ROME - SEPTEMBER 10/12, 2017
UNIVERSITÀ URBANIANA

9TH PROBIOTICS, PREBIOTICS & NEW FOODS, NUTRACEUTICALS AND BOTANICALS

for NUTRITION & HUMAN and MICROBIOTA HEALTH

SCIENTIFIC ORGANISERS

L. Capurso (Italy)

A. Gasbarrini (Italy)

A. Guarino (Italy)

L. Morelli (Italy)

INTERNATIONAL SCIENTIFIC COMMITTEE

G. Barbara (Italy)

R. Berni Canani (Italy)

P. Brigidi (Italy)

M. L. Colombo (Italy)

G. Delle Fave (Italy)

J. Dorè (France)

V. Fogliano (The Netherlands)

F. Guarner (Spain)

M. Rescigno (Italy)

H. Tilg (Austria)

K. M. Tuohy (Italy)

PEDIATRIC DAY

A. Guarino (Italy)

SCIENTIFIC REFEREES

M. Anti (Italy)

G. Capurso (Italy)

M. Koch (Italy)

UNDER THE PATRONAGE OF

Consiglio Nazionale delle Ricerche

SIGE, Società Italiana di Gastroenterologia

FMD Fondazione per le Malattie Digestive

Fondazione Aldo Torsoli per le Malattie dell'Apparato Digerente del Fegato e del Pancreas

UEAGEN

European Association for Gastroenterology, Endoscopy & Nutrition

Associazione Ginecologi Universitari Italiani AGUI 1992

SINut Società Italiana di Nutraceutica

NFI Nutrition Foundation of Italy

UNDER THE PATRONAGE OF

AGGEI
Associazione Giovani Gastroenterologi ed Endoscopisti Italiani

Associazione Giovani Gastroenterologi ed Endoscopisti Italiani

LILT
LEGA ITALIANA PER LA LOTTA CONTRO I TUMORI
prevenire è vivere

SIGENP
Società Italiana di Gastroenterologia Epatologia e Nutrizione Pediatrica

federSalus
Associazione Nazionale Produttori e Distributori Prodotti Salutistici

MTCC
Mediterranean

MTCC, Mediterranean Task Force for Cancer Control

PROBIOSTUDIO
ITALIAN ACADEMY FOR THE STUDY OF HUMAN MICROBIOTA

INDEX

SCIENTIFIC PROGRAMME

08.30-10.00 a.m.

YOUNG ITALIAN GASTROENTEROLOGISTS (AGGEI)
MEET THE EXPERT: QUESTIONS AND ANSWERS
Chairs: M. Koch (Italy), F. Scaldaferri (Italy)

Experts: L. Morelli (Italy), L. Putignani (Italy),
C. Scarpignato (Italy), G. Capurso (Italy), K. M. Tuohy (Italy)

Discussants: F. Facciotti (Italy), L. R. Lopetuso (Italy), I. Marafini (Italy)
L. Pastorelli (Italy), V. Petito (Italy)

10.00-11.30 a.m.

MASSIMO CRESPI MEMORIAL - MTCC
(MEDITERRANEAN TASK FORCE CANCER CONTROL)
Chairs: A. Montori (Italy), P. G. Natali (Italy)

To remember Massimo Crespi
A. Montori (Italy)

Probiotics in IBD. Running between excitement and disappointment
N. Tozun (Turkey)

Role of probiotic in treatment of nonalcoholic fatty liver disease (NAFD)
R. Malekzadeh (Iran)

How we can modify microbioma and prevent cancer
A. Saggioro (Italy)

11.30-01.00 p.m.

BOTANICALS
Chair: M. L. Colombo (Italy)

Our microbiome cloud and us: common destiny and conflicts of interest
R. K. Aziz (Egypt)

Artichoke and ginger promote digestive function and gastrointestinal motility
A. Giacosa (Italy)

Melatonin, an ubiquitous and evolutionary hormone: its identification in plants
A. Conti (Switzerland)

Plant probiotics for the production of healthy foods
M. Giovannetti (Italy)

01.00-02.00 p.m.

Lunch

02.00-03.00 p.m.

