], Valerio Nobili[2]
[1] *Molecular Genetics of Complex Phenotypes Research Unit, Bambino Gesù Hospital, IRCCS, 00146 Rome, Italy.*
[2] *Hepato-Metabolic Disease Unit, Bambino Gesù Hospital, IRCCS, 00146 Rome, Italy.*

Non-alcoholic fatty liver disease (NAFLD) has rapidly emerged as one of the most prevalent liver diseases worldwide. NAFLD is set to achieve virtually epidemic proportions in parallel to obesity trends. This problem is particularly awareness in children in whose the prevalence of NAFLD has been estimated to be between 3–11%, but this rate can be as high as 70–80% among those children who are considered obese. The abnormalities include steatosis (intra-hepatic fat accumulation) and non-alcoholic steatohepatitis (NASH), which is characterized by steatosis, inflammation, hepatocellular ballooning and eventually fibrosis [1].
NAFLD and metabolic syndrome (hepato-metabolic syndrome) are commonly associated, therefore they create a multi-organ disease that currently is an increasing health concern and hot topic in medical research.
A considerable volume of data from animal experiments has revealed the magnitude of contribute of both gut microbiota dysbiosis and disordered microbial population on the development of obesity and its complications, including NAFLD [2].
During last five years, considerable progress has been made in exploring the pathogenic role of the microbiome also in paediatric NAFLD and NASH, even if there are still many issues to be resolved [3,4]. Among these, the nature and location of the altered microbiome in specific population; the specificity of deficits in intestinal integrity to NAFLD/NASH versus metabolic pathways that are central to the influence of the microbiome; and finally, the therapeutic interventions that are likely to be of benefit to our patients [5].
Current studies hypothesize the use of probiotics for modulating gut dysbiosis in paediatric hepato-metabolic syndrome. Probiotics are defined as live microorganisms, which when consumed in adequate amounts, confer health effects on the host by modulating gut microbiome composition, intestinal permeability, and inflammatory response. Studies in children with NAFLD showed that probiotic supplements is associated with reduced liver damage, decreased concentrations of lipopolysaccharide, as well as improved aminotransferase levels, body mass index, and insulin resistance [6,7].
Therefore, the use of probiotics seems to be a promising option for the treatment of hepato-metabolic syndrome in children. However, future research is expected to further explore the physiopathological links between intestinal microbiota and NAFLD, to identify clinically relevant gut microbiota profiles that could indicate the best probiotic. While waiting for these further findings, we could already try to modulate gut microbiota using the mixtures of agents that have already demonstrated beneficial effects in human studies.

References

1. Nobili V, Alisi A, Valenti L, Miele L, Feldstein AE, Alkhouri N. NAFLD in children: new genes, new diagnostic modalities and new drugs. Nat Rev Gastroenterol Hepatol. 2019 Jul 5. doi: 10.1038/s41575-019-0169-z.
2. Moschen AR, Kaser S, Tilg H. Non-alcoholic steatohepatitis: a microbiota-driven disease. Trends Endocrinol Metab. 2013 Nov;24(11):537-45.
3. Del Chierico F, Nobili V, Vernocchi P, Russo A, Stefanis C, Gnani D, Furlanello C, Zandonà A, Paci P, Capuani G, Dallapiccola B, Miccheli A, Alisi A, Putignani L. Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. Hepatology. 2017 Feb;65(2):451-464.
4. Schwimmer JB, Johnson JS, Angeles JE, Behling C, Belt PH, Borecki I, Bross C, Durelle J, Goyal NP, Hamilton G, Holtz ML, Lavine JE, Mitreva M, Newton KP, Pan A, Simpson PM, Sirlin CB, Sodergren E, Tyagi R, Yates KP, Weinstock G, Salzman NH. Microbiome Signatures Associated with Steatohepatitis and Moderate to Severe Fibrosis in Children With Nonalcoholic Fatty Liver Disease. Gastroenterology. 2019 Jun 27. pii: S0016-5085(19)41037-8.
5. Putignani L, Alisi A, Nobili V. Pediatric NAFLD: the Future role of Patient-Tailored Probiotics Therapy. J Pediatr Gastroenterol Nutr. 2016 Jul;63 Suppl 1:S6-8.
6. Famouri F, Shariat Z, Hashemipour M, Keikha M, Kelishadi R. Effects of Probiotics on Nonalcoholic Fatty Liver Disease in Obese Children and Adolescents. J Pediatr Gastroenterol Nutr. 2017 Mar;64(3):413-417.
7. Alisi A, Bedogni G, Baviera G, Giorgio V, Porro E, Paris C, Giammaria P, Reali L, Anania F, Nobili V. Randomised clinical trial: The beneficial effects of VSL#3 in obese children with non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2014 Jun;39(11):1276-85.

GUT MICROBIOTA, BILIARY SALTS AND FXR

Domenico Alvaro,
Sapienza University of Rome, Division of Gastroenterology,
Department of Translational and Precision Medicine,
Viale Università 37, 00185 ROME, ITALY.

The gut microbiota produces several bioactive compounds that can signal by activating cognate receptors in different cells. One example is represented by bile acids (BA), which are synthesized from cholesterol in the liver and then metabolized by the gut microbiota. The amphipathic structure of BA provides them with detergent properties that enable digestion and absorption of dietary lipids and fat-soluble vitamins. Ii is now clear that the interaction between BA and gut microbiota is a key player in the regulation of intestinal barrier integrity, gut motility and secretory activities other than a very important regulator of host metabolism. BA act as signaling molecules in the liver, gut and in peripheral tissue, through the activation of receptors involved in metabolic pathways such as the nuclear farnesoid X receptor (FXR) and TGR5. FXR is a central transcriptional sensor of BA metabolism, and one of its key target genes is intestinal FGF19 which encodes an enterokine released into portal circulation following BA activation of FXR. FGF19 inhibits BS synthesis in the liver while exerting a number of effects on cell proliferation and inflammation. In addition, we cannot neglect the role of intestinal FXR on the gut microbioma and intestinal barrier integrity and this is mediated by different mechanisms including the secretion of antibacterial peptides and the regulation of tight junctions. Therefore, a bidirectional interaction exists between BA and microbiota since BA modulate microbiota composition and viceversa microbiota modulate BA metabolism. FXR activation by BA, in turn, modulates either microbiota composition or BA metabolism. FXR is activated by the primary bile acids chenodeoxycholic acid (CDCA) and cholic acid (CA), the secondary bile acid deoxycholic acid (DCA) and to a lesser extent by the secondary bile acid lithocholic acid (LCA). The conjugated forms of these bile acids (amidated with taurine (T) or glycine (G)) can also activate FXR. The gut microbiota deconjugates and subsequently further metabolises the primary bile acids into secondary bile acids in the gut and thereby changes FXR activation and signalling. Based on these interactions between microbiota, BA and FXR changes of microbiota composition my influence the qualitative and quantitative composition of BA pool thus affecting the BA-dependent signaling functions. On the other hands, the changes of BA composition associated with different pathologies (cholestasis, liver disease, malabsorption etc.) may influence microbiota composition with relevant effects on the progression and clinical manifestations of the underlying disease. Finally, modulation of intestinal FXR by specific ligands is currently under investigation as a therapeutic strategy to manage diseases of intestinal, liver and biliary tract.

References

1. Wang H, et al. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. Mol Cell 1999; 3: 543–553.
2. **Wahlström A, et al.** Crosstalk between Bile Acids and Gut Microbiota and Its Impact on Farnesoid X Receptor Signalling. Dig Dis. 2017;35(3):246-250.
3. Long SL, et al. Interactions between gut bacteria and bile in health and disease. Molecular Aspects of Medicine 2017; 56: 54-65.

FUNCTIONAL FOODS IN LIVER DISEASES

Morisco M[1], Ascione A[2]
[1] *Dipartimento di Medicina Clinica e Chirurgia, Università di Napoli Federico II*
[2] *Centro per le malattie del fegato, Ospedale Buon Consiglio - Fatebenefratelli, Napoli*

The interest shown in recent years for the relationship between food and health has led to an evolution of the concept of food. Foods, in addition to satisfying nutritional needs, can provide an additional physiological advantage by promoting people's health. Thus the concept of "functional food" was born, a term first introduced in Japan in the mid-1980s. Today the global functional food market is valued at 153600 million USD in 2018 and is expected to reach 260400 million USD by the end of 2025, with an expected annual growth rate of 6.8% during 2019-2025.
There is still no univocal definition of functional food and, over time, each institution has developed its own concept of functional food.
According to the European Commission, "*A food that beneficially affects one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. It is part of a normal food pattern. It is not a pill, a capsule or any form of dietary supplement.*". In any case, a food can be defined as functional if, in addition to its nutritional properties, it is scientifically demonstrated its ability to positively influence one or more physiological functions, helping to improve health and reduce the risk of developing diseases related to the regime food.
Bioactive compounds, molecules naturally present or added or increased to food, are responsible for the beneficial effects on health. They are very many and with very different molecular structures. Some examples are omega-3, polyphenols, carotenoids, fibres and probiotics. The scientific evidence that demonstrates the beneficial effects on health of functional foods concern all sectors of medicine, from metabolic diseases to neurodegenerative diseases up to cancer prevention. In particular, several foods and bioactive compounds have been evaluated in liver diseases. Dietary antioxidants such as carotenoids, flavonoids, resveratrol, lycopene and vitamin E present in many foods prevent and/or reduce liver damage by positively influencing biological systems, for example, through DNA repair mechanisms or acting as free radicals scavenger.
Several studies show that garlic organ sulphur compounds have hepatoprotective properties. In particular, cistamine improves hepatic fibrosis, both *in vitro* and *in vivo,* as it inhibits the activity of tissue transglutaminase and reduces the extracellular matrix. Furthermore, S-allyl cysteine in combination with lycopene suppressed the development of chemically induced gastric cancer by modulation of apoptosis-associated proteins.
The effects of omega-3 in liver disease have been widely evaluated in recent years. It has been that omega-3 improve hepatic steatosis, reduce liver enzymes and markers of inflammation in patients with NAFLD. Furthermore, dietary intake of omega-3 was related to a lower risk of hepatocellular carcinoma.
The use of probiotics and prebiotics in the prevention and treatment of various disorders has increased in recent years. Clinical trials have shown that probiotics improve the metabolic and hepatic parameters of patients with NAFLD, given the close relationship between the gut and the liver called the gut-liver axis. It is well documented that dietary fibres and prebiotics favourably modulate the intestinal microbiota and indirectly improve glucose and lipid metabolism and improve hepatic steatosis. Recent studies suggest that prebiotics may represent a therapeutic strategy not only for chronic liver disorders but also for hepatocellular carcinoma.
Coffee is rich of antioxidants and other bioactive compounds such as chlorogenic acid, melanoidins and diterpenes and its consumption has been associated with several health benefit and improvement of chronic liver diseases. In particular, it has been shown that coffee improves hepatic steatosis by reducing fat oxidation and improving metabolic parameters. Furthermore, coffee reduces intestinal permeability by modulating tight junction proteins, preventing the flow of toxic substances that would otherwise reach the liver. Finally, epidemiological studies correlate the consumption of coffee, with or without caffeine, to the reduction of the risk of hepatocellular carcinoma.

PHARMACOMICROBIOMICS: THE MICROBIOME CLOUD AS A DOUBLE-EDGED SHIELD AGAINST DRUGS AND NUTRIENTS

Ramy K. Aziz,
Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University

The human microbiome consists of trillions of microbial cells that reside in and on the human body. Its composition varies according to dietary, spatial, temporal, hormonal, and other individua factors, making it best described as a cloud of genes and genomes associated with different human organs and cavities. This set of microbes and their genomes offers tremendous functional potential, in particular in metabolic pathways, which consequently affects human health positively and negatively. Moreover, the variability and uncertainty of a microbiome's composition affects human metabolic capabilities, including the ability to detoxify xenobiotics and metabolize dietary components and drug molecules.

Pharmacomicrobiomics is a nascent field exploring drug-microbiome interactions, and has lately expanded to show how the human microbiome not only alters the fate of drugs, but also affects their action in a host genetic, diet-, and environment-dependent manner. This nascent field is rapidly moving from anecdotal or individual cases to systematics studies, and from observational, descriptive studies, to studies addressing causality and pinpointing the main microbial players as well as their genetic elements involved in microbiome-drug interactions.

Here, examples of the microbiome's effect on health, disease, and drug disposition and action will be presented. The PharmacoMicrobiomics database (http://pharmacomicrobiomics.org) will be introduced, and its usage statistics will be briefly assessed. The growth of the field and its movement towards systematic and mechanistic studies will be evaluated, and future prospects in diagnosis and intervention will be projected and discussed in the light of practical, ethical, and legal concerns.

APPROPRIATE USE OF "PROBIOTIC": BECAUSE WORDS MATTER

Dr Sylvie Binda, IPA EU scientific chairman
Colin Hill, Professor APC Microbiome Ireland, University College Cork

Probiotics are the subject of investigative research, innovative product design, and regulatory scrutiny, not to mention successful marketing, focused consumer interest and use by healthcare practitioners on a global scale. The original FAO/WHO definition of probiotics for foods, beverages, and dietary supplements is still widely recognized and relevant, but like any short definition it can be open to some interpretation as to which microorganisms meet the criteria to be called a probiotic. Practically speaking, this has led to confusion and even misuse regarding the application of the word "probiotic". To address this, we have translated the FAO definition into four simple and pragmatic criteria that will allow one to conclude whether or not specific strains of microorganisms qualify as probiotics. This original paper, currently under consideration by IPAEU and ISAPP members, describes the minimum criteria that apply to probiotic strain(s) that will be used in food. The wide adoption of these criteria is encouraged to ensure the proper use of the word probiotic in scientific publications and in communication with regulators and the general public.

Probiotic strains should be:

(i) Sufficiently characterised,
(ii) Safe for the intended use,
(iii) Supported by at least one positive human clinical trial according to generally accepted scientific standards,
(iv) Alive in adequate numbers in the product throughout shelf life and when consumed.

It is essential for all probiotic stakeholders to clearly understand the criteria and requirements needed for the word "probiotic" to be responsibly used. More specifically, the strain(s) must be identified using recognized scientific methods, named according to valid current nomenclature, named with a retrievable strain designation, and deposited in an international culture collection. The strain(s) must have demonstrated safety and the amount of live probiotic organisms in the final product should be consistent with the scientifically demonstrated amount required to achieve the desired benefit up to the end of shelf-life. Furthermore, any statements with regard to the survival of the probiotic through the gastrointestinal tract should be demonstrated by human studies. All probiotic products should be manufactured according to applicable good manufacturing requirements to assure safety, purity, and stability, and labeled in a manner that communicates essential information on product contents (specific strains, level of live probiotic delivered at end of shelf life, and statement about health benefit as allowed) to the end-user. Such criteria are relevant to the final product that contains the probiotic. Adherence to these principles will promote transparency and good science and assure consumers that the marketplace does not contain products that misuse the term "probiotic".

PROBIOTIC DEFINITION AND ELEMENTS OF THE PROPOSAL ON HARMONISED CODEX APPROACH

Bourdichon, F.[1,2], IDF Action team on CCNFSDU Work Item Probiotics, Morelli, L.[2].
[1] *Food Safety, Microbiology, Hygiene, 74 Boulevard Blossac, 86100 Chatellerault, France.*
[2] *Facoltà di Scienze agrarie, alimentarie ambientali, Università Cattolica del Sacro Cuore, Piacenza-Cremona, Italy*

Following the business meetings prior to the World Dairy Summit in Daejon, South Korea, in October 2018, the International Dairy Federation (IDF) dedicated action team on "Guidelines for Probiotics" made following comments to CX/NFSDU 18/40/12: Discussion paper on harmonized probiotic guidelines for use in foods and dietary supplements:
"In principles, IDF supports the proposal of Argentina to establish guidelines for the use of probiotics in foods and dietary supplements.
Subject to approval by the committee to develop guidelines for the use of probiotics in foods and dietary supplements, IDF would like to offer its participation in the work, as it is believed modifications are needed to the proposed document.
It should be more explicit on its scope, purpose, and limits. The definition of a probiotic strain, inactivated or not, a probiotic food product should be established for food and not medical purpose. IDF would like to see the focus on the labelling rules regarding the use of "probiotic".
Safety and health benefit demonstration guidelines should not be detailed in the document but referred to scientific evidence presently available. Of note, a positive list approach such as IDF's one for food culture, does not apply to probiotic strain candidates."
While IDF expressed some reserve on modifications needed to the proposed document, such was the decision taken in November 2018 during the 40th session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU):
"The delegations in support of the work noted that harmonized global guidelines would benefit the Codex community in light of the significant increase in global trade of probiotics for use in foods and dietary supplements in recent years and would assist national authorities in evaluating foods/supplements containing probiotics. One observer (Note: IDF) also supported new work on harmonized guidelines that would establish a definition with minimum characterization requirements as well as quality and labelling criteria for probiotics for use as an ingredient in foods and dietary supplements.
The delegations and an observer (Note: International Special Dietary Foods Industries - ISDI) not in favour of starting new work at this point, expressed the following view or concerns:
There was no perceived need for such work.
This work might not have the priority taking into account the current heavy workload of the Committee.
The paper needed to be revised to provide more clarity especially on the scope of the work.
Collection of information and data from Members should be first conducted to identify a globally applicable definition of probiotics.
Infant foods should be excluded since safety was of concern due to a limited number of studies.
As a conclusion, the Committee agreed that Argentina should redraft the discussion paper for consideration at its next session elaborating further on the sections on scope, definition as well as health and trade concerns in particular."
Considering the topic of probiotic, IDF action team has aligned its position on the statement of the FAO in the 2006 Food and Nutrition Paper #85, that is a compendium of 2001 (Cordoba, Argentina) expert consultation and 2002 (London, Ontario, Canada) expert working group on the topic. These reports provide scientific advice in relation to the safety assessment, general guidance for their evaluation on their functional and nutritional properties.
The CCNFSDU document, either standard or guidelines, should be aligned on the initial proposal of those documents, especially the definition of probiotic strain and the limitation of use to food products for generally healthy population. The use of probiotic strains for specific population (infants, medical purposes) requires specific safety demonstration and health efficacy that were not initially considered in the 2001 and 2002 documents. There are also numerous implementation guidelines and regulations, national and regional currently in place. All are based on the initial FAO/WHO reports with updated scientific knowledge.
IDF is supportive of the joint IPA and Argentina initiative, and IDF members of the action team believed that the Codex document is an opportunity to define the scope, definition, use and limitation of probiotic strains either as ingredient of a food product or as a food product itself (in an Over the Counter – OTC product e.g.).

References and Discussion document on safety evaluation of microorganisms in the food chain (Microbial Food Cultures, Probiotic)

1. Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J.C., Gerds, M.L., Hammes, W.P., Harnett, J., Huys, G., Laulund, S., Ouwehand, A., Powell, I.B., Prajapati, J.B., Seto, Y., Ter Schure, E., Van Boven, A., Vankerckhoven, V., Zgoda, A., Tuijtelaars, S., Hansen, E.B., 2012a. Food fermentations: microorganisms with technological beneficial use. International Journal of Food Microbiology 154 (3) 87-97.
2. Bourdichon, F., Alper, I., Bibiloni, R., Dubois, A., Laulund, S., Miks, M., Morelli, L., Zuliani, V., Yao, S., 2018. Inventory of Microbial Food Cultures with safety demonstration in fermented food products. Update of the Bulletin of the IDF 455-2012. Bulletin of IDF 495.
3. FAO, 2006. Probiotics in food. Health and nutritional properties and guidelines for evaluation. FAO Food and Nutrition Paper #85. Available at: http://www.fao.org/3/a-a0512e.pdf
4. FEEDAP Panel, EFSA. 2018. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018; 16(3) 5206
5. Herman, L., Chemaly, M., Cocconcelli, P.S., Fernandez, P., Klein, G., Peixe, L., Prieto, M., Querol, A., Suarez, J.E., Sundh, I., Vlak, J., and Correia, S., 2018. The qualified presumption of safety assessment and its role in EFSA risk evaluations: 15 years past. FEMS Microbiology Letters, 366, 2019, fny260.

Bibliography

François is delegate of France at the International Dairy Federation (IDF) since 2010.
He is presently chair of the standing committee on microbial hygiene (SCMH), member of the food standards steering group (FSSG), the standing committee on harmonization of microbial method (SCHMM) and analytical methods for dairy microorganisms (SCAMDM). He is the action team leader for IDF Action Team - CCNFSDU – Guidelines Probiotics.
He represents IDF at ISO, WHO, FAO and Codex Alimentarius on food safety related topics.

FUNCTIONALITY OF NEXT GENERATION PROBIOTICS IN HUMANS: A GUT-BRAIN PERSPECTIVE

Robert-J M Brummer MD PhD, Nutrition-Gut-Brian Interaction Research Centre, Örebro
University, Sweden

The lecture will start to describe and explain the bidirectional signaling between the gut, its microbiome and the brain and how microbial modulation and probiotics can beneficially affect this interplay. Decreased mental health is regarded as one of the main health issues of modern society. It has recently been acknowledged that the intestinal barrier plays a pivotal role in gut-brain interactions and minor aberrations in barrier function may not only have a negative impact on gut health but also on the entire body including mental health and wellbeing. It is assumed that local immune responses and inflammatory reactions in the gut epithelium play a central role.
Another route by which probiotics can positively affect the gut-brain axis is that by the metabolites produced by the microbiome in its specific ecosystem. In this context the recent changes in dietary preferences of consumers will be discussed in conjunction with the role of next generation probiotics and "precision nutrition".
One of the major challenges research is facing in validating functionality of functional food, and specifically probiotics, is the identification of valid biomarkers or "surrogate" markers of the functional impact of these compound on the gut and brain. Dedicated mode-of-action studies are pivotal to move the research frontiers beyond state-of-the-art.

MICROBIOMES EXPLOITATION FOR THE SUSTAINABLE FOOD PRODUCTION, THE CIRCLES APPROACH

Marco Candela (PhD)
Associate Professor, Department of Pharmacy and Biotechnology, University of Bologna, Italy

A **food system** is defined as a system that embraces all the actors (e.g. environment, people, inputs, procedures, processes, infrastructures, markets and trade organizations) and activities that are related to the production, processing, distribution, marketing, preparation, packaging and consumption of foods. Activity outputs, including environmental and socio-economic aspects, also need to be included in the food system as a whole.
Food systems play a Janus-faced role in the growth and development of the planet: while providing human societies with food, they exert strong pressures on natural resources such as energy, land, oceans, freshwaters, nutrients and biological diversity. Dramatically, the related-costs of these pressures on the environment are growing exponentially: climate change is occurring faster than expected and natural resources are being degraded more severely than previously thought (European Commission 3rd SCAR Foresight Exercise, "Sustainable food consumption and production in a resource-constrained world", 2016). In parallel, common practices in food chains, such as the preventive antibiotic usage and/or pesticide utilization, with the reduction of animal and plant immunological performances, have a negative feedback on food safety, particularly in terms of selection and spread of antimicrobial resistance (AMR) and new or previously neglected spoilage and zoonotic microbes.
By 2050, the planet will be inhabited by 9.7 billion people, a figure ~30% larger than the current world population. This implies an unprecedented challenge for mankind: food systems need to boost the production of safe and nutritious food while drastically reducing their footprint on natural resources. Europe will be at the forefront of this challenge and as such, it will need to witness disruptive changes to its food systems ("Food 2030, European research & innovation for food & nutrition security"). Exploiting the micro-bial communities inhabiting and inter-actting with animals and plants, collec-tively referred to as the microbiome, has been proposed as one of the strategies underpinning these disruptive changes.
Recent advances on sequencing and com-putational approaches empowered scientists with the necessary tools to decipher the metabolic potential of these microbial com-munities. This has opened new perspec-tives on the exploita-tion of this renewable resource of beneficial functions for the environment. Not surprisingly, **microbiome gained centre-stage for human, plant, animal and ultimately, planet health.** When translated to the food systems, studies unequivocally point at the **microbiome as an untapped resource of probiotic functions for the main actors implicated in food systems** (i.e. soil, plant/animal, feed, food, farm, processing, wastewaters, sediments, workers and consumers) ultimately determining the food system productivity, quality, safety and sustainability (Fig. 1).

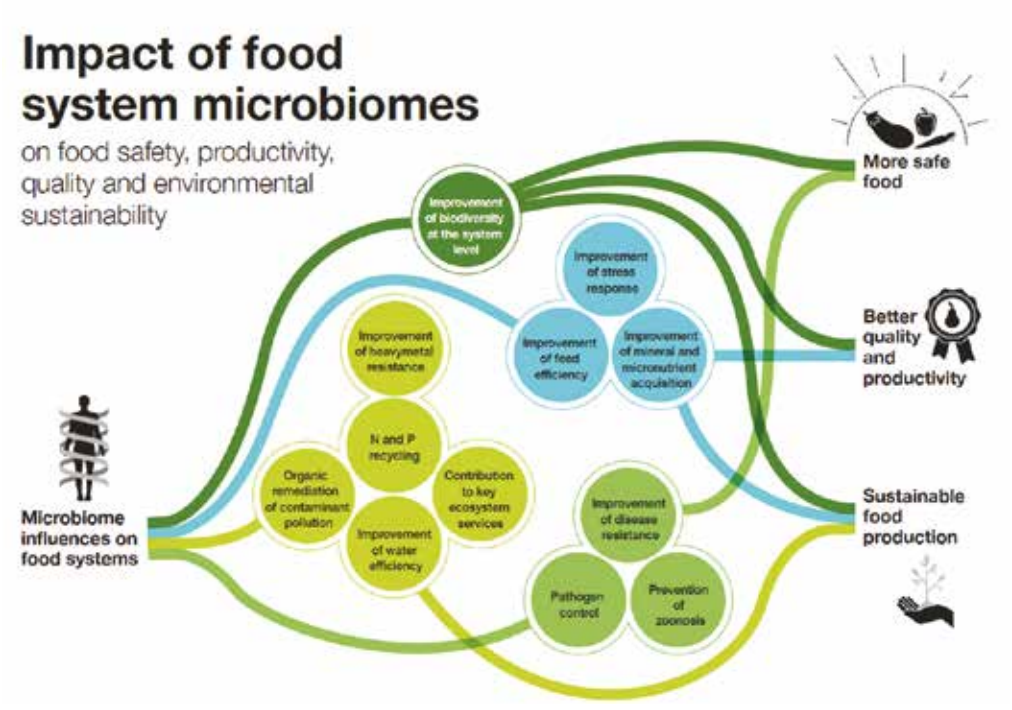


Fig. 1: Animal and plant microbiomes are of strategic importance to improve food productivity and quality, thanks to their positive action on host physiology, energy homeostasis and feed efficiency. On the other hand, environmental microbiomes can be crucial to achieving sustainable intensification of food systems, by enhancing the ecosystem capacity to buffer food system pressures on the environment. Finally, by definition, microbiomes are central in contrasting the biodiversity crash, ensuring a large panel of functional diversity and functional redundancy strategic for the resilience of each actor in the food systems.

However, despite this increased recognition for the microbiome, there is an urgent need of **studies providing decision-makers, academia and companies with an integrated vision of and testing microbiomes impact on food systems, from farm to fork.**
CIRCLES main goal is to fill this knowledge gap by **discovering, translating and communicating innovative, microbiome-based applications to enhance food system performances and their sustainability.** To achieve this goal, CIRCLES foresees 3 main phases: **i) discovery phase,** aimed at improving microbiome knowledge, **ii) translation phase,** aimed at exploiting knowledge to design and test concrete and innovative microbiome applications, and **iii) communication phase,** aimed at increasing the awareness of the general public on microbiome innovations.
The core of the overall methodology of the CIRCLES project are the real-world labs in the field of 7 food systems relevant to the EU: plants (spinach, tomato), poultry, pigs, Atlantic salmon and seabream aquaculture and fisheries (wild Atlantic salmon and seabream). The labs in the field will sustain both the experimental observation and intervention (translation) project phases, providing a unique opportunity to explore, understand and modulate the food system microbiomes across all the food system actors to achieve food security and sustainability for the future (Fig. 2). The setup of real-world labs in the field has involved several strategic methodological choices. First, the sites and real-world models for each food system have been carefully selected, putting attention to the representativeness of real EU food chains, even for regional and geographic specificities. Of note, the selected CIRCLES food systems will serve as a paradigmatic example on how microbiomes impact on different food chains

Fig. 2: Labs in the field will allow a longitudinal charact-erization of food system microbiomes and the paral-lel assessment of food system covariates. This will nurture the modelling phase, the SMM formulation and imple-mentation into integrative circular actions, which will be validated in the field.

MICROBIOMES AND DYSBIOSIS: NEW APPROACHES FOR HEALTH & PERSONAL CARE

Federica Carlomagno,
R&D Manager, Personal Care
ROELMI HPC

Microbiome is today a hot topic for health and personal care market. This growing interest is going beyond the simple marketing approach. It could represent the future for research and innovation applied to cosmetic and food supplement formulas targeting consumers' wellness.

Healthy status of people, in fact, is often linked to the homeostasis of microbiome populating different body districts, meaning that specific microorganisms like bacteria, fungi and viruses colonizing different sites of our body, create complex but balanced living communities, leading our healthy status.

Recent studies have highlighted the link between inflammatory or allergic diseases (for example atopic dermatitis or acne) and localized microbiome dysbiosis.

ROELMI HPC, starting from its deep knowledge of probiotics, has developed different approaches to interact with localized microbiomes. First, by investigating secondary effects of probiotics on the gut-brain-skin axis and secondly by exploring cutting-edge technologies for a new era of ingredients application on microbiome.

With a strong know-how on bio-fermentation, ROELMI HPC discovered how to protect probiotics and make them more suitable for food application by encapsulation technology, in order to preserve them during product preparation and administration. Later, the activity of probiotics has been investigated not only within the intestine, but also by looking at their effect on skin inflammation, cognitive function, metabolic pathways, the urogenital system, etc. As a step forward, ROELMI HPC moved to a different market, from food to cosmetics, designing specific ingredients for the skin. First by using deactivated probiotics thanks to their booster and immune-mimetic effect, then by fermenting metabolites aiming at rebalancing the skin microbiome affected by external stresses like air pollution or salty/chlorinated water.

Every new development has been clinically studied *in-vivo* by analyzing the metagenomics of microbiomes and measuring target parameters. Subjects belonging to both European and Asian ethnicities have been selected for the studies, to globally enlarge ROELMI HPC expertise on these leading-edge topics.

PSYCHOBIOTICS AND THE MICROBIOME-GUT-BRAIN AXIS: MOVING THE GOALPOSTS IN PSYCHIATRY?

Gerard Clarke[1,2,3]

[1] Department of Psychiatry and Neurobehavioural Science, University College Cork, Cork, Ireland
[2] APC Microbiome Ireland, University College Cork, Cork, Ireland
[3] INFANT Research Centre, University College Cork, Cork, Ireland

Experimental evidence continues to accumulate from both preclinical and clinical studies to support the concept that the gut microbiota can regulate brain function, structure and behaviour. Within this conceptual framework, the gut microbiome can signal along the gut-brain axis to influence many fundamental aspects of the central nervous system. This includes a major influence on host stress physiology as well as a broad range of behaviours relevant to stress-related psychiatric disorders including depression and anxiety, and functional gastrointestinal disorders such as irritable bowel syndrome. Stress exposures across the life span can also alter microbiome-gut-brain axis signalling, including gut microbiome composition and function, illustrating the bidirectional nature of this host-microbe dialogue.

Research efforts continue to identify the precise mechanisms underpinning these effects and possible routes of communication include the vagus nerve, the neuroendocrine system and immune factors. There is now an increasing focus on microbial regulation of tryptophan metabolism and the serotonergic system arising from a number of key observations taken from a variety of strategies used to parse the role of microbiota in brain function. These include germ free animals, antibiotic treatments, dietary manipulations, probiotics and prebiotics. Microbiota-deficient animals in particular consistently show alterations in the availability of tryptophan as well as serotonergic alterations in the gastrointestinal tract and the CNS. Many of the behaviours influenced by the gut microbiota rely on intact serotonergic neurotransmission and serotonin is a key stress-responsive neurotransmitter at both terminals of this bidirectional communication network.

Taken together, these studies support the possibility of microbial-directed management strategies to improve brain function and behaviour. An important option in this regard is the use of psychobiotics to modulate signalling along the microbiome-gut-brain axis. To date, this approach has met with some success mixed with failures to translate in healthy study participants. Further translational insights and well-controlled human studies in stress-related disorders will be critical as we transition from promising preclinical research towards mechanisms and therapeutic targeting of the gut microbiome in the clinical setting.

Research Support: GC is a faculty member of APC Microbiome Ireland and an associate investigator in the INFANT research centre which are funded by Science Foundation Ireland (SFI; grant numbers: SFI/12/RC/2273 and SFI/12/RC/2272 respectively). Other funding sources include the Health Research Board (HRB) through Health Research Awards (grant numbers HRA_POR/2011/23 and HRA-POR-2-14-647). GC has also been supported by a NARSAD Young Investigator Grant from the Brain and Behavior Research Foundation (Grant Number 20771) and by the by European Office of Aerospace Research and Development/Air Force Office of Scientific Research (grant number FA9550-17-1-0016).

PHYSIOPATHOLOGY AND DYNAMICS OF HUMAN GUT MICROBIOTA

Massimo Clementi
UNIVERSITÀ VITA-SALUTE SAN RAFFAELE, MILANO
LABORATORIO DI MICROBIOLOGIA E VIROLOGIA
OSPEDALE SAN RAFFAELE, MILANO

The human gut is a complex ecosystem; a great microbial community collectively designated as gut microbiota, including bacteria, archaea, fungi, and viruses, reside in human gastrointestinal tract. Increasing body of data suggests that the microbiota has a central role in many physiological processes, but also that its alteration may pave the way to many diseases in intestine and other organs. Physiologically, the gut microbiota is a crucial organ, intimately tied to the maturation of our immune system, our methabolism, and even our psychology. The gut microbiota of healthy individuals is composed of six bacterial phyla: Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria and Verrucomicrobia, which include more than 100 species that are further divided into ~200 bacterial strains. However, normally 90% of the gut microbiota is represented by members of the Firmicutes and Bacteroidetes (*eubiosis*). The relative levels of bacterial phyla change (*dysbiosis*) in individuals with pathological conditions associated to the gut microbiota such as obesity, inflammatory bowel disease, diabetes and neurological disorders. From this point of view, the one-pathogen–one-disease paradigm, a major focus of medical microbiology for more than two centuries, has been complicated by the evidence of the role of the human microbiome.

THE HEALTHY MICROBIOTA

The gut microbiota represents a crucial physiological organ for normal intestinal function, (GALT maturation, tissue regeneration, gut motility and permeability), metabolism (energy expenditure, nutrient accessibility, short-chain fatty acids and adiposity), intestinal vessel formation (TF glycosylation, thrombin cleavage, ANG1 expression, vascularization), bone homeostasis, and behaviour (synaptic connectivity, anxiety, pain perception). Despite these important fuctions, identification of the determinants of gut community formation, organization, stability, and function across the life span of a host presently remains an important scientific and medical challenge. Each person contains a unique set of bacterial strains obtained from the environment, and this population remain relatively stable for years. This suggests that the gut microbiome is a self-assembled "organ" specifically tailored to each of us. The ability of a commensal to colonize a given subject is controlled by a variety of factors, including the immune system, availability of metabolic niche space, and competition with indigenous microrganisms. Since bacterial species in the gut maintain similar abundance levels across humans, this suggests that species-specific niche volumes are conserved within the host population. Interestingly, longitudinal diversity studies have shown that at a broad taxonomic level the gut microbiota can be stable for years, but it is highly dynamic and variable at the species level. Dietary interventions in healthy individuals and comparisons between the gut microbiota of twins indicate that diet is a crucial driving driving force of bacterial diversity in the gut, although host genetics remains important. Finally, the interplay between the gut microbiota and human immune system is central and only partially understood. The immune system has evolved to maintain a symbiotic relationship between host and microbiota, and its disruption leads to profound effects in human health.

MICROBIOTA AND PATHOLOGY

Alterations of normal microbiota are caused by change in the composition, change in microbial methabolic activity, and shift in local distribution of microbial communities. Principally these conditions are determined by inappropriate diet, antibiotic exposure, and presence of pathogens. Of note, these conditions lead to chronic inflammation, metabolic dysfunction and metabolic diseases, diabetes, atherosclerosis, colon cancer, autoimmune diseases. More recently, it has been observed that the gut microbiota can impact central nervous system physiology and neurochemistry (*the gut-brain axis*). Germ-free mice that are devoid of associated microflora exhibit neurological deficiencies in learning, memory, recognition, and emotional behaviour. They display variations in important neurotransmitters compared to conventional mice. In humans, evidence for interplay between gastrointestinal pathology and neuropsychiatric conditions has been reported in diseases such as anxiety, depression, and autism.

There is a clear association between low gut microbiota diversity and susceptibility to pathogen invasion and infectious disease. In the case of *Clostridium difficile*, we observe that patients with severely depleted species diversity are predisposed to recurrent infections. Ecological repair of the gut environment through fecal microbiota transplantation (FMT) from a healthy donor has proven to be an effective treatment for recurrent *Clostridium difficile* infections. However, we do not fully understand the mechanisms underlying FMT efficacy, as yet. This makes it difficult to translate such treatments to other disease. In particular, microbial therapeutics that work in one individual are often ineffective in another, suggesting that a different or personalized approach is necessary for many conditions. Before we can integrate ecological therapeutics into modern medicine, we must understand the basic rules governing the ecology and evolution of gut commensals within a host. Furthermore, we should have a complete map for how variation in the microbiota is associated with host physiology, immunity, and disease.

DIAGNOSTIC POTENTIAL OF MICROBIOME ANALYSIS (MICROBIOME IN THE CLINICS)

Has microbiome analysis a present diagnostic role in different pathological conditions? Can this analysis meet specific clinical needs at present? Or, it is primarily a tool for pathogenic assessment.

In the last few years, we addressed in our lab the diagnostic potential of gut and vaginal microbiome analysis in two parallel studies. Firstly, we evaluated if gut microbiome analysis could be an early predictor of outcome in hematopoietic stem-cell transplantation. Interestingly, we observed that dysbiosis at time 0 before transplant (including levels higher than 5% of enterobacteriaceae, or lower than 10% of lachnospiraceae) influence significantly the probability of developing bacteriemia and severe sepsis as well as the overall survival (Mancini N. et al., OFID, 2017). The study is proceeding with an assessment of the correction of dysbiosis at time 0 in the subpopulation of crtically ill patients. In the second study, we investigated vaginal and seminal microbiome in infertile couples. The data obtained support the hypothesis that infertility condition is associated with an alteration of the vaginal flora and represents the first step toward its correlation with intrauterine insemination (IUI) pregnancy outcome (Amato V. et al. in press, 2019).

Although more experience is clearly necessary, it is currently believed that careful analysis of human microbiome at different body sites can open a new way to interpret many pathological conditions in the modern medicine.

EXCLUSION DIETS AND GUT MICROBIOTA MODULATION IN PATHOLOGICAL CONDITIONS

Del Chierico Federica
Human Microbiome Unit, Bambino Gesù Children's Hospital, IRCCS

Gut microbiota provides the host with unique and specific enzymes and biochemical pathways. A large proportion of these metabolic processes are involved in either nutrient acquisition, undigested carbohydrates metabolism, and vitamin biosynthesis. Moreover, gut microbiota performs essential protective, structural, and metabolic functions for host health.
The equilibrium within microbial communities and between the microorganisms and the host is called eubiosis. Failure to achieve or maintain a eubiotic status, leads to negative consequences on health, causing intestinal diseases and/or disorders.
The gut microbiota composition can be affected by several factors, among them dietary habits may be considered the most important.
Distinct dietary regimens are considered necessary or adjuvant treatments for patients affected by specific pathological conditions, as irritable bowel syndrome (IBS), celiac disease (CD), or neurological disorders (ND).
These dietary regimens are characterized by a reduction or exclusion of a specific nutrient from the entire dietary pattern. In particular, in low fermentable, oligo-, di-, mono-saccharides and polyols (FODMAPs) diet, recommended for IBS, the poorly absorbed or indigestible short-chain carbohydrates are reduced. In the ketogenic diet (KD), recommended in epileptic patients, carbohydrates are reduced. Finally, the gluten is excluded from the gluten-free diet (GFD) administered in CD patients. These alimentary regimens, protracted for a long time can affect microbiota composition favoring the selective enrichment of microorganisms capable of adapt to different availability of some nutrients, leading to the alteration of the microbiota eubiosis. Then the identification of specific eubiotic or dysbiotic profiles associated with diet therapy is necessary to develop personalized dietary intervention protocols for patients. Promising results are reported in studies exploring the effect of a GFD supplemented with probiotics in CD patients. In these studies, an improvement of persistent gastrointestinal symptoms coupled with an increase of Bifidobacteria and a re-establishment in the physiological Firmicutes/Bacteroidetes ratio were reported. Regarding KD, some authors recommend pre- or probiotics treatment for patients with a prolonged dysbiosis KD-induced.
In conclusion, even if further studies are needed to confirm and/or expand these findings, the evidence encourages the use of dietary patterns coupled to probiotics to avoid the alteration of the intestinal microbiota, especially in pathological subjects already characterized by intestinal dysbiosis.

DIETARY FLAVAN-3-OLS, GUT MICROBES, AND HEALTH: HOW DOES IT WORK?

Daniele Del Rio
The School of Advanced Studies on Food and Nutrition, University of Parma

Flavan-3-ols are the most complex subclass of flavonoids, with structures of various molecular weight ranging from simple monomers to oligomers and polymers of up to 190 units. The monomeric flavan-3-ol presents two stereogenic centers at C2 and C3 that produce four isomers for each level of B-ring hydroxylation and shows two hydroxyl group in C5 and C7. The hydroxyl groups in the B ring can range from one to three, yielding different structures: (epi) afzelechin, with one hydroxyl at C4′; (epi)catechin, two hydroxyls at C3′ and C4′; and (epi)gallocatechin, three hydroxyls at C3′, C4′, and C5′. (+)-Catechin and (–)-epicatechin are the most common flavan-3-ol monomers, being widespread in nature. Monomers can also undergo esterification with gallic acid, forming (epi)(gallo)catechin derivative. Differently from other flavonoids, flavan-3-ols exist *in planta* predominantly as aglycones rather than glycosides.
Flavan-3-ols undergo an extensive metabolism once introduced into the gastrointestinal (GI) tract. After ingestion, they pass through the oral cavity and the stomach remaining almost unchanged, thus reaching the small intestine. Here, some monomers are absorbed in the enterocytes by passive diffusion and subjected to some degree of phase II enzymatic metabolism. Sulfotransferases (SULT), uridine-5′-diphosphate-glucuronosyl-transferases (UGT) and catechol-O-methyltransferases (COMT) originate sulfated, glucuronidated and O-methylated metabolites respectively, which pass through the portal vein to the liver or efflux back into the lumen mediated via members of the adenosine-binding cassette (ABC) family of transporters. Unlike simple monomers, flavan-3-ols with a 3-gallate moiety do not necessarily undergo conjugation by phase II enzymes since they have been detected in the circulation unmetabolized. In addition, a very small percentage of oligomers (< 1%, mainly dimers) seems to be absorbed in the small intestine, getting into the portal bloodstream in unconjugated forms. Absorbed flavan-3-ols rapidly reach the liver, where they may be subjected to further phase II metabolism prior to entering the systemic circulation and eventually undergoing renal excretion. A small amount of phase II metabolites may also return from the liver to the small intestine via enterohepatic recirculation in the bile. However, most of the ingested flavan-3-ols (> 70%) are not absorbed in the upper part of the GI tract and reach the colon, where they are extensively metabolized by the host microbiota.
The microbial derivatives phenyl-γ valerolactones (PVLs) and their related phenylvaleric acids (PVAs) are the main circulating metabolites of flavan-3-ols. Despite their presumed importance, these gut microbiota-derived compounds have, to date, been considered subordinate to their parent dietary compounds, flavan-3-ol monomers and proanthocyanidins. The role and prospects of PVLs and PVAs as key metabolites in the understanding of the health features of flavan-3-ols will be critically assessed and, among the covered topics, the formation, bioavailability and pharmacokinetics of PVLs and PVAs from different types of flavan-3-ols will be discussed, taking into account *in vitro* and animal studies, as well as inter-individual differences and the existence of putative flavan-3-ol metabotypes. Figure 1 shows a schematic representation of the main frames and outcomes considered for the assessment of PVL and PVA bioactivity.