OPENING CEREMONY
Chairs: L. Capurso (Italy), A. Gasbarrini (Italy), A. Guarino (Italy), L. Morelli (Italy)

WELCOME ADDRESS
L. Capurso (Italy)

E. Giorgetti - Farmindustria (Italy)
R. Pecere - IPA Europe (Belgium)
M.C. Parodi - FISMAD (Italy)
C. Agostoni - SIGENP (Italy)
A. Costa - FederSalus (Italy)

03.00-05.00 p.m.

OPENING LECTURES: PAST, PRESENT AND FUTURE

Prebiotics - *R. A. Rastall (UK)*
Probiotics - *L. Morelli (Italy)*
Fecal microbiota transplant - *A. Gasbarrini (Italy)*
New foods - *V. Fogliano (The Netherlands)*
Pediatric:
Nutrition - *J. A. Vanderhoof (USA)*
Use of probiotics in diseases - *A. Guarino (Italy)*

05.00-07.00 p.m.

PROBIOTICS, PREBIOTICS AND HUMAN HEALTH
Chair: K. M. Tuohy (Italy)

Trans-omics technologies for linking microbiota structure and metabolic output with human health
J. R. Marchesi (UK)

Food structure and microbiota metabolic function
W. Russell (UK)

Gut microbiota composition, bile acid signalling and human health
K. M. Tuohy (Italy)

Dietary modulation of the gut microbiota and its metabolic output in celiac disease
M. De Angelis (Italy)

Gut microbiota and tryptophan metabolism - implications for human health
F. Mattivi (Italy)

07.00-08.00 p.m. **LECTURES**

Chair: V. Fogliano (The Netherlands)

Symbiotic relationship between probiotic, food matrix and host
L. Morelli (Italy)

Nutraceutical health enhancing functional foods (NUTRHEFF): the CNR network
P. Lavermicocca (Italy)

Probiotics market: development and trend. The regulatory framework
M. Carnassale (Italy)

08.00 p.m. **WELCOME COCKTAIL**

10.30-11.00 a.m.

ORAL COMMUNICATIONS
ASSESSMENT OF THE BIOACTIVITY OF FOOD COMPONENTS

Chair: M. L. Colombo (Italy)

OC. 1 - THE EFFECT OF DIETARY FIBRE SUPPLEMENTATION ON CLINICAL MANIFESTATIONS AND OESOPHAGEAL FUNCTION IN NON-EROSIVE REFLUX DISEASE PATIENTS WITH LOW DIETARY FIBRE INTAKE

Mariya Konovalova [1], Sergey Morozov [1], Vasily Isakov [1]

[1] *Federal Research Center of Nutrition and Biotechnology, Gastroenterology and Hepatology, Moscow, Russian Federation*

OC. 2 - PREBIOTIC POTENTIAL EVALUATION OF AGAVE FRUCTAN FRACTIONS USING AN EX VIVO SYSTEM: A COMPARISON BASED ON POLYMERIZATION DEGREE

Ricardo García Gamboa [1], Marisela Gonzalez Ávila [1]

[1] *Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C., biotecnología medica y farmacéutica, GUADALAJARA, Mexico*

OC. 3 - THE MOLECULAR IDENTIFICATION AND ISOLATION OF PROBIOTICS PRESENT IN SOME DAIRY PRODUCTS AND THE EFFECT OF AURICULARIA POLYTRICHA ON THESE PROBIOTICS

Gamze Cagatay [2], Gulcin Alp Avci [1], Burcin Ozcelik [3], Aslı Kara [3], Emre Avci [2], Menderes Suicmez [2]

[1] *Hitit University, Department Molecular Biology and Genetics, Molecular Microbiology and Biotechnology, Çorum, Turkey*
[2] *Hitit University, Department Molecular Biology and Genetics, Çorum, Turkey*
[3] *Hitit University, Department Biology, Çorum, Turkey*

OC. 4 - ORGANOLEPTIC & SENSORY EVALUATION OF A NOVEL FOOD PRODUCT FROM CARICA PAPAYA

Dr. Jyoti Vora [1], Sneha Pednekar [1]

[1] *Ramnarain Ruia College, Department of Biochemistry & FSQC, University of Mumbai, Mumbai, India*

OC. 5 - DISCOVERY OF NOVEL PROBIOTIC STRAINS WITH SUPERIOR PROPERTIES IN THE PRESENCE OF VIRAL GASTROENTERITIS AGENTS

Gulcin ALP AVCI [1], Emre AVCI [1]

[1] *Hitit University, Molecular Biology and Genetics, Çorum, Turkey*

11.00-12.00 a.m.