Keywords
flavan-3-ols, phenyl-γ valerolactones.

References

1. Mena P, Bresciani L, Brindani N, Ludwig IA, Pereira-Caro G, Angelino D, Llorach R, Calani L, Brighenti F, Clifford MN, Gill CIR, Crozier A, Curti C, Del Rio D. Phenyl-γ-valerolactones and phenylvaleric acids, the main colonic metabolites of flavan-3-ols: synthesis, analysis, bioavailability, and bioactivity. Nat Prod Rep. 2019 May 22;36(5):714-752

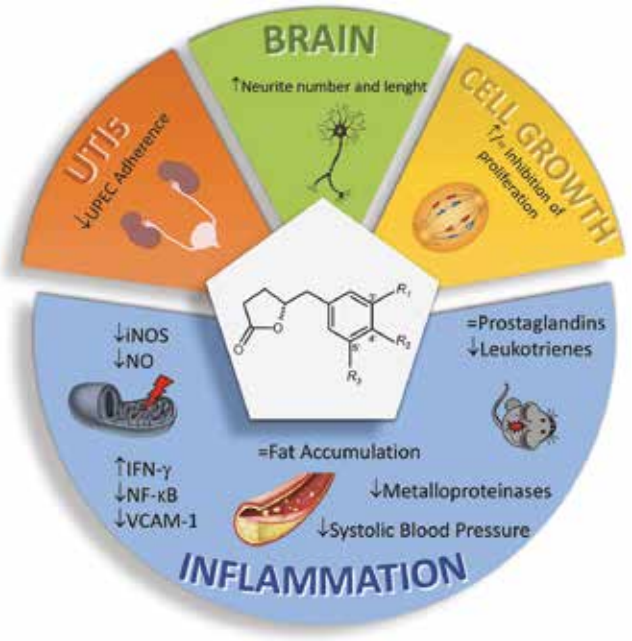


Figure 1 (modified from reference 1)

FOOD MATRIX AND HOST FACTORS INFLUENCE BIOAVAILABILITY AND METABOLISM OF POLYPHENOLS FROM BOTANICALS

Mario G. Ferruzzi, Ph.D.
Plants for Human Health Institute, North Carolina State University
600 Laureate Way, Kannapolis, NC 28081 USA

Epidemiological and clinical evidence support the notion that polyphenol rich diets including fruits, vegetables and whole grains are associated with a decreased risk of select chronic diseases and overall mortality. With potential for health benefits, interest in the underlying mechanism(s) of action have grown to include a focus on the ability of select polyphenol forms and/or their metabolites to modulate oxidative and inflammatory stress, impact gut microbial communities, and positively impact glycemic properties, blood flow and cognitive performance. A key factor in understanding underlying mechanism at play continues to be the characterization of polyphenol bioavailability and specifically, the absorption, formation and tissue distribution of biologically relevant metabolites generated by both host and microbial metabolism so that biological activities can be investigated in alignment with protective or therapeutic actions.

Polyphenol bioavailability is a complex multistep process that is subject to both host and food related influences. As subject of intense investigations over the years it has been possible to characterize critical paths for delivery of select biologically relevant polyphenol metabolites and identification of select food, dietary and select host factors that can influence this process. Key factors include (1) polyphenol chemical structure (2) form and composition of the food/botanical matrix; (3) sensitivity to gastrointestinal digestion; (4) efficiency of intestinal transport; (5) metabolism by host and microbial communities to small molecular weight phenolics and/or conjugated forms (i.e. glucuronidation, sulfonation or methylation) and (6) distribution and excretion from the body[1]. In investigating food factors impacting bioavailability, a focus can be placed on the digestive breakdown of the food/supplement matrix and resulting release/solubilization in the gut lumen. This fraction of polyphenols made available for subsequent uptake in the intestine is defined as *bioaccessibile*, and is a measure of the (poly)phenols potential for absorption[2]. While not accounting for host or microbial metabolism, bioaccessibility remains a metric by which food matrix effects on bioavailability may be assessed in a highly controlled in vitro or preclinical environment providing insights into polyphenol availability for absorption in the upper intestine as well as availability for interaction of gut microbial communities in the lower intestine.

Through a combination of preclinical and clinical studies we have investigated the role of food matrix factors including food form and formulation on digestive stability, bioaccessibility and ultimate bioavailability of select polyphenols from tea, cocoa, grapes and berries. Both food form, (e.g. liquid, foam, gels and whole food), as well as macro/micronutrient interactions within the food matrix can modify bioaccessibility of polyphenols. For example, presence of ascorbic acid and carbohydrate were found to positively influence flavan-3-ol bioaccessibility and ultimate absorption from cocoa and tea while protein only had a modest impact to overall absorption in both animal models and humans despite the ability to enhance digestive stability of flavan-3-ols,[3,4,5]. Equally as critical, food physical form appears to impact polyphenol bioaccessibility. Polyphenol-protein interactions assessed in model sodium-caseinate foams, emulsions and gels formulated with green tea and grape seed flavan-3-ols found that protein-flavonoid binding modulated both protein functionality and flavonoid bioaccessibility[6]. More recently, we have completed a comparison of 100% fruit juice and whole fruit to understand the impact of form and processing on bioaccessibility of a broader range of polyphenols (anthocyanins, flavan-3-ols, flavonols and phenolic acids). In vitro bioaccessibility of anthocyanins, flavan-3-ols and phenolic acids from 100% Concord grape juice was more consistently higher compared to whole Concord grapes suggesting that juice processing may serve to enhance release of polyphenols in fruit tissue compartments such as skin and seeds beyond that observed through normal mastication and digestion of whole fruit. This implies that processing may be leveraged to positively impact the availability of polyphenols in finished consumer products.

It is also important to consider food matrix effects in the context of broader dietary and host related factors known to influence polyphenol absorption and metabolism. In this regard, we have explored the impact of background diet (e.g. high versus low fat), repeated exposure (10 days or greater) and presence of risk factors (obesity and diabetes) on polyphenol absorption and metabolism from grape, tea, berry fruits. While background diet had only a modest influence on polyphenol absorption[7], differences in pharmacokinetic responses and metabolism have been observed in lean versus obese rodents and human volunteers[8,9]. Changes in absorption and metabolism of polyphenols during periods of longer term exposure were particularly significant for grape derived flavan-3-ols (monomers and polymers) while less evident for anthocyanins for blackberry and blueberry supporting the notion that polyphenol form/source may be a factor even in longer term dietary patterns [10,11]. In regards to shifts in microbial metabolism of polyphenols, we explored impacts of blueberry and grape seed extract dose at low to modest dietary levels (<100mg/kg bw) versus supplemental levels (>300mg/kg bw) in the context of repeated exposure in a rodent models. For polymer rich grape seed, evidence of monomer release and changes in small molecular weight phenolic metabolites derived from polymeric flavan-3-ols after repeated exposure suggest that alteration of microbial metabolism over time is a modifiable factor affecting metabolite profiles in target tissues[12]. In comparison of dietary or high supplemental blueberry doses, shifts in urinary content and qualitative profiles of small molecular weight phenolic metabolites were observed between groups fed low (60 mg), medium (300 mg), and high (1100 mg) levels of total blueberry polyphenols[13]. These results suggest that circulating metabolite profiles derived from higher supplemental doses would not reflect those from typical dietary exposure which are often associated with health benefits. In conclusion, evidence supports the notion that both food and physiological factors can alter bioavailability and metabolism of polyphenols, and that understanding these factors may serve to facilitate the design of foods and diets consistent with delivery of bioactive polyphenols and their desired health benefits.

Acknowledgments
Funding for these studies was provided by the NIH, USDA and Welch's Foods

References
1. Neilson AN and Ferruzzi MG. *Annual Review of Food Sci and Tech.* 2:125–151. **2011.**
2. Neilson AN, Goodrich KM and Ferruzzi MG. *Nutrition in the Prevention and Treatment of Disease 4th* ed. (pp. 301-319) **2017.**
3. Peters CM, et al. *Food Research International.* 43:95–102. **2010.**
4. Neilson AP et al. J*ournal of Agriculture and Food Chemistry.* 58(11):6685-6691. **2010.**
5. Moser SM et al. *Food Research International.* 66:297-305. **2014.**
6. Elegbede J et al. *Journal of Food Science.* 83(5) 1229-1236. **2018**
7. Wang J et al. *Front Aging Neurosci.* 6:42. **2014.**
8. Chen Y et al. *Molecular Nutrition & Food Research.* 61:10. **2017.**
9. Novotny J. et al. *Molecular Nutrition & Food Research.* 61:11. **2017.**
10. Wang J et al. *Journal of Neuroscience. 32(15):5144-50.* **2012.**
11. Redan BW et al. Journal of Agriculture and Food Chemistry. 65(13):2694-2701. **2017.**
12. Wang D et al. *Molecular Nutrition & Food Research.* 59(6)1025-40. **2015.**
13. Cladis D et al. *Current Developments in Nutrition.* 3(s1). **2019**

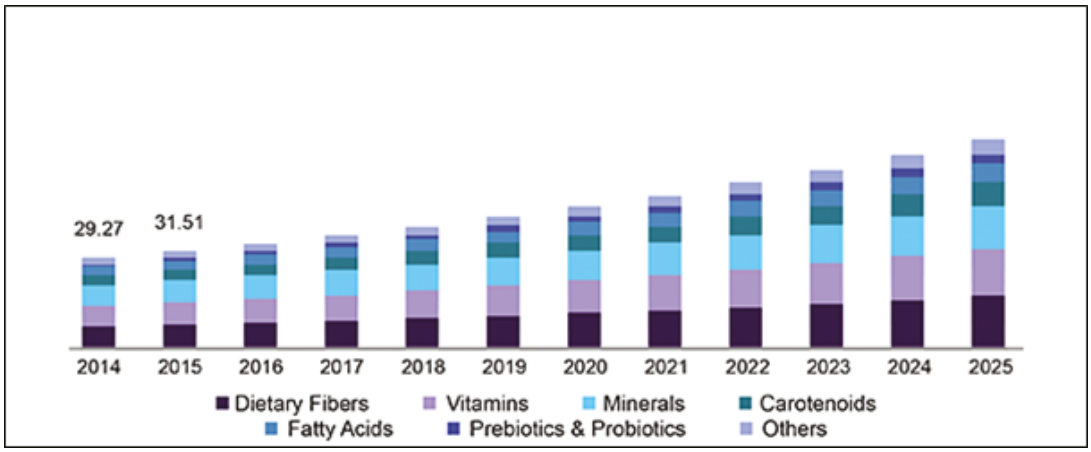
INVERNIZZI FOUNDATION AND THE MARKET FOR FUNCTIONAL FOODS

Vitaliano Fiorillo, Ph.D.
Director AGRI Lab – Romeo and Enrica Invernizzi Agribusiness Research Initiative
SDA Bocconi School of Management

As part of the research interests spur from the support of the Romeo and Enrica Invernizzi Foundation, the probiotics market offers Bocconi the opportunity to explore different phenomena in their making. On the one hand, the shifting interest of consumers towards an innovative product category with a strong functional content, on the other the mechanisms of innovation and research and development that influence the adoption of a product and its evolution on the market.

The World Health Organization defines health as "a state of complete physical, mental and social well-being and not a mere absence of disease or infirmity". The increase in life expectancy combined with the commitment to work until old age, puts the quality of life and aging at the center of consumer attention. Acting to promote one's own health is a primary need that involves an increasingly wide range of consumers, inclined to resort massively to self-diagnosis and self-prescription (the so called *self healthcare*). This group of consumers is particularly attentive to the prevention of cardiovascular diseases, as they represent the first cause of mortality in advanced societies so much to be considered a social emergency, and to the prevention of gastrointestinal disorders, because it is strongly symptomatic and because it is linked to psychosis of diseases caused by incorrect practices in food production.

In this scenario, the new and wide area of the market of nutraceutical products (food supplements and functional foods) is developing, transversal to the pharmaceutical and food industries, characterized by a continuous strong worldwide growth. Nutraceuticals and nutrigenetics have common characteristics worldwide: they are sectors in the initial development phase and have had a strong growth, constant for about ten years. Worldwide, from 2009 to 2014, the supplement market grew by 37.2%, that of functional foods by 44.7%1. Since then, the growth of functional foods slowed down with a global market value of USD 161.49 billion in 2018 CAGR of 7.9% during the forecast period. Neverthless, increasing demand for nutritional and fortifying food additives is anticipated to drive the growth. Positive outlook on food and beverage industry, particularly in Brazil, Russia, India, China and South Africa (BRICS), is expected to continue driving the market over the forecast period.



1:US FUNCTIONAL FOODS MARKET SIZE, BY INGREDIENT, 2014-2025 (USD billion). Source: Functional Foods Market Size, Share & Trends Analysis Report By Ingredient (Carotenoids, Prebiotics & Probiotics, Fatty Acids, Dietary Fibers), By Product, By Application, And Segment Forecasts, 2019 - 2025. Grand View Research, 2018.

FOCUS ON PROBIOTICS SUPPLEMENTS

Probiotic supplements also witnessed sustained strong growth at around the same time, which despite early interest in Asia Pacific and Western Europe, quickly became a marketplace dominated by the US, which sustained a scalding 10% constant value CAGR from 2004 to 2016[2]. The industry was buoyed at this time by broader changes to consumer preferences, such as widespread interest in dietary supplements, a stronger focus on nutrition and healthy ageing, and interest in healthy living from younger and more affluent consumers. In addition, the category was supported by the rise of speciality health stores and higher-end grocery retailers such as Whole Foods at the front-end of this period and the explosion of internet retailing sites such as Amazon and direct-to-consumer outlets at the back-end. As a result, the US represented 42% of global sales of probiotic supplements in 2016, up from just 15% in 2004.

Meanwhile, growth in probiotic supplements was steady but more muted in other developed regions (6% CAGR in Asia Pacific and 5% in Western Europe), due in the former to the sustained popularity of dairy-based probiotics and in the latter to the regulatory burden that hampered growth in all probiotic categories during that time. By the end of 2016 probiotic supplements market value was around US$ 6 bln out of US$ 106 bln for supplements overall, whereas dairy-based probiotics value was around US$ 30 bln[3].

Since 2016, however, the market for probiotic supplements has markedly slowed its growth. Despite the over-excitement for a 3.7% y/y between 2018 and 2019 in Italian drugstores, and the fact Italy is the best performing market in Europe hovering around 6%, this data substantially does not deny the overall deceleration.

We could say that this slowdown has caught the sector, somehow, by surprise, at a time of growing attention to the gastrointestinal microbiome and its correlations with other vital functions. It is not uncommon to witness "*rollercoaster*" phenomena during the initial phases of a newborn sector, and precisely because of its intrinsic novelty, even analysts who pay more attention to the probiotic sector struggle to indicate clear trends for future development. What the market highlights, however, is a consumer moving away from this type of product. Most probably, the main reason is to be found in the observation of the adjacent sector of functional foods, incorporating probiotics, and fermented beverages; easier for the consumer to understand and with similar functions, at least according to the multiple claims, this type of product could cannibalize the probiotic supplement sector.
But because of the novelty that probiotic supplements constitute, the market is still very young and all the possible developments are still open and possible, as often happens in the early stages of a new business. The market could continue to grow at this "slow pace", it could rebound thanks to new products and the advancement of scientific research, it could collapse on itself when supplements are overcome by functional foods that already incorporate the functions of probiotics.
The first hypothesis, that of a slow but constant growth, is realistic and, with an analysis limited to the indicators, even probable. In practice, what is already happening is projected forward and the market is assumed to stabilize at around 4/5% of aggregate annual growth. This conservative hypothesis is accompanied by another in which the effects of what is observed between 2016 and 2018 becomes more evident and the market slows down its course even faster. Are there any grounds for this hypothesis? Yes, there are above all the precedents in history.
First, probiotics could undergo premature market development, a sort of "Werner Syndrome" in the market not uncommon in FMCG. How does it reveals itself usually? Consumer awareness increases exponentially with the proliferation of products and brands and the category begins to take on the characteristics of a mature category, growing little and moving towards commoditization. This is the case, for example, of what happened with coffee capsules. However, contrary to what happens in medicine, in the specific case this syndrome actually has positive and negative effects: on the one hand, a wide diffusion of the product and, therefore, a general awareness on the subject of prevention, on the other, due to the effect of commoditization, a steep reduction in prices and a reduction in research and development funding. If compared to the case of coffee capsules, there are many *caveats* to be considered: to date, the behavior of probiotics on the market has been different and the product is far from being a mainstream product, unlike coffee.
Secondly, the downward trend could be justified by the fact that there has been no real change in consumer habits towards probiotics and the prevention of certain diseases, it was just a passing fad instead and the bubble of interest has popped. In this case, the case of Stevia makes a good point, where a growth of 134% in 2012 was followed by a growth of 0.01% in the following year, meaning that consumption had already reached the asymptote. Also in this case, the parallel with Stevia suffers many "buts" and "ifs", since the interest of the food industry for probiotics is still very high, perhaps in alternative forms and shapes.
And so here is the third reason why degrowth could turn from a transient phenomenon to a trend: the cannibalization by adjacent categories. This phenomenon, which I mentioned earlier, is actually what makes the degrowth scenario very probable in the face of a growth of prebiotic, symbiotic and products incorporating probiotic. See in this sense the spread of Kombucha.
Nevertheless, beyond these reasons that could lead to a decline in the probiotic supplement market, there are also reasons that support the idea of the other scenario, that is, the market will rebound and start growing again. This scenario is also very likely given the still high degree of innovation in the sector. Although it is not possible today to determine the magnitude of such a rebound, it is noticeable that, for most probiotic supplement, consumers focus on the beneficial products for the digestive tract and the gastrointestinal appartus in genera. Such products, therefore, dominate the shelves reinforcing the perception of consumers that probiotics are for the gut. The innovations of probiotics on various aspects of health, however, are proliferating in format (non-pill delivery), in segmentation (for athletes, for the elderly, for men and for women, etc.) and in positioning, extending to an almost infinite range of applications (heart, child development, pregnancy, etc). With these assumptions, if we were to imagine the innovation that will lead to the true rebound of the category, we should look at that probiotic affecting the "brain-belly" connection, those phenomena of regulation of emotional states that some studies, which are also very popular in the mass audience, place in the gastrointestinal apparatus.
In conclusion, beyond the development scenarios that may be more or less probable depending on how we combine and recombine the factors that determine them, there is no doubt that all the market that revolves around probiotics, prebiotics and symbiotics can find its main barriers to development in three factors:
- The lack of education, of consumers on what still creates a lot of confusion (care vs. prevention, conservation of products, etc.), of the general healthcare practitioners who are the first to be addressed by undecided consumers.
- The loss of interest: in addition to consumer education, it is still important to support the interest in nutrition that different groups and segments of the population are showing;
- The balance between price and commoditization policies: price is certainly still a barrier for many consumers but a premature product commoditization could slow down research and development in the face of tight margins.

1. Passport, Euromonitor International “Probiotics Supplements Market”, October 2018
2. Ibid.
3. Passport, Euromonitor International “Probiotics Supplements Market”, October 2018

EVIDENCE BASED MANAGEMENT OF ABDOMINAL SYMPTOMS WITH PROBIOTICS

Pierluigi Fracasso
Gastroenterology Unit, Ospedale Sandro Pertini, Rome Italy

In 2013, a systematic review and Delphi consensus reported that specific probiotics can benefit adult patients with irritable bowel syndrome (IBS) and other gastrointestinal (GI) problems.
The consensus has been updated in 2017 and published in 2018, with new evidence.

Methods

A systematic review identified randomised, placebo-controlled trials published between January 2012 and June 2017. Evidence was graded, previously developed statements were reassessed by an 8-expert panel, and agreement was reached via Delphi consensus.

Results

A total of 70 studies were included (IBS, 34; diarrhoea associated with antibiotics, 13; diarrhoea associated with *Helicobacter pylori* eradication therapy, 7; other conditions, 16). Thirty seven studies were already included in the 2013 study; 33 have been published in the 2013-2017 period. Of 15 studies that examined global IBS symptoms as a primary endpoint, 8 reported significant benefits of probiotics vs placebo. Consensus statements with 100% agreement and "high" evidence level indicated that specific probiotics help reduce overall symptom burden and abdominal pain in some patients with IBS and duration/intensity of diarrhoea in patients prescribed antibiotics or *H. pylori* eradication therapy, and have favourable safety. Statements with 70%-100% agreement and "moderate" evidence indicated that, in some patients with IBS, specific probiotics help reduce bloating/distension and improve bowel movement frequency/consistency.

Conclusions

This updated review indicates that specific probiotics are beneficial in certain lower GI problems, although many of the new publications did not report benefits of probiotics, possibly due to inclusion of new, less efficacious preparations. Specific probiotics can relieve lower GI symptoms in IBS, prevent diarrhoea associated with antibiotics and *H. pylori* eradication therapy, and show favourable safety. This study will help clinicians recommend/prescribe probiotics for specific symptoms.

References

1. Hungin AP, Mitchell CR, Whorwell P, Mulligan C, Cole O, Agréus L, Fracasso P, Lionis C, Mendive J, Philippart de Foy JM, Seifert B, Wensaas KA, Winchester C, de Wit N; European Society for Primary Care Gastroenterology.
   Systematic review: Probiotics in the management of lower gastrointestinal symptoms- an updated evidence-based international consensus.
   Aliment Pharmacol Ther 2018; 47: 1054-1070
2. Hungin AP, Mulligan C, Pot B, Whorwell P, Agréus L, Fracasso P, Lionis C, Mendive J, Philippart de Foy JM, Rubin G, Winchester C, de Wit N; European Society for Primary Care Gastroenterology.
   Systematic review: probiotics in the management of lower gastrointestinal symptoms in clinical practice -- an evidence-based international guide.
   Aliment Pharmacol Ther. 2013 Oct;38(8):864-86.

POLYPHENOL RICH DIET AND CARDIOVASCULAR DISEASES

Rosalba Giacco
Institute of Food Science, National Research Council, Avellino, Italy

A diet based on foods or beverages naturally rich in polyphenols is closely associated with a reduction in the risk of developing type 2 diabetes (T2D), cardiovascular diseases (CVD) and some types of cancer. Polyphenols are a class of heterogeneous bioactive molecules with antioxidant properties contained in small amounts in plant-based foods, in particular dark chocolate and some beverages such as tea, coffee and red wine. The Mediterranean diet is an important source of polyphenols due to its high consumption of fruit, vegetables, whole grains, legumes, oilseeds and extra virgin olive oil together with a moderate consumption of wine.
The polyphenols chemical structure is characterized by the presence of one or more phenolic rings with one or more hydroxyl groups attached. Polyphenols are classified in ***flavonoids***, which include anthocyanins, flavonols, flavones, flavanones, isoflavones and flavan-3-ols, and ***not flavonoids***, which include phenolic acids, stilbenes, lignans, phenolic alcohols and tannins. Some polyphenols have a low bioavailability and are poorly absorbed. In fact, only 5-10% of ingested polyphenols pass into the bloodstream after being largely metabolized in the intestine by the bacterial flora, and in the liver, to be then rapidly eliminated in the urine.
Most of the beneficial properties of polyphenols have been highlighted by in vitro experiments and animal models, using native polyphenols at concentrations much higher than usual dietary intake. However, only few studies assessed the effect of polyphenols on cardio-metabolic risk factors in humans, often providing conflicting results.

Evidence from epidemiological studies

Epidemiological studies showed that higher dietary polyphenols intake associated to lower risk for all-causes and CVD mortality. The PREDIMED study, performed on 7447 participants and aimed at assessing the effects of the Mediterranean Diet in the primary prevention of CVD, reported a reduction by 37% in all-cause mortality in the highest (>750 mg/d) versus the lowest quintiles (<600mg/d) of total polyphenol intake over a mean 4.8 years of follow-up. Among the polyphenol subclasses, stilbenes and lignans were significantly associated with reduced all-cause mortality (*Tresserra-Rimbau et al., 2014*). This finding was confirmed in the CHIANTI-study that, using a nutritional biomarker and not just a food frequency questionnaire, observed a reduction by 30% in all-causes mortality in an elderly, free-living population in the highest tertile of total urinary polyphenol (TUP) excretion compared with the lowest tertile (*Raul Zamora-Ros et al., 2013*). Finally, a recent meta-analysis of prospective cohort studies reported a reduction by 14% for both CVD and all-causes mortality in people with the highest flavonoid intake (*Kim & Je, 2017*).
As for new CVD events, meta-analyses of prospective studies reported a significant reduction in CVD risk (10-13%) (*Wang et al, 2014*) and coronary heart disease (CHD) risk (15%) in the highest flavonoids intake compared with the lowest intake (*Jiang et al, 2015*).
Finally, the recent SUN cohort study (*Mendonça RD et al., 2019*) reported a 47% lower incidence of cardiovascular events in the highest quintile of flavonoids intake compared to those in the lowest quintile in a prospective cohort of Spanish middle-aged adult university graduates over 10 years of follow-up.
Therefore, evidence from epidemiological studies strongly suggest a beneficial role of dietary polyphenols in cardiovascular prevention.

Evidence from clinical trials

In recent years, results of clinical trials showed that the consumption of tea polyphenols, as well as those of cocoa and dark chocolate, extra virgin olive oil or red fruits were able to improve the main CVD risk factors such as glucose and lipid metabolism, blood pressure and inflammation, particularly in individuals at high CVD risk.
Overall, these studies have explored the effects of polyphenols contained in specific foods while the effects of naturally polyphenol-rich diets have been poorly investigated. This is a relevant issue since polyphenols differ in terms of metabolic characteristics, i.e. bioavailability and activity of their metabolites (*Visioli et al, 2011*). Furthermore, very few data are available on the effects of polyphenols on postprandial lipids, an independent CVD risk factor. To answer these questions our research group carried out a medium-term (8 weeks) clinical trial aimed to evaluate the effects of naturally polyphenol-rich diets (3g/day), containing different classes of polyphenols, on glucose and lipid metabolism, and oxidative stress in overweight/obese subjects with high cardio-metabolic risk. The main results showed a significant reduction in fasting and postprandial triglycerides, and oxidative stress (*Annuzzi et al, 2014*). In addition, an improvement of glucose tolerance after OGTT-challenge was observed, likely mediated by the increase of early phase of insulin secretion and insulin sensitivity (*Bozzetto et al, 2015*). Interestingly, the improvement of each outcome was significantly associated with a different subclass of polyphenols (*Vetrani et al, 2018*).
Furthermore, many studies have pointed out the beneficial role of grape and red wine on CVD prevention. This effect has been linked mainly to resveratrol. In fact, the meta-analysis by Zhu et al, (*2017*) showed that resveratrol reduced significantly fasting blood glucose, insulin levels and the HOMA index in subjects with T2D. However, recently, an acute study carried out by our group on healthy subjects, reported a reduction by 31% of postprandial insulin response and an improvement by 36% of insulin sensitivity index after a standard meal preceded by a grape pomace-based drink, rich in anthocyanidins but without resveratrol (*Costabile et al, 2018*). This finding suggests that beneficial effects of grape and wine polyphenols on glucose metabolism depend on different classes of polyphenols and not on individual fractions, in particular resveratrol.

In conclusion, epidemiological evidence supports the beneficial effects of polyphenol-rich diets in CVD prevention. Data from clinical trials suggest that the cardio-protective effect of polyphenols is mediated by the improvement of several cardio-metabolic risk factors, i.e. glucose and lipid metabolism at fasting and in the postprandial period, and oxidative stress.
However, the benefits of polyphenols are particularly evident when they are consumed in the context of a whole diet, rich in different polyphenol subclasses. The hypothesis is that the combination of several phenolic subclasses may exert pleiotropic effect on different cardio-metabolic risk factors.

PROBIOTICS AND FUNCTIONAL GI DISORDERS

Stefano Guandalini, MD
Professor Emeritus of Pediatrics
University of Chicago Medicine

Among the various fields in which probiotics have been tried or utilized, functional gastrointestinal disorders (FGID) are arguably the most common. Infantile colic, constipation, irritable bowel syndrome (IBS) and constipation are the most common FGID. While the etiology of FGID is considered multifactorial, the importance of intestinal microbiota in their development has been repeatedly emphasized. Hence, the potential role of probiotics in their treatment is being increasingly scrutinized.

Colic. Infantile colic is an extremely common problem in otherwise healthy infants and is characterized by inconsolable crying, irritability and fussing. The symptoms peak typically at 4-6 weeks and then regress by 12 weeks. The inconsolable crying often causes distress for the parents. While the cause(s) of colic are unknown, there is evidence that infants with colic have an intestinal microbiota that differs from that of healthy controls, showing less diversity and higher amounts of coliform bacteria. Thus, probiotics have been investigated as a means to influence the intestinal microbiota in babies with colic and thus ameliorating their symptoms. A few randomized clinical trials have been performed with various species and doses of probiotics. A recent meta-analysis of these studies [1] concluded that the probiotic *Lactobacillus reuteri* DSM 17938 at a dose of 10^8CFU/day significantly reduced the duration of crying episodes. Of note, its superiority was evident even when compared to other types of intervention such as dietary or manipulative interventions, reassurance, massage, herbal treatment, acupuncture and drugs. Of interest however, *Lactobacillus reuteri* DSM 17938 appears to have been efficacious only in breast fed infants, and much less in artificially fed ones.

Irritable bowel syndrome. Irritable bowel syndrome (IBS) is a functional bowel disorder with a recurrent natural history. The global prevalence of the condition in the community is approximately 10%, depending on the criteria used to define its presence. It ranges between 6% and 14% in children and it is even higher in adolescents [2]. Although usually considered very benign, IBS can significantly decrease quality of life to the point of being perceived as damaging as some severe organic gastrointestinal diseases, like inflammatory bowel disease.
The pathophysiology of the disorder remains incompletely understood, and likely to be complex and multifactorial. The concept that alterations in the gut microbiota might be relevant to IBS arose from observations that symptoms of IBS often developed after an infection, (the so-called "post-infectious IBS"). In fact, children with IBS have a greater proportion of *Proteobacteria*, and of genera such as *Dorea, Haemophilus*, *Ruminococcus*, *Clostridium* spp. They also have been found to have an increased ratio of *Firmicutes* to *Bacteroidetes* [3,4] Thus these changes might theoretically influence visceral perception, gut motility, permeability and intestinal gas production, eventually ;leading to the development of pain-predominant FGIDs such as IBS and functional abdominal pain. As recently reviewed and summarized by Abbot et al. [5], treatment options for children with IBS are limited, and in fact scanty data are available for pharmacological treatments, for which no firm recommendation are possible [5]. Hence, the interest in probiotics appears obvious. Indeed, after several RTCs investigated the effect of probiotics in IBS, it can be concluded that the most single species probiotics studied are *Lactobacillus rhamnosus* GG (LGG) and *L. reuteri* DSM 17938. A meta-analysis of published RCTs showed that the use of LGG significantly decreases the post-intervention pain intensity (MD -0.44, 95% CI -0.82 to -0.05) [6]. It must be stressed that the efficacy was evident only for children with IBS. In addition to LGG and *L. reuteri* DSM 17938, multi-strain preparations have also been investigated in two separate, double-blinded, placebo-controlled, cross-over trials in children with IBS: VSL#3 [7], a proprietary specific mixture of Lactobacilli, Bifidobacteria and a Streptococcus at high concentration; and a combination of 3 different strains of *Bifidobacterium* [8]. In both trials these multi-strain preparations were able to significantly reduce intensity, duration and frequency of pain. Of note, a significant reduction in parents' perceived health issues of their children was reported in the VSL#3 trial[7].

Constipation. A remarkably common problem in infants and children, functional constipation is thought to arise as a result of various factors, eventually leading to a condition of dysmotility of the colonic-rectum segments of the GI tract, maintained in most cases by a withholding behavior. In recent years, and after studies in animal have provided evidence for a role of microbiota in the onset of slow transit constipation, human investigations seem to have also provided support for this concept by showing overall a different microbial composition when compared to healthy age-matched controls. However, whether the dysbiosis is responsible for constipation or only represents its consequence remains unclear[9]. A recent trial in adults[10] with a yeast fermentate was able to modulate the composition of the gut microbiome, resulting in improvement of constipation-associated symptoms. In children, several randomized trials have been performed with a variety of probiotics including LGG, *L. reuteri* DSM 17938, *B. lactis* DN- 173 010, strains of *L. casei rhamnosus* and various *Bifidobacteria*. A recent meta-analysis of these trials [11] concluded however that there was lack of evidence of efficacy for probiotics in the treatment of functional constipation in children. A joint ESPGHAN/NASPGHAN statement of 2014 already concluded that the routine use of probiotics in the treatment of constipation in children was not recommended; and very recently a highly critical review and meta-analysis[12] more strongly also concluded that "the current evidence does not support the use of probiotics as a single or coadjuvant therapy for treatment of functional constipation in children and refutes recently published reviews reporting favorable effects of probiotics". Of course, as we have seen many times in the past, medicine is an ever evolving science, and future studies may indicate otherwise.

References

1. Gutierrez-Castrellon P, Indrio F, Bolio-Galvis A, Jimenez-Gutierrez C, Jimenez-Escobar I, Lopez-Velazquez G. Efficacy of Lactobacillus reuteri DSM 17938 for infantile colic: Systematic review with network meta-analysis. *Medicine (Baltimore).* 2017;96(51):e9375.
2. Giannetti E, Staiano A. Probiotics for Irritable Bowel Syndrome: Clinical Data in Children. *Journal of pediatric gastroenterology and nutrition.* 2016;63 Suppl 1:S25-26.
3. Rajilic-Stojanovic M, Biagi E, Heilig HG, et al. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology.* 2011;141(5):1792-1801.
4. Saulnier DM, Riehle K, Mistretta TA, et al. Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome. *Gastroenterology.* 2011;141(5):1782-1791.
5. Abbott RA, Martin AE, Newlove-Delgado TV, et al. Recurrent Abdominal Pain in Children: Summary Evidence From 3 Systematic Reviews of Treatment Effectiveness. *Journal of pediatric gastroenterology and nutrition.* 2018.
6. Horvath A, Dziechciarz P, Szajewska H. Meta-analysis: Lactobacillus rhamnosus GG for abdominal pain-related functional gastrointestinal disorders in childhood. *Alimentary pharmacology & therapeutics.* 2011;33(12):1302-1310.
7. Guandalini S, Magazzu G, Chiaro A, et al. VSL#3 improves symptoms in children with irritable bowel syndrome: a multicenter, randomized, placebo-controlled, double-blind, crossover study. *J Pediatr Gastroenterol Nutr.* 2010;51(1):24-30.
8. Giannetti E, Maglione M, Alessandrella A, et al. A Mixture of 3 Bifidobacteria Decreases Abdominal Pain and Improves the Quality of Life in Children With Irritable Bowel Syndrome: A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Crossover Trial. *J Clin Gastroenterol.* 2017;51(1):e5-e10.
9. Dimidi E, Christodoulides S, Scott SM, Whelan K. Mechanisms of Action of Probiotics and the Gastrointestinal Microbiota on Gut Motility and Constipation. *Adv Nutr.* 2017;8(3):484-494.
10. Pinheiro I, Robinson L, Verhelst A, et al. A yeast fermentate improves gastrointestinal discomfort and constipation by modulation of the gut microbiome: results from a randomized double-blind placebo-controlled pilot trial. *BMC Complement Altern Med.* 2017;17(1):441.
11. Wojtyniak K, Szajewska H. Systematic review: probiotics for functional constipation in children. *Eur J Pediatr.* 2017;176(9):1155-1162.
12. Harris RG, Neale EP, Ferreira I. When poorly conducted systematic reviews and meta-analyses can mislead: a critical appraisal and update of systematic reviews and meta-analyses examining the effects of probiotics in the treatment of functional constipation in children. *Am J Clin Nutr.* 2019.

RESPIRATORY INFECTIONS: WHAT ARE WE TRYING TO PREVENT?

Iva Hojsak, MD, PhD
Children's Hospital Zagreb, Croatia

Infectious diseases are the most important cause of morbidity in children where respiratory infections encounter for majority of them (1). Recurrent respiratory tract infections are common problem in preschool age, mainly due to the presence of unfavorable environmental conditions including early socialization in daycare centers and the physiologic immaturity of the immune system. There are two major settings where children acquire respiratory infections and those are hospital and day care centers. Children who attend daycare centers have 2-3 times more infections than children who stay at home, they have more outpatient doctor and emergency room visits and increased usage of prescribed antibiotics (2). Furthermore, they cause an substantial economic burden not only for child's family, but healthcare in general. Taking all that into account together with possible complications, respiratory tract infections are important health care problem for pediatricians who are facing a real task to discriminate the children who are at higher risk and to try to offer preventive measures; good hand hygiene, absenteeism of ill child from daycare center in order to prevent spreading of infection and vaccination for influenza and rotavirus. However, all those measures often are ineffective leaving a place for possible new modalities, like probiotics. In the last two decades, there have been an increasing number of trials investigating the role of probiotics on the prevention of common infections in children.

There are several trials which evaluated probiotics in the prevention of respiratory tract infection in children attending daycare centers. Interestingly, majority of studies beyond infancy found positive effect on the lowering of respiratory tract infections (3-10). Recent meta-analysis reviewed available literature and found that probiotics (in general) reduce the risk of respiratory tract infections (RR 0.89, 95% CI 0.82 to 0.96) (11). Unfortunately, this meta-analysis included all age groups, was not strain specific and was not stratified based on the type of facility where probiotics were used. However, it was reported that although there was no effect on the duration of illness, absenteeism from the kindergarten was decreased (11). Based on the evidence it be concluded that probiotics could have a place in the prevention of upper respiratory tract infections. However, questions that remain are what strain to use, in which dose and when. Based on well-designed RCTs in children, LGG was examined in 3 studies (3, 6, 8) involving all together 1375 children and all studies reported positive effect on the lowering the incidence of respiratory tract infections. Other strain *Bifidobacterium* (B) *animalis* subsp. *lactis* was evaluated in 4 RCTs (12-15) from which all found negative results.

Recent meta-analysis found that none of the probiotic strains was able to reduce the risk for respiratory tract infections, but LGG was able to shorten the respiratory tract infection if infection occurred (16).

Literature

1. Monto AS. Epidemiology of viral respiratory infections. Am J Med 2002;112 Suppl 6A:4S-12S.
2. Silverstein M, Sales AE, Koepsell TD. Health care utilization and expenditures associated with child care attendance: a nationally representative sample. Pediatrics 2003;111:e371-5.
3. Hatakka K, Savilahti E, Ponka A, et al. Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. BMJ 2001;322:1327.
4. Lin JS, Chiu YH, Lin NT, et al. Different effects of probiotic species/strains on infections in preschool children: A double-blind, randomized, controlled study. Vaccine 2009;27:1073-9.
5. Hojsak I, Abdovic S, Szajewska H, et al. Lactobacillus GG in the prevention of nosocomial gastrointestinal and respiratory tract infections. Pediatrics 2010;125:e1171-7.
6. Hojsak I, Snovak N, Abdovic S, et al. Lactobacillus GG in the prevention of gastrointestinal and respiratory tract infections in children who attend day care centers: a randomized, double-blind, placebo-controlled trial. Clin Nutr 2010;29:312-6.
7. Merenstein D, Murphy M, Fokar A, et al. Use of a fermented dairy probiotic drink containing Lactobacillus casei (DN-114 001) to decrease the rate of illness in kids: the DRINK study. A patient-oriented, double-blind, cluster-randomized, placebo-controlled, clinical trial. Eur J Clin Nutr 2010;64:669-77.
8. Kumpu M, Kekkonen RA, Kautiainen H, et al. Milk containing probiotic Lactobacillus rhamnosus GG and respiratory illness in children: a randomized, double-blind, placebo-controlled trial. Eur J Clin Nutr 2012;66:1020-3.
9. Leyer GJ, Li S, Mubasher ME, Reifer C, Ouwehand AC. Probiotic effects on cold and influenza-like symptom incidence and duration in children. Pediatrics 2009;124:e172-9.
10. Gutierrez-Castrellon P, Lopez-Velazquez G, Diaz-Garcia L, et al. Diarrhea in preschool children and Lactobacillus reuteri: a randomized controlled trial. Pediatrics 2014;133:e904-9.
11. Wang Y, Li X, Ge T, et al. Probiotics for prevention and treatment of respiratory tract infections in children: A systematic review and meta-analysis of randomized controlled trials. Medicine (Baltimore) 2016;95:e4509.
12. Weizman Z, Asli G, Alsheikh A. Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents. Pediatrics 2005;115:5-9.
13. Merenstein DJ, Smith KH, Scriven M, et al. The study to investigate the potential benefits of probiotics in yogurt, a patient-oriented, double-blind, cluster-randomised, placebo-controlled, clinical trial. Eur J Clin Nutr 2010;64:685-91.
14. Merenstein D, Gonzalez J, Young AG, et al. Study to investigate the potential of probiotics in children attending school. Eur J Clin Nutr 2011;65:447-53.
15. Hojsak I, Mocic Pavic A, Kos T, Dumancic J, Kolacek S. Bifidobacterium animalis subsp. lactis in prevention of common infections in healthy children attending day care centers - Randomized, double blind, placebo-controlled study. Clin Nutr 2016;35:587-91.
16. Laursen RP, Hojsak I. Probiotics for respiratory tract infections in children attending day care centers-a systematic review. Eur J Pediatr 2018;177:979-994.