ORAL COMMUNICATIONS
PROBIOTICS HEALTH EFFECTS

Chair: K. Tuohy (Italy)

OC. 6 - EXPOSURE OF LACTOBACILLUS BULGARICUS AND LACTOBACILLUS CASEI TO 2.4 GHZ WI-FI RADIOFREQUENCY RADIATION ENHANCES THE GROWTH OF THESE PROBIOTIC BACTERIA

SMJ Mortazavi [1]

[1] *Fox Chase Cancer Center, FCCC, Philadelphia, United States*

OC. 7 - NOVEL STRATEGIES THAT ENHANCE THE BIOAVAILABILITY OF PROBIOTICS FOR THERAPEUTIC USE DURING INFLAMMATORY BOWEL DISEASE

Sandeep K Gill [1], Artem Godovannyi [1], Jacqueline A Barnett [1], Candice Quin [1] Sanjoy Ghosh [1], Deanna L Gibson [1]

[1] *University of British Columbia Okanagan, Biology Unit 2, Kelowna, Canada*

OC. 8 - EX VIVO AND NUTRITIONAL EVALUATION OF A SYNBIOTIC ON THE IMPACT OF GUT MICROBIOTA AND BODY COMPOSITION IN OBESE SUBJECTS: STUDY REFERENCED IN BLOOD SIBLING

Nad´xeli Saharay Galvez Alvarez [1], Marisela Gonzalez Ávila [1]

[1] *Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A.C., Biotecnología Médica y Farmacéutica, Guadalajara, Mexico*

OC. 9 - EFFECT OF MATRIX MODIFICATION ON DRYING AND SYMBIOTIC SYNERGY OF MICRO-SPHERES CONTAINING PROBIOTICS

Anil Pandey [1], Neelam Yadav [2], Atul Kumar Mishra [3]

[1] *Centre of Food Technology, University of Allahabad, Allahabad, India*
[2] *Centre of Food Technology Centre of Food Technology University of Allahabad, Allahabad*
[3] *Centre of Food Technology University of Allahabad*

OC. 10 - NEW PROBIOTIC WHEY PROTEIN FORTIFIED BEVERAGE ENRICHED WITH BIFIDOGENIC FIBERS

Silvia De Candia [1], Laura Quintieri [1], Leonardo Caputo [1], Francesca De Leo [2], Antonio F. Logrieco [1], Federico Baruzzi [1]

[1] *National Research Council, Institute of Sciences of Food Production, BARI, Italy*
[2] *NATIONAL RESEARCH COUNCIL, Institute of Biomembranes, Bioenergetic and Molecular Biotechnologies, BARI, Italy*

OC. 11 - THERAPEUTIC PROSPECTS OF LACTOBACILLUS RHAMNOSUS GG (LGG) POSTBIOTICS ON INFECTIVE DAMAGE OF HUMAN COLONIC MUSCLE

Alessia Cicenia [1], Valentina Totino [2], Lucia Pallotta [1], Massimo Marignani [3], Annunziata Scirocco [1], Guglielmo Tellan [4], Marilia Carabotti [1], Serena Schippa [2], Enrico Corazziari [1], Carola Severi [1]

[1] University Sapienza Rome, Dip.Med. Int.e Spec. Med., Rome, Italy
[2] University Sapienza Rome, Dip.Sanità Pubblica e Malattie Infettive, Rome, Italy
[3] University Sapienza Rome, UOC Malattie dell'Apparato Digerente e Fegato, Rome, Italy
[4] University Sapienza Rome, Dip.di Chirurgia "F.Durante", Rome, Italy

OC. 12 - EFFECT OF "IFLORA" AS A PROBIOTIC ON PENTYLENETETRAZOL-INDUCED SEIZURES IN RATS

Parvaneh Jafari [1], Aram Gharebaghi [2], Hamidreza Mohajerani [2] Saeed Tahmasebi [2], Neda Akbari [2], Maryam Tajabadi Ebrahimi [3]