INNOVATIONS IN DAIRY THROUGH PRODUCT-PROCESS INTERACTIONS: THE FOOD MATRIX EFFECT

Thom Huppertz[1,2]
[1] *FrieslandCampina, Amersfoort, The Netherlands*
[2] *Wageningen University and Research, Wageningen, The Netherlands*

The primary aim of any food product is the provision of essential nutrients in a bio-available manner. Food safety may be ensured through various processing steps, e.g., heat treatment or filtration, resulting in the inactivation or removal of pathogenic micro-organisms as well as the micro-organisms and enzymes responsible for spoilage. The efficacy of such processes however, is strongly dependent on the type of product matrix, e.g., liquid, gel or solid and properties such as pH and moisture content. Likewise, these product properties also strongly affect the availability and release of nutrients from food products. Such effects were already reported in the 1970s, when Haber (1977) showed that human responses to carbohydrate intake varied considerably whether these were from apples, apple puree or apple juice. Four decades of subsequent research have highlighted that such food matrix effects are not only important in relation to the uptake of and responses to carbohydrates, but also lipids, proteins and minerals from many food products. These findings have not only deepened our understanding of digestion and nutrient release, but have also got to the forefront of innovations in the food industry, including the dairy industry.
Like for many other product categories, the proteins in dairy products have also been at the fore-front in many recent innovations, with notable aim on protein quality. For this, key aspects are: (1) the concentrations of essential (unmodified) amino acids, and (2) the digestibility of the proteins. Both of which are strongly affected by the product matrix and processing intensity. Studies on post-prandial amino acid release after consumption of the same amount of dairy protein from different product show considerable differences in the kinetics of release of amino acids into the blood stream. Release from yoghurt was faster than that from milk, which was faster than from cheese. For milk, further differentiation could be made as a result of heat treatment; post-prandial amino acid release was faster from sterilized milk than from pasteurized milk. Effects observed appear related to the coagulation behavior of samples under gastric conditions. When firm and large curd particles form, gastric passage is delayed, which in turn leads to delayed uptake of amino acids in the bloodstream. Hence, tailored application control of basic processes in dairy technology, e.g., heat treatment, homogenization and gelation can form the basis of innovative product concepts aimed at targeted protein release kinetics.

BREAST MILK: PRO-, PRE- AND POST-BIOTIC FEATURES

Erika Isolauri and Seppo Salminen[1]
Department of Paediatrics, and [1]Functional Foods Forum, Institute of Clinical Medicine, University of Turku, and Department of Paediatric and Adolescent Medicine, Turku University Hospital, Finland

The model of infant feeding is the healthy breast-fed child, who also remains healthy long-term. Breast milk confers nutrients optimal for growth and development, as well as protection to compensate for the immature and inexperienced defence mechanisms at mucosal surfaces. The mucosal surfaces, again, are the major portal of entry of external antigens, including allergens and potential pathogens. However, antigen contact is necessary for the maturation of immuno-physiological regulation. Extremely important here is the initial establishment of the gut microbiota; the microbe contact in the perinatal period represents the most massive antigen exposure educating the physiological adaptation to the immediate environment.

Since aberrant compositional development of the gut microbiota at an early age has been linked to a heightened risk of both infectious and non-communicable diseases, host-microbe crosstalk is currently attracting active research interest (reviewed in: Rautava *et al* 2012). One important element behind the health benefits of breastfeeding is associated with promotion of age-appropriate and environment-adjusted gut colonization. Human milk is rich in bioactive compounds including health-promoting microbes (model for probiotics) and their optimal growth factors, human milk oligosaccharides (exemplary of prebiotics), and bioactive compounds derived thereof (recently characterized as a uniform entity, postbiotics). These elements are instruments of the concept of personalized nutrition designed for the child at risk of infectious and non-communicable diseases.

Scientific evidence from experimental and clinical studies documents that specific probiotics can be used to strengthen and maintain intestinal homeostasis and thereby treat gastrointestinal conditions and reduce the risk of infectious and non-communicable disease with an intestinal dysbiotic involvement. Specific probiotics have been shown to reverse increased intestinal permeability and to enhance gut-specific IgA responses, frequently defective in children. Promotion of gut barrier functions by specific probiotics basically involves degradation/ structural modification of enteral antigens, normalization of the properties of aberrant indigenous microbiota and of gut barrier functions, regulation of the secretion of inflammatory mediators, and direction of the development of the immune system during the critical period of life when the risk of allergic or inflammatory disease is heightened. Clinical and experimental studies indicate that certain probiotics alleviate the inflammatory response both locally and systemically. Moreover, recent evidence from experimental and clinical studies indicates that the gut microbiota is also involved in the control of body weight and energy metabolism, affecting the two main causes of obesity: energy acquisition and storage, and contributing to insulin resistance and the inflammatory state characterizing obesity. These findings tend to add weight to the hypothesis that manipulation of the early gut microbiota by specific probiotics offers a valid strategy to reduce the risk of non-communicable diseases (Isolauri *et al* 2016).

The definition of probiotics is presented as '*live microorganisms that, when administered in adequate amounts, confer a health benefit on the host*' (Hill *et al* 2014). Prebiotic is '*a substrate that is selectively utilized by host microorganisms conferring a health benefit*' (Gibson *et al* 2017). Postbiotics aim to mimic the beneficial therapeutic effects of probiotics while avoiding the risk of administering live microorganisms to vulnerable infants with immature intestinal barriers or impaired immune defences. One early example of postbiotic potential was reported for *Lactobacillus rhamnosus* GG (ATCC 53103): the homogenates of probiotic bacteria suppressed lymphocyte proliferation (Sütas *et al* 1996; Pessi *et al* 1999). When assessing cytoplasmic and cell wall extracts separately, it was detected that these bacteria possess heat-stable antiproliferatice components located in the cytoplasm. Clinically, the same strain (as probiotic) promoted acquiring tolerance in cow milk allergy (Berni Canani *et al* 2013). Moreover, a culture supernatant of the strain is able to accelerate the maturation of neonatal intestinal defence and prevent experimental oral infection (Gao *et al* 2019). Postbiotics have recently tentatively defined as '*components produced by a fermentation process (including microbial cells, constituents and metabolites) that, when administered in adequate amounts, support health and/or wellbeing*' (Collado *et al*, in press).

The safety, avoiding the use of live microorganisms, is taken as the key aspect that will favour the development of prebiotic or postbiotic products particularly for children. However, gut barrier dysfunction has been reported in experimental studies for some prebiotics (Ten Bruggencate *et al* 2004), and a probiotic strain effective if in control of atopic eczema and local and systemic inflammatory responses was associated with adverse gastrointestinal symptoms and diarrhoea when used in heat-killed inactive form (Kirjavainen *et al* 2003).

It needs to be acknowledged that probiotics for clinical use and health claims have to be assessed for efficacy in strain-specific studies, and extrapolation of data from one strain to another is virtually impossible (Hill *et al* 2014); each probiotic strain is inherently different from others and that similar health effects cannot therefore be expected even from closely related strains (Isolauri and Salminen 2015). Species-level effects are more frequent necessitating evidence from well-conducted human studies supporting general beneficial effect for the taxonomical category concerned (Hill *et al* 2014). An equivalent documentation is mandatory for the documentation of safety.

Thus assumption of the mimicry of the pre-, pro- and postbiotics does not permit generalizations in terms of efficacy and safety. Each product with a specific strain, species or combinations (combinations of strains or use of synbiotics or postbiotics) need to be assessed in well-controlled human intervention studies (Isolauri and Salminen 2015). Any proof of causality requires clinical intervention studies in humans in different populations (Zhao 2013). The validation of pre-, pro- or postbiotic properties for health claims in paediatric population calls for systematic research and needs to be also reported as such in order to enhance reproducibility, facilitate meta-analyses and comprehensive reviews.

References

1. Berni Canani R, Nocerino R, Terrin G, Frediani T, Lucarelli S, Cosenza L, Passariello A, Leone L, Granata V, Di Costanzo M, Pezzella V, Troncone R. Formula selection for management of children with cow´s milk allergy influences acquisition of tolerance: a prospective multicenter study. J Pediatr 2013; 163: 771-7.
2. Collado MC, Vinderola G, Salminen S. Postbiotics: facts and open questions. A position paper on the need for consensus definition. Benef Microb, in press.
3. Gao J, Li Y, Wan Y, Hu T, Liu L, Yang S, Gong Z, Zeng Q, Wei Y, Yang W, Zeng Z, He X, Huang SH, Cao HA. A novel postibiotic from Lactobacillus rhamnosus GG with a beneficial effect on intestinal barrier function. Front Microbiol 2019; 10: 477.
4. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, Verbeke K, Reid G. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol 2017; 14: 491-502.
5. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014; 11: 506-14.
6. Isolauri E, Salminen S. The impact of early gut microbiota on risk of child health- alert to accuracy in probiotic studies. Benef Microb 2015; 6: 167-71.
7. Isolauri E, Rautava S, Collado MC, Salminen S. Early microbe contact in defining child metabolic health and obesity risk. In: Parental Obesity: Intergenerational Programming and Consequences. Physiology in Health and Disease. Green LR, Hester RL, eds. The American Physiological Society, Springer, 2016.
8. Kirjavainen PV, Salminen SJ, Isolauri E. Probiotic bacteria in the management of atopic disease- underscoring the importance of viability. J Pediatr Gastroenterol Nutr 2003; 36: 223-7.
9. Pessi T, Sütas Y, Saxelin M, Kallioinen H, Isolauri E. Antiproliferative effects of homogenates derived from five strains of candidate probiotic bacteria. Appl Environ Microb 1999; 65: 4725-8.
10. Rautava S, Luoto R, Salminen S, Isolauri E, 2012. Perinatal microbial contact and the origins of human disease. Nat Rev Gastroenterol Hepatol 2012; 9: 565- 76.
11. Sütas Y, Soppi E, Korhonen H, Syväoja EL, Saxelin M, Rokka T, Isolauri E. Suppression of lymphocyte proliferation in vitro by bovine caseins hydrolysed with Lactobacillus GG-derived enzymes. J Allergy Clin Immunol 1996; 98: 216-24.
12. Ten Bruggencate SJ, Bovee-Oudenhoven IM, Lettink-Wissink ML, Katan MB, Van Der Meer R. Dietary fructo-oligosaccharides and inulin decrease resistance of rats to salmonella: protective role of calcium. Gut 2004; 53: 530-5.
13. Zhao L. The gut microbiota and obesity: from correlation to causality. Nature Rev Microbiol 2013; 11: 639- 47.

INTRATISSUE COMMENSAL BACTERIA MEDIATES BALANCING ACT BETWEEN GUT SYMBIOSIS AND INFLAMMATION

Hiroshi Kiyono, D.D.S., Ph.D.
Department of Mucosal Immunology
International Research and Development Center for Mucosal Vaccines
The Institute of Medical Science, The University of Tokyo
Department of Immunology, Graduate School of Medicine, Chiba University
Department of Internal Medicine, Chiba University -UCSD Center for Mucosal Immunology, Allergy and Vaccines (cMAV), University of California San Diego (UCSD)

The digestive tract is constantly exposed to infinite beneficial and harmful antigens including commensal and pathogenic microbe via the large surface of mucosal epithelium. The intestinal mucosa is thus equipped with multi-complexed but harmonized biological system composed with epithelial-mesenchymal cells, mucosal immunocompetent cells and commensal microbiota, which form "Gut Multi-ecological system (GME)" for the establishment of beneficial symbiotic condition as well as cooperative defense force. As an example, our study identified that commensal bacteria, *Alcaligenes* species can create "intra-tissue co-habitation niche" in the inside of Peyer's patches (PPs), an example of commanding tissue for the induction and regulation of a balanced mucosal immunity. These intra-tissue commensal bacteria (ICB) enter PPs via M cells located in the follicle associated epithelium and newly identified Aifi-1 plays a critical role for the M cell transcytosis of commensal bacteria. Innate lymphoid cells (ILCs) type3 have been shown to play critical role by the cooperative interaction with epithelial cells for the creation of intra-tissue co-habitation niche. These results suggested that ICB is a key element for the creation and regulation of GME-mediated healthy gut environment for the balancing act between elimination and symbiosis.

SELECTED PROBIOTICS FOR SUPPORT OF IRON STATUS AND BONE HEALTH WHEN NEEDED MOST

Niklas Larsson, PhD
Research Director at Probi AB
Sweden

Certain health issues primarily affect women. Two such areas are osteoporosis, common in postmenopausal women, and iron deficiency, common in women of reproductive age. Bone loss increases the risk of developing osteoporosis, leading to a higher risk of fractures. For women, bone loss accelerates in the years prior to menopause, and peaks during the first years after menopause. Pre-clinical studies in rodents have shown beneficial effects on bone loss after intake of a combination of three selected probiotic strains (*L. paracasei* DSM 13434, *L. plantarum* DSM 15312 and DSM 15313, Probi® Osteo) compared to control (Ohlsson C. et al., 2014 and unpublished data). The impact of a daily supplement of Probi® Osteo for 12 months was further evaluated on lumbar spine bone loss in postmenopausal women. A randomized, double blind, placebo-controlled clinical study including 234 healthy postmenopausal women was conducted with the primary endpoint being change in lumbar spine bone mineral density (LS-BMD). Intake of Probi® Osteo for 12 months significantly reduced bone loss as compared to placebo ($p<0.05$). The LS-BMD loss over time was significant in the placebo group with -0.77% ($p<0.001$) but not in Probi® Osteo, -0.17% ($p=0.40$). Moreover, the difference between the groups was even more prominent in women with less than the median of 6 years from menopause ($p<0.05$). In this predefined subgroup the LS-BMD loss was -1.21% ($p=0.0001$) in the placebo group and -0.18% ($p=0.56$) in Probi® Osteo.
Iron deficiency is the most common nutrient deficiency in the world with pregnant women being especially at risk. Iron deficiency anemia is associated with increased risk of adverse pregnancy outcomes such as preterm labor, low neonatal weight, cognitive dysfunctions and increased perinatal mortality. Iron deficiency is generally treated using oral iron supplements. However, a large proportion of the ingested iron remains unabsorbed in the intestine causing adverse gastrointestinal effects and may limit the ability to efficiently replete iron stores. Strategies to efficiently increase iron absorption are therefore warranted. It has previously been shown that the probiotic strain *Lactobacillus plantarum* 299v (DSM 9843) significantly increase iron absorption in meal studies in women of reproductive age (Bering *et al.*, 2016, Hoppe *et al.*, 2015, Hoppe et *al.*, 2017). The impact of *Lactobacillus plantarum* 299v together with a carefully balanced dose of iron (FerroSorb®) was further evaluated in a randomized, double blind, placebo-controlled, multi-center clinical study including 326 healthy pregnant women with the primary endpoint being serum ferritin, a marker of iron stores. Intake of FerroSorb® significantly attenuated the decrease in serum ferritin ($p<0.01$) during pregnancy compared to placebo. Furthermore, other markers of iron status were also improved after intake of FerroSorb®. In addition, intake of FerroSorb® resulted in a lower prevalence of iron deficiency and iron deficiency anemia ($p<0.05$) in late pregnancy compared to placebo.
In conclusion, we show scientific data that supports the daily use of FerroSorb® to improve iron status during pregnancy and the daily use of Probi® Osteo to protect against bone loss in postmenopausal women.

ADVANCED IN VITRO MODELS: STUDYING ORGAN AND TISSUE CROSS-TALK AT THE MICROSCALE

Chiara Magliaro
Research Center E. Piaggio, University of Pisa, Italy
Fondazione Umberto Veronesi, Milan, Italy

The most relevant diseases that afflict humans are those associated with ageing, altered metabolism and environmental factors. Many of disease processes do not occur in single cells, but are associated with changes in the microenvironment and alterations in signaling between cells via released mediators or direct cell-cell contacts, and even distant tissues or organs, leading to systemic pathologies such as neurodegenerative diseases and metabolic disorders.

The current state-of-art for studying complex human diseases is to either use mouse models or human induced pluripotent stem cells (hiPSC) in classical 2D cultures. While animals are naturally characterized by species-specific differences that often limit their predictive value towards human disease [1, 2, 3], 2D cultures typically represent the main cell type affected in the disease (e.g. dopaminergic neurons in PD) but neglect all other cell types involved as well as the complex interactions between multiple organs. Between the two, there is a gap in our knowledge and understanding of disease processes as far as systemic and extracellular interactions are concerned. Bridging this gap requires a shift in experimental biology paradigms towards interdisciplinary methods which combine state of the art technology in cell manipulation and assembly with advanced engineering and computational sciences. In this light, the development of new *in vitro* models able to truly capture human pathophysiology, i.e. organoids are the closest near-physiological 3D models to accurately study a range of *in vivo* biological processes (i.e. tissue responses to drugs, mutation or damage [4, 5]). There is the need to bring these novel models into microfluidic devices in order to allow their parallelization, standardize their characteristics and allow an easy handling, and to combine them with advanced imaging. Moreover, the complex interplay between organs can be recapitulated functionally connecting different devices together.

The "*In vitro* models and cell imaging" group of the Research Center "E. Piaggio" – University of Pisa, lead by prof Arti Ahluwalia, is mainly focused on the development 3D smart biomaterials, modular bioreactor and advanced imaging combined with organoid and organ printing technology to create innovative *in vitro* models of systemic physiology and organ cross-talk. These models can be applied for drug testing, for evaluating systemic side effects and for implementing disease models for mechanistic studies. The use of hiPSC technology, derived from mature cells of a specific individual, enable the development of personalized therapy. The results obtained through these advanced *in vitro* models will have an important socio-ethical impact that may accelerate the adoption of the 3 Rs (Replacement, Refinement and Reduction) principle of animal experimentation in basic research.

A striking example of systemic tissue and/or organ interaction to be modeled *in vitro* is the gut-brain axis, i.e. the bidirectional communicational system between the gastrointestinal tract and the central nervous system, which plays a crucial role in the pathophysiology of many neurodegenerative diseases. This is particularly crucial for Parkinson's Disease (PD), a neurological disorder characterized by the loss of dopaminergic neurons in the mid-brain, affecting 7 to 10 million people worldwide [6]. Its etiology is unknown, although increasing attention has been devoted to the concept that gut micro-organisms have an integral role in both triggering and driving the progression of the pathology [7, 8, 9, 10]. However, the biological complexity of these interactions remains poorly understood. In fact, despite researchers claim a relation between gut microbiota alterations and alpha-synuclein clumping, i.e. the primary structural component of the Lewy bodies (a marker for dopaminergic neuron degeneration in PD), the underlying mechanisms remains unclear. Learning how to modulate the gut microbiome (i.e. therapeutically or nutritionally) to modulate disease pathogenesis, as well as elucidating the abnormal formation of alpha-synuclein in the mid-brain, may be crucial to the design of new therapeutic approaches to control the PD progression at its early stages or at least to treat the gastro-intestinal dysfunctions involved in the disease.

In this scenario, the iPAD, "*In vitro* Parkinson in A Dish" project aims at developing a multi-organ platform to mimic the brain-gut axis and to investigate the mechanisms of the inter-organ cross talk occurring in PD. This project is carried out thanks to Post-Doctoral Fellowships by Fondazione Umberto Veronesi and the host Institution, the Research Center "E. Piaggio" - University of Pisa. The idea beyond iPAD is to use a novel approach, integrating micro-fluidic systems, imaging and image processing algorithms to study gut-to-brain communication and the effects of metabolic disequilibrium in PD. In particular, mid-brain organoids and intestinal epithelial cultures using media spiked with and without metabolites can be functionally connected *in vitro*, using a series of inter-connected bioreactors to elucidate the inter-organ cross-talk between gut and brain. The connected systems will allow mimicking complex human physiology as well as pathological process at a system level with an unprecedented completeness. The combination of advanced imaging techniques and real-time metabolite monitoring allow for in-depth insight into physiological processes and their regulatory mechanisms. In order to assess cell function and viability, small samples of media can be withdrawn from the mixing chamber at fixed intervals and biochemical assays were performed to measure the change over time in the metabolite. Moreover, at different time-points, the experiment can be stopped, and the integration of clarification protocols, advanced imaging techniques and image processing algorithms, as well as immune-staining procedures will be applied to mid-brain organoids to monitor both alpha-synuclein clumping and the dopaminergic neuron degeneration (see Fig 1 for an example). The monitoring of metabolite concentrations and alpha-synuclein clump formation may allow disentangling the alterations in signaling between the two organs due to endogenous or exogenous factors in both healthy individuals and PD patients.

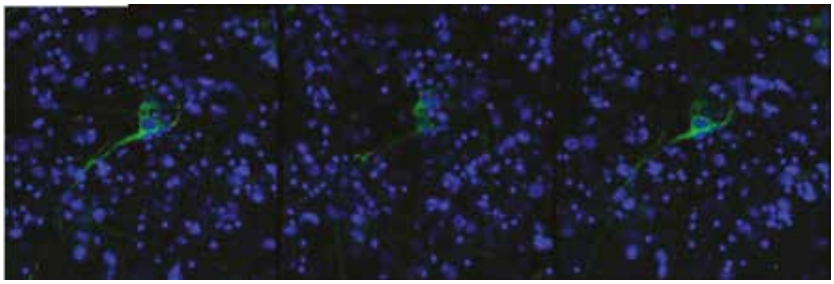


Fig 1 A clarified organoid at different optical sections. Dopaminergic neurons are immune-labeled with anti-Thy primary antibody with Alexa Fluor 488 secondary one.

In this perspective, iPAD platform could recapitulate salient features of PD observed in humans at different stages of the disease. Thanks to this novel multi-disciplinary approach, which integrates biological, engineering and computational methodologies, iPAD proposes solutions with high technological content to study and understand organ-to-organ communication and the effect of the metabolic disequilibrium on dopaminergic neuron degeneration in the mid-brain. In fact, iPAD will provide meaningful human-specific relevant data using advanced *in vitro* models. As the systems are based on hiPSC lines, they represent an important step toward future personalized medicine, in which pharmaceutical companies conduct their drug screening approaches with connected human organoid systems as personalized pre-testing before prescription to the patient. Thereby, firstly clinical trials will be cheaper and more efficient and secondly patients will only receive compounds that really work for them. The approach proposed could speed up the research on the disorder, reducing the gap between animal testing and standard i*n vitro* cultures, unraveling the etiology of the PD and disentangling the gut-brain connection at a physiological level.

1. Scott, Sean, et al. "Design, power, and interpretation of studies in the standard murine model of ALS." *Amyotrophic Lateral Sclerosis* 9.1 (2008): 4-15.
2. Tsilidis, Konstantinos K., et al. "Evaluation of excess significance bias in animal studies of neurological diseases." *PLoS biology* 11.7 (2013): e1001609.
3. Van der Worp, H. Bart, et al. "Can animal models of disease reliably inform human studies?." *PLoS medicine* 7.3 (2010): e1000245.
4. Fatehullah, Aliya, Si Hui Tan, and Nick Barker. "Organoids as an in vitro model of human development and disease." *Nature cell biology* 18.3 (2016): 246.
5. Kelava, Iva, and Madeline A. Lancaster. "Dishing out mini-brains: Current progress and future prospects in brain organoid research." *Developmental biology* 420.2 (2016): 199-209.
6. Parkinson Disease Foundation, 2016
7. Perez-Pardo, Paula, et al. "The gut-brain axis in Parkinson's disease: Possibilities for food-based therapies." *European Journal of Pharmacology* (2017).
8. Mayer, Emeran A., et al. "Gut microbes and the brain: paradigm shift in neuroscience." *Journal of Neuroscience* 34.46 (2014): 15490-15496.
9. Cryan, John F., and S. M. O'Mahony. "The microbiome-gut-brain axis: from bowel to behavior." *Neurogastroenterology & Motility* 23.3 (2011): 187-192.
10. Sampson, Timothy R., et al. "Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease." *Cell* 167.6 (2016): 1469-1480.

THE LUNG MICROBIOME

Michele Miraglia del Giudice
Professor of Pediatrics - Department of Woman, Child and of General and Specialized Surgery, University of Campania "Luigi Vanvitelli," Naples, Italy.

The pulmonary microbiome the new methods of investigation demonstrated that the lungs are not sterile, but that in healthy subjects there is a pulmonary microbiome characterized by the presence of bacteria, fungi and viruses. In the normal subjects the microbiome is different than in pathological conditions as asthma, cystic fibrosis, pulmonary bronchodispalysis and COPD. The pulmonary microbiota is determined in the first years of life and changes with age, diet, living environment and the use of antibiotics. Early changes in both intestinal and pulmonary microbiota can promote the onset of wheezing and asthma in later life. The connections between pulmonary microbiota and intestinal microbiota are very important for the development of a lot of pulmonary illness.

HOW TO OBTAIN NOWADAYS RELIABLE HIGH-QUALITY MULTI-STRAIN PROBIOTIC FOR CLINICAL PRACTICE: THE CASE OF VSL#3®

Rossella Filardi, Stefania Arioli, Diego Mora*
Department of Food Environmental and Nutritional Sciences (DeFENS), University of Milan, Italy

Introduction

According to the definition by a consensus panel of experts in 2014 probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014). Therefore, probiotics products represent a uniqueness in the food supplement market because there are designed to deliver live microorganisms to the end user to confer a health benefit, but which are the quality criteria that should be applied to guarantee their efficacy during the consumption and in clinical practice? Other microbial-based product in the market, *e.g.* those used for food fermentations are under a strict microbiological quality control because their activities are fundamental for the success of the production process. On the other hands, probiotics are controlled only at the taxonomic level of the microorganisms used (and quite often limiting the investigation to the species level) and at the number of viable cells. The need of a serious quality assessment of commercialized probiotic products is particularly requested due to the growing commercial interest in food-supplements based on probiotics (Kolacêk *et al.,* 2017; Jackson *et al*., 2019). In the absence of a standardized quality criteria the risk is a market colonization with products which could not respond to the minimal requested microbiological standards. Moreover, in terms of quality assessment, single- and multi-strains probiotic products should be considered differently. Single-strain probiotics could be easily characterized and controlled whereas multi-strain products are more complex both in term of richness and evenness. In terms of richness, multi-strain could be composed by two or more than ten different species/strains; in terms of evenness, the relative abundance of the species/strains blended in the products is often a trade secret, and the species/strains in a blend can have counts that differ for more than two-three logs. Therefore, considering that the minimal microbiological quality criteria must be satisfied by all probiotic products, the assessment of those quality criteria in a multi-strain product showed technical difficulties that are much more problematic compared to their evaluation in a single-strain product.
In the absence of probiotic products whose quality is rigorously controlled, it will be extremely difficult, if not impossible, compare the results obtained in different human intervention trials or to allow retrospective explanations in case of deviating clinical or health outcomes, and contribute to further assessing the role of probiotics in human health.

Which microbiological characterizations are necessary to address the quality of multi-strain probiotics?

We propose the following microbiological quality criteria (Qc) as the minimum standard for marketed probiotics. Qc-1 - *Taxonomy*. Correct identification of species and strains used in the product according to the latest scientific literature and supported by genomic analysis. For multi-strain products metagenomic characterizations should be performed to control the taxonomy of the microbial community at species/strain level. Qc-2 - *Viability*. Cell viability should be performed by culture independent methods because microbiological media composition and quality, and culture condition could strongly affect the total bacterial count. In this context, single cell analysis carried out by flow cytometry according to the ISO 1934 IDF 232 should be the elective counting method, even if, for multi-strain products it may be difficult/impossible to differentially quantify the species/strains blended in the formula. Qc3 - *Safety*. According to EFSA recommendations probiotic strains should satisfy the safety issues in terms of antibiotic resistances. While these quality criteria could be easily measured in single-strain product using a phenotypic approach (MICs evaluation) the metagenomic approaches should be the elective method to address this Qc in a multi-strain product. Qc-4 – *Level of biological contaminant*. Contaminants are considered pathogens, microbial species not declared in the label including lactic acid bacteria and bifidobacterial, and environmental microorganisms in the manufacturing plants. Not all the contaminants represent a safety concerns, but their presence and abundance is directly linked to the quality level of the production process. Here, beside the standard microbiological methods, a metagenomic approach could provide useful information. Qc-5 – *Reproducibility of biomass production.* Industrial production of microbial cells is rigorously controlled, nevertheless, changes in product composition in order to adapt the probiotic product to the consumer requirement may occur, *e.g.* when allergens are reduced or avoided in the media composition used to prepare the microbial biomass. In this context, variation in the production process of microbial cells could affect the microbial proteome and the level of enzymatic activities associated with one or several strains blended in the probiotic product. Here, metaproteomic approach and/or the measurement of some enzymatic activities could be used as marker to monitor the overall reproducibility of the fermentation processes.

The case of VSL#3®

The multi-strain VSL#3® product is a high dosage probiotic blend consisting of 8 different strains belonging to 7 *Lactobacillus, Bifidobacterium* and *Streptococcus* species. The design that was originally adopted to develop such complex product allowed to obtain a mixture of food- ad gut-associated bacteria and a high potency (450 billion per sachet), one of the highest in the probiotic market. VSL#3® composition is an equilibrated formula based on fermented-food-associated lactic acid bacteria strains (*L. helveticus* BD08, *L. paracasei* BP07, *L. plantarum* BP06, *Streptococcus thermophilus* BT01) together with human gut associated bacterial species (*L. acidophilus* BA05, *B. animalis*. subsp. *lactis* BL03 and BI04, *B. breve* BB02) (Douillard et al., 2018). Due to the complex microbial composition, VSL#3® was used as model to develop a quality control assessment based on the Qc1-5 above described. Table 1 summarizes the responses to the quality control assessment carried out during the last three years.

Table 1 – Quality assessment of the multi-strain VSL*#3*® product

Quality criteria	Number of lots analyzed	Methodology	Outcome of the evaluation
Qc-1 *Taxonomy*	3	shotgun metagenomics, species-specific PCR assays, strain-specific PCR assay, sequencing of short genome sequences to address the strains identity	Identification at species and strain level of VSL*#3*® strains
Qc-2 *Viability*	12	flow cytometry following ISO-1934, IDF 232 protocol B	Quantification of live, damaged and dead cells. Live cells always above 76% of the total cell populations in different stages of the shelf life of the product
Qc-3 *Safety*	3	shotgun metagenomics	Absence of transferrable antibiotic resistance genes
Qc-4 *Level of biological contaminant*	3	shotgun metagenomics	Reads not ascribed to VSL*#3*® species below 0.007% of total metagenomic reads
Qc-5 *Reproducibility of biomassproduction*	2/12	metaproteomics/measurements of βgalactosidase activity	Over 1600 protein groups belonging to all VSL*#3*® strains identified. Only 3.2 % proteins showed significant differences in relative abundance between lots. βgalactosidase activity normalized on the number of live cells measured by flow cytometry showed standard deviation among lots < 20%

The microbiological quality assessment based on the Qc 1-5 above described, revealed a high level of the VSL*#3*® production standards. While some Qc can be routinely verified in every production lots (Qc-1, Qc-2, Qc-4, Qc-5) using product-design simplified methodologies (species/strain-specific PCR assay and sequencing, enzymatic assays), other high throughput technologies such as metagenomics and metaproteomics could be used to address Qc-3 and Qc-5 only periodically or when changes in the production processes are planned.
These Qc, and related methodological approaches, could be used by producers, customers and regulators to control the quality of probiotic products, with the aim of increasing end-user confidence and scientifically supporting clinical practices involving the use of probiotics.

References

1. Douillard, F.P., Mora D., Eijlander, R.T., Wels, M., and de Vos, W.M. (2018). Comparative genomic analysis of the multispecies probiotic-marketed product VSL*#3*. *PLoS One* **13**: e0192452.
2. Jackson, S.A., Schoeni, J.L., Vegge, C., Pane, M., Stahl, B., Bradley, M., *et al.* (2019). Improving end-user trust in the quality of commercial probiotic products. *Front Microbiol* **10**: 739.
3. Kolacêk, S., Hisak, I., Berni Canani, R., Guarino, A., Indrio, F., Orel, R., *et al.* (2017) Commercial Probiotic Products: A Call for Improved Quality Control. A Position Paper by the ESPGHAN Working Group for Probiotics and Prebiotics. *J. Pediatr Gastroenterol Nutr.* **65**: 117-124.

PROBIOTICS IN HELICOBACTER PYLORI INFECTION AND IN GUT MICROBIOTA DISEQUILIBRIUM

G. Gasbarrini[1], C. Mosoni[2], F. Bonvicini[3]
[1] Emeritus Professor of Internal Medicine, Catholic University of the Sacred Heart, Rome, Italy
[2] Department of Internal Medicine and Gastroenterology, Catholic University of the Sacred Heart, Rome, Italy
[3] Alma Mater-School of Medicine, University of Bologna, Bologna, Italy

The community of microorganisms that colonize the human intestinal tract, driving an elaborate, mutualistic and symbiotic interplay with the host, is currently defined as Gut Microbiota and is now well known to play a major role in health and disease, being involved in both digestive and extra-digestive pathological conditions. The extreme variability of these germs produces a wide and variegated scenario, in which the interactions among micro-organisms are more important than the action of a single one. This concept represents a milestone in medicine, overcoming the "single germ theory" postulated by Koch in the XIX century.

The modulation of the Gut Microbiota is under the influence of many factors, such as diet, age, environment, lifestyle and many others and represents an important clinical target in Microbiota-related diseases. The benefits of Microbiota modulation were probably somehow known also to our ancestors: in fact, traces of this practice can be found in human history since the Neolithic Age, when the changes in human habits to a lifestyle based on farming and breeding led many populations to the consumption of aliments containing fermented milk. Many years later, according to the legend, the prophet Muhammad donated Kefir, a drink rich in lactic acid, bacteria and probiotics, to the ancestors of the mountaineers of Caucasus. A comparable drink called "Chemmisi" was also described by Marco Polo in his journey to China[1]. The first scientific studies concerning the benefits of the consumption of probiotics were conducted by I'ja Il'ič Mečnikov (Charkiv, 05/16/1845 – Paris, 07/16/1916), that investigated a potential link between the consumption of yogurt and the longevity of Bulgarian sheperds, opening the way to the research regarding probiotics role in human health[1].

Nowadays, probiotics, prebiotics and antibiotics are most studied and commercially available options to treat dysbiosis and dysbiosis-related pathologies. The use of antibiotics is well known to often determine several alterations, including the reduction of beneficial bacteria, on the Gut Microbiota. Among antibiotics, rifaximin seems to have non-traditional effects on the Gut Microbiota, showing anti-inflammatory properties and an "eubiotic" function[2]. These characteristics make it an important therapeutic option in patients with dysbiosis. In our clinical experience, the treatment of dysbiosis with probiotics administration is more effective when preceded by rifaximin-induced Microbiota modulation. Still to define are the proper administration times of rifaximin, however our clinical experience, based on clinical symptoms reported by patients and lactulose breath test results, suggests that rifaximin eubiotic effects are more evident and lasting when administered in bimonthly cycles compared to monthly cycles.

Probiotics are defined as living micro-organisms that can provide health benefits when administered in adequate amounts. Most of them contain Lactobacilli and Bifidobacteria, with good results reported also with therapies based on Saccharomyces boulardii. The clinical use of probiotics aims to restore a homeostasis in Gut Microbiota to treat Microbiota-related disease; to this purpose, probiotics have been used in clinical practice to treat many pathological conditions, even without solid evidence. In fact, high variability in strain administration, dosage, length and type of treatment make it very difficult to clearly assess probiotics efficacy[3].

Prebiotics, namely non-digested foods components that can modulate the Gut Microbiota improving the host health as inulin and trans-galacto-oligosaccharides, are also a therapeutic possibility, but further studies regarding their efficacy are required[3].

Another therapeutic chance to treat Microbiota-related diseases is represented by Fecal Microbiota Transplantation, mostly used in refractory and recurrent C. Difficile infections and currently an emergent research topic for other pathologies[3].

The administration of probiotics was studied also in Helicobacter pylori (Hp) infection. Hp is one of the most studied germs that can be isolated in human Microbiota, and Hp infection is at present time an emergent clinical problem, with elevated prevalence rates especially in developing countries. Over 80% of infected individuals show no symptom, but the chronic infection is involved in the pathogenesis of critical illnesses ranging from chronic gastritis to peptic ulcer, gastric cancer and gastric lymphoma, making its eradication a primary clinical goal. However, growing antibiotic resistance and drug related adverse events such as diarrhea, vomit and abdominal pain, determined a decrease in eradication rates. Furthermore, also the impact of Helicobacter Pylori on gastric and intestinal microbiota must be considered. In fact, Helicobacter Pylori is an important perturbing factor in the equilibrium of the bacterial gastric community, in which most represented phyla are Actinobacteria, Bacterioidetes, Firmicutes and Proteobacteria, and affects also the intestinal homeostasis. Furthermore, recent studies suggested that the pathological manifestations of Helicobacter Pylori may be ascribed both to Hp infection and to Hp induced microbiota changes. Antibiotics and Proton Pump Inhibitors, cornerstone in Helicobacter Pylori eradicating therapy, are also responsible of complex changes in the Gut Microbiota that are currently an emergent research topic. In particular, the role of other microbiota components including viruses and bacteriophages must be considered. Focused therapies aimed to Microbiota modulation are strongly needed[4].

In fact, as living beings, probiotics may competitively inhibit Hp growth and improve the tolerability and efficacy of the eradication treatment restoring the equilibrium in gastric mucosa.

In Italy, the III Working Group Consensus Report stated that some probiotics reduce the adverse events of Hp eradication therapy, but further evidence is needed to better define this issue[5].

A recent metanalysis revised clinical trials that compared placebo and probiotics when supplemented in triple therapy, sequential therapy and quadruple therapy. This metanalysis evidenced that, compared to placebo, most probiotics resulted to be more effective when supplemented in Hp eradication therapy. Compared to single use, the administration of combined probiotics was not associated with a significant improvement in tolerability and efficacy terms[6].

A recent paper by Fallone et al. evidenced that, based on several meta-analysis, the evidence supporting the administration of single or multi-strain probiotics in Hp eradication therapy is still low, primarily because of the low quality of most of the trials investigating this topic and variability and poor definition of the strains of probiotic given. Nowadays, the Toronto guidelines do no encourage the probiotics administration if given just to increase eradication rates; ACG, despite recognizing strong potentialities of probiotic for this purpose, do not advocate their administration. European guidelines suggest a case by case evaluation, with the prescription of probiotics in certain patients categories[7]. In conclusion, probiotic administration in Hp infection, in particular as an adjunct to eradication therapy, may offer significant potential, but further studies are needed to clearly identify which strains, and in which settings, may have beneficial role.

Bibliography
1. Gasbarrini G et al., Probiotics history, J Clin Gastroenterol. 2016 Nov/Dec;50 Suppl 2, Proceedings from the 8th Probiotics, Prebiotics & New Foods for Microbiota and Human Health meeting held in Rome, Italy on September 13-15, 2015:S116-S119.
2. Ponziani FR et al., Eubiotic properties of rifaximin: Disruption of the traditional concepts in gut microbiota modulation, World J Gastroenterol. 2017 Jul 7;23(25):4491-4499. doi: 10.3748/wjg.v23.i25.4491.
3. Cammarota et al., Gut microbiota modulation: probiotics, antibiotics or fecal microbiota transplantation? Intern Emerg Med. 2014 Jun;9(4):365-73. doi: 10.1007/s11739-014-1069-4. Epub 2014 Mar 25.
4. Lopetuso L et al., Considering gut microbiota disturbance in the management of Helicobacter pylori infection. Expert Rev Gastroenterol Hepatol. 2018 Sep;12(9):899-906. doi: 10.1080/17474124.2018.1503946. Epub 2018 Aug 7.
5. Zagari RM et al., Guidelines for the management of Helicobacter pylori infection in Italy: The III Working Group Consensus Report 2015. Dig Liver Dis. 2015 Nov;47(11):903-12. doi: 10.1016/j.dld.2015.06.010. Epub 2015 Jul 6.
6. Wang et al, Probiotics in Helicobacter pylori eradication therapy: Systematic review and network meta-analysis. Clin Res Hepatol Gastroenterol. 2017 Sep;41(4):466-475. doi: 10.1016/j.clinre.2017.04.004. Epub 2017 May 25
7. Fallone et al., Reconciliation of Recent Helicobacter pylori Treatment Guidelines in a Time of Increasing Resistance to Antibiotics. Gastroenterology. 2019 Jul;157(1):44-53. doi: 10.1053/j.gastro.2019.04.011. Epub 2019 Apr 15.

THE MEDITERRANEAN TASK FORCE FOR CANCER CONTROL: AN EVERGREEN COMMITMENT IN FIGHTING CANCER

Pier Giorgio Natali MD, Secretary General Mediterranean Task force for Cancer Control - Rome

Cancer research is undoubtedly advancing at a rapid pace, but a paradoxical gap remains between what is known about improving cancer control and what is implemented as best practices.
Although cancer-related deaths are decreasing by combined preventive/early diagnosis and therapeutic advancements, a number of emerging factors, including changing epidemiology, are conducive to decreasing effectiveness of present cancer control policies.
As a result, the occurrence of hard to treat advanced disease is likely to remain too high in view of the expected increase in cancer incidence, thus undermining the sustainability of health care systems.
In the present scenario, prevention and early diagnosis cost-effective strategies are not fully exploited, people awareness is not yet pursued at educational level and alarmingly disseminated through uncensored information. In addition, financial resources are progressively scanty, even in "rich "economies, and cultural and social disparities are progressively becoming irredeemable. Key territorial medical figures such as Family doctors and GPs[IL1] are becoming an endangered species in national health policies. The figure of Family Nurse is rarely present among the health providers.
As back as 2005, the founding Members have forethought the above issues in MTCC mission, recognizing that health and wealth are closely interconnected. Indeed, since its inception and upfront of very limited resources, MTCC has constantly contributed to raising voices about prevention and early diagnosis in medical Congresses, social and political arenas. This has been done also by providing highly qualified faculties to national congresses, organizing training courses, publishing multilingual primary and secondary prevention brochures delivering out the paradigms messages, raising awareness through collaboration with Universities, Associations, Foundations, National and International Agencies. Further, MTCC has advocated the need for training and maintaining GPs as key advisors between patients and qualified specialists and Centers, for further disease management.
Since cancer "*cannot be fought in solitude*" (*Chart of Paris, 2002*), MTCC is launching a renewed Call for Action leading to a stronger alliance between medical sciences, legislators and Governments, educators, families, religious centers, charitable/cultural associations, for an increasing effort to develop and disseminate relevant and sustainable policies to face the ongoing cancer global epidemic.
At MTCC, we strongly believe that much can be done also with limited budgets but recognizing that we have medical as well as ethical obligations to prioritize public health.
In this framework we advocate that "*health should be regarded as a safe space for dialogue between Countries of different cultures, faiths, having the power to unite because of common goals and challanges*".

MULTI-STRAIN OR SINGLE-STRAIN PROBIOTICS

Arthur C. Ouwehand
Global Health and Nutrition Sciences; DuPont Nutrition and Biosciences, 02460 Kantvik, Finland

Probiotics are marketed both as single- and multi-strain preparations. There may exist the expectation that a multi-strain product may be more efficacious than a single strain product. Beyond the general perception that more is better, there are some more structured thoughts behind this:

1. More strains may provide more chances of success
2. More strains provides a greater diversity and thus more potential niches to fill
3. More strains may provide a broader range of health benefits
4. There is the expectation of at least an additive, and maybe even a synergistic effect
5. Neglected is often that there may also be antagonistic effects

Under ideal conditions, studies would have investigated these hypotheses. Unfortunately, that is not the case. This may be explained by the fact that it is actually quite complicated to investigate whether a combination of strains is more efficacious then a single strain.