[1] Founding member of Iranian society of probiotics and functional foods and Faculty member of IAU, Microbiology department, Science faculty, Arak branch, Arak, Iran
[2] IAU, Microbiology department, science faculty, Arak branch, Arak, Iran
[3] IAU, Biology department, science faculty, Tehran central bracnh, Tehran, Iran

OC. 13 - OBTAINING EFFECTS OF AN ANTI-CANCER FORMULATION BASED ON A PROTEIN SOLUBLE HYDROLYSATE (PSH) OBTAINED FROM THE PROBIOTIC BACTERIA L. FERMENTUM DSM32448

Erica Castro [1]

[1] Universidad San Sebastián, Facultad de Medicina, Concepción, Chile

01.00-02.00 p.m. **Lunch**

03.30-05.00 p.m. **NUTRHEFF-CNR MEETING (NUTRACEUTICAL HEALTH ENANCING FUNCTIONAL FOOD - NATIONAL RESEARCH COUNCIL OF ITALY)**

Chair: P. Lavermicocca (Italy)

Artichoke head, in vitro intestine models and probiotic bacteria: bioaccessibility, bioavailability, antioxidant and immunomodulatory activity exerted by polyphenols content
F. Minervini (Italy)

Use of ancient wheat crops for the diet of non-celiac gluten sensitivity
G. Mazzarella (Italy)

NMR based plant metabolomics: saffron as a source of bioactive compounds
R. Consonni (Italy)

Valorization of the neglected P. mahaleb. L. fruits as a source of functional molecules
C. Gerardi (Italy)

Functional spaghetti supplemented with supercritical CO_2 extracted pumpkin oil encapsulated in α-cyclodextrins
M. Durante (Italy)

05.00-06.00 p.m.

SINUT MEETING (ITALIAN SOCIETY OF NUTRACEUTICALS)
CARDIOVASCULAR AND AGING PREVENTION:
A ROLE FOR PROBIOTICS?
Chairs: A.F.G. Cicero (Italy), G. Scapagnini (Italy)

Peptides derived from lactic fermentation and blood pressure control
A.F.G. Cicero (Italy)

Probiotics and heart failure: a new challenge
A. Colletti (Italy)

New perspective in neuroprotection by probiotics
G. Scapagnini (Italy)

06.00-07.00 p.m.

POTENTIAL ROLE OF NUTRACEUTICAL PEPTIDES
Chair: G. Antonini (Italy)

Molecular models of peptides with nutraceutical properties
G.L. Gianfranceschi (Italy)

Lactoferrin nutraceutical peptides
F. Giansanti (Italy)

Bioactive peptides in dairy food products
M. Albenzio (Italy)

08.30-10.30 a.m.

NEW FOODS
Chair: V. Fogliano (The Netherlands)

Sulphur compounds
D. Huang (Japan)

Mediterranean diet and microbiome
D. Ercolini (Italy)

Food design & low calorie intake
N. Pellegrini (Italy)

Food for mood and cognition
M. Dekker (The Netherlands)

LECTURES
Chair: G. Gasbarrini (Italy)

Skin gut brain axis. A microbiome related link?
M. Picardo (Italy)

Vitamin D: immunomodulatory aspects
M. Miraglia del Giudice (Italy)

10.30-12.00 a.m.

BENEFITS OF A MULTISTRAIN BACTERIA FORMULATION FOR HEALTH: FROM MICROBIOLOGY TO CLINICAL TRIALS
Chairs: L. Morelli (Italy), A. Gasbarrini (Italy)

Microbial composition and cell viability of a multi-strain bacteria formulation
D. Mora (Italy)

Genomic analysis of bacteria strains - impact on quality control and function prediction *(video presentation)*
W. M. de Vos (Finland)

Scientific literature review, state of art
A. Gasbarrini (Italy)

Multi-strain bacteria formulation, a new therapeutic opportunity for the treatment of osteoporosis
R. Pacifici (USA)

12.00-01.30 p.m. **GI TRACT DISEASES: FOCUS ON GUT MICROBIOTA AND PROBIOTICS**
Chairs: R. Caprilli (Italy), G. Barbara (Italy)

Gut microbiota, probiotics and intestinal motility
E. S. Corazziari (Italy)

IBS
G. Barbara (Italy)

IBD
D. Gilardi (Italy)

Microbial signature in Crohn's disease
C. Manichanh (Spain)