To investigate the efficacy of a single-strain probiotic, one needs two study arms; a probiotic and a placebo arm. The simplest form of a multi-strain is a two-strain probiotic; it requires four study arms, two arms to test both strains separately, one to test the combination and a placebo arm. Thus, to investigate the value of a multi-strain probiotic over their composing single-strain versions, requires a number of study arms equal to the number of strains plus the multi-strain probiotic and a placebo. Obviously, this quickly becomes prohibitive.

In addition to this, one has to consider the dose. When the same counts for a single-strain probiotic is used as the total count for the multi-strain, the compounding strains are present in lower amounts. Conversely, if strains are in the same counts in the single-strain as each strain in the multi-strain product, the latter will have higher total counts. As an example, when a single-strain product has 10^9 CFU a two-strain product with the same counts for each strain would have a total count of $2x10^9$ CFU. The more strains there are in the product the bigger the difference between the single- and multi-strain total counts get. Or, if the total counts are the same, the difference for the compounding strains gets ever bigger. There is no simple solution how to address this.

There are thus practical limitations and challenges on how to test single- vs. multi-strain probiotics.

Nevertheless, there is a wealth of data from studies with single or multi-strain probiotics. And although these may be consisting of different strains, they could provide us an indication of the comparison. Most notably, meta-analyses that have performed a sub-group analysis for strain number can give us some insights into the topic as they focus on the same health end-point.

To this end, PubMed was searched using the terms: meta-analysis AND probiotic AND (multi OR combination OR blend). This yielded 86 articles. Of these, 17 evaluated the effect of single- and multi-strain probiotics on a particular health end-point but did not assess the difference between the two. A further 15 meta-analyses evaluated the efficacy of single- and multi-strain probiotics and made a statistical comparison between the two. Two additional articles investigated single- and multi-strain probiotics but concluded that too little data was available to do a meaningful analysis.

Overall, necrotising enterocolitis (NEC; n=7), supplemental treatment to Helicobacter pylori eradication (n=5), antibiotic associated diarrhoea risk (AAD, n=4) and *Clostridioides difficile* associated diarrhoea risk (CDAD, n=2) were the most commonly investigated health outcomes comparing single- and multi-strain probiotic efficacy. In NEC and NEC-associated sepsis and mortality, most studies suggest either no difference or a similar efficacy for both single- and multi-strain probiotics. Similar in supplemental treatment with probiotics in *H. pylori* eradication, most studies either indicate no difference or suggest a higher eradication rate with multi-strain probiotics as compared to single-strain probiotics. For both AAD and CDAD, studies report a better efficacy for both single-strain and for multi-strain probiotics as well as no difference. It should be noted that in several of the comparisons, there is a disbalance in the number of single-strain vs. multi-strain probiotic studies and it would be more correct to state that no meaningful conclusion can be drawn. For most other health benefits meta-analyses do not report a difference in efficacy between single and multi-strain probiotics. A relatively consistent point is, however, that it is rare for a multi-strain product to underperform compared to a single-strain product; indicating that an antagonistic effect between strains in one formulation is rather the exception.

Thus, despite the availability of data, no definite conclusion can be drawn. This is likely due to the heterogeneity of the studies included in the meta-analyses. Nevertheless, there is value in considering the multi-strain approach. For this, however, a better mechanistic understanding of the mechanisms of probiotic action would be desirable. This way, a multi-strain product can be formulated where strains that either reinforce each other's mechanism or address different mechanisms for the same health benefit.

Until then, our choice of probiotic should not be guided by the number of strains (or their counts), but by their clinical documentation.

PROBIOTIC FORMULATIONS FOR HEALTHY SKIN: FROM LAB TO SLAB

Professor Indu Pal Kaur
University Institute of Pharmaceutical Sciences
Panjab University, Chandigarh – 160014
India

Designing live probiotic formulations for dermal applications is both exciting and challenging. Concept has a potential scope, especially in view of the fact that we now understand and appreciate the important role that the commensal bacteria play in the development of immune system and reactions of the human body to external stimulus.

Skin is the primary interface of the human body with the external environment and in a way adapts and monitors the whole body physiology. The skin ecosystem constitutes a complex and dynamic habitat reflecting the wider ecosystem in which reside more than a million organisms ($10^6/cm^2$). These resident microbes are now viewed as an integral part of the functional unit of skin. Humans are thus multicellular organisms i.e. metaorganisms which comprise both the macroscopic host and symbiotic commensal organism. In the same context skin is thus a complex composite of microbes coupled with the structural, hormonal, nervous and immunological networks of the host.

Skin-gut-brain axis is now a well-established fact where modifying gut flora can alter the skin function. However, it may be highlighted that there are some distinct difference between the gut and the skin flora, mainly because gut is a rich nutrient site while skin is nutrient-poor, less hospitable site. The association of host and microorganism in the latter, depends on factors like skin surface (topography) and its environment i.e. pH, temperature, moisture and sebum content. Skin is cool, acidic, desiccated and bathed in sweat, with sebum and skin stratum corneum protein being the only sources of nutrition. Sweat contains high concentration of salt and antimicrobial peptides (AMPs) not amenable to microbial growth. However, various microorganisms (virus, fungi, bacteria) cover the skin surface and reside in its various appendages (hair follicle, sebaceous glands, and sweat glands).

Skin is also a highly sophisticated system of immune surveillance and is a rich network of epithelial cells and billions of lymphocytes. Its innate immunity arm probably arises from early mediators of host-skin interaction. As a consequence of the effect of microbiota on innate immunity there is an increase in lymphocytes and hence increase in adaptive immunity. Any damage to the skin activates the keratinocytes to release AMPs. Some AMPs are constitutively expressed while resident bacteria trigger others, along with lymphocytes-IL1 and IL-17A production. This directly or indirectly enhances skin immunity by promoting cytokine production, enhancing cell microbicidal function, and promoting the recruitment of effector cells. Enhanced production of IL-17 by the microbiota promotes keratinocyte effector function against invading microbes. Overall the skin flora controls the immune homeostasis and responds to infection in an autonomous manner independent of the gut flora.

Exposure to natural environment or environmental microdiversity, and air and water pollution can disrupt the balance between skin and mucosal ecosystem which can further result in dysbiosis, immune dysregulation, local tissue damage and increased risk of inflammatory skin diseases like atopic dermatitis, psoriasis, acne and Rosacea. As resident commensals control the innate and adaptive immunity so they are the primary drivers and amplifiers of skin pathology.

The potential mechanism by which the skin microbiota may initiate or amplify skin disorders are:

(i) Host predisposition factors e.g. barrier defect, and regulatory pathway defect. Enhanced sensing or translocation of the microbiota can also be mediated by host genetic predispositions e.g. filaggrin, IL-23, IL-10 mutations;
(ii) Change in microbial composition e.g. in case of metabolic diseases like diabetes (associated with alteration of nutrients) and acne can lead to enhanced microbial density and dysbiosis;
(iii) Contextual pathogens e.g. *S. aureus, C. albicans* – which though the normal constituents of the skin microbiota could become pathogenic under certain conditions;
(iv) Co-infection: Under certain conditions skin microbes may contribute to inflammation and tissue damage;
(v) Increase of defined bacteria e.g. *S. aureus*: Microbes with inflammatory potential may dominate an event that can be promoted by antibiotic treatment or environmental alternatives.

Combinations of these scenarios are likely to trigger various disease pathologies. In turn, the inflammatory tissue response can also alter microbial communities, a process that can further amplify tissue damage.

Presently practiced approaches for prevention and control of these skin ailments are antibiotics, antiseptics, antimicrobials and certain herbal agents. Antibiotics/antimicrobials are associated with side effects like diarrhea, GIT distress, disturbed gut ecology, allergies, toxicity, development of antibiotic-resistant strains, hepatotoxicity, renal failure, agranulocytosis, alteration of the normal flora and poor patient compliance.

Probiotics are emerging as a potential therapy for a variety of conditions including skin infections. They act by multiple mechanisms including production of antimicrobial substances, inhibition of pathogen adhesion, and stimulation of immune respons. These properties coupled with their capacity to colonise the skin surface can be used to control the invasion of healthy or traumatized skin by pathogenic organisms. Existence of a gut-brain–skin axis indicates that altering the gut flora, which is quintessential for maintaining the normal and healthy skin state, may help in the management of various skin ailments including infections. The inflammatory skin diseases like acne can be suitably manipulated by oral administration of probiotics, however this pathway is majorly immune system mediated. Thus it may have a limited scope for the control of infections, while a direct manipulation/modification of skin flora by topical application of probiotics to restore its robustness may be a more favorable option. The transiently administered oral probiotic on the other hand may not survive adverse conditions of the gut, and also fail to establish themselves in the gut for long-term effects.

Therefore, delivering the probiotics locally to the skin surface for an enhanced effect is proposed presently. Topical route is the most suitable and easily acceptable route of application for any medication.

A system for delivery of probiotics is considered as suitable, which will
(i) *Preserve the viability of probiotic cells* on storage,
(ii) *Maintain them in a sporulated/vegetative state,*
(iii) Help in elicitation of their physiological action by allowing their *successful germination* to the vegetative form or to multiply following application on the skin surface,
(iv) *Retain probiotic on the site for longer durations - sufficient time for release, growth and adherence to produce effects , and*
(v) *Prevent fast* ***wash out*** *of probiotic otherwise observed with free probiotics.*

My endeavours include development of a gelatin-based stable, patient compliant gel formulation/vaginal suppository containing whole cell probiotic (*Bacillus coagulans*) for diverse skin and vaginal conditions.
Bacillus coagulans was selected as a suitable probiotic as it is Gram-positive rod, spore-forming, motile and facultative anaerobe, exhibit probiotic activity under aerobic conditions - skin & mucous membrane, optimally grow from 30° C to 55° C, and its spores can withstand higher manufacturing temperature.
Microscopic images of the developed formulation indicated honeycomb network with presence of probiotic spores enmeshed in void spaces. Key of the work was to develop a formulation with low water activity (a_w) of < 0.6, so that the *B. coagulans* do not germinate during storage of the formulation. This also assigned a self-preservative capacity to the formulation (0.541 a_w) i.e. there was no need to add any preservative. Challenge test as per Indian Pharmacopoeia confirmed that the formulation was protected against contamination during storage. The probiotic count was maintained on storage for three months (5°C±3°C and 30°C±2°C/ 65%RH±5%RH) confirming stability and cell viability. *B. coagulans* spore were confirmed to germinate within 6 h of their application on rabbit skin/rat vagina and only few non-germinated spores were left at 24 h after application. The developed formulation was safe in terms of cytotoxicity, acute dermal irritation and permeation/ translocation as NMT 0.16±.03% of $8±4×10^6$ cfu applied per day translocated to systemic circulation. Patient compliance in terms of ease of application and removal was confirmed by texture and rheological studies which matched with those reported suitable for topical products. The *in vivo* therapeutic effect of the developed formulation was confirmed in terms of wound healing and control of vulvovaginal candidiasis (for vaginal suppositories). Probiotic-loaded formulations were significantly better than the marketed antibiotic/ antifungal preparations.
The developed formulation containing whole cell probiotic (*Bacillus coagulans*) can thus be considered as a potential option for wound healing and vulvovaginal candidiasis.

NUTRACEUTICALS AND BIOMARKERS

Marcella Renis
Department of Drug Sciences- University of Catania, Italy

The approaches to the management of well-being are today substantially modified since the use of new personalized dietetic strategies, adequate to meet the metabolic needs or to regulate the metabolism at epigenetic and molecular level, contributing to the improvement of human health.
In recent years, research on naturally occurring bioactive compounds, mainly plant extracts, has been growing rapidly. These extracts and their components are studied for their phytochemistry, phytopharmacology and phytotherapy and evaluated for efficacy and safety. These compounds, also referred to as herbal supplements, are called, with a term coined in 1989 by Dr. Stephen: "Nutraceuticals", a combination of two words: "nutrient" (beneficial food component) and "pharmaceutical" (a medical drug). Nutraceuticals, being inexpensive, easily accessible and not requiring a physician's prescription, are very interesting for clinical use and widely used, compared to modern drugs, for non-specific biological therapies focused on general well-being, diseases prevention and treatment, especially chronic non-communicable diseases with high social and health costs (i.e. diabetes, obesity, cardiovascular diseases, respiratory diseases and intestinal diseases). These functional food components, standardized in nutraceuticals and produced according to good manufacturing practices, include vitamins, minerals, prebiotics, probiotics, postbiotics, proteins, amino acids, polyunsaturated fatty acids, rich compounds and polyphenols (Gupta S, et al. 2010; Rivellese et al. 2019).
In the last decade the knowledge that connects specific pathologies with the nutraceutical role of different phytochemicals, in particular polyphenols, has rapidly grown due to their role as antioxidant and anti-inflammatory molecules, also showing a preventive effect against specific diseases (Vamanu E., 2019). However, the management of Nutraceuticals, with the increase in new clinical applications, requires accuracy and scrupulousness, avoiding excess in dosage and clinical consequences. Furthermore they must be utilized with specific reference to the diseases, age, lifestyle of the patients, individual metabolic-deficiencies and health areas to which they are addressed as products with biologically active ingredients, i.e. cosmeceuticals, with skincare efficacy, and sportceuticals, with support for physical activity (Gupta R.C. et al. 2018).
A series of complications can threaten the efficacy and safety of nutraceutical compounds with therapeutic potential: I) the presence of more than one hundred phyto-components with biological and pharmacological potential; II) their possible contamination with metals, pesticides, mycotoxins, as well as with illegal drugs; III) the lack of information, compared to medicines, regarding the interaction with other Nutraceuticals, with some therapeutic drugs and the related biomarkers. In this scenario, basic and translational research are encouraged, in order to determine and validate, in human body fluids and tissues, biomarkers related to food compounds, phytochemicals, and/or their metabolites, stressing on the identification of novel biomarkers of disease, useful to define innovative therapeutic targets, both in the pharmacological and nutraceutical area.
The use of Biomarkers, "wet" or "dry", in clinical research as well as in clinical practice, has a long history as one of the most objective and quantifiable medical signs. The clinical information represented by biomarkers, measurable in biological samples such as total blood, plasma, cerebrospinal fluid, urine, saliva or biopsies, are of enormous value in all physiopathological conditions. Modern technology, laboratory sciences and easy access to quantifiable and reproducible biomarker data have consistently encouraged their use and routine acceptance as primary endpoints in clinical trials, as well as positively interfering with the drug development process (Peluso I. et al. , 2017).
Biomarkers are roughly divided into different classes according to their characteristics and/or source: metabolic, genetic, DNA and Exosome-based; biomarkers of exposure, susceptibility or biological effects, able to provide indications of the status of the organism, environment or ecosystem as well as biomarkers of dietary intake and nutritional status. These are useful to assess the effect of some dietary components on health and also to counteract the risk of various pathologies, such as cancer and cardiovascular diseases.
We tested, *in vitro,* the efficacy of some Nutraceuticals compounds such as *Brassica Oleracea* e.s. tit.11% Sulforaphane glucosinolate and Betula etnensis Raf. (Betulaceae) polyphenols enriched extract, on human cancer cell lines, together with sugars/minerals enriched fraction from olive mill wastewater on human fibroblast cell line (HFF1) for skin protection and an enriched extract of blood oranges (Citrus sinensis (L.) Osbeck on pre-adipocytes 3T3 cell line. Biomarkers examined were: dose dependent cytotoxicity (MTT and wound healing assay), global DNA methylation (methylation sensitive Comet assay), DNMT1 expression (Qiagen kit) and nuclear sirtuin activities (ab156915 kit, Abcam); redox homeostasis modulation, adipogenesis related genes, direct/indirect immunofluorescence analysis of cytoskeleton microfilaments (IF) (Di Mauro M.D. et al 2017; Tomasello et al., 2018; Malfa G.A. 2018; Tomasello B. et al, 2019).
Biomarkers, further, can be divided into diagnostic (when a disease is present) , prognostic (indicative of the development of the disease), predictive (treatment- related), pharmacodynamic (indicative of potential drug efficacy), toxicological (informating about some possible side effects) and surrogate (usually indicative of a replacement of the primary clinical endpoint).
DNA-based markers may be gene mutations or polymorphisms, as well as transcripts, proteins, peptides or metabolites. Many individuals may have mutations in heterozygosis or in homozygosis, in several enzymes involved in micronutrients metabolism affecting the distribution and the effect of the nutraceutical compounds. The knowledge of these alterations is of significant importance for the therapeutic strategy to be adopted. A striking and well-known example is represented by the enzyme MHTFR (methylenetetraidrofolate reductase) which, if mutated (in heterozygosis and/or in homozygosis), can increase the concentration of homocysteine, a sign of possible cardiovascular disease requiring the appropriate therapeutic administration of folic or folinic acid.
Genomic, exomic, epigenomic and proteomic data have played a crucial role in the discovery of biomarkers and translational medicine in recent years. Today a promise for the future is represented by metabolomics data, which, measuring small endogenous and exogenous molecules, substrates and products of chemical cellular reactions, provide indications of the activity and the state of cellular and organism metabolism, giving essential information about the underlying biological state of the examined system (Li B. et al., 2017). Metabolomics allow us to profile thousands of unknown features and to perform both "targeted" screening and "non-targeted" screening. Today the new challenges for researchers are represented by the acquisition of metabolomic data at single cell level and, in particular, sub-cellular compartmentalization, since the cellular data reflect the sum of the metabolites in different cellular organelles.
However, a new research strategy in particularly complex clinical situations, is the integration of multi-Omix, metabolic, leaky gut biomarkers and microbioma analysis (Mazahery H. et al. 2019; Vamanu E., 2019). These approaches are surely valid for studying small groups of patients and above all in the field of research, they are not easily applicable in examining large groups of patients.

We applied, in a complex clinical case, the "integrated approach", utilizing metabolic and multi-omix biomarkers. We worked in a multidisciplinary team (clinical biochemist, pediatrician, psychologist, nutritionist, neuropsychiatrist, gastroenterologist, bioinformatics) and in patient-centered way, sharing decisions on the diagnostic analytical tests, on the therapeutic plane and on the psycho-attitudinal treatments to be performed.
Clinical case: 12-year-old child, with a diagnosis of Autistic disease syndrome (ASD) when he was 4 years old and presenting epilepsy crisis and other co-morbidities.

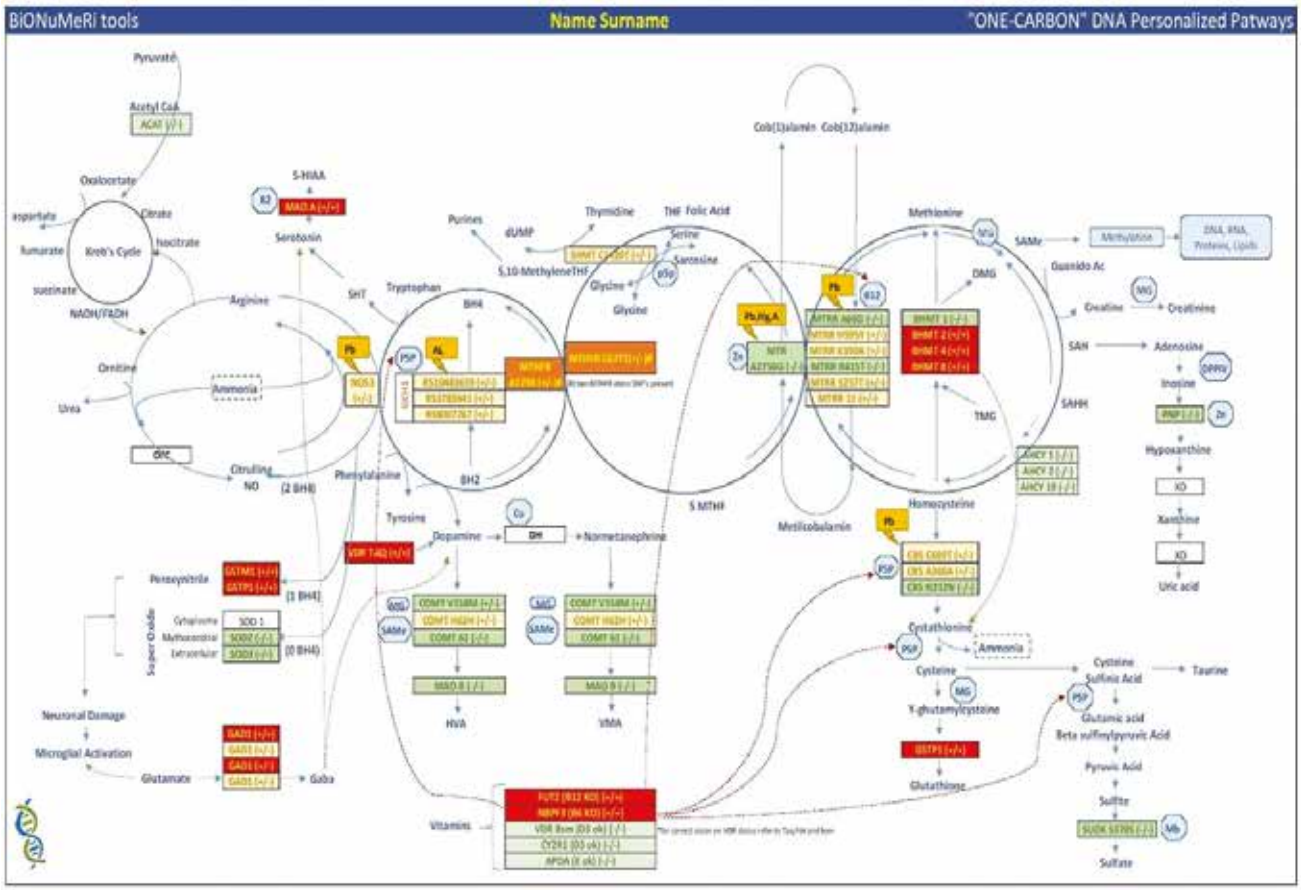


Figure 1. Example of personalized One Carbon pathway. The raw results of the sample used in this work, related to one child with ASD diagnosis, were donated to ONLUS BiONuMeRi by parents who had spontaneously acquired 23andMe kit for exome analysis.
BIOESOnet is freely available visiting http://www.bionumeri.org/joomla/restricted-area/onecarbon-tool

We evaluated the following selected biomarkers: hematic levels of amino acids, short fatty acids, vitamins (A, B6, B9, B12, C, and D), ATP, CoQ10. In addition some key factors envolved in vitamine D metabolome were measured: magnesiun, phosphates, calcium, PTH blood level along with polymorphisms of VDR (vitamin D receptor) and CYP450. Hair mineral test and the urinary level of gluteo/caseomorphine were tested , in order to define also the dietary plan, whether with or without gluten and casein, nutraceutical and / or pharmacological treatments. Serum level of zonulin, occludin, LPS and PCR as marker of intestinal barrier function and microbioma analysis was also performed. Furthermore, Exome analysis was accomplished by BIOESOnet, a new software specially shaped by our bioinformatics and was utilized for the filtering and visualization of Exome raw data into customized one carbon cycle pathway (Fig.1). The tool enables a fast and extensive overview of possible polymorphisms (red in homozygosis and yellow in heterozygosis) present in the enzymes of the examined pathways, helping us to locate possible interventions (i.e. dietary changes and/or integration) and to improve patient's quality of life. Finally, "non target" metabolomics data were acquired (Pennisi M. et al. 2018).
The accurate analysis of all data obtained, provided us with a very interesting feedabck on the clinical history of the young patient and allowed us to perform a fine adjustment on some metabolic pathways.

References
1. Di Mauro MD et al. Sugar and mineral enriched fraction from olive mill wastewater for promising cosmeceutical application: characterization, in vitro and in vivo studies.Food Funct. 2017, 8(12):4713-4722.
2. Gupta S, et al . An overiew of nutraceuticals: current scenario. J Bas.Clin Pharm. 2010, 1(2):55-62.
3. Gupta RC et al. Toxicity Potential of Nutraceuticals. Methods Mol Biol. 2018;1800:367-394.
4. Li B et al.Novel Applications of Metabolomics in Personalized Medicine: A Mini-Review. Molecules. 2017 13;22(7).
5. Malfa GA et al. *Betula etnensis* Raf. (Betulaceae) Extract Induced HO-1 Expression and Ferroptosis Cell Death in Human Colon Cancer Cells.Int J Mol Sci. 2019, 3;20(11)
6. Mazahery H, et. al. A randomised controlled trial of vitamin D and omega-3 long chain polyunsaturated fatty acids in the treatment of irritability and hyperactivity among children with autism spectrum disorder.J Steroid Biochem Mol Biol. 2019;187:9-16.
7. Peluso I et al. Biomarkers of Oxidative Stress in Experimental Models and Human Studies with Nutraceuticals: Measurement, Interpretation, and Significance 2017. Oxid Med Cell Longev. 2017; 3457917.
8. Pennisi M. et al. BIOESOnet: A Tool for the Generation of Personalized Human Metabolic Pathways from 23andMe Exome Data International Conference on Intelligent Computing.ICIC 2018: Intelligent Computing Theories and Application pp 345-352. Part of the Lecture Notes in Computer Science book series (LNCS, volume 10955)
9. Rivellese AA, et al. The Possible Role of Nutraceuticals in the Prevention of Cardiovascular Disease.High Blood Press Cardiovasc Prev. 2019,26(2):101-111.
10. Tomasello B. et al. Effects of Sulforaphane and Myrosynase blend on human astrocytoma cells. Oral Communication. International Conference Integrative Oncology, 23/24/25 March 2018, PALAZZO DEI CONGRESSI – FLORENCE-Italy
11. Tomasello B. et al. Anti-adipogenic and anti-oxidant effects of a standardized extract of Moro blood oranges (Citrus sinensis (L.) Osbeck) during adipocyte differentiation of 3T3-L1 preadipocytes" Natural Product Research 2019 IN PRESS,
12. Vamanu E.Polyphenolic Nutraceuticals to Combat Oxidative Stress Through Microbiota Modulation.Front Pharmacol. 2019,3;10:492.

ADVERSE EFFECTS TO PLANT FOOD SUPPLEMENTS

Patrizia Restani, Francesca Colombo, Simone Biella, Chiara Di Lorenzo
Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano

Products containing botanical ingredients are widely accepted by consumers and are increasingly part of the daily dietary habits in Europe. Products with vegetable ingredients or their derivatives (botanicals) fall into various categories, which can be regulated differently in among countries, including those belonging to the European Community.
Most European countries classify products containing botanicals as food supplements (Plant Food Supplements or PFS) that, commercialized in pharmaceutical form (capsules, tablets, drops, etc.), intend to improve the daily intake of nutritional or functional substances. They should not be considered as a substitute of healthy diet and lifestyle approach.
In the last decades, Plant Food Supplements have received an increasing interest by consumers for their possible beneficial effects but, in parallel, concerns have been raised by international bodies responsible for consumers' safety.
The quality of a food product is always important for consumers' safety. Supplements containing botanicals, being mainly classified as food, must comply with the legislation of the sector in terms of production and control. Since the botanicals are ingredients of plant origin, the problem of residues of environmental contaminants is of particular importance.
To minimize the risks for the consumers, farmers must follow the rules of good agricultural practices (GAP) for the use of pesticides and fertilizers or adapt to the legislation for organic farming. Among other parameters, the quality of final PFS includes the content in those active compounds, which are responsible for the desired physiological effect. The quality of PFS can be regulated by national/international limits for both active molecules and/or other undesired compounds having toxicological concerns. Table 1 lists the most important problems, associated with the raw botanical material, having a possible role on consumers' safety; some of them are not intentional, other come from illicit activities.

Table 1 – Problems of raw material having a role in adverse effects

Quality of ingredients	Contaminants	Counterfait	Illicit additions
Unsatisfactory quality of raw material	Pesticides	Intentional use of low quality botanicals	Conventional drugs such as antibiotics, amphetamines, etc.
Misidentification	Heavy metals	Use of botanicals different from those declared in the label (lower cost)	Stimulating molecules (ephedrine, active amines, sildenafil, etc.)
Wrong part of botanical (leaves instead of root, etc.)	Mycotoxins		Doping (hormones)
Low quality of extracts/derivative	Pollutants (PCB, PAH, etc.)		

Adverse effects to PFS can be due to different problems, where quality is only one of the most important contributors. Very important is the information provided both to the general population and to group at risk: consumers must know that botanical ingredients could be responsible for adverse events. Pregnant or lactating women, as well as children, should avoid the use of products with plant derivatives apart from those products specifically formulated and, in any case, recommended by a medical doctor. Particular attention must then be given to consumers suffering from food-related diseases, such as celiac disease and allergy. "Social cofactors" can also contribute to the onset of an adverse event as described in Table 2.

Table 2 – "Social cofactors" contributing to the onset of adverse events

Consumers' factors	Information	Interaction	Irregular products
Consumer perception that "natural is always safe"	Consumers are not sufficiently informed on PFS. Advise from friends	Consumers are not always informed on interaction between PFS and nutrients	Consumers do not take due account of the risks of illicit additions
Consumer perception that natural products have always high quality	PFSs do not require a prescription and are therefore self-administered	Consumers are not always informed on interaction between conventional drugs and nutrients	The unusual effectiveness of a natural extract does not cause alarm although this is often due to the presence of undeclared pharmacologically active molecules
Distrust of conventional medicine in favor of alternative medicines	Nutritional claims are strictly regulated in EU and label are only rarely informative		Poor public disclosure of PFS withdrawn due to irregularities unlike common foods
Belief that natural products can effectively replace drugs (distorted messages from internet)	"Alternative markets" are very active and dangerous (Gym, internet, etc.)		

The most critical issues, relating to adverse events, lie in the fact that the average consumer considers the "natural" safe and therefore does not consider botanical products as a possible source of discomfort; furthermore, it rarely tells the doctor to use it in the case of short or long term therapy.
On these bases, to reply to the EU concerns on PFS safety, an important gathering of information was carried out during and after the period of the European research project PlantLIBRA. Risk associated with PFS consumption, was analyzed collecting information from different sources:
1) critical review of the adverse effects reported in the scientific literature (case reports and human clinical studies);
2) retrospective study involving several European and a Brasilian Poison Centers;
3) assessment of adverse effects self-reported by people participating to the PlantLIBRA PFS consumer survey.
4) collection of adverse effects made available by the Pavia Poison Centre (after the end of the EU Project), ANSES and FDA.

According to PlantLIBRA results and the new collected data, *Valeriana officinalis* and *Camellia sinensis* were the most frequently cited in Europe for their role in adverse effects. Data from FDA listed *Silybum marianum* and *Serenoa repens* in the corresponding first positions. Although most case reports showed minor symptomatology, some severe events occurred, including fatalities. Symptoms involved mainly liver, gastrointestinal and nervous systems. The evaluation of the whole data confirmed that some plants are more frequently involved in adverse effects than others with a certain difference between continents. These data are very important for family doctors and other health professionals to become aware about the possible consequences of the increasing use of food supplements containing botanicals.
This information is also necessary to educate the public to prevent an unsuitable use of PFS and the possible associated adverse events.

RECOMMENDED REFERENCES

1. C. Di Lorenzo, A. Ceschi, H. Kupferschmidt, S. Lüde, E. De Souza Nascimento, A. Dos Santos, F. Colombo, G. Frigerio, K. Nørby, J. Plumb, P. Finglas, **P. Restani.** *Adverse effects of plant food supplements and botanical preparations: a systematic review with critical evaluation of causality.* Br J Clin Pharmacol 79, 578-592, 2014, DOI:10.1111/bcp.12519
2. P. Restani, C. Di Lorenzo, A. Garcia-Alvarez, M. Badea, A. Ceschi, B. Egan, L. Dima, S. Lüde, F.M. Maggi, A. Marculescu, R. Milà-Villarroel, MM. Raats, L. Ribas-Barba, L. Uusitalo, L. Serra-Majem. *Adverse effects of Plant Food Supplements self-reported by consumers in the PlantLIBRA survey involving six European countries.* PLoS One 11: e0150089, 2016, doi:10.1371/journal.pone.0150089
3. S. Lüde, S. Vecchio, S. Sinno-Tellier, A. Dopter, H. Mustonen, S.Vucinic, B. Johnsson, D. Müller, LV. Gimenez Fruchtengarten, K. Hruby, E. De Souza Nascimento, C. Di Lorenzo, P. Restani, H. Kupferschmidt, A. Ceschi. *Adverse effects of Plant Food Supplements and plant consumed as food: results from the Poisons Centres-Based PlantLIBRA Study.* Phytotherapy Research, 2016, doi:10.1002/ptr.5604, Published online in Wiley Online Library
4. C. Di Lorenzo, A. Dopter, S. Vecchio, S. Lüde, F. Colombo, F. Orgiu, **P. Restani.** *The other face of the moon: Side effects, interactions and molecules of concerns*. In: "Food supplements containing botanicals: benefits, side effects and regulatory aspects. The scientific inheritance of the EU Project PlantLIBRA". Ed.: P. Restani. Springer International Publishing AG, 2018, ISBN 978-3-319-62228, pp.141-176 - https://doi.org/10.1007/978-3-319-62229-3_5

MATERNAL ANTIBIOTIC PROPHYLAXIS AFFECTS BIFIDOBACTERIUM SPP. COUNTS IN THE HUMAN MILK, DURING THE FIRST WEEK AFTER DELIVERY

Susana Marta Isay Saad (Brazil – University of Sao Paulo, School of Pharmaceutical Sciences)
Authors: Marina Padilha[1,2], Julia M. Iaucci[1], Vanessa P. Cabral[1,3], Edna M.A. Diniz[4], C.R. Taddei[1,5] and Susana M.I Saad[1,2]
[1] *School of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, 05508-000, Brazil.*
[2] *Food Research Center (FoRC), University of São Paulo, São Paulo, SP, 05508-000, Brazil.*
[3] *School of Public Health, University of São Paulo, São Paulo, SP, 03178-200, Brazil.*
[4] *Medical School, University of São Paulo, São Paulo, SP, 01246-903, Brazil.*
[5] *School of Arts, Sciences and Humanities, University of São Paulo, São Paulo, SP, 03828-000, Brazil.*

Infant gut colonization is affected by several factors, including maternal prenatal conditions, type and conditions of labour, and if they are or not exclusively breastfed. *Bifidobacterium* spp. levels of the intestinal microbiota of exclusively breastfed infants are high, probably due to their presence in human milk, as well as to the presence of oligosaccharides. The application of maternal antibiotic prophylaxis (MAP) is increasing aiming at the prevention of Group B *Streptococcus* infection during labour, decreasing risk of maternal and new-born peripartum infections. However, the MAP might affect *Bifidobacterium* spp. levels in human milk and, consequently, in the intestinal microbiota of infants. The study to be presented will discuss the practical results obtained in a clinical study with human milk from 55 healthy lactating women volunteers (21 and 34, respectively, had and had not received MAP), at days 4-10 and 26-34 after vaginal delivery, regarding *Bifidobacterium* spp. and total bacterial levels,

IMPACT OF DIETARY FIBERS ON METABOLIC CROSS TALK BETWEEN THE SPLANCHNIC AREA AND THE MUSCLE/ADIPOSE IN MINI PIGS

Isabelle Savary-Auzeloux
INRA Clermont Ferrand Theix 63122 St Genès Champanelle

Context

Hypercaloric diets are one of the causes of the increased obesity prevalence worldwide (and particularly in western countries). Conversely, dietary fibers consumption is associated with a better health status. Yet, dietary fibers consumption in western countries (20 g/d in France or Italy) is below the values of dietary fiber intake recommended by health care organizations (30 g/d). One of the ways to increase fiber intake in populations is to supply fiber-enriched foodstuffs, and particularly food products enriched with fibers capable to improve metabolic health (i.e. fermentable fibers). The aim of our work is to determine if, in overfed minipigs, dietary fermentable fibers supplementation included in a regularly consumed food (i.e. bread), was capable to limit the obesity-linked metabolic disorders induced by overfeeding.

Methods

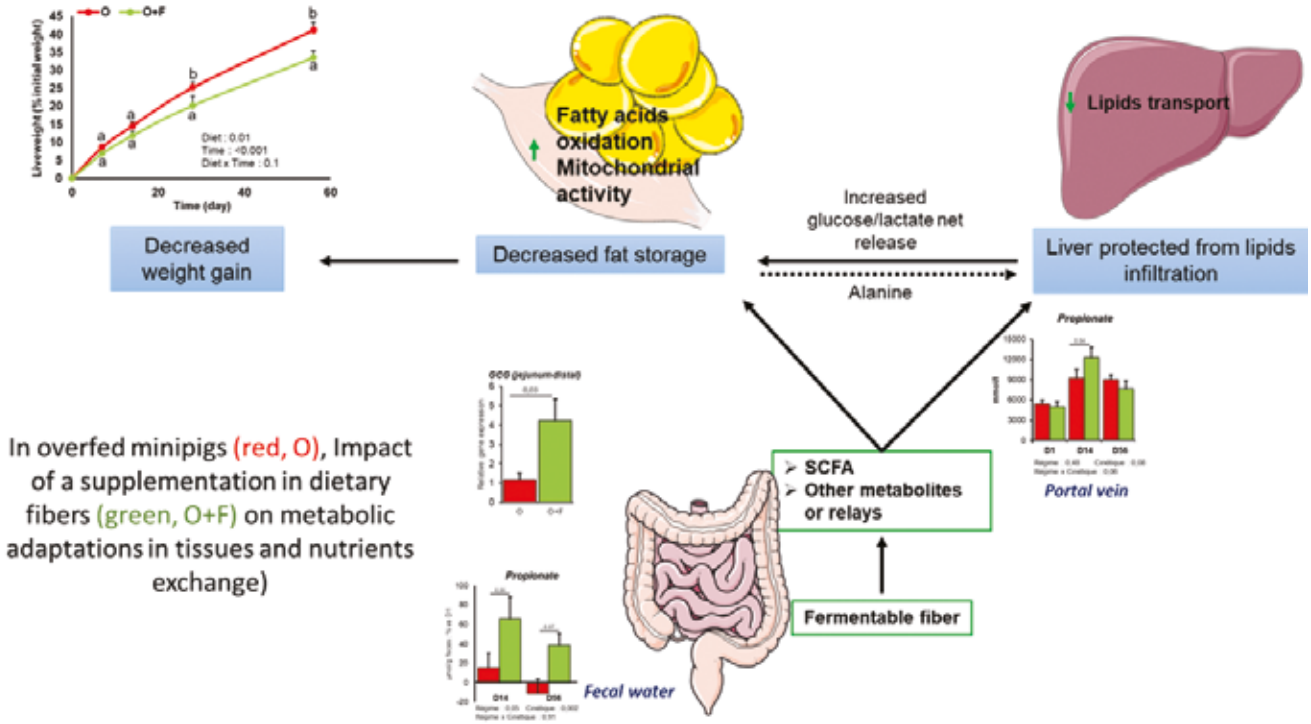
To do this, fourteen female minipigs were overfed for 2 months with a diet supplemented with sucrose (10% w/w) and palm oil (10% w/w) associated with a supplementation (or not) of fibers (25 g/d, pectin, inulin & resistant starch, Cargill®) included in a specially designed bread (250 g/d ingested). Animals were multicatheterized at the gut and hepatic levels for net splanchnic nutrients fluxes measurements over the experimental period. Feces were also sampled for microbiota composition and activity analysis. Animals were euthanized after 56 days of nutritional treatment and tissues sampled (caecum, jujunum, liver, muscle, adipose) for metabolic phenotyping (histology, qRT-PCR, proteomics).

Key results

We have shown that fermentable fibers supplementation, in a situation of overnutrition for 56 days, was capable to limit body weight gain and lipids droplets accumulation in the liver. We also observed a stimulation of the oxidative capacity in peripheral tissues (particularly the muscle). The decreased lipids droplets accumulation within the liver was due to a decreased entry of lipids (Fabp1) and a probable decreased lipogenesis (Srebp-1c). This mechanism, confirmed by the analysis of net nutrients fluxes, induced an increased bioavailability of energy nutrients to peripheral tissues. This excess of nutrients was handled by the muscle that increased its oxidative capacity (® mRNA Pgc1®, Ppar®, Nrf2, Acox, Ucp2, sdha, Cpt1-m). This altered liver-muscle cross talk could be regulated by an increased release of GLP1 and / or GLP2 by intestinal L cells (↑ mRNA jejunum GCG) and stimulation of short chain fatty acids receptor (SCFA) GPR41 in caecum (® mRNA). A direct action of SCFA directly on peripheral tissues is highly improbable and then should require relays (via activation of GPR receptors or GLPs/glucagon). These are some hypothesis we will investigate in the months to come.

Conclusion

Fermentable fibers supplementation, even during overfeeding, was beneficial for metabolic health via an impact on tissues cross talk and metabolic activities regulations. The next step would be to test a similar nutritional strategy in human being.



Keywords :
fermentable fiber, short chain fatty acid, liver, muscle, adipose tissue, obesity.

THE DEVELOPMENT OF GUT MICROBIOME-IMMUNE SYSTEM AXIS

Laura Stronati
Department of Molecular Medicine, Sapienza University of Rome, Italy

The gut microbiota (GM) is a major factor influencing both health and disease. The GM is characterized by distinctive compositional and functional features in different periods of life. The accumulated evidence has shown that the microbes found in the human body are essential for the development and maturation of the child's immune system. Moreover, they play fundamental roles in nutrition and resistance to pathogens at all ages.
The GM and the immune system codevelop around the time of birth. Each of these "organ systems" displays plasticity. The immune system can mount highly specific adaptive responses to newly encountered antigens, and the GM is affected by changes in the environment.
Attempts to reset the GM as a therapy for disease have met with varied success. Therefore, how these codeveloping systems become established is a central question relevant to our overall health.

PERSON-SPECIFIC GUT MUCOSAL COLONIZATION RESISTANCE TO PROBIOTICS AND THEIR INHIBITORY EFFECT ON POST-ANTIBIOTICS MICROBIOME RECOVERY

Jotham Suez
Immunology department, Weizmann Institute of Science, Rehovot, Israel

Empiric probiotics are commonly consumed by healthy individuals as means of life quality improvement and disease prevention. However, evidence of probiotic gut mucosal colonization efficacy remains sparse and controversial. We metagenomically characterized the murine and human mucosal-associated gastrointestinal microbiome and found it to only partially correlate with stool microbiome. A sequential invasive multi-omics measurement at baseline and during consumption of an 11-strain probiotic combination or placebo demonstrated that probiotics remain viable upon gastrointestinal passage. In colonized, but not germ-free mice, probiotics encountered a marked mucosal colonization resistance. In contrast, humans featured person-, region- and strain-specific mucosal colonization patterns, hallmarked by predictive baseline host and microbiome features, but indistinguishable by probiotics presence in stool. Consequently, probiotics induced a transient, individualized impact on mucosal community structure and gut transcriptome. Colonization resistance to probiotics is partially alleviated following antibiotics treatment in mice and humans, a common scenario in which probiotics are widely prescribed for prevention of antibiotics-associated dysbiosis and related adverse effects. However, probiotic impact on post-antibiotic reconstitution of the gut mucosal host-microbiome niche remains elusive. We invasively examined the effects of multi-strain probiotics or autologous fecal microbiome transplantation (aFMT) on post-antibiotic reconstitution of the murine and human mucosal microbiome niche. Compared to spontaneous post-antibiotic recovery, probiotics induced a markedly delayed and persistently incomplete indigenous stool/mucosal microbiome reconstitution and host transcriptome recovery toward homeostatic configuration, while aFMT induced a rapid and near-complete recovery within days of administration. In vitro, Lactobacillus-secreted soluble factors contributed to probiotics-induced microbiome inhibition. Collectively, empiric probiotics supplementation may be limited in universally and persistently impacting the gut mucosa, and is associated with a previously underappreciated inhibitory effect on post-antibiotics gut mucosal recovery, highlighting a need of developing aFMT or personalized probiotic approaches achieving mucosal protection without compromising microbiome recolonization in the antibiotics-perturbed host.

METAGENOMIC ANALYSIS OF COLORECTAL CANCER DATASETS IDENTIFIES CROSS-COHORT MICROBIAL DIAGNOSTIC SIGNATURES AND A LINK WITH CHOLINE DEGRADATION

Adrian James Tett PhD
Department of Cellular, Computational and integrative Biology (CIBIO)
University of Trento, TN, 38123

Several studies have investigated links between the gut microbiome and colorectal cancer (CRC), but questions remain about the replicability of biomarkers across cohorts and populations. We performed a meta-analysis of five publicly available datasets and two new cohorts and validated the findings on two additional cohorts, considering in total 969 fecal metagenomes. Unlike microbiome shifts associated with gastrointestinal syndromes, the gut microbiome in CRC showed reproducibly higher richness than controls ($P < 0.01$), partially due to expansions of species typically derived from the oral cavity. Meta-analysis of the microbiome functional potential identified gluconeogenesis and the putrefaction and fermentation pathways as being associated with CRC, whereas the stachyose and starch degradation pathways were associated with controls. Predictive microbiome signatures for CRC trained on multiple datasets showed consistently high accuracy in datasets not considered for model training and independent validation cohorts (average area under the curve, 0.84). Pooled analysis of raw metagenomes showed that the choline trimethylamine-lyase gene was overabundant in CRC ($P = 0.001$), identifying a relationship between microbiome choline metabolism and CRC. The combined analysis of heterogeneous CRC cohorts thus identified reproducible microbiome biomarkers and accurate disease-predictive models that can form the basis for clinical prognostic tests and hypothesis-driven mechanistic studies.

GUT DYSBIOSIS AND LIVER DISORDERS

Herbert Tilg
Department of Internal Medicine I, Gastroenterology, Hepatology, Metabolism & Endocrinology
Corresponding author:
Herbert Tilg, M.D., Department of Internal Medicine I, Gastroenterology, Hepatology, Metabolism & Endocrinology, Medical University Innsbruck, Innsbruck, Austria

The human intestinal tract contains an enormous number of bacteria, archaea and viruses[1]. The development of modern molecular and sequencing technologies allowed recently deeper insights into this world[2]. Whereas it has been thought that the genes of this microbial world mainly control functions regarding digestion of complex carbohydrates, it became evident that the intestinal microbiota has major functions in directing metabolic, immune and inflammatory pathways in health and disease[3]. Furthermore, and this seems crucial for most liver diseases the gut microbiota also contributes to the integrity of the intestinal epithelial barrier[4].
Recent research efforts have enabled to gain further insights into the role of the intestinal microbiota in various liver diseases. Non-alcoholic fatty liver disease (NAFLD) is one of the leading liver diseases worldwide. The pathophysiology of this disorder is rather complex involving diverse immunological and metabolic pathways. Recent evidence suggests that the intestinal microbiota and related metabolites are also crucially involved in this disorder. Preclinical studies have shown that germ-free mice do not develop obesity and hepatic steatosis. Several large studies suggest that certain bacteria are overwhelmingly present in these patients such as Proteobacteria or *Escherichia coli*. In contrast, rather protective bacteria such as *Faecalibacterium prausnitzii* are rather missing in NAFLD patients. A change in the intestinal microbiota has been observed also in other chronic liver diseases such as alcoholic liver disease [5] and especially in liver cirrhosis and associated complications such as hepatic encephalopathy (HE) and hepatocellular carcinoma (HCC). Various studies have now investigated the intestinal microbiome landscape in liver cirrhosis convincingly showing that especially bacteria with pro-inflammatory features are dominating. This also suggests that such bacteria might contribute to the pro-inflammatory nature of various advanced liver diseases. The main complications of liver cirrhosis such as HE and HCC are also associated with dysbiosis and especially in HE it is well established that the use of various pre- and prebiotics is clinically effective. This is also supported by first data suggesting that fecal microbial transplantation could be of clinical benefit in this disorder[6]. Overall, data from the last years highlight the crucial role of the intestinal microbiota in early and advanced liver diseases independent of etiology of underlying liver disease.

References

1. Cani, P. D. Human gut microbiome: hopes, threats and promises. Gut 2018;67:1716-25.
2. Pasolli, E., Asnicar, F., Manara, S., et al. Extensive Unexplored Human Microbiome Diversity Revealed by Over 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle. Cell 2019;176:649-62 e20.
3. Gilbert, J. A., Blaser, M. J., Caporaso, J. G., et al. Current understanding of the human microbiome. Nat Med 2018;24:392-400.
4. Bischoff, S. C., Barbara, G., Buurman, W., et al. Intestinal permeability--a new target for disease prevention and therapy. BMC Gastroenterol 2014;14:189.
5. Grander, C., Adolph, T. E., Wieser, V., et al. Recovery of ethanol-induced Akkermansia muciniphila depletion ameliorates alcoholic liver disease. Gut 2018;67:891-901.
6. Bajaj, J. S., Fagan, A., Gavis, E. A., et al. Long-term Outcomes of Fecal Microbiota Transplantation in Patients With Cirrhosis. Gastroenterology 2019;156:1921-3 e3.

VIRAL MICROBIOTA AND IBD

Federica Ungaro[1,2], Luca Massimino[3], Silvia D'Alessio[1,2], and Silvio Danese[1,2]
[2] *Department of Biomedical Sciences, Humanitas University, Milan, Italy*
[3] *Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy*

During birth and since the first days of our life, the gastrointestinal (GI) tract starts being colonized by microbial species, constituting the gut microbiota. The microbiota composition unceasingly changes up to adulthood, when it becomes more stable. Nevertheless, environment, diet, daily habits, and antibiotic treatments impact on this stability, modulating the microbiome composition throughout the lifespan and eventually influencing the host in health and disease. The existence of a microbiota populating our GI has favorable effects on our health as in the case of *Bifidobacterium* and *Lactobacillus*, that have been shown to protect enterocytes from an acute inflammatory response counteracting enteropathogen infections. Moreover, the microbiota ensures gut homeostasis by modulating immune functions, and contributes to the absorption of nutrients from food, sometimes cooperating with the host for their metabolism. Therefore, it is easy to imagine that the interruption of the balance between the gut microbiota and the host may lead to pathological conditions, such as Inflammatory Bowel Disease (IBD).
IBD is a class of diseases, comprising Crohn's disease (CD) and ulcerative colitis (UC), mainly characterized by an exacerbated immune response due to the gut epithelial barrier disruption and bacterial translocation from the lumen to the mucosal tissue. This event leads to the activation of both innate and adaptive immune responses, ultimately failing to be resolved and leading to chronic inflammation.

NGS: the breakthrough for microbiota discovery

Although the consistent attention was given to the microbiota contribution in IBD pathogenesis in the past, more exhaustive works elucidating this aspect have come out only in the recent years after Next Generation Sequencing (NGS) has established.
Even if bacterial culture-based systems allowed to define quite extensively and with high accuracy the bacterial genus and species abundance, they were time-consuming because of protocol optimization and low-throughput. Moreover, many entities populating the gut microbiota, such as viruses, archaea, and yeasts, were neglected because of the impossibility to create a dedicated composite culture condition *in vitro* sustaining the growth of different microorganisms at the same time.
The advent of system biology posed the first milestone for the composite and detailed study of the microbiota.
First studies exploiting NGS defined the bacterial diversity within microbiota by prokaryotic 16S rRNA gene targeted-sequencing (metataxonomics). These are highly evolutionary conserved genes displaying hypervariable regions that allow researchers to analyze the differential microbial enrichment within a biological sample.
A more unbiased approach, exploiting untargeted sequencing, was the shotgun metagenomics, that explores the whole DNA in an environment. Differently from the metataxonomics, where barcoded primer sets target the 16S variable regions, the metagenomic approach is based on the fragmentation of the DNA, its barcoding with random primers, sequencing, and analysis via dedicated bioinformatic pipelines.
Perhaps the great limitation of metagenomics is represented by the analysis of DNA sequences only, thus ignoring RNAs. To date, the metatranscriptomics embodies the unique useful approach to overcome this issue. In fact, by analyzing the RNA composition of a biological sample (such as gut mucosa) and by exploiting cutting-edge NGS technologies, the analysis of the whole transcriptome may point out new microbial entities (such as RNA-based viruses).
It is worth to note that metataxonomics, metagenomics, and metatranscriptomics share the same limitations: *i) they must rely on accurate databases featuring the different genomes and their annotations, otherwise the analysis remains elusive and may lose some important information; ii) they need to be performed on purified RNA and DNA samples, but sometimes the RNA or DNA yields are not sufficient to cover some less represented entities of the microbiota; moreover DNA and RNA residual molecules from the host may remain in the sample after purification, producing misleading results; iii) the depth of the sequencing has to be by very high, mainly for metatranscriptomics, to ensure reliable results, and this might be costly.*
Nevertheless, although this field needs improvements, the results obtained are very promising. In fact, being the advances in NGS very quick, in few years all these limitations will be overcome, and better and more accurate results may come soon. Once the viral species infecting patients in the early phase of intestinal inflammation will be identified and characterized, it will be possible on one hand to identify early biomarkers predictive of the disease, and on the other to engineer specific siRNAs or antiviral drugs to treat IBD patients thus representing a real break-through in the field.

Neglected actors of the gut microbiota in intestinal chronic inflammation

More commonly, the human intestine is meant to be populated by bacteria, being the majority of the microbes colonizing our GI. With a variety of more than 1000 species, the main bacterial *phyla* composing the adult gut microbiota are the *Bacteroidetes* and *Firmicutes*, followed by *Actinobacteria* and *Proteobacteria*. Here, substantial shifts in the overall microbiota composition have been associated with IBD etiopathogenesis, whose most relevant feature is the reduction of bacterial diversity in the microbiota structure and lower proportions of *Bacteroidetes* and *Firmicutes*, coupled with higher levels of *Proteobacteria*.
A key aspect that deserves consideration is whether the intestinal microbiota changes associated with IBD pathogenesis are causes or consequences of this disease. For example, in transgenic mouse strains, like *T-bet–/– x Rag2–/–* animals, used for spontaneous colitis investigation, *Proteus mirabilis* and *Klebsiella Pneumoniae* correlated with colonic inflammation symptoms, whereas in *Il10-/-* mice, *Bilophila Wadsworthia*, normally found as a minor component of the gut commensalism, was associated with colitis development. More interestingly, *Fusobacterium Varium* isolated from the colonic mucosa of UC patients, displayed the capability of disrupting epithelial barrier in mice, raising the questions whether some microbiota components may trigger colitis.
Alongside bacteria, another consistent but neglected proportion of the gut microbiota is made of other species colonizing the GI, such as fungi, eukarya, and viruses, all actively interacting with each other and impacting on bacterial composition. Fungi normally colonize GI of healthy subjects, namely the gut mycome, impacting on the immune system and producing specific metabolites, and some of them may have a beneficial role, such as *Saccharomyces Cerevisiae*, used as probiotic to treat GI disorders. By contrast, the fungal entity *Candida tropicalis* was linked to CD dysbiosis and the presence of circulating anti-*Saccharomyces cerevisiae* antibodies correlated with its abundance.

Another big portion of the gut microbiota is composed of viruses, infecting both prokaryotic and eukaryotic cells, that form the gut virome. While prokaryotic viruses actively interact with and impact on the bacterial composition of the microbiome, eukaryotic viruses can also integrate into the host genome, ultimately influencing its intestinal cells. Prokaryotic-infecting viruses are 10-fold more abundant than bacteriawhich metagenomic analysis shows are mostly unique to each individual. To investigate the origin and evolution of the human gut virome, we analyzed the viral community of one adult individual over 2.5 y by extremely deep metagenomic sequencing (56 billion bases of purified viral sequence from 24 longitudinal fecal samples, thus meaning that their existence within the microbiota strongly contributes to the modification of the different proportions of bacterial strains that live within the intestine. This mutual commensalism between bacteria and viruses has growingly attracted interest so that some studies are now dedicating more attention to the role that viruses may have in regulating gut homeostasis and in GI disorders. For example, by targeted deep-sequencing analysis of stools from patients with CD and UC, Norman and colleagues demonstrated not only that the virome composition was disease- and cohort-specific, but also that its variations contributed to intestinal dysbiosis. However, this study based on metagenomic analysis of DNA sequences, ignored RNA viruses, such as norovirus and astrovirus, previously suggested as potential precipitators or triggers of intestinal inflammation.

Alongside prokaryotic-infecting viruses, eukaryotic-targeting viruses have been demonstrated to be associated with IBD pathogenesis. The most investigated eukaryotic viral candidates are the cytomegalovirus (CMV) and the Epstein–Barr virus (EBV). To date, their involvement in IBD pathogenesis has yet to be elucidated as their reactivation may result from immunosuppression or stressing conditions common in IBD patients and therefore might be mere bystanders instead of real triggers for this disease. In this regard, the effective role of virome in IBD aetiogenesis has yet to be discovered, even if some evidence depicting eukaryotic viruses as potential initiators of intestinal inflammation are available. In fact, in the il10 knockout model of spontaneous colitis, the Norovirus infection was discovered as a potent colitogenic factor, strongly depending on the presence of enteric microbiota. Similarly, results from IBD-susceptibility gene *Atg16L1*^{HM} mouse models demonstrated that Norovirus infection contributes to the development of intestinal inflammation. Both studies thus showed the synergistic effect between genetic background and Norovirus infection as a precipitator of intestinal inflammation, fastening colitis development. Notably, these studies emphasized only enterotropic viruses, normally localizing at the level of the GI. By contrast, our recent study exploiting state-of-the-art metagenomic pipelines on a large cohort of IBD patients showed that RNAs of eukaryotic viruses, with a physiological hepatic tropism, were surprisingly detected within intestinal mucosa of IBD patients. In detail, CD patients' intestinal mucosa was colonized by RNAs belonging to *Hepeviridae*, a family of RNA eukaryotic viruses normally causing hepatitis in mammalians, while UC patients displayed *Hepadnaviridae* transcripts (Hepatitis B Virus belongs to this DNA eukaryotic viral family). These two families were defined to be likely in charge of initiation of intestinal inflammation since the metagenomics described in this work was performed on patients in *i*) young age, without previous GI infections, *ii*) treatment naïve and *iii*) at their first diagnosis. All these inclusion criteria allowed to i) reduce lifespan frame during which active, but also silent viral infections may occur, and which may influence the study outcome; *ii*) highlight only viruses activated independently of immunosuppression, thus excluding any pharmacological effects on virome composition; iii) better identify those viral entities responsible for the initial stages of the disease. These findings introduce a quite new concept in the field and the occurrence of not enterotropic viral infections in intestinal cells opens new perspectives in IBD aetiopathogenesis. The notion stating that viruses may be the early triggers for a disease has been already explored in previous studies, and clinical evidence is already available. For example, the role of viruses in causing and sustaining diseases has been confirmed for tumors, so that viral infections are known to be responsible for about 20% of the global cancer burden. In fact, HBV, hepatitis C virus, human papillomavirus, human herpes virus 8, Merkel cell polyomavirus, and HTLV-1 are responsible for the 80% of hepatocellular carcinomas. In all these cases, however, viral infections occur in the tissues in which cancer develops, because their opportunistic lifecycle leads to uncontrolled proliferation, and finally to tumorigenesis. By contrast, based on our last work, not enterotropic eukaryotic viral entities are supposed to infect mucosa apparently without symptomatic manifestation. However, their presence probably confers to the host a virotype that might latently stimulate its mucosal immune response and, in turn, initiates IBD aetiogenesis. To the best of our knowledge, neither scientific nor clinical demonstrations confirming these novel and exceptional insights for IBD aetiogenesis are available. Once the viral species infecting patients in the early phase of intestinal inflammation will be identified and characterized, it will be possible on one hand to identify early biomarkers predictive of the disease, and on the other to engineer specific siRNAs or antiviral drugs to treat IBD patients thus representing a real break-through in the field.

PROCESSING OR ULTRAPROCESSING: DOES IT MAKE A DIFFERENCE FOR HEALTH?

Tiny van Boekel
Food Quality & Design Group, Wageningen University & Research, the Netherlands

In many countries, food production has been largely industrialized over the past century and from the viewpoint of food security, food safety and food quality (in short: nutrition security) the situation has much improved. Many of the foods that are consumed nowadays are processed one way or the other. The benefits of processing are obvious from a scientific point of view (see, for instance, van Boekel et al, 2010): it improves food safety, prolongs shelf life, improves palatability and digestion, prevents waste, makes it relatively cheap. However, the public perception of processing is rather different in that processed foods should be avoided as much as possible. This is, at least partly, caused by the very complex and untransparent food chain resulting in distrust towards the food industry. Even though nutrition security has much improved, there are also undeniable problems to tackle, mainly with respect to obesity and sustainability of food production. In this lecture, we pay attention to over- as well as undernutrition in relation to processing. Globally, many people are still undernourished, but at the same time obesity is one of the biggest problem, also coming up strongly in developing countries. While it is certainly true that there is an association between the advent of industrially produced food and the rise of obesity, the question is whether there is also a direct causal relation with the food. This lecture will try to address that question.
Recently, a nutritionist group in Brazil (Monteiro et al, 2017) has published a classification system that categorizes food in four groups, the so-called Nova classification, see Table 1.

Table 1. An impression of the Nova classification (after Monteiro et al, 2017)

Category	Definition and examples
Unprocessed or minimally processed foods	Edible parts of plants and animals, algae, fungi (e.g., fruits and vegetables, grains, eggs, milk, nuts, seeds)
Processed culinary ingredients	Substances obtained from unprocessed foods (e.g., butter, salt, sugar)
Processed foods	Processed to prolong shelf life and enhancing of sensorial properties (e.g., canned vegetables, cured products, bread, beer, wine)
Ultra-processed foods	Industrial formulations with more than 5 ingredients (e.g., ready-to-eat products, snacks, sodas, instant sauces, infant formulas, pizza's, burgers, whiskey, rum

This classification scheme receives quite some attention from nutritionists as a possible way to give nutrition advice. Especially, the fourth category of ultra-processed food is seen as the cause of many problems and the advice is then to avoid such products as much as possible. A recent study of Hall et al (2019) drew quite some attention; they did an intervention study with volunteers who were put on a diet of ultra-processed food for two weeks and on a diet of unprocessed foods for another two weeks. The result was that eating ultra-processed foods resulted in weight increase (1 kg over two weeks) while the group consuming unprocessed food lost weight (1 kg over two weeks). This seems to prove the statement that ultra-processed foods should be avoided if the goal is to combat obesity. On the other hand, the results were not unexpected because the diet containing ultra-processed food was energy-dense. Some criticism appeared shortly afterwards (Ludwig et al., 2019). The Nova classification as such was earlier also criticized (Gibney et al, 2017, 2019). The main problem is that the classification makes a link with processing, resulting in the advice that people should avoid ultraprocessed foods as much as possible. This is in conflict with the earlier mentioned benefits of processing in terms of nutrition security. It is rather obvious that a diet containing energy-dense foods with little fresh fruit and vegetables will eventually lead to weight gain. So, the problem seems to be much more linked to food composition and an unbalanced diet than the fact that food is processed (Pelligrini & Fogliano, 2017). The question is thus what it has to do with processing. Of course, food technology makes it possible to produce foods that are not really contributing to a healthy diet. It concerns foods that have a high caloric content, are high in sugar and fat, and lack essential nutrients, but are appealing to consumers. But the Nova classification also puts foods in the ultra-processed category that do have an essential contribution, for instance, infant foods, protein-enriched foods, meat-alternatives. So, the problem seems to be not so much in the processing itself, but in the composition of certain foods that, if consumed at high amounts, lead to unbalanced nutrition. A much bigger problem than processing seems to be the fact that foods are available at any time of the day, everywhere and at a relatively low price. This creates an obesogenic society where people are tempted to eat anytime.
So, to answer the question whether or not (ultra)processing does affect health, the following can be said. Processing does have an effect on health in the sense that foods can be made safe, palatable and better digestible, so processing has a positive effect on health. Besides that, processing helps also to combat food waste and to achieve sustainable food production. The term 'ultraprocessing' is a misnomer by creating a negative connotation about processing with the public. However, processing has made the obesogenic society possible, and that has a negative impact on health, so the focus should be on how to combat that phenomenon. It is clear that the food industry has a role to play here, not by moving away from food processing but by actively contributing in helping consumers to compose a healthy diet, that can be achieved by moderate consumption and a varied offer on foods. Some examples on how that can be achieved is by developing products that contain less sugar and less salt (Mozaffarian, 2018), less calories, and that require more oral processing (Aguayo-Mendoza et al, 2019) so that people do not eat too much. Another action could be to reduce portion size. All this requires product and process design, so that in the end processing does indeed contribute to a healthy diet.

References

1. Aguayo-Mendoza, M. G., Ketel, E. C., van der Linden, E., Forde, C. G., Piqueras-Fiszman, B., & Stieger, M. (2019). Oral processing behavior of drinkable, spoonable and chewable foods is primarily determined by rheological and mechanical food properties. *Food Quality and Preference*, *71*, 87-95. https://doi.org/10.1016/j.foodqual.2018.06.006
2. Gibney, M. J, Forde, C.G. , Mullally, D., Gibney, E.R. Ultra-processed foods in human health: a critical appraisal. *Am. J. Clin. Nutr*. 106(2017)717-724
3. Gibney, M.J. Ultra-processed foods: definitions and policy issues. *Current Developments in Nutrition* 3(2019)nzy077
4. Hall, K.D. et al. Ultra-processed diets cause excess calorie intake and weight gain: an inpatient randomized controlled trial of *Ad Libitum* food intake. *Cell Metabolism* 30(2019)1-11
5. Ludwig,D.S., Astrup,A., Bazzano, L.A., Ebbeling, C.B., Heymsfield, S.B., King, J.C., Willett, W.C.. Ultra-Processed Food and Obesity: The Pitfalls of Extrapolation from Short Studies. Cell Metabolism Volume 30, Issue 1, 2 July 2019, Pages 3-4. https://doi.org/10.1016/j.cmet.2019.06.004
6. Monteiro C.A., Cannon G,, Moubarac J.C., Levy R.B., Louzada, M.L.C., Jaime, P.C. The UN Decade of Nutrition, the NOVA food classification and the trouble with ultra-processing. *Public Health Nutr* 21(2018)5–17.
7. Mozaffarian, D. History of modern nutrition science: implications for current research, dietary guidelines, and food policy. *BMJ* 361(2018)1-6
8. Pellegrini, N., & Fogliano, V. (2017). Cooking, industrial processing and caloric density of foods. *Current Opinion in Food Science*, *14*, 98-102. https://doi.org/10.1016/j.cofs.2017.02.006
9. Van Boekel, M.A.J.S., Fogliano, V., Pellegrini, N., Stanton, C. Scholz, G., Lalljie, S., Somoza, V., Knorr, D., Rao Jasti, P., Eisenbrand, G. A review on the beneficial aspects of food processing. *Mol. Nutr. Food Res.* 54(2010) 1215–1247

THE MICROBIOME IN CYSTIC FIBROSIS-PROGNOSTIC ASPECTS

Michael Wilschanski
Director, Pediatric Gastroenterology, Hadassah University Hospitals, Mt. Scopus POBox 24035, Jerusalem, 91240 Israel

One of the most exciting developments in research in recent years relate to our understanding of the gut microbiota and how they contribute to health and disease states.

The microbiome supplies important ecosystem services, which benefit the microbes as well as the host[1]. There are inter-species and inter-kingdom interactions, influencing the burden and the physiology of microbial cells [2]. These interactions lead to the production of important resources, bioconversion of nutrients, and protection against pathogenic microbes [3]. The microbiome may play a role in a variety of diseases, potentially when a microbial community is deficient in a beneficial function or because of the presence of a detrimental microbial activity. Strategies of restoration of a beneficial microbial structure or function could potentially offer a novel treatment in the era of personalized medicine. Several strategies have been proposed, although success to date has been restricted to a few conditions and therapies [3].

Cystic Fibrosis is a genetic disease with shortened life expectancy associated with progressive lung disease, gastrointestinal (GI) involvement and an excessive inflammatory response.

The gastrointestinal tract is the earliest system to be affected by CF with a significant proportion of neonates having pathology. Most CF infants are born with exocrine pancreatic insufficiency. Loss of bicarbonate –rich pancreatic fluids alter the intestinal milieu. The lack of this major power of neutralisation contibutes to gastrointestinal symptoms in CF. A major consequence of the altered luminal environment is the accumulation of mucus in the CF intestine. Most CF patients complain of abdominal pain, bloating, changes in bowel habit and these common symptoms are probably secondary to the changed microbiota. In this lecture I will show that the unique symptom of CF named Distal Intestinal Obstruction Syndrome may also be secondary to dysmotility and altered microbiome.

Several studies assessed CF microbiome in sputum and in stool. CF lung microbiome contains a diverse community and changes in the structure and activity of these communities influence pulmonary disease progression [4]. In a serial collection of 126 sputum cultures from six adult CF patients, those with a relatively stable pulmonary status exhibited stable bacterial community and diversity; while those with a progressive decline in pulmonary function exhibited a parallel loss of airway community diversity [5]. Changes in the lung microbiome were patient-specific 6. There is also increasing evidence that the CF-GI microbiome is altered, and that this dysbiosis contributes to disease manifestations in many organs, both within and beyond the GI tract [7,8]. Two studies characterized the microbiota in CF and found relative depletions in several taxa in the *Bifidobacterium* and *Clostridium* genera as well as lower overall species richness [9,10]. Isolates of Enterobacteriaceae cultured from the stool of children with CF tended to be less susceptible to beta-lactams, highlighting the markedly higher antibiotic exposure that children with CF experience. Schippa et al. [11] found in CF patients that fecal microbiota correlated with severity of CFTR mutation. Expansions of potentially harmful Proteobacteria species (such as *Escherichia coli* and *Eubacterium biforme*) and depletions in health-associated taxa (such as *Eubacterium limosum, Faecalibacterium prasunitzii*, and *Bifidobacterium* spp.) were found [12]. One of the therapeutic strategies is to replace the "missing" elements. Probiotics, when administered in adequate amounts, may confer a health benefit on the host [3]. Potential mechanisms of action include changes in respiratory and stool microbiome, thereby affecting respiratory and GI symptoms and systemic inflammation [13]. A systematic review examined the effect of probiotics on respiratory, GI and nutritional status in CF patients, and showed a reduction in pulmonary exacerbations with an improvement in subjective GI symptoms [13]. Two studies measured the effect of probiotics on fecal microbiota composition, and showed a significant increase in the proportion of Bacteroidetes and Firmicutes [14] and a significant increase in Bacteroides [15]. Recently, in a pilot study from the ESPGHAN Working Group, probiotic supplementation did not influence fecal calprotectin, pulmonary function, pulmonary exacerbations and microbiome. However, 13% of patients normalized their gut permeability [16].

The aim of this lecture is to review the literature on changes in the microbiome in Cystic Fibrosis and to predict the effect of probiotics administration on stool and sputum microbiome, as well as respiratory, GI and inflammatory parameters in CF patients.

Characterization of the microbiome will enable personalized medicine. Strategies of restoration of a beneficial microbial structure or function could potentially offer novel treatment options

References

1. Johnson-King B, Terry SF. Future of Microbiomes Through the National Microbiome Initiative. *Genet Test Mol Biomarkers.* 2016;20(10):561-562. doi:10.1089/gtmb.2016.29022.sjt
2. Lynch S V. The lung microbiome and airway disease. *Ann Am Thorac Soc.* 2016;13(December):S462-S465. doi:10.1513/AnnalsATS.201605-356AW
3. Young VB. The role of the microbiome in human health and disease: an introduction for clinicians. *Bmj.* 2017:j831. doi:10.1136/bmj.j831
4. Huang YJ, LiPuma JJ. The Microbiome in Cystic Fibrosis. *Clin Chest Med.* 2016;37(1):59-67. doi:10.1016/j.ccm.2015.10.003
5. Zhao J, Schloss PD, Kalikin LM, et al. Decade-long bacterial community dynamics in cystic fibrosis airways. *Proc Natl Acad Sci U S A.* 2012;109(15):5809-5814. doi:10.1073/pnas.1120577109
6. Whelan FJ, Heirali AA, Rossi L, Rabin HR, Parkins MD, Surette MG. Longitudinal sampling of the lung microbiota in individuals with cystic fibrosis. *PLoS One.* 2017;12(3):1-17. doi:10.1371/journal.pone.0172811
7. Flass T, Tong S, Frank DN, et al. Intestinal lesions are associated with altered intestinal microbiome and are more frequent in children and young adults with cystic fibrosis and cirrhosis. Beekman JM, ed. *PLoS One.* 2015;10(2):e0116967. doi:10.1371/journal.pone.0116967
8. Werlin SL, Benuri-Silbiger I, Kerem E, et al. Evidence of intestinal inflammation in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr.* 2010;51(3):304-308. doi:10.1097/MPG.0b013e3181d1b013
9. Duytschaever G, Huys G, Bekaert M, Boulanger L, De Boeck K, Vandamme P. Cross-sectional and longitudinal comparisons of the predominant fecal microbiota compositions of a group of pediatric patients with cystic fibrosis and their healthy siblings. *Appl Environ Microbiol.* 2011;77(22):8015-8024. doi:10.1128/AEM.05933-11
10. Duytschaever G, Huys G, Bekaert M, Boulanger L, De Boeck K, Vandamme P. Dysbiosis of bifidobacteria and Clostridium cluster XIVa in the cystic fibrosis fecal microbiota. *J Cyst Fibros.* 2013;12(3):206-215. doi:10.1016/j.jcf.2012.10.003
11. Schippa S, Iebba V, Santangelo F, et al. Cystic fibrosis transmembrane conductance regulator (CFTR) allelic variants relate to shifts in faecal microbiota of cystic fibrosis patients. Vij N, ed. *PLoS One.* 2013;8(4):e61176. doi:10.1371/journal.pone.0061176
12. Hoffman LR, Pope CE, Hayden HS, et al. Escherichia coli dysbiosis correlates with gastrointestinal dysfunction in children with cystic fibrosis. *Clin Infect Dis.* 2014;58(3):396-399. doi:10.1093/cid/cit715
13. Anderson JL, Miles C, Tierney AC. Effect of probiotics on respiratory, gastrointestinal and nutritional outcomes in patients with cystic fibrosis: A systematic review. *J Cyst Fibros.* 2017;16(2):186-197. doi:10.1016/j.jcf.2016..004
14. del Campo R, Garriga M, Pérez-Aragón A, et al. Improvement of digestive health and reduction in proteobacterial populations in the gut microbiota of cystic fibrosis patients using a Lactobacillus reuteri probiotic preparation: a double blind prospective study. *J Cyst Fibros.* 2014;13(6):716-722. doi:10.1016/j.jcf.2014.02.007
15. Bruzzese E, Callegari ML, Raia V, et al. Disrupted intestinal microbiota and intestinal inflammation in children with cystic fibrosis and its restoration with Lactobacillus GG: a randomised clinical trial. Bereswill S, ed. *PLoS One.* 2014;9(2):e87796. doi:10.1371/journal.pone.0087796
16. Van Biervliet S, Hauser B, Verhulst S, et al. Probiotics in cystic fibrosis patients: A double blind crossover placebo controlled study: Pilot study from the ESPGHAN Working Group on Pancreas/CF. *Clin Nutr ESPEN.* 2018;27:59-65. doi:10.1016/j.clnesp.2018.06.008

ORAL COMMUNICATIONS

EFFECTS OF RESVERATROL IN A MOUSE MODEL OF ALCOHOL ADDICTION

Marco Fiore [1] - Carla Petrella [1] - Fausta Natella [2] - Mauro Ceccanti [3]
Institute of Cell Biology and Neurobiology, IBCN-CNR, Rome, Italy [1] - CREA-AN, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Food and Nutrition Research Centre, Rome, Italy [2] - Centro Alcologico della Regione Lazio (CRARL), Department of Clinical Medicine, Sapienza University of Rome, Italy [3]

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

PUTATIVE PROBIOTIC LACTIC ACID BACTERIA ISOLATED FROM GREEK PROTECTED DESIGNATION OF ORIGIN CHEESES USING TRADITIONAL AND METAGENOMIC MICROBIOLOGICAL ANALYSIS

Jonathan Rhoades [1] - Sofia Michailidou [2] - Iro Anastasiou [1] - Anagnostis Argiriou [2] - Eleni Likotrafiti [1]
International hellenic university, food science and technology, thessaloniki, greece [1] - center for research and technology hellas, institute of applied biosciences, thessaloniki, greece [2]

Objective

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

Methods

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

Results

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

PRODUCTION OF FRUIT BASED DRINKS AS CARRIERS OF PROBIOTIC BACTERIA LACTOBACILLUS RHAMNOSUS SP

Perihan Kubra AKMAN [1] - Fatih Törnük [1] - Hasan Yetim [2]
Yildiz Technical University, Food Engineering Department, Istanbul, Turkey [1] - Sabahattin Zaim University, Food Engineering Department, Istanbul, Turkey [2]

Objective

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

Methods

Sour cherry, apricot and peach juices were pasteurized at 65 °C for 30 min after adjusting the fruit concentration to 35%, 40% and 50% (w/v), respectively and adding sugar of 12% (w/v). The pasteurized juices were inoculated with probiotic *Lactobacillus rhamnosus* 99, which was precultured at 37 °C overnight in MRS broth, at a targeted level of 10^9-10^{10} cfu/mL, and then they were fermented at 37 °C for 48 h. During the fermentation, survival of *L. rhamnosus* 99, pH changes and sensory properties were evaluated at 24h intervals.

Results

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

Conclusions

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

GUT MICROBIOTA COMPOSITION AND IMMUNITY OF AGEING MICE SUPPLEMENTED WITH COW MILK CONTAINING A DIFFERENT CASEIN PROFILE

Barbara Guantario [1] - Paola Zinno [1] - Alberto Finamore [1] - Vincenzo Motta [2] - Paolo Trevisi [2] - Marzia Giribaldi [3] - Laura Cavallarin [4] - Marianna Roselli [1] - Chiara Devirgiliis [1]
CREA, Centro Ricerca Alimenti e Nutrizione, Roma, Italy [1] - Università di Bologna, Dipartimento di Scienze e Tecnologie Agro-alimentari, Bologna, Italy [2] - CREA, Centro ricerca Ingegneria e Trasformazioni Agroalimentari, Torino, Italy [3] - CNR, Istituto di Scienze delle Produzioni Alimentari, Torino, Italy [4]

Objective

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

Methods

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

Results

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

Conclusions

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

VITAMIN D RECEPTOR CONTRIBUTES TO THE HEALTH BENEFITS OF PROBIOTIC CONSUMPTION

Carolina Battistini [1] - Yong-guo Zhang [2] - Ishita Chatterjee [2] - Rong Lu [2] - Jilei Zhang [2] - Susana M I Saad [1] - Jun Sun [2]
University of São Paulo, School of Pharmaceutical Sciences / Department of Pharmaceutical and Biochemical Technology / FoRC - Food Research Center, São Paulo, Brazil [1] - University of Illinois at Chicago, Department of Medicine, Chicago, United States [2]

Objective

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

Methods

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

Results

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

Connclusions

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

FORTUNELLA MARGARITA: THE CITRUS FRUIT OF THE MOMENT? INVESTIGATION OF BIOACTIVE COMPOUNDS: VITAMINS, MACROELEMENTS AND POLYPHENOLS

Clarice Silva e Souza [1] - Pamella Cristine Anunciação [2] - Ceres Mattos Della Lucia [2] - Rosana Gonçalves Rodrigues das Dôres [3] - Regina Célia Rodrigues de Miranda Milagres [2] - Helena Maria Pinheiro-Sant'Ana [2]
Institution, Szent István University, Gödöllő, Hungary [1] - Institution, Universidade Federal de Viçosa, Viçosa, Brazil [2] - Institution, Universidade Federal de Ouro Preto,, Ouro Preto, Brazil [3]

Objective

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

Methods

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

Results

In peel and pulp of Kinkan were detected the presence of vitamin C (2.32 ± 44.24 mg.100 g^{-1}). The most expressive component of vitamin E were α-tocopherol (569.00 ± 10.20 µg.100g^{-1}) and ß-tocotrienol (66.89 ± 39.93 µg.100g^{-1}). The polyphenol content was (98.55 ± 1.93 mg GAE.100g^{-1}). The majority flavonoids detected were apigenin (38.1573 ± 0.53 mg.100g^{-1}) and eriodyctiol (36.8809 ± 0,38 mg.100g^{-1}) The antioxidant activity was (62.01%). In Kinkan fruit, the macroelements most expressive were K, Ca and Mg.

Connclusions

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

EFFECT OF GLUTEN FREE DIET ON INTESTINAL MICROBIOTA IN PEDIATRIC CELIAC PATIENTS

Yasemin Ertaş Öztürk [1] - Efsun Karabudak [1] - Ödül Eğritaş Gürkan [2] - Buket Dalgıç [2]
Gazi University, Department of Nutrition and Dietetics, Ankara, Turkey [1] - Gazi University, Division of Pediatric Gastroenterology, Ankara, Turkey [2]

Objective

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

Methods

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

Results

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

Conclusions

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

DETECTION OF NEW PROBIOTICS IN THE PRESENCE OF VIRAL GASTROENTERITIS

Gulcin Alp Avci [1]
Hitit University, Molecular Biology And Genetics, Corum, Turkey [1]

Objective

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

Methods

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

Results

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

Conclusions

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

ANTI-CAMPYLOBACTER EFFECTS OF PROBIOTICS IN VITRO AND IN VIVO

Elena Ermolenko [1] - Konstantin Ermolenko [2] - Elvira Martens [3] - Sergey Sidorenko [3] - Marina Kotyleva [1] - Alexander Suvorov [1] - Guo Danyang [4]
Institute of Experimental Medicine, Department of experimental microbiology, Saint-Petersburg, Russian Federation [1] - Pediatric Research and Clinical Center for Infectious Diseases, Department of intestinal infections, Saint-Petersburg, Russian Federation [2] - Pediatric Research and Clinical Center for Infectious Diseases, laboratory of medical microbiology, Saint-Petersburg, Russian Federation [3] - Agro-food Science and Technology, SAAS, Shandong, China [4]

Objective

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

Methods

The antagonistic activity of prorobiotic cultures *Enterococcus faecium* L3 (Laminolact) and Lactobacillus acidophilus (Acipol) against 5 strains of *Campylobacter coli* and *C.jejuni* taken the bacterial cultures collection of Pediatric Research and Clinical Center for Infectious Diseases was studied using the double-layer agar method with different doses of probiotics 5 - 8 lg CFU /ml.
The experiments in vivo were performed on white outbreed mice, which were injected with *C. jejuni* taken in a dose of 10 lgCFU per animal (i.p.). One day before and for 5 days after infection the animals got probiotics Laminolact (group E) or Acipol (group L) in dose 7 lg CFU /mouse for 5 days. Animals of the control group (C) did not get probiotic.

Results

Probiotic enterococci and lactobacilli in minimal concentrations 5 and 6 lgCFU/ml, correspondently, inhibited growth of all calmylobacter strains.
After infection, dyspepsia symptoms were not noted. But the weight in the control group gained worse than in groups with probiotic therapy. On the 14th day of observation, *Campylobacter jejuni* were found in 50,0 %, 33,1% and 62, 5 % in E, L and C groups, respectively.

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

MICROBIOTA CHANGES IN CHILDREN WITH VIRAL GASTROENTERITIS

Konstantin Ermolenko [1] - Natalia Gonchar [1] - Yurii Lobzin [1]
Pediatric Research and Clinical Center for Infectious Diseases, Intestinal infection, Saint-Petersburg, Russian Federation [1]

Background

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

Methods

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

Results

FGD were diagnosed in 14 (23.3%) convalescents of RI. Changes in microbiological and immunological parameters were found. Microbiota changes in RI were characterized with a significant increase in the level of opportunistic *Bacteroides fragilis*, decrease in the number of symbiotic Lactobacillus spp., as well as the obligate microorganisms *Bacteroides thetaiotaomicron*, *Faecalibacterium prausnitzii*, which are difficult to cultivate on ordinary nutrient media. This parameters has also shown high level of statistical differences between children with and without FGD. In the period of convalescence, the ratio of *Bacteroides fragilis* / *Faecalibacterium prausnitzii*, which reflects the degree of severity of anaerobic imbalance, was highly informative. These parameter was significantly increased in convalescents who had formed FGD (p=0,01).
Study of immune status, has shown that in acute period of RI more frequently children had virus-induced IFN production suppression (p=0.03), and CD8 lymphocytes level decrease (p=0.05). Children with FGD after RI had an increase in the level of CD25 + lymphocytes (p=0.05), and relatively lower levels of serum IgA (p=0.04).

Conclusions

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

NOD2 PROMOTES HOST DEFENSE AND RECOVERY FROM CANDIDA TROPICALIS INFECTION DURING EXPERIMENTAL COLITIS

Kristine Ann Buela [1] - Theresa Pizarro [1]
Case Western Reserve University, Department of Pathology, Cleveland, United States [1]

Objective

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

Methods

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

Results

Ct did not exacerbate colitis in DSS-treated WT mice; however, *Ct*-infected *Nod2*$^{-/-}$ mice had a higher fungal burden and delayed recovery vs. *Ct*-infected WT controls, suggesting a protective role of NOD2 in colitic mice with *Ct* infection. *Ct*-infected *Nod2*$^{-/-}$ mice also expressed a dramatic reduction in colonic IL-22 and IL-17, cytokines important in maintaining epithelial barrier integrity, and IL-17^{+}ILC3s, known to clear opportunistic fungal infections, were significantly decreased in colons/MLNs of *Ct*-infected, DSS-colitic *Nod2*$^{-/-}$ vs. WT mice, indicating that delayed fungal clearance and recovery of *Nod2*$^{-/-}$ mice may be due to their inability to mount appropriate, protective type-3 immune responses.

Conclusions

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

HEALTHY PROTECTION OF BERGAMOT IS LINKED TO THE MODULATION OF MICROBIOTA

Giovanna Petrangolini [1] - Valeria Longo [1] - Davide Berlanda [1] - Pietro Allegrini [1] - Giulia Masetti [2] - Sara Botti [2] - Antonella Riva [1]
Indena SpA, R&D, Milano, Italy [1] - PTP Science Park, -, Lodi, Italy [2]

Ojective

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

Methods

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

Results

In this experimental model the modulation of microbiome diversity was observed after *ex-vivo* Bergamot Phytosome treatment. 25 different phyla were identified, of which 4 major phyla were modulated: *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*. A decreased ratio of *Firmicutes/Bacteroidetes* and increased of *Proteobacteria* were observed indicating an intestinal microbial and cardiovascular health positive modulation.
Regarding genus levels, 418 different genera were identified. 8 major genera (62%) were modulated: *Escherichia*, *Serratia*, *Bacteroides*, *Prevotella*, *Enterococcus*, *Bifidobacterium*, *Blautia* and *Faecalibacterium*.