LECTURE
Chair: L. Orfeo (Italy)

The role of gut microbiota in zonulin-mediated instestinal permeability, antigen trafficking and onset of chronic inflammatory diseases
A. Fasano (USA)

01.30-02.30 p.m. **Lunch**

02.30-03.30 p.m. **LECTURES**
Chair: G. Delle Fave (Italy)

Evidence Based Medicine and probiotics
M. Koch (Italy)

Antibiotic and modulation of microbiota: a new paradigm?
A. Gasbarrini (Italy)

03.30-04.30 p.m. **MICROBIOTA AND GUT BRAIN AXIS: THE EU MYNEWGUT PROJECT**
Chair: P. Brigidi (Italy)

Mechanisms underlying host-microbiota crosstalk insights from preclinical studies
M. Beaumont (Belgium)

The microbiome-host-diet dialogue in the obesity epidemic
A. Benítez-Páez (Spain)

Infant gut microbiota and implications for host health
C. Stanton (Ireland)

04.30-05.10 p.m.

LECTURES
Chairs: M. Koch (Italy), M. Cicala (Italy)

Metagenomic and metaproteomic aspects of autistic and Panda children
L. Putignani (Italy)

Potential role of gut microbiota in Amyotrophic Lateral Sclerosis pathogenesis and possible novel therapeutic strategies
F. De Marchi (Italy)

05.10-06.30 p.m.

GUT MICROBIOTA/PROBIOTICS IN TUMORIGENESIS AND IMMUNOTHERAPY
Chairs: M. Rescigno (Italy), P. Nisticò (Italy)

Microbiota and cancer
P. Nisticò (Italy)

Microbiota and colon cancer
M. Rescigno (Italy)

Microbiota and pancreatic cancer: an evidence-based association?
G. Capurso (Italy)

Cancer immunotherapy
M. Maio (Italy)

Diet and probiotics in cancer: from pathogenesis to cancer treatment
M. Libra (Italy)

PEDIATRIC DAY

09.00-09.30 a.m. **LECTURE**
Stewardship in clinical practice. Lessons from the antibiotic world
L. Galli (Italy)

09.30-11.00 a.m. **PROBIOTICS STEWARDSHIP IN PEDIATRICS**
Chair: Y. Yamashiro (Japan)

Evaluation tools for probiotics in clinical practice
H. Szajewska (Poland)

Position papers and guidelines: the role of scientific societies
B. Koletzko (Germany)

Quality of probiotics
S. Kolacek (Croatia)

Implementation science applied to probiotics
A. Guarino (Italy)

11.00-11.30 a.m. **Break**

11.30-01.00 p.m. **PROBIOTICS IN CLINICAL PRACTICE: CONSOLIDATED INDICATIONS**
Chair: H. Szajewska (Poland)

Acute gastroenteritis
A. Lo Vecchio (Italy)

Antibiotic associated diarrhea
Y. Vandenplas (Belgium)

Nosocomial infections
I. Hojsak (Croatia)

Infantile colics
F. Indrio (Italy)

01.00-01.30 p.m. **Discussion**

01.30-02.30 p.m. **Lunch**

02.30-04.00 p.m. **PROBIOTICS IN PAEDIATRIC GASTROENTEROLOGY: EMERGING INDICATIONS**
Chair: S. Kolacek (Croatia)

IBDs
E. Scarpato (Italy)

Functional intestinal disorders
M. M. Tabbers (The Netherlands)

NEC
J. B. H. van Goudoever (The Netherlands)

NAFLD/NASH
V. Nobili (Italy)

04.00-04.30 p.m. **Break**

04.30-06.00 p.m. **PROBIOTICS FOR PAEDIATRIC EXTRAINTESTINAL DISORDERS: WHERE WE ARE NOW**
Chair: J. A. Vanderhoof (USA)

Respiratory tract infections
E. Bruzzese (Italy)

Allergy
R. Berni Canani (Italy)

Obesity
E. Isolauri (Finland)

06.00-06.30 p.m. **Conclusions**

08.30-10.00 a.m.