Conclusions

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

EFFECTS OF B-FRUCTANS FIBER ON BOWEL FUNCTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

Le Bourgot Cindy [1] - Respondek Frédérique [1] - Calame Wim [2] - de Vries Jan [3]
Tereos, R&D department, Moussy-Le-Vieux, France [1] - StatistiCal BV, StatistiCal BV, Wassenaar, Netherlands [2] - De Vries Nutrition Solutions Inc., De Vries Nutrition Solutions Inc., Gorssel, Netherlands [3]

Objective

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

Methods

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

Results

The search resulted into 2343 hits that were screened on relevance. Forty-seven publications were analyzed in detail which resulted into 31 cases on stool frequency, 6 cases on stool consistency, 12 cases on stool wet weight and 6 cases on stool dry weight. All beta-fructans contributed to increase in stool frequency (+0.28 ± 0.06 defecations per day). This was mostly explained by short-chain fructans (DP < 10) and not by long-chain fructans (DP ≥10). The meta-regression describes a relationship between the frequency of bowel movements and the BMI.
In addition, stool consistency, as reported by the Bristol Stool Scale, was positively impacted by beta-fructans. The effect was more outspoken with short-chain beta-fructans. No effect was observed on stool dry weight while stool wet weight was increased when using beta-fructans, with most prominent effect due to beta-fructans with DP < 10.

Conclusions

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

COMPLIANCE TO PROBIOTIC THERAPY IN IRRITABLE BOWEL SYNDROME IN CLINICAL PRACTICE: A REAL-LIFE STUDY

Lucrezia Laterza [1] - Marco Napoli [1] - Valentina Petito [1] - Franco Scaldaferri [1] - Eleonora Gaetani [1] - Antonio Gasbarrini [1]
Università Cattolica del Sacro Cuore, Digestive Disease Center, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy [1]

Introduction

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

Aims and methods

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

Results

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

Conclusions

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

PROBIOTICS IN METABOLIC SYNDROME

Emre Avci [1]
Hitit University, Molecular Biology And Genetics, Corum, Turkey [1]

Objective

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

Methods

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

Results: Eight Lactobacilli and 2 Bifidobacteria strains were isolated from 42 feces samples with metabolic syndrome. 16 Lactobacilli and 8 Bifidobacteria strains were isolated from 20 feces samples of healty individulas. The low pH resistance and bile tolerance of strains were determined. EPS production ranges were 31.10-88.52 mg/L. The cholesterol assimilation rates ranged between 8.66-52.06%. A positive and strong correlation was determined between EPS production and cholesterol assimilation ($r=0.674$, $P<0.005$). The glycocholate rates was higher than the taurocholate in all strains. Lactobacillus spp. had higher EPS production, deconjugation and cholesterol assimilation compared to in the Bifidobacterium spp..

Conclusions

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

OBESITY MANAGEMENT WITH PROBIOTICS: MODULATION OF GUT MICROBIOTA COMPOSITION AND BODY WEIGHT CONTROL

Maria Magdalena Coman [1] - Cinzia Cecchini [1] - Maria Vittoria Micioni di Bonaventura [2] - Stefania Silvi [3] - Carla Orpianesi [1] - Alberto Cresci [1] - Carlo Cifani [2] - Maria Cristina Verdenelli [1]
Synbiotec Srl, Spin-off of University of Camerino, Camerino, Italy [1] - School of Pharmacy, University of Camerino, Camerino, Italy [2] - School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy [3]

Objectives

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

Methods

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

Results

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

Conclusions

Probiotics supplement can represent a valuable support in obesity management

A RANDOMIZED CONTROLLED TRIAL ON THE EFFECTS OF A SYNBIOTIC COMPOUND ON MARKERS OF INFLAMMATION IN OBESE ADOLESCENTS

Roya Kelishadi [1]
Child Growth and Development Research Center,Research Institute for Primordial Prevention of Non Communicable Disease, Isfahan University of Medical S, Pediatrics, Isfahan, Iran (islamic Republic Of) [1]

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

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

Results
Overall, 55 participants completed the study, i.e. 28 in the synbiotic group and 27 in the placebo group. At the end of the trial, higher decrease was documented in BMI Z-score, waist circumference, and waist-to-hip ratio of the synbiotic group than in the placebo group. Moreover, the synbiotic group had significant decrease in mean levels of high-sensitive C-reactive protein and interleukin-6. These changes remained significant after adjustment for potential confounding factors including anthropometric indices.

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

PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM FERMENTED CEREAL FOODS

Ilkin Yucel Sengun [1] - Kivanc Atlama [1] - Gulden Kilic [1]
Ege University, Engineering Faculty, Food Engineering, Izmir, Turkey [1]

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

Keywords: cereal, probiotic, lactic acid bacteria, fermentation

THE HIGH POLYMERIZATION DEGREE INFLUENCES THE INULIN BIOACCESSIBILITY IN DURUM WHEAT SPAGHETTI

Angela Cardinali [1] - Antonella Garbetta [1] - Isabella D'Antuono [1] - Maria Grazia Melilli [2] - Vito Linsalata [1] - Salvatore Scandurra [2]
National Research Council, Institute of Science of Foods Production (ISPA), Bari, Italy [1] - National Research Council, Institute for Agricultural and Forest Systems in the Mediterranean (ISAFOM), Catania, Italy [2]

Objective

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

Methods

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

Results

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

Conclusions

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

EFFECTS OF SYNBIO® PROBIOTIC FORMULATION ON PATHOGENS ISOLATED FROM CHRONIC ULCERATIVE LESIONS: IN VITRO STUDY

Lucia Mazzotti [1] - Maria Magdalena Coman [1] - Carla Orpianesi [1] - Alberto Cresci [1] - Stefania Silvi [2] - Alessandro Scalise [3] - Maria Cristina Verdenelli [1]
Synbiotec Srl, Spin-off of University of Camerino, Camerino, Italy [1] - School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy [2] - Department of Plastic and Reconstructive Surgery, Marche Polytechnic University, Ancona, Italy [3]

Objective

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

Methods

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

Results

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

Conclusions

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

SKIN MICROBIOTA AND BACTERIAL BIOFILMS IN PATIENTS WITH ATOPIC DERMATITIS AND IN HEALTHY HUMAN HOSTS

Oksana Rybalchenko [1] - Olga Orlova [1] - Valentina Kapustina [1]
Saint-Petersburg State University, Medical department, Saint-Petersburg, Russian Federation [1]

Introduction

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

Aim

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

Materials and Methods

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

Results

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

Conclusion

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

PERIODONTAL DISEASES & SYSTEMIC ORGANS INTERACTION

Dr Piero Simeone [1]
[1] Active member of Italian Academy of Prosthetic Dentistry

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

Oral Microbiota & Periodontal diseases

Periodontal diseases frequently occur in human mouth, and can be divided into two categories, gingival diseases and periodontitis. Periodontal diseases cause destruction of periodontium (tooth-supporting tissues such as gingiva and alveolar bone) and constitute a potential risk factor for certain systemic diseases. Oral cavity is a natural microbial culture medium, in which periodontal tissue has complex anatomy and organizational structure, physical and chemical properties, which indeed provides good conditions for growth of microorganisms.

Oral Microbiota & Gastrointestinal system diseases

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

Oral Microbiota & Diabetes

Diabetes mellitus is characterized by hyperglycemia, inflammation and high oxidative stress, which can lead to systemic complications. There is a bidirectional relationship between periodontal disease and diabetes. Microbiome plays a key role in homeostasis and affects several pathologic processes, including diabetes.
Diabetes is a risk factor for periodontitis and increases disease severity. In type I diabetics, an increase in the severity of periodontal diseases has been shown across most age ranges. Age itself has been shown to be a risk factor for periodontitis, and is likely to be a confounder. Type II diabetes has also been shown to be a risk factor for periodontal diseases. A study of association between diabetic status and periodontal conditions in 1,342 individuals showed increased risk for periodontitis

Interdisciplinary Therapy

The new classification of periodontal diseases and conditions also includes systemic diseases and conditions that affect the periodontal supporting tissues. Such conditions are grouped as "Periodontitis as a Manifestation of Systemic Disease", and classification should be based on the primary systemic disease.
There are, however, common systemic diseases, such as uncontrolled diabetes mellitus, with variable effects that modify the course of periodontitis. These appear to be part of the multifactorial nature of complex diseases such as periodontitis and are included in the new clinical classification of periodontitis as a descriptor in the staging and grading process. Although common modifiers of periodontitis may substantially alter disease occurrence, severity, and response to treatment, current evidence does not support a unique pathophysiology in patients with diabetes and periodontitis.

Full-mouth scaling and root planing (FM-SRP) resulted in clinical and microbiological improvement 6 weeks post-treatment, but produced a moderate systemic acute-phase response including elevated inflammatory mediators 1 day post-treatment.
However this dental clinical approach should be accompained by the reduction of systemic risk factors (smoke, nutrition, diabetes) and in cooperation with the interdisciplinary medical disciplines.
All periodontal patients must be included in a systematic recall programme to maintain the achieved outcomes.

POSTERS

NANOEMULSION OF PASSIFLORA EDULIS AS DIETARY PRESERVATIVE PROPOSAL

Juliana Cristina dos Santos Almeida [1] - Rosana Gonçalves Rodrigues-das-Dôres [2] - Thascilaine Souza Ribeiro [1] - Luan Silvestro Bianchini Silva [1] - Fernanda Barçante Persoli [1] - Bruna Inez Carvalho de Figueiredo [1] - Gustavo Henrique Bianco de Souza [1] - Orlando David Henrique dos Santos [1]
UFOP, Departamento de Farmácia, Ouro Preto, Brasil [1] - UFOP, Centro de Saúde, Ouro Preto, Brasil [2]

There is serious growing concern about the effects of the use of various chemical additives in food products. This situation has encouraged the research of new natural agents that are more efficient and healthier to complement the food preservation. *Passiflora edulis* has a variety of antioxidant compounds responsible for its bioactives properties, which justifies its use in this work to develop an oil-in-water nanoemulsion for natural preservative in foods. The main objective of this work was to study potential of *P. edulis* extract for food preservation and develop an oil oil-in-water nanoemulsion. The formulation was composed by Hydrogenated PEG-40 Castor oil; *P. edulis* extract at 0.5%(w/w); Span 60 and Sunflower oil and ultrapure water. Physicochemical characteristics as size, polydispersion index (PdI) and zeta potential were analysed in Zetasizer. Thermal stress and stability tests were conducted too. Contents of flavonoids compounds were determined, the phenolic compounds was determined by Folin-Ciocalteau method and antioxidant activity was analysed using DPPH method. The average size of nanoemulsion was 70 nm and mean zeta potential value was – 22 mV. The formulation showed physicochemical stability after 2 months and when submitted to 70 ° C, with no statistically significant variation of size and zeta potential. Nanoemulsion was considered monodisperse since values of PdI were lower than 0.3. Analyses of extract indicates that total phenolic values were 51,25(±0,41) mg GAE/g, flavonoids content was 14,52 (±0,25) mg/g and antioxidant activity was EC50= 25,2mg/mL. These results suggest the use of *P. edulis* nanoemulsion as a natural preservative in foods.

Keywords
DPPH, preservative, food

CORN (ZEA MAYS L). EXTRACTS AS SOURCE OF ACTIVE COMPOUNDS WITH PROMISING EFFECTS IN REDUCING TRIGEMINAL PAIN THROUGH MICROBIOTA MODULATION

Giulia Magni [1] - Chiara Di Lorenzo [1] - Francesca Colombo [1] - Simone Biella [1] - Alessandra Marinelli [2] - Davide Lecca [1] - Christian Milani [3] - Marco Ventura [3] - Maria Pia Abbracchio [1] - Chiara Tonelli [2] - Patrizia Restani [1] - Katia Petroni [2] - Stefania Ceruti [1]
Università degli Studi di Milano, Dept. Pharmacological and Biomolecular Sciences, Milan, Italy [1] - Università degli Studi di Milano, Dept. Biosciences, Milan, Italy [2] - Università degli Studi di Parma, Dept. of Chemistry, Life Sciences and Environmental Sustainability, Parma, Italy [3]

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

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

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

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

EFFECTS OF THE DIETARY INTERVENTION WITH GREEN DWARF BANANA FLOUR ON THE TNBS RELAPSE MODEL OF INTESTINAL INFLAMMATION

Ana Quaglio [1] - Gabriela Batista [2] - Ligia Sassaki [2] - Luiz Di Stasi [1]
São Paulo State University (UNESP), Department of Pharmacology, Institute of Biosciences, Botucatu, Brazil [1] - São Paulo State University (UNESP), Department of Medical clinics, School of Medicine, Botucatu, Brazil [2]

Objective

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

Methods

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

Results

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

Conclusion

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

THE USE OF FERMENTED SOUR SOBYA RICH IN PREBIOTICS, PROBIOTICS AND POSTBIOTICS AS PART OF SUSTAINABLE DIET. RECENT FINDING BASED ON RANDOMIZED CONTROLLED TRIALS (RCT).

Laila Hussein [1]
National research center, Nutrition, Giza, Egypt [1]

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

Results

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

Conclusion

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

COMPARATIVE STUDIES CONCERNING THE BIOACTIVITY OF PEPTIDES OBTAINED BY KEFIR-KOMBUCHA FERMENTATION OF BOVINE COLOSTRUM

Anca Oancea [1] - Elena Utoiu [1] - Teodora Ciucan [1] - Oana Craciunescu [1] - Alexandra Gaspar-Pintiliescu [1] - Viorica Coroiu [1] - Ana Maria Stanciuc [1] - Laura Mihaela Stefan [1]

National Institute of Research and Development for Biological Sciences, Cellular and Molecular Biology Department, Bucharest, Romania [1]

Objective

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

Methods

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

Results

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

Conclusions

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

Acknowledgments

PNIII-P3-3.5-EUK-2017-02-0026

PHYTOCHEMICAL AND NUTRITIONAL CHARACTERIZATION OF PSIDIUM GUAJAVA L. (GUAVA) LEAVES EXTRACT

Daiane Cristina de Assis Braga [1] - Rosana Gonçalves Rodrigues-das-Dôres [2] - Gustavo Henrique Bianco de Souza [3] - Juliana Cristina dos Santos Almeida Bastos [3] - Lisandra Brandino de Oliveira [1] - Andréia Carvalho Alzamora [1] - Vagner Roberto Antunes [4] - Leonardo Máximo Cardoso [1]

Federal University of Ouro Preto, Department of Biological Sciences, Ouro Preto, Brazil [1] - Federal University of Ouro Preto, Health Center, Ouro Preto, Brazil [2] - Federal University of Ouro Preto, Department of Pharmacy, Ouro Preto, Brazil [3] - University of Sao Paulo, Department of Physiology and Biophysics, São Paulo, Brazil [4]

Introduction

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

Methods

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

Results

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

Discussion

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

Financial support

UFOP, Capes, CNPq, FAPEMIG, personal resources.

TARGETED SHORT-TERM PROBIOTIC INTERVENTION EFFECTIVELY RESTORES THE FUNCTION AND STRUCTURE OF DAMAGED KIDNEY IN GOUT AS DETECTED BY ULTRASONOGRAPHY

Mykola Spivak [1] - Rostyslav Bubnov [1]
Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Interferon department, Kyiv, Ukraine [1]

Objective

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

The Aim

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

Methods

We included twelve patients (age 32-68 years) with BMI>30, waist circumference (WC)>110, who met criteria of MetS with hyperuricemia (the levelsof uric acid over 400 µmol/L) and increased blood preassure. All patients underwent extensive general clinical, lab tests; multiparameter US of kidneys, joints, liver, measuring visceral fat (FV). The typical symptoms of gouty nephropathy in sonographic display were precisely documented. Patients were given probiotics (B. animalis VKB / B. animalis VKL strains at a dose 10^8 CFU daily during 10 days); in cases of liver fibrosis - L. delbrueckii subsp. bulgaricus IMV B-7281, B. animalis VKB, L. casei IMV B-7280 (considered individually according to the knowledge on treatment mechanics obtained from in vivo in vitro studies and existing evidence).

Results

We registered ultrasound signs of symptoms of gouty nephropathy in all patients, namely detection of small hyperechoic inclusions in parenchyma (chalk-stone), increase in resistive index (RI) in segmental vessels over 0.7, thinning of parenchyma (less than 13 mm), fibrotic changes in parenchyma, hilly kidney margins, anaechoic strips under the capsule. Weight, BMI, WC and VF decreased, liver structure, blood pressure improved, joints tophacae decreased in size, normalized creatinine levels and uric acid levels (was 273 µmol/L) in all patient after focused probiotic administation. Most signs of gouty nephropathy improved, namely increasing in kidneys size and parenchyma, decreased IR under 0.7, decreased petrifications, small cystic lesions; fibrotic changes retained in parenchyma after short-term treatment. In 6 patients microsplenia was detected, size improved to norm after treatment.

Conclusions

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

PROTEOLYTIC ACTIVITY OF ENTEROCOCCUS FAECALIS OB15, A PROBIOTIC STRAIN ISOLATED FROM RIGOUTA TUNISIAN DAIRY PRODUCT

Olfa Baccouri [1] - Neyssene Aissaoui [1] - Leila Ben Farhat [1] - Nathalie Connil [2] - Ferid Abidi [1]
LIP-MB, INSAT, Université de Carthage, Tunis, Tunisia [1] - LMSM, Université de Rouen, Evreux, France [2]

Objective

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

Methods

The following parameters were studied:

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

Results

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

Conclusions

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

THERMAL OR MEMBRANE PROCESSING FOR INFANT MILK FORMULA- EFFECT ON PROTEIN DURING SIMULATED GASTROINTESTINAL INFANT DIGESTION.

Simona Lucia Bavaro [1] - Fiona O'Mahony [2] - Yihong Chen [3] - Michael J. Callanan [2] - Andre Brodkorb [4] - Linda Giblin [1]
Teagasc, Food Bioscience, Fermoy, Co.Cork, Ireland [1] - Cork Institute of Technology, School of Biological Sciences, Cork, Ireland [2] - Teagasc, Chemistry, Fermoy, Co.Cork, China [3] - Teagasc, Chemistry, Fermoy, Co.Cork, Ireland [4]

Objective

Infant Milk Formula (IMF) is designed as a breast milk substitute to satisfy the nutritional requirements of infants during the first months of life. We have recently produced a new IMF by cascade membrane filtration (CMF) as an alternative to thermal processing, with equivalent safety but with enhanced bio accessibility, bioavailability and digestion of proteins. Herein we investigate the fate of milk proteins during in vitro gastro-intestinal digestion from pilot scale IMF produced by CMF versus standard thermal processing.

Methods

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

Results

SDS-PAGE revealed different protein patterns with intact proteins notably visible in G60 of CMF compared to the thermal treatment. At I60 there were significant differences ($P<0.05$) in the amino acid composition of CMF-IMF and thermally treated IMF compared to their G0 time. The incubation of Caco-2 cells for 4 h with IMF digested samples at a concentration of 200 µg/mL revealed significant ($P < 0.05$) alterations in trans epithelial electrical resistance (TEER) between the two different treatments.

Conclusion

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

STRESS HORMONES INCREASE BIOFILM FORMATION OF ENTEROCOCCUS FAECALIS

Flore Nilly [1] - Mélyssa Cambronel [1] - Amine Boukerb [1] - Olfa Baccouri [2] - Marc Feuilloley [1] - Nathalie Connil [1]
LMSM, Université de Rouen, Evreux, France [1] - LIP-MB INSAT, Université de Carthage, Tunis, Tunisia [2]

Objective

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

Methods

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

Results

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

Conclusions

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

METABARCODING ANALYSIS OF GUT MICROBIOTA OF HEALTHY INDIVIDUALS REVEALS IMPACT OF PROBIOTIC CONSUMPTION

Matteo Calgaro [1] - Mattia Pandolfo [1] - Eleonora Sarno [1] - Angela Marotta [2] - Ilaria Larini [1] - Elisa Salvetti [3] - Marco Pane [4] - Antonio Del Casale [3] - Nicola Vitulo [1] - Mirta Fiorio [2] - Giovanna E. Felis [1]

University of Verona, Department of Biotechnology, Verona, Italia [1] - University of Verona, Department of Neurosciences, Biomedicine and Movement Sciences, Verona, Italia [2] - MICROBION SRL, Open Innovation Department, Verona, Italia [3] - BIOLAB RESEARCH SRL, R&D, Novara, Italia [4]

Objective

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

Methods

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

Results

The probiotic cohort was characterized by *Akkermansia* and *Lactobacillus rhamnosus* after 3 weeks of treatment, shifting to a more diverse microbial composition comprehensive of *Romboutsia*, *Lactobacillus, Ruminococcus* and *Akkermansia* towards the end.
Amplicons related to *L. rhamnosus* and *B. longum* confirmed the presence of these two probiotic species throughout the assumption.

Conclusions

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

Reference

Marotta A, Sarno E, Del Casale A, Pane M, Mogna L, Amoruso A, Felis GE, Fiorio M. Effects of Probiotics on Cognitive Reactivity, Mood, and Sleep Quality. Front Psychiatry. 2019 Mar 27;10:164.

INCREASED INCIDENCE OF CLOSTRIDIUM-LIKE SPECIES AND LOWER DIVERSITY OF COMMON COMMENSAL BACTERIA IN GUT MICROBIOTA OF CHILDREN WITH NEURODEVELOPMENTAL DISORDERS

Djurdjica Ignjatovic [1] - Svetlana Sokovic Bajic [2] - Katarina Bojovic [3] - Danijela Vojnovic Milutinovic [1] - Mirko Tomic [1] - Natasa Golic [2] - Maja Tolinacki [2]

Institute for Biological Research "Siniša Stanković", University of Belgrade, Department of Biochemistry, Belgrade, Serbia [1] - Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Laboratory for Molecular Microbiology, Belgrade, Serbia [2] - Psychiatry clinic "Dr Selakovic", Psychiatry clinic "Dr Selakovic", Belgrade, Serbia [3]

Objective

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

Methods

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

Results

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

Conclusions

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

EFFECT OF LACTOBACILLUS FARCIMINIS SUPPLEMENTATION IN PREVENTING LPS-INDUCED HIPPOCAMPAL NEUROINFLAMMATION, IN MICE

Carla Petrella [1] - Georgios Strimpakos [2] - Francesca de Santa [2] - Marco Fiore [1] - Stefano Farioli-Vecchioli [2]
Institute of Cell Biology and Neurobiology, CNR, Rome, Italy [1] - Institute of Cell Biology and Neurobiology, CNR, Monterotondo, Italy [2]

Objective
The discovery of adult neurogenesis in human dentate gyrus of the hippocampus opens new challenges on the role of a virtuous lifestyle (i.e. diet) aimed at counteracting7slowing the decay of new neurons production in the aged brain. The use of probiotics could represent an intriguing approach for the prevention of specific diseases. We studied the potential neuroprotective effect of prolonged consumption of Lactobacillus *farciminis* (LF), in a mouse model of acute neuroinflammation

Methods
C57B6/J male mice (Charles River Laboratories) were divided in the following groups: (1) controls (2) mice supplemented with LF (10^9CFU/day, for 14 days in drinking water); (3) mice treated with LPS (1mg/kg, ip); (4) mice supplemented with LF and injected the 14th day with LPS. Study of changes in adult neurogenesis was conducted by immunofluorescence technique, 24 hours after LPS injection.

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

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

INTERACTIONS AND CROSS-TALK BETWEEN MICROBIOTA AND HUMANS: A PRELIMINARY STUDY ON THE MOLECULAR RESPONSES OF ENTEROCOCCUS FAECIUM NCIMB10415 TO BIOACTIVE COMPOUNDS AND FEEDBACK SIGNALS

Rossella Scardaci [1] - Nathalie Connil [2] - Enrica Pessione [1]
Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Torino, Italy [1] - LMSM, Université de Rouen, Evreux, France [2]

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

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

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

Conclusions
As previously demonstrated for pathogenic bacteria, also this probiotic strain can respond to human bioactive molecules, supporting the idea of an 'interkingdom signalling'. Work is in progress to evaluate bacterial metabolic pathways modifications exerted by these compounds (proteomic approach) and possible feed-back effects induced on Caco-2/TC7 (IL-8) and other types of cells present in the gut, as immune cells (dendritic cells) and enteroendocrine cells by treated *in- toto* bacteria and their supernatants.

BRUSH BORDER ENZYME ACTIVITY IN PATIENTS WITH THE SYNDROME OF BACTERIAL OVERGROWTH IN THE SMALL INTESTINE

V.P. Novikova [1] - A.P. Listopadova [1] - O.M. Tsekh [1] - E.Yu. Kalinina [2] - I.Z. Samorukova [2] - E.A. Orishak [3]

St. Petersburg State Pediatric Medical University, laboratory of medical and social problems in Pediatrics SIC, St. Petersburg, Russian Federation [1] - North-Western State Medical University named after I.I. Mechnikov, Department of Pathological Anatomy, St. Petersburg, Russian Federation [2] - North-Western State Medical University named after I.I. Mechnikov, Department of Medical Microbiology, St. Petersburg, Russian Federation [3]

Objective

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

Methods

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

Results

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

Conclusions

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

NOVEL INSIGHTS INTO THE STRAIN-LEVEL VARIATIONS AND METABOLIC POTENTIAL OF THE UNIQUE INDIAN GUT MICROBIOME

Vineet K. Sharma [1] - Vishnu Prasoodanan PK [1] - Darshan Dhakan [1] - Ashok K. Sharma [2] - Shruti Mahajan [1]

Metagenomics and Systems Biology Laboratory, Department of Biological Sciences,, Indian Institute of Science Education and Research Bhopal, Bhopal, India [1] - Department of Animal Science, University of Minnesota, Minnesota, United States [2]

Objective

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

Methods

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

Results

7,714 genome bins were identified from ten different populations, of which 1,988 were India-specific. Interestingly, 88 out of the total 163 Prevotella bins belonged to India, which reaffirms the abundance (up to 80%) of Prevotella in Indian gut. The functional analysis of gut microbiome genes showed an enrichment of carbohydrate-metabolism and plant-degrading enzyme genes, which aligns well with the carbohydrate and plant-rich diet of the Indian population.

Conclusions

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

PLASTICITY OF CHILD MICROBIOTA IN THE DEVELOPMENT OF OBESITY

Emanuel Vamanu [1]
Faculty of Biotechnology, University of Agronomic Science and Veterinary Medicine, Bucharest, Romania [1]

Objective

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

Methods

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

Results

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

Conclusions

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

THE ANALYSIS OF FECAL MICROBIOTA AND INSULIN PRODUCTION IN DIABETIC RATS AFTER ORAL ADMINISTRATION OF PROBIOTIC LACTOBACILLUS PARAPLANTARUM BGCG11

Milica Zivkovic [1] - Svetlana Sokovic Bajic [1] - Maja Tolinacki [1] - Emilija Brdaric [1] - Jelena Djokic [1] - Nikola Popovic [1] - Jovana Rajic [2] - Marija Djordjevic [2] - Natasa Golic [1]
Institute of Molecular Genetics and Genetic Engineering, Laboratory for Molecular Microbiology, Belgrade, Serbia [1] - Institute for Biological Research "Sinisa Stankovic", Department of Molecular Biology, Belgrade, Serbia [2]

Objective

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

Methods

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

Results

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

Conclusions

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

EXTENDED-SPECTRUM-BETA-LACTAMASES IN ESCHERICHIA COLI OF INTESTINAL MICROBIOTA OF PIGLETS

Maria Manuel Donato [1] - Sofia Osório [2] - Fernando Ramos [3] - Olga Cardoso [4]

CIMAGO, Faculty of Medicine, University of Coimbra, Coimbra, Portugal [1] - Augusto Virgílio Sousa & Filho, Lda, None, Aveiro, Portugal [2] - REQUIMTE/LAQV; Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal [3] - CIEPQPF; Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal [4]

Objective

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

Methods

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

Conclusions

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

IDENTIFICATION OF NOVEL BIOACTIVE METABOLITES PRODUCED DURING THE COCULTIVATION OF PROBIOTIC STRAINS AND CLOSTRIDIUM DIFFICILE IN IN VITRO SETUP.

Thiyagarajan Gnanasekaran [1] - Carmen Saenz [1] - Thomas Moritz [1] - Manimozhiyan Arumugam [1]

Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark [1]

Objective

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

Methods and results

For this project, we adapted a QuadSHIME with a modified configuration containing 8 distal colon compartments. Using short chain fatty acid (SCFA) analysis as surrogate for community stability, we successfully established the gut microbiota from a healthy individual in the SHIME system, and a subsequent colonization of the probiotic strains. Using qPCR and colony counts specific to *C. difficile*, we verified the successful colonization of *C. difficile* during the infection period and gradual decrease of *C. difficile* population during the treatment period. By employing multi-omics (metagenomics, metatranscriptomics and metabolomics) analysis on the *in vitro* cultures we identified different signaling molecules and their biosynthetic pathways. In addition, the integration of multi-omics data helped us to understand community dynamics of different individual bacterial populations in the *in vitro* conditions using SHIME®.

Conclusion

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

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF LACTIC ACID BACTERIA STRAINS ISOLATED FROM NEWBORN FECES

Ditu Lia-Mara [1] - Lazar Veronica [1] - Preda Madalina [2] - Lescaru Anca [1]
Faculty of Biology, University of Bucharest, Bucharest, Romania [1] - Cantacuzino National Institute of Research, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania [2]

Objective

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

Methods

In the present study, ten feces samples were collected from newborns of different ages (between 0-5 days), born both naturally and by caesarean. A total of eight isolated LAB strains were identified using MALDI-TOF Mass Spectrometry system, after cultivation on selective medium (MRS with $CaCO_3$ and M17). In order to establish their pathogenic potential, the isolated strains were screened for soluble virulence factors production like hemolysin, lipase, gelatinase and DN-ase (using cultivation methods on specific substratum) and for adherence pattern on the cellular substrate represented by HeLa cells (using Cravioto's adapted method). The study continued with phenotypic determination of antibiotic susceptibility spectrum using disc diffusion standard method (according with CLSI standard, 2019) and genetic identification of potential resistance markers for *Enterococcus* isolated strains using Simplex PCR for the *van*A gene and multiplex PCR for the *van*B and *van*C genes.

Results

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

Conclusions

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

Acknowledgment

The authors gratefully acknowledges the financial support of the research project PD 148/2018 (PN-III-P1.1-PD-2016-1798).

IMPACT OF NUTRIENT STARVATION ON THE MODULATION OF THE INFLAMMATORY RESPONSE BY LACTOBACILLUS STRAINS IN INTESTINAL EPITHELIAL CELLS AND MACROPHAGES

Alissar Al Tarraf [1] - Aurélie Rieu [1] - Sylvie Nguyen [1] - Deirdra Flaherty [1] - Jean Guzzo [1] - Pierre Lapaquette [1]
Institut Jules Guyot De Vigne et de Vin IUVV, Université de Bourgogne Franche comté, Dijon, France [1]

A chronic inflammation state is associated with aging and with the onset and progression of many human diseases, including inflammatory bowel diseases, obesity, cardiovascular diseases and cancer. Among strategies developed to dampen inflammation, the use of probiotic bacteria with anti-inflammatory properties, from *Lactobacillus* and *Bifidobacterium* genus, represents a safe and low-cost promising treatment. However, despite encouraging *in vitro* and *in vivo* results, probiotic bacteria remain poorly effective in the treatment of human inflammatory-related diseases. Emerging evidence suggests that the immune system function might be heavily influenced by the sensing of nutrient, reinforcing the idea that diet can influence the inflammatory response. This may be particularly true for the intestine that face dynamic changes to nutrient bioavailability.
The purpose of this study is to investigate whether nutrient deprivation of host cells may potentiate the anti-inflammatory effects of *Lactobacillus* probiotic strain. Inflammatory responses were induced in intestinal epithelial cells (HT-29) using *Salmonella* infection and in macrophages (RAW, THP1 and J774A1) using lipopolysaccharide (LPS) treatment, in the presence or absence of nutrients in the host cells media. Inflammatory responses were analyzed by qRT-PCR and ELISA to follow the expression and secretion of various pro- and anti-inflammatory cytokines.
Our *in vitro* data shows that nutrient starvation allows to potentiate the abilities of *Lactobacillus* strains to downregulate proinflammatory responses in intestinal epithelial cells and macrophages, suggesting that diet may influence positively the efficiency of probiotics.

Keywords

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

AGE AND GENDER PROFILES OF SELECTED GUT MICROBIOTA AMONG EGYPTIAN CHILDREN AND THE SIBLING-SPECIFIC VARIATION

Hagar Elbakry [1] - Sa-ngapong Plupjeen [2] - Massalin Nakphaichit [2] - Sunee Nitisinprasert2 [2] - Laila Hussein [1]

National research center, Nutrition, Giza, Egypt [1] - Department of Biotechnology, Faculty of Agro-industry, Kasetsart University, Bangkok, Thailand [2]

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

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

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

USE OF A NEW PROBIOTIC FORMULATION IN ALLERGIC PATIENTS: A PILOT STUDY

Patrizia Malfa [1] - Giuliana Zisa [2] - Davide Raineri [3] - Luca Gigliotti [3] - Marcello Manfredi [4] - Annalisa Chiocchetti [3]

Proge Farm Srl, Largo Donegani 4/A- Novara, Italy [1] - Allergic Unit, University of Piemonte Orientale (UPO) – Novara, Italy [2] - Department of Health Science and IRCAD and CAAD, University of Piemonte Orientale (UPO) -Novara, Italy [3] - ISALIT Srl, Via Canobio 4/6 – Novara, Italy [4]

Objective

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

Methods

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

Results

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

Conclusions

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

TYPING OF SURFACE GLYCOSILATION OF MICROORGANISMS BY LECTINS WITH IN HOUSE ELISA

Luka Dragacevic [1] - Brizita Djordjevic [2] - Marija Gavrovic-Jankulovic [3] - Vesna Ilic [4] - Rajna Minic [5]
Institute of Virology, Vaccines and Sera, Torlak, Vojvode Stepe 458, *Department of Research and Development, Belgrade, Serbia [1] - Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, Department of Bromatology, Belgrade, Serbia [2] - Faculty of Chemistry, University of Belgrade , Studentski trg 16, Department of Biochemistry, Belgrade, Serbia [3] - Institute for Medical Research, University of Belgrade, Dr Subotica 4, Belgrade, Serbia [4] - Institute of Virology, Vaccines and Sera, Torlak, Vojvode Stepe 458, Department od Research and Development, Belgrade, Serbia [5]*

Objective

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

Methods

Lectin molecules with previously defined specificities were WGA, SBA, LCA, RCA_{120}, MAA, UEA, all commercially available and recombinant banana lectin[i]. Lectins were biotinylated and biotinylation efficiency was assessed by dot blotting and titration against a mixture of microorganisms in ELISA to select appropriate dilutions. Subsequently analysis was performed with direct ELISA. 0,5 M monosaccharide mixture (glucose, mannose and galactose) was used as inhibitor.

Results

All but one lectin binding was removed with 0,5 M monosaccharide solution, implying a specific lectin-sugar interaction. WGA could not be inhibited with this mixture. This lectin also showed lowest selectivity as it bound most of the bacteria tested. Of the tested microorganisms *L. helveticus* LAFTI and *L. acidophilus* Vivag showed highest binding to 4 of the 7 tested lectins.

Conclusions

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

REDUCED FOOD DIVERSITY IN SIBO PATIENTS

Vladimir Pilipenko [1] - Maya Naidenova [1] - Vasily Isakov [1]
Institute of Nutrition, Gastroenterology & Hepatology, Moscow, Russian Federation [1]

Objective

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

Methods

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

Results

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

Conclusions

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

EFFECT OF PROBIOTICS IN CHILDREN WITH INFANTILE ECZEMA : A DOUBLE-BLIND RANDOMIZED CONTROL TRIAL.

Tamara Sabbi [1]
SIP SIGE SIGENP, ASL RMG, Palestrina, Italy [1]

Objective

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

Patients and Methods

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

Results

LGG, but not placebo, caused a significant reduction of both frequency ($P < .01$) and severity ($P < .01$) of eczema. These differences still were significant at the end of follow-up ($P < .02$ and $P < .001$, respectively).

Conclusions

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

ADDITION OF MUCIN TO GROWTH MEDIUM STIMULATES ADHESIVITY AND THERAPEUTICAL POTENTIAL OF LACTOBACILLUS REUTERI E

Boris Dudík [1] - Hana Kiňová Sepová [1] - Andrea Bilková [1] - František Bilka [1]
Faculty of Pharmacy, Comenius University, Bratislava, Slovakia (Slovak Republic) [1]

Objective

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

Methods

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

Results

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

Conclusions

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

PREBIOTICS: FROM BENCH TO BEDSIDE

Dorna Davani-Davari [1] - Iman Karimzadeh [2] - Manica Negahdaripour [3] - Milad Mohkam [4] - Younes Ghasemi [3]
Pharmaceutical Biotechnology Incubator, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran (islamic Republic Of) [1] - Department of Clinical Pharmacy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran (islamic Republic Of) [2] - Department of Pharmaceutical Biotechnology and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran (islamic Republic Of) [3] - Biotechnology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran (islamic Republic Of) [4]

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

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

THE HUMAN PROTECTIVE BASIC LECTIN SUPERSYSTEM WITH PROBIOTIC PROPERTIES

Mikhail Lakhtin [1] - Vladimir Lakhtin [1]
G.N. Gabrichevsky Research Institute for Epidemiology & Microbiology, Medical Biotechnology, Moscow, Russian Federation [1]

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

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

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

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

PROBIOTIC SCREENING OF TEN LACTIC ACID BACTERIA FROM TRADITIONAL ALGERIANS DRIED MEAT (KHLIAA)

Mostefa Naimi [1] - Omar Abid [1] - Méghit Boumédiène Khaled [2]
Nour Bachir University Center, Biology, El-Bayadh, Algeria [1] - Djillali Liabes University, Biology, sidi bel abbes, Algeria [2]

Objective

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

Methods

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

Results

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

Conclusions

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

Key words

meat, lactic acid bacteria, probiotic potential, screening

BIO-CONTROL OF VIBRIO SPECIES IN CULTURED MILK BY IN SITU BACTERIOCIN PRODUCTION FROM LACTIC ACID BACTERIA

Samuel Temitope Ogunbanwo [1] - Oluwadamilola Rashidat Odubanjo [1] - Caleb Oladele Adegoke [2] - Chigozie Oramadike [3]
University of Ibadan, University of Ibadan, Ibadan, Nigeria [1] - Ogun State College of Health Technology, Ogun State College of Health Technology, Ijebu Ode, Nigeria [2] - Nigeria Institute for Oceanography and Research, Nigeria Institute for Oceanography and Research, Lagos, Nigeria [3]

Objective

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

Methods

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

Results

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

Conclusions

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

GUT DISEASES, BIRTH CHARACTERISTICS, NUTRITION AND PHYSICAL DEVELOPMENT: CASE-STUDY ON CHILDREN AGED 0-3 YEARS, ADMITTED INTO A PEDIATRIC HOSPITAL FROM TIMISOARA, ROMANIA

Cristina Petrescu [1]
Victor Babes University of Medicine and Pharmacy Timisoara, Hygiene Department, Timisoara, Romania [1]

Objective

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

Methods

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

Results

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

Conclusions

Children hospitalized for gut diseases (allergic, infectious) present certain birth characteristics (caesarian, APGAR=9, 1st-order child), nutrition (artificial-NAN/natural=0-2 months) and physical development (normal).

ANTIOXIDANT ACTIVITY OF LACTIC ACID BACTERIA AS POTENTIAL PROBIOTICS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

Katarina B. Veljović [1] - Marija M. Stanković [2] - Svetlana S. Soković Bajić [1] - Amarela I. Terzić-Vidojević [1] - Dušanka D. Popović [1] - Miroslav R. Dinić [1] - Nataša E. Golić [1]
Institute of Molecular Genetics and Genetic Engineering, Laboratory for Molecular Microbiology, University of Belgrade, Belgrade, Serbia [1] - Institute of Molecular Genetics and Genetic Engineering, Laboratory for Molecular Biology, University of Belgrade, Belgrade, Serbia [2]

Objective

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

Methods

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

Results

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

Conclusions

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

ESOPHAGEAL VARICES ARE ASSOCIATED WITH GUT DYSBIOSIS IN CIRRHOSIS

Roman Maslennikov [1] - Maria Zharkova [1] - Marina Maevskaya [1] - Anna Kudryavtseva [2] - Vladimir Ivashkin [1]

Sechenov University, Clinic of internal medicine, gastroenterology and hepatology, Moscow, Russian Federation [1] - Engelhardt Institute of Molecular Biology, Laboratory of postgenomic studies, Moscow, Russian Federation [2]

Objective

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

Methods

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

Results

Patients had age 48.9±12.9 years and gender distribution 24 male/26 female. Cirrhosis was Child–Turcotte–Pugh class A in 19 patients, B in 19, and C in 12. Etiology was alcohol (18 persons), viral (17), autoimmune liver diseases (11), and unclear (4). Esophageal varices Grade I was in 16 patients, Grade II - in 14, Grade III - in 10. Ten patients had not esophageal varices. In gut microbiome in cirrhosis with large esophageal varices (Grade II-III) in comparison with cirrhosis without these (Grade I and no varices), the abundance of Bacilli and Streptococcaceae increased (5.5%[1.8%-17.8%] vs. 0.7%[0.2%-7.3%], p=0.005; and 4.6%[0.4%-14.4%] vs. 0.3% [0.1%-3.0%], p=0.011), but the abundance of Clostridia decreased (66.9%[53.2%-83.7%] vs. 81.3%[70.5%-85.4%], p=0.049), the abundance of Proteobacteria and Bacteroidetes didn't significantly change (0.8%[0.2%-3.1%] vs. 1.7 %[0.2%-4.0%], p=0.541; and 6.8%[4.5%-13.2%] vs. 5.7% [1.8% -7.8%], p=0.241).

Conclusions

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

GUT MICROBIOME ANALYSIS IN BIOPSIES FROM SLOVAK COLORECTAL ADENOMAS AND CARCINOMAS PATIENTS

Lenka Wachsmannova [1] - Katarina Soltys [2] - Viola Stevurkova [1] - Sona Ciernikova [1]

Cancer Research Institute, Biomedical Research Center, University Science Park for Biomedicine, Slovak Academy of Sciences, Bratislava, Slovakia (slovak Republic) [1] - Comenius University Science Park, Comenius University in Bratislava, Bratislava, Slovakia (slovak Republic) [2]

Objective

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

Methods

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

Results

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

Conclusion

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

HYPOLIPIDAEMIC EFFECTS OF OGI, A TRADITIONAL ADULT AND WEANING CEREAL GRUEL FERMENTED WITH PROBIOTIC LACTIC ACID BACTERIA AND YEASTS IN RATS FED HIGH CHOLESTEROL DIET.

Oluwaseun G Kolawole [1] - Kolawole Banwo [1] - Abiodun I Sanni [1]
University of Ibadan, Department of Microbiology, Ibadan, Oyo State, Nigeria [1]

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

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

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

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

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

DETECTION OF NEW PROBIOTICS IN THE PRESENCE OF VIRAL GASTROENTERITIS

Gulcin Avci [1]
Hitit University, Molecular Biology And Genetics, Corum, Turkey [1]

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

Methods
Lactobacillus spp. identification was performed in stool samples of 188 children with gastroenteritis and 40 healthy children who underwent rotavirus and norovirus research by ELISA.
Lactobacilli were selected according to gram reaction, morphology, classical identification (API) and molecular (16sRNA) tests. Acid resistance (pH 1.5-8.2), bile tolerance (0.01-0.4 %), antimicrobial activity against *E.coli* ATCC 11229, exopolysaccharide (EPS) production, bile acid deconjugation and cholesterol removal of isolates were identified.

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

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

PROBIOTIC AS A POTENTIAL THERAPEUTICS IN PREVENTION AND TREATMENT OF ACUTE DIARRHOEA WITH GASTROINTESTINAL INFECTIONS IN CHILDREN; A CONVENTIONAL THERAPEUTICS APPROACH"

Prabhurajeshwar C [1] - Kelmani Chandrakanth R [2]
Department of Microbiology, Viral Research and Diagnostic Laboratory, ICMR, Gulbarga Institute of Medical Sciences, Gulbarga, Karnataka [1] - Department of Biotechnology, Gulbarga University, Gulbarga, Karnataka [2]

In medicine, "diarrhoea" means "a flow through", also defined as "the passage of three or more loose or liquid stools per day, more frequently than is normal for the personality". If left untreated, diarrhoea can lead to severe dehydration, which can result in hospitalisation or even death. It is usually the symptom of gastrointestinal infection, most cases of diarrhoea in children result from infection caused by a variety of viruses, bacteria or parasites, which disturb the normal fluid and nutrient assimilation of the intestines. As per UNICEF data: Monitoring the situation of Children and Women report June 2016, diarrhoeal diseases accounting for 9 percent of all deaths among the children under five years of age making them the second largely common cause of child deaths worldwide. *Shigella* species is the second most widespread bacterial agents causing diarrhoea after *Escherichia coli*. Shigellosis is endemic to many developing countries and also occurs in outbreak causing substantial morbidity and mortality. Most *Shigella* infections result sporadically, but huge *Shigella* epidemic have been traced to contaminated food and water. In local Gulbarga district and surrounding region of Gulbarga (Karnataka state), diarrhoea has been estimated to be responsible for approximately 11-13% of all childhood illness, with a population of about 5,32,031. Among the four species of *Shigella*, *Shigella dysentriae* and *Shigella flexnerii* were more predominant one. At present, multi-drug resistance has complicated the assortments of empirical agents used for treatment of shigellosis, particularly in children. The emergence of fluoroquinolone resistance in *Shigella* sps and their dissemination across the countries, so the practicing the prevention and develop into protocols with natural pattern consider to be essential. The present research work is efforts made to develop alternative to conventional drugs. Now, the application of *Lactobacillus* species as a probiotic to the prevention, management and became possible option is use to probiotic *Lactobacillus* species as a biotherapeutics against enteric infections. Our results and its importance suggest that the *Lactobacillus* treatment is potentially useful for treatment of *Shigella* infections, especially in children. Scientific proof of the *in-vivo* efficacy and safety of probiotic bacteria as biotherapeutics has validated its clinical use in real-life situations for human health benefits. Hence, we conclude that, our *Lactobacillus* species as a probiotic showed more efficient results/ reports. Also this study provides the support for the formulation of novel probiotic as a biotherapeutic agent or supplements that can play a role in the prevention of gastro intestinal infection especially infantile diarrhoea and other related enteric infection.

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

PREVENTIVE ROLE OF FOUR-COMPONENET PROBIOTIC IN ICU PATIENTS WITH CDI AND DETECTION OF ENVIRONMENTAL SOURCES

Katja Popovska [1] - Kartalov A [2] - Trajkovska Dokic E [1] - Andonovska B [2] - Shosholcheva M [2]
Institute for microbiology UKIM Skopje RM [1] - Clinic for anesthesiology, intensive care and reanimation TOARIL [2] - Medical Faculty Ukim, Institut For Microbiology, Skopje, Macedonia [1]

Introduction
Laboratory manipulation of *C. difficile* requires no oxygen contamination, and enriched nutrient media with reductive agents (Thioglycolate and L-cistine) and antibiotic mixture to inhibit other environmental microbial agents.

Aim of study
Detection of *C. difficile* from hospital environment in neurosurgical intensive care unit after CDI patients were confirmed in this unit.

Design of study
A study was performed in ICU of the University Clinic of Neurosurgery. The first step of the study included 32 senior and debilitated patients with antibiotic therapy in order to detect CDC/CDI associated with/without probiotic administration. Stools were collected twice from each patient (first 3 /7-10th day). Total of 120 samples were collected with moistened rayon swabs from environment/patients' palms. Inoculation of samples was performed by Evans recommendations in selective (TB and CDFA) pre-reduced media (1).

Results and discussion
GUT flora was reduced in 100% when antibiotic therapy was not combined with probiotics. Acquisition of CDC was significantly lower in probiotic group 12.5% *vs.* 25% in the first 3 days and 6.25% *vs.* 37.5% after 7-10 days (Table 1).
Fifty samples (24.5%) collected with rayon swabs from patients' beds were positive to facultative anaerobic microorganisms: *Klebsiella aerogenes*, *Enterococcus spp., MRSA* and *Candida albicans*. Only 6.6 samples in TSB presented germinative shapes of CD spores. None of patients' hand samples was positive for opportunistic microorganisms.

Conclusion
Pre-reduced anaerobic selective media by Evans in combination with strictly controlled anaerobic conditions during sampling can be effective in detecting some spores *of C. difficile* in environmental samples.

Key words
Clostridium difficile, environment, anaerobic cultivation

PREVENTION OF RECURRENT URINARY TRACT INFECTIONS: EFFICACY OF A FORMULATION CONTAINING THE SELECTED LACTOBACILLUS PARACASEI LC11, CRANBERRY AND D-MANNOSE

Filippo Murina [1] - Patrizia Malfa [2] - Letizia Fracchia [3] - Ariella Annoni [4] - Franco Vicariotto [1]

Lower Genital Tract Unit-Obst. And Gyn, Dept. V.Buzzi Hospital- University of Milan- Milan, Italy [1] - Proge Farm Srl, Largo Donegani 4/A- Novara, Italy [2] - Department of Pharmaceutical Sciences, Università del Piemonte Orientale "A. Avogadro", Largo Donegani 2, 28100, Novara, Italy [3] - Montefarmaco OTC S.p.A., Via IV Novembre 92/94, Bollate, Italy [4]

Objective

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

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

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

Methods

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

Results

the recurrent cystitis episodes were significantly lower in both group 1 and group 2 (53% vs 16%, p<0,01) compared to control. During the 6 months follow up there were no differences in recurrent cystitis episodes in group 1 and group 2 (66% vs 69%, p<0,02).

Conclusions

Lactoflorene®Cist was effective in the prevention of recurrent cystitis episodes.

THE ROLE OF A LOW PROTEIN DIET (LPD) WITH AND WITHOUT PROBIOTICS IN PATIENTS WITH ADVANCED RENAL FAILURE.

Luca Mogna [1] - Andreana De Mauri [2] - Doriana Chiarinotti [2] - Marco Pane [1] - Angela Amoruso [1] - Mario Del Piano [3]

Biolab Research Srl, Ricerca & Sviluppo, Novara, Italy [1] - Maggiore della Carità University Hospital, Nephrology and Dialysis Unit, Novara, Italy [2] - Santa Rita Hospital - Policlinico di Monza, Gastroenterology Department, Vercelli, Italy [3]

Objective

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

The aim of our study was to evaluate the association of LPD and selected probiotics, namely $5x10^9$ CFU of *Bifidobacterium longum* (mix DLBL) and 1 x 10^9 CFU of *Lactobacillus reuteri* LRE02 (DSM 23878), in reducing the levels of PC, IS and Lipoprotein-associated Phospholipase A_2 (Lp-PLA_2) in patients with advanced renal failure.

Methods

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

Results

9 drop-outs were recorded. A significant reduction of uric acid (5.9 ± 1.4 vs 6.4 ± 1.4 mg/dl, p=0.07), triglycerides (172 ± 71 vs 239 ± 210 mg/dl, p=0.08), C-reactive protein (71.4 ± 18.8 vs 60.9 ± 16.2 g/24h, p=0.0077) and Lp-PLA_2 (170.2 ± 52 vs 158.5 ± 51.9 nmol/ml/min, p=0.04) was recorded after T5 or T2 compared with T0. Lp-PLA_2 and PC were reduced by the LPD but were not affected by probiotics, while IS metabolism was affected by probiotics.

Conclusions

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

CORRELATION OF ANORECTAL MOTILITY AND SENSITIVITY CHARACTERISTICS WITH SMALL INTESTINAL BACTERIAL OVERGROWTH IN PATIENTS WITH DIARRHEA-PREDOMINANT IRRITABLE BOWEL SYNDROME

Anatoly Ulyanin [1] - Olga Storonova [1] - Vladimir Ivashkin [1] - Elena Poluektova [1] - Alexander Trukhmanov [1] - Oleg Shifrin [1]
Sechenov University, Chair of internal diseases propedeutics, medical faculty, Moscow, Russian Federation [1]

Introduction

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

Aim of the study

to assess prevalence of SIBO in IBS-D patients and to assess correlation between SIBO and anorectal motility and sensitivity abnormalities in IBS-D patients.

Materials and methods

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

Results

SIBO was found in 24 patients with IBS-D (68,5 %). According to HRAM IBS-D patients with SIBO had decreased absolute anal squeeze pressure (p=0,012), maximum absolute anal squeeze pressure (p=0,011), threshold for intense urge to defecate (p=0,022) and maximum tolerable volume (p=0,025) comparing to non-SIBO IBS-D patients.

Conclusions

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

COMPARISON OF SPECIFIC IGG AND IGA SUBCLASS LEVELS TO LACTOBACILLUS AND STREPTOCOCCUS IN YOUNG HEALTHY ADULTS

Luka Dragacevic [1] - Rajna Minic [1] - Danica Michalickova [2] - Vesna Ilic [3] - Brizita Djordjevic [4]
Institute of Virology, Vaccines and Sera "Torlak", Department of Research and Development, Belgrade, Serbia [1] - Institute of Pharmacology, First Faculty of Medicine, Charles University, Prague, Czech Republic [2] - Institute for Medical Research, University of Belgrade, Belgrade, Serbia [3] - Department of Bromatology, University of Belgrade, Faculty of Pharmacy, Belgrade, Serbia [4]

Objective

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

Methods

Serum samples were collected from 22 young healthy adults, and were used as a pool.
The methodology consisted of attaching whole bacterial cells to ELISA plate, followed by blocking, the addition of pooled serum sample, which was dilluted in PBS 500 x for IgG, IgG1 and IgG2, 250 x times for IgG3 and IgG4, 100 x for total IgA analysis and 50 x for IgA1 and IgA2. Commercial secondary antibodies were all monoclonal, biotinylated. For statistical analysis bacteria were grouped as *Lactobacillus* (8 strains), *Streptococcus* (5).

Results

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

Conclusions

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

PROSPECTS OF PROBIOTIC LECTINS AS FUNCTIONAL FOOD INGREDIENTS

Mikhail Lakhtin [1] - Vladimir Lakhtin [1] - Valery Davidkin [1]
G.N. Gabrichevsky Research Institute for Epidemiology & Microbiology, Medical Biotechnology, Moscow, Russian Federation [1]

Objective

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

Methods

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

Results

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

Conclusions

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

STUDY OF TWO COMPLEMENTARY METHODS FOR THE QUANTIFICATION OF PEPTIDOGLYCAN IN GRAM-POSITIVE BACTERIA.

Marco Pane [1] - Luca Mogna [1] - Angela Amoruso [1] - Silvia Rapacioli [2] - Vanessa Coghetto [2] - Elisa Breviario [2] - Ilaria Vitangeli [2]
Biolab Research Srl, Ricerca & Sviluppo, Novara, Italy [1] - BiCT Srl, Ricerca & Sviluppo, Lodi, Italy [2]

Objective

The peptidoglycan (PG), also known as murein or bacterial mucopeptide, is a polymer that represents an essential component of the cell wall of bacteria responsible for both shape determination and cellular integrity under osmotic stress.
Its main role is to maintain the structure of the cell, provide a basis of attachment for the outermost proteins and take part in the processes of cell growth and division, so that the inhibition of its synthesis leads to lysis and, therefore, to cell death.
The PG layer is formed by saccharide chains joined by peptide bridges.
A quantitative understanding of the relationships between PG architecture, morphogenesis, immune system activation and pathogenesis can provide molecular-scale insights into the function of this polymer involved in cell wall synthesis and cell growth.

Methods

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

Results

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

Conclusions

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

INTESTINAL EPITHELIAL BARRIER REGULATION WITH PROBIOTICS

Diana Paveljšek [1] - Roman Jerala [2] - Irena Rogelj [1]
University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Ljubljana, Slovenia [1] - National Institute of Chemistry, Department of Synthetic Biology and Immunology, Ljubljana, Slovenia [2]

Objective

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

Methods

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

Results

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

Conclusions

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

ANTICARCINOGENIC ACTIVITY OF B. LONGUM BAA-999 MICROENCAPSULATES AND LYCOPENE AGAINST AZOXYMETHANE-DEXTRAN SULFATE SODIUM (AOM-DSS) INDUCED COLON CARCINOGENESIS IN CD-1 MICE

Nancy Valadez [1] - Eleazar Escamilla [2] - Montserrat Hernández [1] - Blanca García [1] - Minerva Ramos [1]
Universidad Autónoma de Querétaro, Facultad de Química, Querétaro, Mexico [1] - TecNM/Instituto Tecnológico de Celaya, Departamento de Ingeniería Química, Celaya, Mexico [2]

Objective

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

Methods

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

Results

BF and BF+LYC-treated groups had significantly lower inflammation grade, mean tumor number and tumor (13-38%) and adenocarcinoma (13-14%) incidence compared to AOM+DSS-treated animals (80%); with a higher distribution at the distal colon (67-100%), whereas LYC treatment only protected against inflammation grade and incidence. pH and Beta-GA values were significantly higher in the caecum, colon and feces samples of AOM+DSS control group and normalized by BF and/or LYC. Similar to the histopathology analysis, only BF and BF+LYC treatments significantly reduced both positive rate and expression grade of IGF-1 and IGF-1R proteins and normalized IGFBP3 expression, however, LYC co-administration did not further improve these values.

Conclusions

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

PRUNUS MAHALEB FRUIT FERMENTATION FOR NEW FUNCTIONAL FOODS AND BEVERAGES

Federico Baruzzi [1] - Francesco Grieco [2] - Luca Giordano [3] - Patrizia Rampino [3] - Vittorio Capozzi [4] - Carmela Gerardi [2]

Istituto di Scienze delle Produzioni Alimentari - CNR, Department of biology, agriculture and food sciences, Bari, Italy [1] - Istituto di Scienze delle Produzioni Alimentari - CNR, Department of biology, agriculture and food sciences, Lecce, Italy [2] - University of Salento, Department of Biological and Environmental Sciences and Technologies, Lecce, Italy [3] - University of Foggia, Department of the Sciences of Agriculture, Food and Environment, Foggia, Italy [4]

Objective

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

Methods

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

Results

Results indicated that all strains were able to grow and ferment fruits and that all *L. plantarum* strains were able to survive to simulated gastric and pancreatic digestions. However, the starter *L. plantarum* FG69 + *S. cerevisiae* Li180-7 had the best impact on sensory characteristics.

Conclusions

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

TRIBIOTICS DESIGN FROM BOVINE COLOSTRUM BY ARTISANAL AND SELECTED CULTURES WITH MULTIPLE METABOLIC ACTIVITIES

Mihaela Cotârleț [1] - Aida Mihaela Vasile [1] - Alexandra Gaspar-Pintiliescu [2] - Oana Craciunescu [2] - Anca Oancea [2] - Gabriela Elena Bahrim [1]

Dunarea de Jos University, Faculty of Food Science and Engineering, Galati, Romania [1] - National Institute of Research and Development for Biological Sciences, Cellular and Molecular Biology, Bucharest, Romania [2]

Objective

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

Methods

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

Results

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

Conclusions

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

USE OF AUTOCHTHONOUS PROBIOTIC YEASTS TO IMPROVE THE AROMA PROFILE OF FERMENTED CHILI PEPPER SAUCES

Carlos Lara-Hidalgo [1] - Carmela Belloch [2] - Lidia Dorantes-Alvarez [3] - Mónica Flores [1]
Instituto de Agroquímica y Tecnología de Alimentos, Ciencia de Alimentos, Valencia, Spain [1] - Instituto de Agroquímica y Tecnología de Alimentos, Biotecnología, Valencia, Spain [2] - Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Ciudad de México, Mexico [3]

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

Methods

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

Results

A total of 78 volatile compounds were identified during the fermentation. Among them 34 were aroma active compounds detected by GCO. Propanoic acid (cheesy), 3-methylbutanoic acid (sharp, cheese), ethyl 2-methylbutanoate (fruity), and 6-methyl-5-hepten-2-one (strong, citrus) were identified as key aroma contributors produced by the inoculation of the yeasts. All aroma compounds detected were affected by fermentation time, while aldehydes and terpenes, were not affected by yeast inoculation.

Conclusions

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

ARTEMISIA UMBELLIFORMIS SUBSP. ERIANTHA EXTRACT INHIBITS GROWTH AND INVASION OF HEPATOCARCINOMA CELL LINES

Federica Ragusa [1] - Giulia Caioni [1] - Gianluca Solito [1] - Mauro Giorgi [2] - Loretta Giuseppina Pace [1] - Mara Massimi [1]
University of L'Aquila, Department of Life, Health, and Environmental Sciences, L'Aquila, Italy [1] - Sapienza University of Rome, Department of Biology and Biotechnology "Charles Darwin", Rome, Italy [2]

Objective

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

Methods

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

Results

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

Conclusions

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

EFFECT OF INULIN DP ON QUALITY CHARACTERISTICS OF FUNCTIONAL WHOLE-MEAL SPAGHETTI OF DURUM WHEAT OLD VARIETIES

Carla Sillitti [1] - Angela Cardinali [2] - Salvatore Scandurra [1] - Isabella D'Antuono [2] - Vito Linsalata [2] - Amalia Conte [3] - Matteo Alessandro Del Nobile [3] - Maria Grazia Melilli [1]
Institute for Agricultural and Forest Systems in the Mediterranean, National Council of Research, Catania, Italy [1] - Institute of Sciences of Food Production, National Council of Research, Bari, Italy [2] - Department of Agricultural Sciences, Food and Environment, University of Foggia, Foggia, Italy [3]

Objective

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

Methods

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

Results

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

Conclusions

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

BIOCHEMICAL PROPERTIES, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF MULBERRY FRUITS FROM SOUTH APULIA (SOUTH ITALY)

Antonio Miceli [1] - Carmine Negro [1] - Alessio Aprile [1] - Luigi De Bellis [1]
Università del salento, Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Lecce, Italy [1]

Objective

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

Methods

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

Results

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

Conclusions

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

RELEASE, STABILITY AND BIOACCESIBILITY OF BIOACTIVE PEPTIDES DURING IN VITRO DIGESTION

Ljiljana Popovic [1] - Jelena Cakarevic [1] - Tea Sedlar [1]
Faculty of Technology, University of Novi Sad, University of Novi Sad, Novi Sad, Bul. cara Lazara 1, Serbia [1]

Objective

In the scientific literature, which is focused on the research and detection of biological activity of different food components, recently there is a great interest for investigation of their fate in process of gastrointestinal digestion. Biologically active compounds, such as peptides, polyphenols, flavonoids, carotenoids, prebiotics, probiotics, etc., generally, are chemically and thermally unstable, sensitive to oxidation and other environmental conditions. Therefore, their release, stability and availability during the passage through the digestive tract can be affected. As the *in vivo* tests are time and financially demanding, there is a growing interest for *in vitro* tests, which are useful tools for examining and understanding of the changes, interactions, bioavailability and bioaccessibility of different nutrients and biologically active compounds.

Methods

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

Results

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

Conclusions

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

FERMENTATION OF KIWICHA (AMARANTHUS CAUDATUS), A PSEUDOCEREAL ORIGINARY FROM PERU, BY LACTOBACILLUS RHAMNOSUS.

Tatiana Rojas Ayerve [1] - Seppo Salminen [2] - Ritva Reppo-CarrascoValencia [3] - Gabriel Vinderola [4]
Postgraduate School, National University of Agriculture - Chemistry Department, Lima, Peru [1] - Functional Foods Forum, University of Turku, Turku, Finland [2] - Postgraduate School, National University of Agriculture-Food Industry Faculty, Lima, Peru [3] - INLAIN, National University of Santa Fe- Chemical Enginieering Faculty, Santa Fe, Argentina [4]

Objective

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

Methods

Kiwicha (Centenario variety) was suspended (20 % w/v) in water and heat treated (54°Cx30 min, 65 Cx60 min, 72 °Cx30 min). Overnight *L. rhamnosus* cultures were inoculated (v/v) with the following set: 1% CRL1505, 1% ATCC 53013, 0.1% LC705, 1% CRL505/0.1% LC705, and 1% ATCC 53013/0.1% LC705. Samples were incubated (37 °C, aerobiosis) for 10h. Total titratable acidity, pH and cell counts were assessed after 6 and 10h of fermentation and after 6, 12 and 22 days of storage at 4 °C.

Results

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

Conclusions

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

SCREENING OF LACTOBACILLUS SP. ISOLATED FROM WHEAT AND SORGHUM SOURDOUGHS MAY HYDROLYZE GLUTEN DURING THE FERMENTATION OF A GLUTEN-CONTAINING MEDIUM

Marcela Albuquerque Cavalcanti de Albuquerque [1] - Kamel Eddine El Merchefi [2] - Vanessa Bíscola [3] - Antônio Diogo Silva Vieira [1] - Susana Marta Isay Saad [4] - Bernadette Dora Gombossy de Melo Franco [1]

Food Research Center, University of São Paulo, São Paulo, Brazil [1] - UR1268 BIA/Allergy Team, Institut National de la Recherche Agronomique, Nantes, France [2] - Clock-T (Cryolog), R&D department, Nantes, France [3] - School of Pharmaceutical Sciences, University of São Paulo, Department of Biochemical-Pharmaceutical Technology, São Paulo, Brazil [4]

Objective

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

Methods

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

Results

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

Conclusions

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

INVESTIGATING POTENTIAL CHEMOPROTECTIVE ROLE OF POMEGRANATE JUICE THROUGH ANALYZING ITS RECIPROCAL INTERACTIONS WITH THE GUT MICROBIOTA.

Heba Attia [1] - Ibrahim E. Sallam [2] - Amr Abdelwareth [3] - Alex Mira [4] - Rania A. Khattab [1] - Aymen S. Yassin [1] - Mohamed A. Farag [3] - Ramy K. Aziz [1]

Faculty of Pharmacy, Cairo University, Microbiology and Immunology Department, Cairo, Egypt [1] - Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Pharmacognosy Department, Cairo, Egypt [2] - School of Sciences and Engineering, The American University in Cairo, Chemistry Department, Cairo, Egypt [3] - Center for Advanced Research in Public Health, FISABIO Foundation, Department of Health and Genomics, Valencia, Spain [4]

Objective

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

Methods

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

Results

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

Conclusions

This pilot work serves as a standardization step for a model to test the potential chemoprotective effect of pomegranate (and other natural polyphenols) against the carcinogenic effect of selected polycyclic hydrocarbons.

AKKERMANSIA MUCINIPHILA ROBUSTNESS TOWARDS DIFFERENT TEMPERATURES, ATMOSPHERES AND GASTROINTESTINAL CONDITIONS

Ana Cristina Freitas [1] - Daniela Machado [1] - Diana Almeida [1] - Catarina Seabra [1] - José Carlos Andrade [2] - Ana Maria Gomes [1]

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal [1] - CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra PRD, Portugal [2]

Objectives

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

Methods

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

Results

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

Conclusions

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

Acknowledgments

This work was supported by national funds through FCT/MEC (PIDDAC), project IF/00588/2015 and by Operational Program Competitiveness and Internationalization (FEDER), by the Foundation for Science and Technology budget, I.P. in its OE component, project POCI-01-0145-FEDER-031400 with CBQF scientific collaboration with FCT project UID/Multi/50016/2013.

(1)Minekus et al., Food Funct. 5(2014):1113–1124.

POSTBIOTIC CHARACTERIZATION IN THE AMENSALISTIC SYMBIOSIS AND CORRELATION TO THE RESILIENCE OF HUMAN MICROBIOTA

Virginia Fuochi [1] - Desirèe Sharon Vincenti [1] - Luca Laghi [2] - Antonio Rescifina [3] - Pio Maria Furneri [1]

Università degli Studi di Catania, BIOMETEC, Catania, Italy [1] - ALMA MATER STUDIORUM - Università di Bologna, Dipartimento di Scienze e Tecnologie Agro-Alimentari, Bologna, Italy [2] - Università degli Studi di Catania, Dipartimento di Scienze del Farmaco, Catania, Italy [3]

Objective

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

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

Methods

In particular, the production of antimicrobial molecules by varying the culture medium for the greatest yield was achieved. Subsequently, the antimicrobial activity of the cell free supernatans (CFSs) was evaluated by agar well diffusion assay. Furthermore, metabolic characterization was assessed by ^{1}H-NMR analysis.

Results

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

Conclusions

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

ANTIMICROBIAL ACTIVITY OF CELL-FREE SUPERNATANT OF LACTOBACILLUS PLANTARUM IMC 509 AGAINST COMMON FOOD SPOILAGE MICROBES

Xiaohui Huang [1] - Franks Kamgang Nzekoue [1] - Maria Magdalena Coman [2] - Anca Peter [3] - Catalina Mihaela Talasman [4] - Goran Drazic [5] - Antonio Peñas [6] - Maria Cristina Verdenelli [2] - Gianni Sagratini [7] - Stefania Silvi [8]
Università degli Studi di Camerino, School of Advanced Studies, Camerino, Italy [1] - Synbiotec Srl, Synbiotec Srl, Camerino, Italy [2] - Technical University of Cluj Napoca, Department of Chemistry, Baia Mare, Romania [3] - Ceprohart, Ceprohart, Braila, Romania [4] - National Institute of Chemistry, National Institute of Chemistry, Ljubljana, Slovenia [5] - Andaltec, Andaltec, Jaén, Spain [6] - Università degli Studi di Camerino, School of Pharmacy, Camerino, Italy [7] - Università degli Studi di Camerino, School of Biosciences and Veterinary Medicine, Camerino, Italy [8]

Objectives

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

Methods

L. plantarum IMC 509 (10^{11}CFU/g) lyophilized powder was cultured in MRS broth. To eliminate cells, centrifugation and 0.2µm filter membranes were used. CTR-supernatant was without treatment; in OA-supernatant pepsin, catalase, proteinase K were added to test the organic acids effects. HO-supernatant was treated with proteinase K and with neutralized pH to monitor the H_2O_2 effects. In BLS-supernatant catalase was added and pH neutralized to check bacteriocin-like-substances. Several Gram positive and Gram negative bacteria strains and one yeast were selected for the well diffusion test. Mueller-Hinton agars were inoculated with tested microbes and loaded with four supernatants. Plates were incubated and inhibition zones were checked and measured.

Results

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

Conclusions

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

Acknowledgments

Supported by the project GRAFOOD, M-ERA NET call 2016.

VIABILITY AND SYNERGISTIC ANTIBACTERIAL EFFECTS OF INDIGENOUS PROBIOTIC BACTERIA AND HERBAL EXTRACTS IN INVITRO CONDITIONS

Naheed Mojgani [1]
Associate Professor, Razi Vaccine & Serum Research Institute, Biotechnology Department, Karaj, Iran (islamic Republic Of) [1]

In present research we investigated the antibacterial potential of local isolates of probiotic bacteria (*Lactobacillus casei, L. plantarum, L.reuteri, Enterococcus faecium* and *Pediococcus acidilacti*) in the presence of herbal extracts [Chamomile (Matricaria chamomilla), Fennel (Foeniculum vulgare), Peppermint (Mentha piperita) and thyme (thymus vulgaris)]. *Escherichia coli, Salmonella typhimurium* and *Salmonella enteriditis* were used as indicator organisms. The antibacterial activity of the mentioned probiotic isolates and the herbal extracts against the selected pathogens were examined by agar well diffusion method. The synergistic antibacterial effect and the viability (survival percentage) of the probiotic bacteria in combination with different concentrations of the mentioned herbal extracts were determined after 24, 48 and 72 hours. The minimum inhibitory concentrations (MIC) of the herbal extracts on the indicator organism (10^6 cfu/ml) was measured by microdilution assay. All experiments were performed in triplicate and results analyzed statistically. According to the results, all selected LAB strains and thyme extracts were able to inhibit the growth of the tested gram negative However, *L.plantarum* and *E.faecium* showed maximum inhibitory actions towards *S.typhimurium*. Probiotic bacteria mixed with thyme and peppermint showed synergistic effects and their combined use resulted in enhanced antibacterial actions. The viability of the probiotic bacteria in the presence of diluted (above 1:10) thyme and peppermint extracts were above 70%. In conclusion, the antibacterial activity exerted by probiotic bacteria against the pathogens could be significantly enhanced when combined with herbal extracts, and hence might be considered as suitable alternative to antibiotics for use in man and animals in future.

Keywords

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

SHORT CHAIN FATTY ACIDS PROFILE DURING REMISSION OR ACTIVITY PERIODS IN IBD PATIENTS

Ana Quaglio [1] - Gabriela Batista [2] - Luiz Almeida-Jr [1] - Ligia Sassaki [2] - Luiz Di Stasi [1]

São Paulo State University, Department of Pharmacology, Institute of Biosciences, Botucatu, Brazil [1] - São Paulo State University, Department of Medical Clinic, Botucatu, Brazil [2]

Objective

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

Methods

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

Results

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

Conclusions

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

CHARACTERIZATION OF MICROVESICLES OF STRAIN LACTOBACILLUS PLANTARUM BGAN8 AND INTERNALIZATION BY HT29 CELLS

Svetlana S. Sokovic Bajic [1] - Svetlana S. Sokovic Bajic [2] - Maria-Alexandra Cañas [3] - Maria-Alexandra Cañas [4] - Jovanka M. Lukic [1] - Dusan D. Radojevic [1] - Josefa Badia [3] - Josefa Badia [4] - Abelardo Margolles [2] - Natasa E. Golic [1] - Laura Baldomá [3] - Laura Baldomá [4] - Patricia Ruas-Madiedo [2] - Maja S. Tolinacki [1]

Institute of Molecular Genetics and Genetic Engineering (IMGGE), University of Belgrade, Serbia, Laboratory for Molecular Microbiology (LMM), Belgrade, Serbia [1] - Instituto de Productos Lácteos de Asturias, Consejo Superior de Investigaciones Científicas (IPLA-CSIC), Department of Microbiology and Biochemistry of Dairy Products, Villaviciosa, Asturias, Spain, Villaviciosa, Spain [2] - Facultat de Farmàcia, Universitat de Barcelona, Secció de Bioquímica i Biología Molecular, Departament de Bioquímica i Fisiologia, Barcelona, Spain, Barcelona, Spain [3] - Institut de Biomedicina de la Universitat de Barcelona, Institut de Recerca Sant Joan de Déu (IRSJD), Barcelona, Spain, Barcelona, Spain [4]

Objective

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

Methods

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

Results

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

Conclusions

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

PROBIOTIC AND POSTBIOTIC EFFECTS OF L. RHAMNOSUS GG AGAINST RV-INDUCED DIARRHEA

Carla Russo [1] - Vittoria Buccigrossi [1] - Antonella Marano [2] - Sara Terranova [1] - Valentina Cioffi [1] - Alfredo Guarino [1]
University of Naples Federico II, Department of Translational Medical Science, Naples, Italy [1] - University of Naples Fderico II, Department of Translational Medical Science, Naples, Italy [2]

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

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

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

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

Conflict of interest
The study was supported by Dicofarm SpA.

EFFECT OF PROBIOTIC AND AUTOPROBIOTIC ENTEROCOCCI ON CONTRACTILE ACTIVITY OF RATS INTESINUM AFTER CORRECTION OF DYSBIOSIS

Elena Pariyskaya [1] - Lydia Zakharova [1] - Alexander Suvorov [2] - Elena Ermolenko [2]
Department of Physiology, Faculty of Medicine, Saint-Petersburg State University, Saint-Petersburg, Russian Federation [1] - Department of molecular microbiology, Institute of Experimental Medicine, Saint-Petersburg, Russian Federation [2]

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

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

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

Conclusions
Restoration of the tonic motor function of the colon occurred more effectively after exposure of probiotic (L3) and autoprobiotic Enterococcus faecium. The obtained results can be used in the development of new medical technologies for treating irritable bowel syndrome and disfunction of gastrointestinal tract accompanied by other human diseases.
This work was supported by Russian Science Foundation under grant № 16-15-10085.

SECRETOME MODULATION OF CACO2 CELL LINE INDUCED BY A MULTI-STRAIN PROBIOTIC

Valentina Petito [1] - Viviana Greco [2] - Lucrezia Laterza [3] - Cristina Graziani [3] - Loris Lopetuso [3] - Franco Scaldaferri [3] - Andrea Urbani [4] - Antonio Gasbarrini [3]

Università cattolica del Sacro Cuore, Institute of Medical Pathology, ROMA, Italy [1] - Institute of Biochemistry and Clinical Biochemistry, Università cattolica del Sacro Cuore, ROMA, Italy [2] - Gastroenterological Area, Gastroenterological, Endocrino-Metabolical and Nefro-urological Sciences Department, Fondazione Policlinico Universitario Gemelli IRCCS, ROMA, Italy [3] - Department of Laboratory Diagnostic and Infectious Diseases, Fondazione Policlinico Universitario Gemelli IRCCS, ROMA, Italy [4]

Background

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

*Recently reclassified as *L. helveticus.*

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

Aim

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

Methods

Two different lots of VSL#3 (Manufacturer: Nutrilinea Srl, Gallarate (VA) – Italy, lot #802097 and lot #802100) were used. Caco2 cell line were treated with a conditioning media (CM) prepared using 1g of probiotic formula grown in D-MEM cell culture medium (free of serum and antibiotics) at 37°C for 48 hours without shaking and in anaerobic conditions. The CM will be centrifuged at 4,100 rpm for 10 min to separate the bacteria, and the resulting supernatant will be filtered through a 0.2 mm syringe filter to remove any insoluble particles and diluted 1:10 and 1:25. Caco2 were treated with diluted CM at 24 and 48 hours. Media culture for each conditions has been collected and analyzed by a deeper proteomics approach.

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

Results

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

Conclusions

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

1. Schrezenmeir J, de Vrese M (2001) Probiotics, prebiotics and synbiotics approaching a definition. Am J Clin Nutr 73: 361S–364S.
2. Dotan I, Rachmilewitz D (2005) Probiotics in inflammatory bowel disease: possible mechanisms of action. Current Opinion in Gastroenterology 21:426–430.
3. Greco V, Spalloni A (2018) Proteomics and Toxicity Analysis of Spinal-Cord Primary Cultures upon Hydrogen Sulfide Treatment. Antioxidant 2018 Jul 10;7(7)

AUTOPROBIOTICS IN TREATMENT OF IBS PATIENTS

Alexander Suvorov [1]
Institute Experimental Medicine, Molecular Microbiology, Saint-Petersburg, Russian Federation [1]

Objective

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

Methods

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

Results

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

Conclusions

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

ELDERLY PEOPLE INTESTINAL MICROBIOTA AS A TARGET FOR PROBIOTIC FUNCTIONAL FOOD INTERVENTION

Chiara Salvesi [1] - Maria Cristina Verdenelli [2] - Dennis Fiorini [3] - Stefania Silvi [4]
Università degli Studi di Camerino, School of Advanced Studies, Camerino, Italy [1] - Synbiotec Srl, Synbiotec Srl, Camerino, Italy [2] - Università degli Studi di Camerino, School of Sciences and Technologies, Camerino, Italy [3] - Università degli Studi di Camerino, School of Biosciences and Veterinary Medicine, Camerino, Italy [4]

Objective

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

Methods

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

Results

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

Conclusions

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

Acknowledgments

This work was supported by the project PROBIOSENIOR, granted by POR FESR 2014-2020.

INDIVIDUALIZED SHORT-TERM PROBIOTIC THERAPY OF METABOLIC SYNDROME ACCORDING TO THE HOST'S PHENOTYPE

Rostyslav Bubnov [1] - Mykola Spivak [1]
Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Interferon department, Kyiv, Ukraine [1]

Objective

Metabolic syndrome (MetS) requires detection of visceral obesity plus any two of the other four factors (hyperglycemia, dyslipidemia, cardiovascular disease, hypertension). Individualized modulating gut microbiome should effectively improve metabolic health.
The **Aim:** was to study the efficacy of individualized short-term probiotic therapy on MetS.

Methods

We included twenty patients (age 37-65 years) with BMI>30, waist circumference (WC)>110; all underwent extensive clinical examination and were stratified according to patterns of MetS as follows: DMT2; NAFLD; hyperuricemia; atherosclerosis; earlty age blood; and 3 patients with normal / low BMI with detected increased visceral fat (VF) on ultrasound. Selected ten patients were given probiotic strains at a dose 10^8 CFU daily during 10 days individually according to the knowledge obtained from in vivo in vitro studies and existing evidence and pathophysiological aspects of potential clinical effects of probiotic strains against particular symptom; and ten patients remained as controls.

Results

Weight, BMI, WC and VF decreased, liver structure, lipidogram amd glucose levels improved after probiotic administration in all patient after focused probiotic administation. The following strains demonstrated highest efficacy in the following conditions:
mixture of L. casei IMV B-7280 / B. animalis VKB / B. animalis VKL strains was effective in decreasing cholesterol levels; L. casei IMV B-7280 - in DMT2; Th1 / Th2 imbalance; L. delbrueckii subsp. bulgaricus IMV B-7281, B. animalis VKB, L. casei IMV B-7280, B. animalis VKL - in NAFLD; strains L. plantarum LM VK7 and V. animalis VKB - in cholestasis;
L. plantarum - in hypertension; in hyperuricemia, gout - Bifidobacterium spp.; in visceral ischemia - Bifidobacterium spp., L. reuteri and L. plantarm; in underweight individuals with increased VF, hyperglycemia and hypercholesterolemia – strains of Bifidobacterium spp., L. plantarum.

Conclusions

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

EFFECT OF A SYNBIOTIC SUPPLEMENT ON CARDIOVASCULAR RISK FACTORS IN OVERWEIGHT OR OBESE PATIENTS WITH POLYCYSTIC OVARY SYNDROME

Mohammad Javad Hosseinzadeh-Attar [1] - Elham Karimi [1] - Elham Alipoor [2] - Ashraf Moini [3]
Tehran University of Medical Sciences, Department of Clinical Nutrition, School of Nutritional Sciences and Dietetics, Tehran, Iran (islamic Republic Of)[1] - Iran University of Medical Sciences, Department of Nutrition, School of Public Health, Tehran, Iran (islamic Republic Of)[2] - Tehran University of Medical Sciences, Department of Gynecology and Obstetrics, Roointan-Arash Maternity Hospital, Tehran, Iran (islamic Republic Of)[3]

Objective

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

Methods

In this randomized double-blind placebo-controlled clinical trial, 88 women, body mass index ≥ 25 kg/m^2 and age 19-37 years, were enrolled. Patients received either a synbiotic supplement (1000 mg capsule containing seven strains of probiotics and inulin) or placebo for 12 weeks. Serum lipids profile and blood pressure were assessed at baseline and following supplementation.

Results

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

Conclusions

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

LACTOBACILLUS REUTERI E MAY ACTIVATE BROWN ADIPOSE TISSUE BY ENHANCING PPAR GAMMA EXPRESSION

Margareta Marusakova [1] - Boris Dudik [2] - Hana Kinova-Sepova [2] - Andrea Bilkova [2] - Peter Krenek [1] - Jan Klimas [1]

Comenius University, Faculty of Pharmacy/Department of Pharmacology and Toxicology, Bratislava, Slovakia (slovak Republic) [1] - Comenius University, Faculty of Pharmacy/Department of Cell and Molecular Biology of Drugs, Bratislava, Slovakia (slovak Republic) [2]

Objective

Some probiotic bacteria are known to improve gut microbiota composition and represent an interesting target in battling obesity. Brown adipose tissue (BAT) plays a significant role in energy homeostasis by taking up fatty acids and glucose to form heat. It has been proposed that activation of BAT could decrease the risk of developing obesity. PPAR gamma, among other effects, is known to promote adipogenesis in BAT and induce BAT recruitment – leading to increase of tissue mass and improvement of glucose homeostasis.

Methods

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

Results

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

Conclusions

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

PAEDIATRIC FUNCTIONAL GASTROINTESTINAL DISORDERS TREATMENT WITH BIFIDOBACTERIUM ES 1 LONGUM: A CROSSOVER, DOUBLE-BLIND, CONTROLLED TRIAL.

Giorgio Bracaglia [1] - Giulia Franceschini [2] - Simona Guglielmi [3] - Dario Alario [4]

Director of Pediatric department, San Giovanni Evangelista Hospital/ Pediatric department, Tivoli, Italy [1] - Undergraduate, Campus Biomedico University of Rome/ Medicine and Surgery/ Pediatric, Rome, Italy [2] - Physician, San Giovanni Evangelista hospital /Pediatric department, Tivoli, Italy [3] - Physician, San Paolo Hospital, Pediatric department, Civitavecchia, Italy [4]

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

ANTIBIOTIC RESISTANCE OF LACTOBACILLUS IN CHILDREN

E. A. Orishak [1] - L. Y. Nilova [1] - A. P. Listopadova [2] - Yu. S. Karpeeva [2] - V. P. Novikova [2]

North-Western State Medical University named after I.I. Mechnikov, Department of medical Microbiology, St. Petersburg, Russian Federation [1] - St. Petersburg State Pediatric Medical University, laboratory of medical and social problems in Pediatrics SIC, St. Petersburg, Russian Federation [2]

Objective

to assess the prevalence of antibiotic resistance among indigenous and probiotic strains of lactobacilli in children.

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

Results

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

Conclusions

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

EFFECTS OF A PROBIOTIC MIXTURE ON MODERATE ATOPIC DERMATITIS OF CHILDREN: A RANDOMIZED CONTROLLED TRIAL

Parnian Poursafa [1] - Roya Kelishadi [2]

University of Isfahan, Biology, Isfahan, Iran (islamic Republic Of) [1] - Child Growth and Development Research Center ,Research Institute for Primordial Prevention of Non Communicable Disease, Isfahan University of Medical, Pediatrics, Isfahan, Iran (islamic Republic Of) [2]

Objective

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

Methods

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

Results

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

Conclusions

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

IN VITRO STUDY OF THE POTENTIAL FOR INHIBITING HAEMOLYTIC PATHOGENS BY SELECTED PROBIOTIC STRAINS: SAFETY ASPECTS AND NEW EFFICACY HORIZONS.

Marco Pane [1] - Angela Amoruso [1] - Serena Allesina [1] - Francesca Deidda [1] - Teresa Graziano [1] - Annachiara De Prisco [1] - Luca Mogna [1]
Biolab Research Srl, Ricerca & Sviluppo, Novara, Italy [1]

Objective

Haemolysis is the lysis of red blood cells (RBCs) and the subsequent release of their contents into surrounding fluid. There are three types of haemolysis, designated alpha, beta and gamma, that could be caused by different pathogenic microorganisms.
A vast body of scientific literature has demonstrated the ability of certain probiotic bacteria to antagonize Gram-positive and Gram-negative strains by secreting soluble molecules named bacteriocins. Anyway, insufficient data are currently available in relation to haemolytic bacteria. Our study focused on *Enterococcus faecalis* ATCC 19433 (γ-haemolysis), *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 8739 (α-haemolysis), *Staphylococcus aureus* ATCC 25923 (beta-haemolysis), and *Pseudomonas aeruginosa* ATCC 9027.

Methods

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

Results

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

Conclusions

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

ACID TOLERANCE OF LACTIC ACID BACTERIA ISOLATED FROM VARIOUS FERMENTED VEGETABLES

Ilkin Yucel Sengun [1] - Miray Gizem Bingol [1]
Ege University, Engineering Faculty, Food Engineering Department, Izmir/Turkey [1]

Objective

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

Methods

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

Results

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

Conclusion

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

Keywords

fermented vegetables, acid tolerance, probiotic, lactic acid bacteria

MODULATION OF GUT MICROBIOME COMPOSITION: EFFECTS OF FRUITS AND VEGETABLES

Caterina Brasacchio [2] - Francesca Pivari [2] - Shaikha Al Abduljabbar [1] - Dhinoth Kumar Bangarusamy [1] - Sara Zaidan [1] - Selvasankar Murugesan [1] - Souhaila Al Khodor [1] - Mario Cozzolino [2] - Annalisa Terranegra [1] - Laura Soldati [2]
Immunology, Inflammation and Metabolism, Sidra Medicine, Doha, Qatar [1] Health Sciences, Nephrology Division, San Paolo Hospital – Universita' degli Studi di Milano, Italy [2]

Background/Objective

It has been well defined over the last decade that the diet influences the health of an individual, especially plant-based diet has been proposed to improve/maintain people's health. But, it remains unclear until recently is the role of gut microbiome to this effect. So, in this study, we aimed to evaluate the beneficial effects of formulated fruits and vegetables supplementation (FVS) on the gut microbiome composition from healthy population.

Materials and Methods

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

Results

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

Conclusion

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

INVESTIGATION OF THE ANTAGONISTIC ACTIVITY OF ENTEROCOCCI AND LACTOBACILLI AGAINST MULTIDRUG-RESISTANT KLEBSIELLA PNEUMONIAE

Alena Karaseva [1] - Elena Ermolenko [1] - Irina Lazareva [2] - Anna Tsapieva [1] - Danyang Guo [3] - Sergey Sidorenko [2] - Alexander Suvorov [1]
Federal State Budgetary Scientific Institution "Institute of Experimental Medicine", Department of molecular microbiology, Saint-Petersburg, Russian Federation [1] - Pediatric Research and Clinical Center for Infectious Diseases, Department of molecular microbiology and epidemiology, Saint-Petersburg, Russian Federation [2] - Shandong Academy of Agricultural Sciences, Agro-food Science and Technology, Shandong, China [3]

Objective

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

Methods

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

Results

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

Conclusions

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

PROBIOTIC POTENTIAL OF KOMBUCHA

Ilkın Sengun [1] - Aysegul Kirmizigul [1]
Ege University, Engineering Faculty, Food Engineering Department, Izmir, Turkey [1]

Kombucha tea, which has recently become popular among many traditional fermented foods, is a beverage of Manchurian origin, generally prepared from black tea with various bacteria and yeast involved in fermentation. Kombucha, a popular beverage in the World, stands out with its positive effects on health as well as its easy preparation and flavored taste. Acetic acid bacteria (AAB; *Komagataeibacter* spp., *Acetobacter* spp., *Gluconobacter* spp.), lactic acid bacteria (LAB; *Lactococcus* spp., *Lactobacillus* spp.) and yeasts (*Schizosaccharomyces pombe*, *Kloeckera apiculata*, *Saccharomyces cerevisiae*, *Saccharomycodes ludwigii*, *Zygosaccharomyces bailii*, *Torulaspora delbrueckii, Brettanomyces bruxellensis*) involved in Kombucha fermentation. Kombucha is considered as a probiotic drink not only for the presence of the diversity of naturally occurring symbiotic microorganisms instead of single strain probiotics in Kombucha, but also providing short chain fatty acids (SCFAs) and other metabolites which improve immunity. At the end of the fermentation, Kombucha is rich in acetic, gluconic, glucuronic, tartaric, malic and less citric acids and limited amount of ethanol and CO_2. The resulting acids decrease the pH value of Kombucha tea and contribute to the formation of its characteristic sour taste. In the studies, it was determined that the mixed culture involved in the Kombucha fermentation has a strong symbiosis that inhibit the growth of pathogenic bacteria and has antiviral, antibacterial and antifungal effects. In this paper, the studies evaluating the probiotic potential of Kombucha were examined.

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

DYSBIOSIS IN THE SMALL INTESTINE: TOWARDS AN OPTIMAL THERAPY TO NORMALIZE THE INTESTINAL MICROBIOTA

Carolina Mosoni [1] - Loris Lopetuso [1] - Giovanni Gasbarrini [2]
Catholic University of the Sacred Heart, Policlinico Gemelli, Internal Medicine, Gastroenterological and Oncological Area, Gastroenterological and Endocrino-Metabolical Sciences Department, Roma, Italy [1] - Catholic University of the Sacred Heart, Emeritus Professor of Internal Medicine, Policlinico Gemelli, Roma, Italy [2]

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

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

Results
1) All subjects at the start of the test were found to have an intestinal "bacterial contamination": the first H2 peak in expired breath occurred within 40 minutes from the start of the test, reaching average levels of over 40 / million, to then re-emerge in at least 2-3 average peaks of 60 / million, remaining at high levels throughout the examination;
2) the test, repeated after a month, gave very different values in the two groups of cases studied:
- in 3 subjects treated with only a cycle of 5 days a month of Rifaximin, the outcome of the breath test has substantially maintained its (pathological) characteristics. In the other 2 cases the first elevation of H2 occurred around 90 minutes and reached maximum levels around 35 / million, and therefore the correction was very partial;
- in 4 subjects treated with two cycles of 5 days each month the breath test indicated substantially normal results with elevation of H2 after 100 minutes and never higher than 20 / million;
- only in one case there was an almost absent elevation of the H2

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

1. Gasbarrini A, *et al.* Methodology and indications of H2-breath testing in gastrointestinal diseases: the Rome Consensus Conference. *Alimentary pharmacology & therapeutics* 29 Suppl 1, 1-49 (2009).
2. Lauritano EC, *et al.* Small intestinal bacterial overgrowth recurrence after antibiotic therapy. *The American journal of gastroenterology* 103, 2031-2035 (2008).

PROBIOTICATION OF DEHYDRATED STRAWBERRY PUREE USING WINDOWS REFRANTANCE AND MICROENCAPSULATION BY VIBRATING TECHNOLOGY

Esmeralda S Mosquera [1] - Alfredo A Ayala [2] - Liliana Serna [2] - Gianluigi Mauriello [3]

Universidad del Valle, School of Food Engineering, Santiago de Cali, Colombia [1] - Universidad Nacional de Colombia, School of Engineering and Administration, Palmira, Colombia [2] - University of Naples Federico II, Department of Agriculture, Naples, Italy [3]

Objective

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

Methods

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

Results

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

Conclusions

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

PHYSIOLOGICAL ASPECTS OF ENTEROCOCCUS GALLINARUM CRL 1826, A POTENTIAL PROBIOTIC FOR RANACULTURE

María Andreína Acevedo [1] - Andrea Lorenzo [2] - Norma Grimald [2] - Sergio Pasteris [1]

Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán. INSIBIO-CONICET, Tucumán, Argentina [1] - Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Tucumán, Argentina [2]

Objective

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

Methods

the *E. gallinarum* strain was grown in MRS broth (pH 6.8, 37°C, 24 h). Cells were collected by centrifugation, washed and resuspended in PBS pH 7.4. 10^9 CFU/mL of fresh cultures (FC), lyophilized (LC) and stored at 4ºC for 1 year (SC), were subjected to simulated sequential gastrointestinal conditions to determine: cell viability (survival factor-SF), bacteriocin activity (Arbitrary Units-AU/mL) and surface properties (% hydrophobicity and auto-aggregation). Cells were added with 0.6% pepsin followed by a gradual descent of pH (7.4 to 2), 90 min. Then, 1% bile (pH 8) was added (10 min) and finally treated with 0.3% bile+0.1% pancreatin, 90 min.

Results

bacterial viability diminished during the treatment with significant differences among FC, LC and SC; LC showing the highest values (SF=0.92). Under gastric conditions there were no variations in cell viability while it diminished during intestinal treatment, independently of the culture. Low bacteriocin activity (AU/mL) was detected in FC. In LC ~1,300 were determined in gastric conditions, while bile treatment increased it up to ~2,000 and then decreased (~800). In SC, it diminished during the gastric treatment, to then increase up to 1,200 AU/mL. The surface properties did not change during treatments.

Conclusions

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

MYCOTOXIN REMOVAL ABILITY OF LACTOBACILLUS ACIDOPHILUS CIP 76.13 AND L. BREVIS CIP 102806T ISOLATED FROM HUMANS

Chaima Ragoubi [1] - Laura Quintieri [2] - Donato Greco [2] - Amel Mehrez [1] - Imed Maatouk [1] - Vito D'Ascanio [2] - Ahmed Landoulsi [1] - Giuseppina Avantaggiato [2]

Faculté des Sciences de Bizerte, Risques liés aux stress environnementaux, lutte et prévention, Zarzouna, Tunisia [1] - National Research Council (CNR), Institute of Sciences of Food Production (ISPA), Bari, Italy [2]

Objective

Mycotoxins are harmful secondary metabolites produced by fungi which contaminate a wide range of food and feed. Lactic acid bacteria (LAB) show a promising potential to degrade mycotoxins thus reducing their bioavailability in human and animal gastrointestinal systems. This study was aimed to investigate the ability of *Lactobacillus acidophilus* CIP 76.13 and *L. brevis* CIP 102806T, isolated from humans, in reducing AFB_1, OTA, ZEA, FB_1 and DON in order to be used as functional starter for feed and food industry.

Methods

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

Results

Viable cells of CIP 76.13 reduced ZEA, FB_1 and DON by 57±2%, 36±7% and 31±3%, respectively, while CIP 102806T reduced ZEA by 100%. Percentage removal from heat inactivated cells were significantly lower (9±4% for FB_1 and DON, and 28±10% for ZEA, on average). Both strains survived at pH 3, 5 and 7 for 24 h in PBS, and were lipolytic and proteolytic towards milk proteins. In addition, CIP 102806T showed antagonistic activity against *S. aureus* and *L. monocytogenes* strains.

Conclusions

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

DIFFERENT PROBIOTIC STRAINS IMPROVE HELICOBACTER PYLORI ERADICATION AND GUT MICROBIOTA

Mirzabey Rustamov [1]

Belorussian State Medical University, Internal diseases, Mlnsk, Republic Of Belarus [1]

Objective

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

Material and methods

In this study 200 Hp - positive patients with DU were randomized into 4 groups, 50 patients in each. Hp was confirmed histologically in samples obtained from the antrum and corpus of stomach. Intragastric and intraduodenal pH-metry by tool Gastroscan-5M, Investigation of gut microbiota, blood analyses also were performed. The following eradication regimens were recommended:

Group I: 10-days twice daily oral administration of PPIs in standard dose, amoxicillin 1000 mg, clarithromycin 500 mg, then 20-days twice daily PPIs plus twice daily oral administration of P, containing 3,025 billion alive lyophilized Lactobacillus bulgaricus DDS-14, Lactobacillus rhamnosus, Lactobacillus acidophilus DDS-1 and Bifidobacterium bifidum during 1 month; Group II: PPIs and P twice daily and alkaline hydrocarbonate-chloride sodium mineral water (MW) 200 ml trice daily during 1 month;

Group III: PPIs and P twice daily and chloride sodium MW 200 ml trice daily during 1 month; Group IV: P and PPIs once daily during 1 month. Therapeutic success was confirmed by a negative histological examination, performed in 4-12 weeks after therapy.

Results

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

Conclusions

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

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

PREBIOTIC EFFECT OF ONION ON THE SURVIVAL OF VARIOUS PROBIOTICS INOCULATED IN KORUK (UNRIPE GRAPE) JUICE

Ilkın Yucel Sengun [1] - Ahmet Gargi [1] - Berna Ozturk [1]
Ege University, Engineering Faculty, Food Engineering Department, Izmir, Turkey [1]

Objective

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

Methods

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

Results

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

Conclusion

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

Keywords

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

CASHEW AND GRAPE AGROINDUSTRY POMACES AS SOURCES OF ENERGY FOR STARTER AND PROBIOTIC STRAINS.

Igor Ucella Dantas de Medeiros [1] - Luiz Hernique Groto Garutti [1] - Thaina da Silva Domingues [2] - Marcela de Souza Leite [1] - Raquel Bedani [1] - Susana Marta Isay Saad [1]
Food Research Center, University of São Paulo, Department of Biochemical and Pharmaceutical Technology, School of Pharmaceutical Sciences, São Paulo, Brazil [1] - University of São Paulo, Department of Biochemical and Pharmaceutical Technology, School of Pharmaceutical Sciences, São Paulo, Brazil [2]

Objective

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

Methods

Modified MRS broth (mMRS) with 1% of irradiated CP or GP were inoculated with 5 *Lactobacillus* strains (PCC, RC-14, 431 and F19), 3 *Bifidobacterium* strains (BB-02, BB-12 and BB-46); and 3 *Streptococcus thermophilus* starters (TH-4, TA-40, and ST-M6). Fermentation took place during 24 and 48 hours. Controls of pure mMRS broth and with 1% of specific carbohydrate were used. Results were expressed as mean variation in populations after fermentation (V24h and V48h) in log CFU/ mL.

Results

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

Conclusions

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

FERMENTED OLIVE: MICROFLORA AND PROBIOTIC PROPERTIES

Ilkın Yucel Sengun [1] - Yunus Yahsi [1]
Ege University, Engineering Faculty, Food Engineering Department, Izmir, Turkey [1]

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

IN VITRO ADHERENCE AND ANTI-PATHOGENS EFFECT OF A CO-CULTURE OF LACTOBACILLUS PLANTARUM AND LACTOBACILLUS CASEI CULTIVATED IN A MINIMAL MEDIA SUPPLEMENTED WITH ALOE VERA

Monica Boev [1] - Gabriela Bahrim [2] - Mariana Chifiriuc [3] - Lia Dițu [4]
Faculty of Medicine and Pharmacy, Dunarea de Jos University of Galati, Galati, Romania [1] - Faculty of Food Science and Engineering, Dunarea de Jos University of Galati, Galati, Romania [2] - Faculty of Biology, University of Bucharest, Bucharest, Romania [3] - The Research Institute of the University of Bucharest, University of Bucharest, Bucharest, Romania [4]

Objective

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

Methods

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

Results

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

Conclusions

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

CLINICAL TRIALS ON THE ANTAGONISTIC ACTION OF PROBIOTICS AGAINST WOUND PATHOGENS

Sabina Fijan [1] - Anita Frauwallner [2] - Tomaž Langerholz [3] - Bojan Krebs [4] - Jessica Ter Haar (Nee Younes) [5] - Adolf Heschl [2] - Dusanka Micetic Turk [6] - Irena Rogelj [7]

FACULTY OF HEALTH SCIENCES, UNIVERSITY OF MARIBOR, MARIBOR, Slovenia [1] - Institut Allergosan, Pharmazeutische Produkte Forschungs- und Vertriebs GmbH, /, GRAZ, Austria [2] - Faculty of Agriculture and Life Sciences, UNIVERSITY OF MARIBOR, MARIBOR, Slovenia [3] - Department of abdominal surgery, University Medical Centre Maribor, MARIBOR, Slovenia [4] - Terhaar Consulting Inc, /, ONTARIO, Canada [5] - FACULTY OF MEDICINE, UNIVERSITY OF MARIBOR, MARIBOR, Slovenia [6] - Biotechnical Faculty, University of Ljubljana, DOMZALE, Slovenia [7]

Objective

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

Methods

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

Results

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

Conclusions

Exogenous and oral application of probiotics has demonstrated a reduction in wound infections and therefore the potential use of probiotics for wound infections remains worthy of further studies.

DEVELOPMENT AND EVALUATION OF WHOLE CELL PROBIOTIC CONTAINING GEL FOR TOPICAL USE

Garima Sharma [1] - Manuhaar Sharma [1] - Indu Pal Kaur [1]

Panjab University, University Institute of Pharmaceutical Sciences, Chandigarh, India [1]

Objective

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

Methods

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

Results

Microscopic images confirmed presence of probiotic spores enmeshed in honeycomb network. Self-preserving nature was confirmed by low water activity (0.541 a_w) and challenge test as per Indian Pharmacopoeia. The probiotic count was maintained ($92\pm5\times10^6$) on storage for three months (5°C±3°C and 30°C±2°C/ 65%RH±5%RH) confirming stability and cell viability. Spore germination started at 6 h and only few non-germinated spores were left at 24 h after application. The developed formulation was safe in terms of cytotoxicity, acute dermal irritation and permeation/ translocation as NMT 0.16±.03% of $8\pm4\times10^6$ cfu applied per day translocated to systemic circulation. Patient compliance in terms of application and removal was confirmed by texture and rheological studies. The *in vivo* therapeutic effect of developed formulation was confirmed in terms of wound healing and no statistically significant difference was found between marketed and probiotic-loaded formulations.

Conclusions

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

DEVELOPMENT OF A RICE-BASED FERMENTED PROBIOTIC BEVERAGE WITH LACTOBACILLUS SPP. AND STREPTOCOCCUS THERMOPHILUS

Ana Maria Gomes [1] - Francisco Ramalhosa [1] - Sérgio Sousa [1] - Dina Rodrigues [1] - Ana Cristina Freitas [1]
Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório, Associado, Escola Superior de Biotecnologia, Porto, Portugal [1]

Objective

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

Methods

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

Results

Consortium *L. casei/S. thermophilus* demonstrated the highest cell growth rate (2 log cycle) and pH decrease (6.6 to 4.5). Consortium *L. rhamnosus/S. thermophilus* presented a cell growth rate of 1 log cycle and a pH decrease of 6.6 to 4.9 whereas consortium *L. acidophilus/S. thermophilus* didn't reveal cell growth and exhibited the lowest pH variation (6.6 to 5.5). Slurries 1:15 revealed the highest viscosity values. After fermentation both *L. casei/S. thermophilus* and *L. rhamnosus/S. thermophilus* consortia exhibited CFU/mL above the minimum probiotic threshold demonstrating to be good options for the elaboration of a low glycemic index rice-based symbiotic beverage.

Acknowledgments

This work was supported by national funds through project ARROZ-BIG ref.17931 financed by FEDER/POCI with CBQF scientific collaboration with FCT project UID/Multi/50016/2013

Keywords

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

STORAGE STABILITY OF PROBIOTIC WEISELLA CIBARIA N9 IN FREEZE-DRIED FORM

Latife Betul Gul [1] - Osman Gul [2] - Enes Dertli [3] - Ahmet Hilmi Con [1]
Engineering Faculty, Department of Food Engineering, Ondokuz Mayis University, Samsun, Turkey [1] - Yeşilyurt Demir–Çelik Vocational School, Program of Food Technology, Ondokuz Mayis University, Samsun, Turkey [2] - Engineering Faculty, Department of Food Engineering, Bayburt University, Bayburt, Turkey [3]

Objective

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

Methods

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

Results

The optimal protective agent formulation was 5.65 (w/v) skim milk combined with 20 (w/v) lactose and 9.38 (w/v) sucrose, which provided high stability of cell (99 % <) after freezing and freeze-drying. The highest viability was observed at 4ºC (9.11 log cfu/g) with an inactivation rate of $3.37x10^{-3}$ day^{-1}, which indicate that viability of freeze-dried cells was more stable at refrigerated temperature than those stored at ambient temperature. The fastest cell reduction was observed at 70ºC and protective agent effectively decreased thermal death.

Conclusions

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

Keywords

Weisella cibaria N9; Prediction model; Probiotic; Protective agents

WHEY PHAGE ECOLOGY IN ARTISANAL TRADITIONAL ITALIAN "HARD" CHEESE: SUSTAINABILITY IN DAIRY PRODUCTION

Andrea Mancini [1] - Ilaria Carafa [1] - Kieran Tuohy [1] - Elena Franciosi [1]
Fondazione Edmund Mach, Food Quality and Nutrition, San Michele all'Adige, Italy [1]

Natural whey starters can be considered as a lactic acid bacteria culture, indispensable for the production of Italian traditional "hard" cheeses like Trentingrana. Daily maintained in the dairy activity, the main function of this culture is the acidification during the curd formation, having important effects such as whey drainage from the cocked curd and growth inhibition of photogenic and undesirable bacteria. Loss in whey starter fitness is often associated in poor acidification, mainly due to phage infection, that can strongly affect the cheese production and its final quality. Phage contamination in the dairy environment can not be completely eradicated, and there is fundamentally a co-evolution process that drives an ecological balance between phage and its microbial host, especially in whey starters environments. Therefore knowledge on phages community represent an important aspect to reduce lost in cheese production and to maintain quality and excellence in cheese making.
To study the phage ecology in Trentingrana production chain, we have collected 400 samples in six Trentingrana dairies distributed on the autonomous province of Trento, Italy. Almost 1800 lactic acid bacteria have been isolated and more than 150 phages retrieved. Characterization of phage biotypes as well as phages genome sequencing is ongoing. The gaining knowledge together with the isolation of performing lactic acid bacteria resistant to phage infection, will be important to avoid milk and cheese spoilage, potentially leading to a more sustainable cheese production.

LACTOBACILLUS RHAMNOSUS GR-1 - THE BEST STUDIED VAGINAL PROBIOTIC STRAIN

Mariya Petrova [1]
KU Leuven, CMPG, Leuven, Belgium [1]

Objective

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

Methods

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

Results

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

Conclusions

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

SAFETY ASSESSMENT OF A MICROBIAL FOOD CULTURE FOR SPECIFIC USAGE IN THE FOOD CHAIN: *WEISSELLA CONFUSA*

Bourdichon F. [1,2] - Patrone V. [2] - Fontana A. [2] - and Morelli L. [2]
Food Safety, Microbiology, Hygiene, 74 Boulevard Blossac, 86100 Chatellerault, France [1] - Facoltà di Scienze agrarie, alimentarie ambientali, Università Cattolica del Sacro Cuore, Piacenza-Cremona, Italy [2]

Introduction and Scope

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

Materials and Methods

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

Results. Discussion and conclusion

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

References

• Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J.C., Gerds, M.L., Hammes, W.P., Harnett, J., Huys, G., Laulund, S., Ouwehand, A., Powell, I.B., Prajapati, J.B., Seto, Y., Ter Schure, E., Van Boven, A., Vankerckhoven, V., Zgoda, A., Tuijtelaars, S., Hansen, E.B., 2012a. Food fermentations: microorganisms with technological beneficial use. Int J Food Microbiol 154(3) 87-97.
• FEEDAP Panel, EFSA. 2018. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018; 16(3): 5206
• Herman, L., Chemaly, M., Cocconcelli, P.S., Fernandez, P., Klein, G., Peixe, L., Prieto, M., Querol, A., Suarez, J.E., Sundh, I., Vlak, J., and Correia, S., 2019. The qualified presumption of safety assessment and its role in EFSA risk evaluations: 15 years past. FEMS Microbiology Letters, Volume 366, Issue 1, fny260.

PROTECTIVE ACTION OF B.CLAUSII PROBIOTIC STRAINS AND THEIR METABOLITES IN AN IN VITRO MODEL OF ROTAVIRUS INFECTION

Lorella Paparo [1] - Lorella Tripodi [2] - Cristina Bruno [2] - Laura Pisapia [1] - Lucio Pastore [2] - Roberto Berni Canani [1]
Università degli Studi di Napoli Federico II, Dipartimento di Scienze Mediche Traslazionali, Napoli, Italy [1] - Ceinge-Biotecnologie Avanzate, Università degli Studi di Napoli Federico II, Napoli, Italy [2]

Objective

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

Methods

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

Results

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

Conclusions

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

LYCOPROZEN: TOMATO AS A FUNCTIONAL FOOD

Piantelli M [1] - Fogliano V [2] - Iacobelli S [3] - Natali PG [1,2]
Center of Excellence on Aging and Translational Medicine (CeSI-Met), "G. d'Annunzio" University, Chieti, Italy [1] - Wageningen University, Department of Agrotechnology & Food Science, The Netherlands [2] - Janus Pharma S.r.l. [3]

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

PROBIOTICS, PREBIOTICS AND NUTRACEULTICALS IN: PEDIATRICS

THE THERAPEUTIC EFFICACY OF BIFIDOBACTERIUM ANIMALIS SUBSP. LACTIS BB-12® IN INFANT COLIC: A RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED TRIAL

Rita Nocerino [1] - Francesca De Filippis [2] - Gaetano Cecere [1] - Antonio Marino [1] - Maria Micillo [1] - Carmen Di Scala [1] - Lorella Paparo [1] - Carmen De Caro [3] - Antonio Calignano [3] - Cristina Bruno [4] - Linda Cosenza [1] - Giusy Della Gatta [1] - Serena Coppola [1] - Danilo Ercolini [2] - Roberto Berni Canani [1]
Università degli Studi di Napoli Federico II, Dipartimento di Scienze Mediche Traslazionali, Napoli, Italy [1] - Università degli Studi di Napoli Federico II, Dipartimento di Agraria, Portici (NA), Italy [2] - Università degli Studi di Napoli Federico II, Dipartimento di Farmacia, Napoli, Italy [3] - Ceinge-Biotecnologie Avanzate, Università degli Studi di Napoli Federico II, Napoli, Italy [4]

Objective

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

Methods

RCT on otherwise healthy exclusively breastfed infants with infant colic (IC) randomly allocated to BB-12(1x109 CFU/day) or placebo. Infants assumed orally 6 drops/day of the assigned product for 28days. Stool samples were collected to assess gut microbiota structure and butyrate, beta defensin 2(HBD-2), cathelicidin(LL-37), secretory IgA(sIgA), calprotectin(CLP) levels.

Results

80 infants were randomized,40/group. The rate of infants with reduction of ≥50% of mean daily crying duration was higher in infants treated with BB-12 compared to placebo(80.0%vs32.5%,$p<0.05$).The mean number of crying episodes decreased in both groups, but with a higher effect in BB-12 group(-4.7±3.4*vs* -2.3±2.2,$p<0.05$). Mean daily stool frequency decreased in both groups but the effect was significantly higher in the BB-12 group; stool consistency was similar between the 2 groups. A increase in *Bifidobacterium* abundance, butyrate production, and HBD-2, LL-37,sIgA levels associated with a decrease in CLP fecal level was observed in BB-12 group. Increase in *Bifidobacterium* levels correlated significantly with the reduction of crying time.

Conclusions

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

PRECISION NUTRITION

TOLEROGENIC EFFECT ELICITED BY PROTEIN FRACTION DERIVED FROM DIFFERENT HYPOALLERGENIC FORMULAS IN PBMCS FROM CHILDREN WITH COW MILK ALLERGY

Lorella Paparo [1] - Rita Nocerino [1] - Carmen Di Scala [1] - Laura Carucci [1] - Linda Cosenza [1] - Ylenia Maddalena [1] - Cristina Bruno [2] - Serena Coppola [1] - Giusy Della Gatta [1] - Laura Pisapia [1] - Gianluca Picariello [3] - Roberto Berni Canani [1]
UNIVERSITY OF NAPLES FEDERICO II, DEPARTMENT OF TRANSLATIONAL MEDICAL SCIENCE, Naples, Italy [1] - CEINGE ADVANCED BIOTECHNOLOGIES, IMMUNONUTRITONLAB, Naples, Italy [2] - CNR AVELLINO, ISTITUTO SCIENZE DELL'ALIMENTAZIONE, Avellino, Italy [3]

Objective

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

Methods

Four hypoallergenic formulas were compared: extensively whey formula (EHWF), extensively hydrolyzed casein formula (EHCF), hydrolyzed rice formula (RHF), amino acid based formula (AAF). Formulas were reconstituted in water according to manufacturer's instructions, and subjected to *in vitro* infant gut simulated digestion using a sequential gastric and duodenal static model. Resulting protein fractions were purified using C18 reversed phase pre-packed cartridges (Sep-Pak, Waters, Milford, MA, USA),recovered in 70% acetonitrile/0.1% trifluoroacetic acid and finally vacuum-dried. Tolerogenic effects were was evaluated in peripheral blood mononuclear cells (PBMCs) from 6 patients, with challenge-proven IgE-mediated CMA (age range 1-5 yrs, all Caucasians), stimulated with different doses of digested protein fractions (from 0.25 to 250 µg/ml) or b-lactoglobulin (BLG;100µg/ml) or bovine serum albumin (BSA;100µg/ml) as positive and negative control respectively. The production of Th2 (IL-4, IL-5, IL-13) and Th1 (IL-10, IFN-γ) cytokines were assessed by ELISA. Modulatory action was also evaluated on immune (IL-33) and non-immune tolerogenic factors (mucin 5AC, tight-junction proteins ZO-1 and occludin) in human enterocytes (Caco-2 cells) by ELISA and Real Time PCR, respectively.

Results

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

Conclusion

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

MICROBIOME ANALYSIS AT STRAIN-LEVEL: STUDY DESIGN FOR THE PRE-CLINICAL AND CLINICAL DEVELOPMENT OF NUTRITIONAL SUPPLEMENTS AND INTERVENTIONS

Nur Hasan [1] - Arne Materna [1] - Brian Fanelli [1] - Manoj Dadlani [2] - Mo Langhi [2]
CosmosID, Scientific Leadership Team, Rockville, MD, United States [1] - CosmosID, Management Team, Rockville, MD, United States [2]

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

FACULTY

NAME	COUNTRY	PAGE
Agnolucci Monica	*Italy*	6
Alisi Anna	*Italy*	17
Aloi Marina	*Italy*	15
Alvaro Domenico	*Italy*	15
Angioli Roberto	*Italy*	13
Armuzzi Alessandro	*Italy*	2
Ascione Antonio	*Italy*	23
Assorgi Chiara	*Italy*	8
Aureli Paolo	*Italy*	4
Auricchio Renata	*Italy*	18
Aziz Ramy Karam	*Egypt*	20
Bandi Claudio	*Italy*	11
Barbara Giovanni	*Italy*	22
Barone Maria Vittoria	*Italy*	18
Bastetti Carla	*Italy*	9
Benedetti Antonio	*Italy*	15
Berni Canani Roberto	*Italy*	16
Binda Sylvie	*France*	3
Bonvicini Fiorenza	*Italy*	20
Bourdichon Francois	*France*	17
Brescia Paola	*Italy*	15
Brigidi Patrizia	*Italy*	20
Brummer Robert J.M.	*Sweden*	11
Caminero Alberto	*Canada*	18
Candela Marco	*Italy*	5
Cani Patrice	*Belgium*	11
Caprilli Renzo	*Italy*	2
Capuano Edoardo	*Netherlands*	1
Capurso Lucio	*Italy*	1, 3, 11
Capurso Gabriele	*Italy*	20
Carlomagno Federica	*Italy*	15
Caserta Donatella	*Italy*	8
Castellazzi Annamaria	*Italy*	4
Cavallo Ilaria	*Italy*	22
Cecchini Isabella	*Italy*	18

NAME	COUNTRY	PAGE
Cetin Irene	*Italy*	7
Clarke Gerard	*Ireland*	22
Clementi Massimo	*Italy*	4
Colacurci Nicola	*Italy*	7, 8
Corazziari Enrico Stefano	*Italy*	6
Cotter Paul	*Ireland*	5
Cricelli Claudio	*Italy*	15
Cucchiara Salvatore	*Italy*	17
Dal Bello Fabio	*Italy*	19
De Filippo Carlotta	*Italy*	13
De Seta Francesco	*Italy*	7
de Vos Willem M.	*Netherlands*	4, 11
Del Chierico Federica	*Italy*	4
Del Piano Mario	*Italy*	5
Del Rio Daniele	*Italy*	13
D'Eufemia Diletta	*Italy*	7
Di Benedetto Luisa	*Italy*	8
Di Domenico Enea Gino	*Italy*	22
Di Maio Silvia	*Italy*	9
Elli Marina	*Italy*	18
Ercolini Danilo	*Italy*	16
Fasano Alessio	*USA*	1
Faravelli Giusto	*Italy*	19
Federici Massimo	*Italy*	4
Ferreira Isabella	*Portugal*	1
Ferretti Sabrina	*Italy*	9
Ferruzzi Mario	*USA*	13
Fiorani Marco	*Italy*	18
Fiorillo Vitaliano	*Italy*	11
Fogliano Vincenzo	*Netherlands*	1
Fracasso Pierluigi	*Italy*	22
Franciosi Elena	*Italy*	5
Freedman Stephen	*Canada*	13
Gasbarrini Antonio	*Italy*	20
Gasbarrini Giovanni	*Italy*	20

NAME	COUNTRY	PAGE
Giacco Rosalba	*Italy*	6
Giovannini Niccolò	*Italy*	7
Gonzalez Cautela Brunella V.	*Canada*	20
Guandalini Stefano	*USA*	13, 17
Guarino Alfredo	*Italy*	13, 17
Guarino Michele	*Italy*	20
Hill Colin	*Ireland*	3
Hojsak Iva	*Croatia*	16
Huppertz Thom	*Netherlands*	1
Ianiro Gianluca	*Italy*	9, 20
Indrio Flavia	*Italy*	16
Ippolito Giuseppe	*Italy*	4, 5
Isay Saad Susana Marta	*Brasil*	7
Isolauri Erika	*Finland*	16
Keller Josbert	*Netherlands*	20
Kiyono Hiroshi	*Italy*	4
Koch Maurizio	*Italy*	1, 12, 15
Langella Philippe	*France*	11
Larsson Niklas	*Sweden*	15
Laulund Esben	*Denmark*	17
Lavermicocca Paola	*Italy*	13
Lehtinen Markus	*Denmark*	20
Leyer Gregory	*USA*	12
Leser Thomas	*Denmark*	18
Lionetti Paolo	*Italy*	16
Lortal Sylvie	*Spain*	17
Lo Vecchio Andrea	*Italy*	13
Madiai Valeria	*Italy*	18
Magliaro Chiara	*Italy*	22
Maniglio Paolo	*Italy*	8
Marci Roberto	*Switzerland*	8
Matteucci Eleonora	*Italy*	8
Mereta Federico	*Italy*	9
Miraglia del Giudice Michele	*Italy*	5
Mogna Luca	*Italy*	5, 16
Monda Vincenzo	*Italy*	9

NAME	COUNTRY	PAGE
Montori Alberto	*Italy*	23
Mora Diego	*Italy*	20
Morelli Lorenzo	*Italy*	3, 4, 9, 11
Mori Marco	*Italy*	18
Morisco Filomena	*Italy*	23
Moschetta Antonio	*Italy*	13
Mosoni Carolina	*Italy*	20
Murina Filippo	*Italy*	7
Natali Pier Giorgio	*Italy*	23
Nisticò Paola	*Italy*	20
Orfeo Luigi	*Italy*	1
Ouwehand Arthur	*Finland*	15
Pal Kaur Indu	*India*	22
Pane Marco	*Italy*	13, 15
Paraskevakos George	*Canada*	17
Pecere Rosanna	*Belgium*	3
Petricevic Ljubomir	*Austria*	7
Picardo Mauro	*Italy*	22
Pignatti Marco	*Italy*	22
Pot Bruno	*Belgium*	17
Putignani Lorenza	*Italy*	2, 4
Quagliariello Andrea	*Italy*	4
Renis Marcella	*Italy*	13
Rescigno Maria	*Italy*	4
Restani Patrizia	*Italy*	6
Riccardi Gabriele	*Italy*	6
Ruscito Ilary	*Italy*	8
Saggioro Alfredo	*Italy*	23
Salvia Alberto	*Italy*	9
Sanz Yolanda	*Spain*	18
Savary Auzeloux Isabelle	*France*	6
Scaldaferri Franco	*Italy*	1, 2
Scarpa Bruno	*Italy*	3
Scarpignato Carmelo	*Italy*	12
Schimberni Mauro	*Italy*	8
Scotti Luca	*Italy*	13

NAME	COUNTRY	PAGE
Senizza Alice	*Italy*	9
Shaker Mohamed Kamal	*Egypt*	23
Silano Marco	*Italy*	18
Stratta Fabio	*Italy*	19
Stronati Laura	*Italy*	16
Suez Jotham	*Israel*	4
Surono Ingrid S.	*Indonesia*	21
Szajewska Hania	*Poland*	13, 16
Tett Adrian	*Italy*	20
Tilg Herbert	*Austria*	15
Tomás-Barberán Francisco A.	*Spain*	1, 6
Torcia Francesco	*Italy*	8
Troncone Riccardo	*Italy*	18
Tuohy Kieran M.	*Italy*	5
Ungaro Federica	*Italy*	2
Valtuena Martinez Silvia	*Italy*	3
Van Boekel Tiny	*Netherlands*	1
Van Den Wijngaard Rene	*Netherlands*	22
Van Goudoever Hans J. B.	*Netherlands*	16
Vandenplas Yvan	*Belgium*	16, 17
Vasto Sonya	*Italy*	6
Vicariotto Franco	*Italy*	7, 13
Warzée Jean-Pol	*Belgium*	18
Wilschanski Micheal	*Israel*	17
Zitvogel Laurence	*France*	20

INDEX OF ORAL COMMUNICATIONS AND POSTERS AUTHORS

INDEX OF ORAL COMMUNICATIONS AND POSTERS AUTHORS

Algeria
Argentina
Austria
Belarus
Belgium
Brazil
Bulgary
Canada
China
Colombia
Denmark
Egypt
Finland
France
Greece
Hungary
India
Indonesia
Iran
Ireland
Italy
Macedonia
Mexico
Netherlands
Nigeria
Perù
Portugal
Qatar
Romania
Russian Federation
Serbia
Slovakia
Slovenia
Spain
USA
Thailand
Tunisia
Turkey
Ukraine

GENERAL INFORMATION

DATES & TIMES
September 8 - 10, 2019

The Conference will commence at 08.30 (CET) Sunday 8 September and will conclude on Tuesday 10 September at 13.30 (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

LANGUAGE
The Conference 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 8 September: 08.00 - 19.00
- Monday 9 September: 08.00 - 18.00
- Tuesday 10 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
Members Mediterranean Task Force for Cancer Control	€ 150,00
Members FederSalus	€ 150,00
Members IPA Europe	€ 200,00
Members Gruppo Miaf-Federchimica	€ 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
- Open coffee and lunch
- Opening ceremony and welcome cocktail
- Certificate of attendance
- Italian CME certificate (to whom entitled)

ACCOMMODATION

Ergife Palace Hotel, Via Aurelia, 619 - 00165 Rome, Italy

Cancellation Policy

Cancellations must be sent via email to the Organising Secretariat.
In the case of cancellation by July 31, 2019, 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 Conference 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 8 September - A buffet lunch will be served at the Conference venue
Sunday 8 September - Welcome Cocktail at the Conference venue
Monday 9 September - A buffet lunch will be served at the Conference venue
Coffee & snacks are available from the coffee corner area.

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 30km 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 20km 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. 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.

ACCESS TO ERGIFE PALACE HOTEL FROM ROME-LEONARDO DA VINCI INTERNATIONAL AIRPORT

- **Taxi:** The Ergife Palace Hotel is located 25km from the airport. Allow 30 minutes by taxi, depending on traffic. The taxi fare costs €40.00 (approximately).
- **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 station, participants may take the Metro A line to Cornelia (approximately 20 minutes). Tickets cost €1.50 and can be purchased at the train station. Tickets are valid for 75 minutes and can be used on both metros and buses. The Ergife Palace Hotel is a 15-minute walk from Cornelia Metro station.

ACCESS TO ERGIFE PALACE HOTEL FROM ROME-CIAMPINO INTERNATIONAL AIRPORT

- **Taxi:** The Ergife Palace Hotel is located 30km from the airport. Allow 35 minutes by taxi, depending on traffic. The taxi fare costs €40.00 (approximately).
- **Public transport:** Service Provider Terravision https://www.terravision.eu/airport_services.html?noredirect=en_US. Participants may 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 station, participants may take Metro A line to Cornelia (approximately 20 minutes). Tickets cost €1.50 and can be purchased at the train station. Tickets are valid for 75 minutes and can be used on both metros and buses. The Ergife Palace Hotel is a 15-minute walk from Cornelia Metro station.
- 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 Conference 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.
Conference 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, refered 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 aWorld Heritage Site.

ORGANISING SECRETARIAT

For additional information or queries, please address all correspondence to the Organising Secretariat:

eMEETING&CONSULTING

Via Michele Mercati, 33 - 00197 Rome, Italy
Phone +39 06 80693320 - Fax +39 06 3231136
E-mail: probiotics2019@emec-roma.com
Website: www.probiotics-prebiotics-newfood.com
www.emec-roma.com

SCIENTIFIC INFORMATION

ORGANISING SECRETARIAT DESK AT THE MEETING VENUE WILL BE OPEN AS FOLLOWS:

DAY	DATE	FROM	TO
Sunday	September 8	08.00	20.00
Monday	September 9	08.00	19.00
Tuesday	September 10	08.00	14.00

ORAL COMMUNICATIONS

Oral communications sessions are scheduled as follows:

September 8 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 9 AULA MAGNA from 11.45 to 12.00 - from 16.15 to 16.30

September 10 AULA MAGNA from 12.50 to 13.20

AULA METCHNIKOFF from 09.20 to 09.30 - from 11.15 to 11.20

POSTERS

Poster authors are kindly requested to hang the poster in the poster area from 10.30 on September 8 and remove it after 13.00 on September 10. Your position will be indicated in the poster area

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:

"10th 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. 267737 - Crediti assegnati 6

Obiettivo formativo - Appropriatezza 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 e superare il test di apprendimento seguendo le seguenti informazioni:
1. Aprire dal proprio smartphone/tablet l'App Store (se in possesso di un dispositivo iOS) o Google Play (se in possesso di un dispositivo Android)
2. Nella barra di ricerca inserire "deep ecm"
3. Touch su Ottieni (App Store) o Download (Google Play) per scaricare e installare l'App
4. Creare un account personale
5. Accedere all'evento tramite il seguente pin PROBIOTICS2019

Come da Comunicato Agenas del 23 giugno 2014, si specifica inoltre che è possibile effettuare una sola (e non ripetibile) compilazione del test di verifica in modalità on-line. Per ulteriori informazioni http://ape.agenas.it/

ROME, SEPTEMBER 2021

11TH

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

2ST SCIENCE & BUSINESS SYMPOSIUM