NOVEL ANTI-INFLAMMATORY STRATEGIES TO COUNTERACT GUT AND LIVER INFLAMMATION
Chair: S. Cucchiara (Italy)

Probiotics and omega-3 cooperate in reducing inflammation
L. Stronati (Italy)

Novel strategies for culturing probiotics
V. Cesi (Italy)

Microbiota and fatty liver: a new therapy target?
V. Nobili (Italy)

LECTURE
Gender medicine: sex and microbiota influences in functional gastrointestinal disorders
C. Severi (Italy)

10.00-11.30 a.m.

MORE IS BETTER
Chair: P. Malfertheiner (Germany)

Probiotics dose-response
C. Scarpignato (Italy)

Bifidobacteria
S. D. Guglielmetti (Italy)

Multi strains effectiveness
A. Ouwehand (Finland)

11.30-12.50 p.m. **NEW GENERATION PROBIOTICS**

Chairs: J. Dorè (France), G. Ippolito (Italy)

Faecalibacterium prausnitzii
P. Langella (France)

Akkermansia muciniphila
C. Belzer (The Netherlands)

Eubacterium hallii
C. Schwab (Swirzerland)

Bacteroides fragilis
C. González De Los Reyes-Gavilán (Spain)

Archaea: great expectations from a forgotten island in next generation probiotics
J. F. Brugere (France)

12.50-01.30 p.m. **LECTURES**

Probiotics in foods
R. Berni Canani (Italy)

Probiotics and sport: from clinical evidence to agonistic performance
M. Pane (Italy)

08.00-09.30 a.m.

AGUI MEETING
(ITALIAN ASSOCIATION OF ACADEMIC GYNAECOLOGISTS)
Chairs: N.Colacurci (Italy), D.Caserta (Italy)

Vaginal microbiota and HPV infection.
A new perspective on cervical cancerogenesis?
A. Perino (Italy)

Changes of vaginal microbiota and high estrogen levels
in controllated ovarian stimulation (COS)
V. De Leo (Italy), P. Piomboni (Italy)

Probiotics in the treatment of bacterial vaginosis
A. Cianci (Italy)

Nutraceutical approach to menopausal compliants
P. De Franciscis (Italy)

09.30-11.00 a.m.

PROBIOTICS FOR VAGINAL HEALTH
Chairs: F. Vicariotto (Italy), S. Lello (Italy)

Early assembly of the microbiome and L. *rhamnosus* HN001 in pregnancy
and the potential to influence fetal immune parameters
A. Ouwehand (Finland)

Molecular mechanisms of probiotic action
S. Lebeer (Belgium)

Murine model of suppurative hysteritis to select vaginal probiotics
G. Pozzi (Italy)

Current clinical use of probiotics
N. Giovannini (Italy)

11.00-12.00 a.m.

MINI LECTURES

Chairs: E. S. Corazziari (Italy), M. Anti (Italy)

Stress and immune function: there is a role for the gut microbiota?
A. Castellazzi (Italy)

An innovative and remarkable activity of micronized cells of the probiotic strain B. lactis BS01 on macrophage activation and polarization: a new vaccine strategy
L. Mogna (Italy)

Influences of dietary fat and probiotic administration on zebrafish microbiota core: effects on glucose and lipid metabolism
O. Carnevali (Italy)

Metabolic effects of fiber and prebiotics: role of microbiota
M. Vitale (Italy)

Which role of nutraceuticals in functional intestinal disease?
E. Lahner (Italy)

12.00-12.45 a.m.

ORAL COMMUNICATIONS
NUTRHEFF - CNR HOT TOPICS: FOOD BIOACTIVE COMPOUNDS AND HEALTH

Chairs: P. Lavermicocca (Italy), V. Fogliano (The Netherlands)

OC. 12 - NEUROTROPHINS' MODULATION BY OLIVE POLYPHENOLS

Valentina Carito [(1)], Mauro Ceccanti [(2)], Marco Fiore [(1)]

[(1)] IBCN-CNR, Institute of Molecular Biology and Neurobiology, Rome, Italy
[(2)] Centro Riferimento Alcologico Regione Lazio,, Università di Roma "La Sapienza", Rome, Italy

OC. 13 - CORYLUS AVELLANA IMMUNOMODULATORY EFFECT ON HUMAN MONOCYTE-DERIVED-MACROPHAGES

Giulia Cappelli [(1)], Daniela Giovannini [(1)], Anna Lisa Basso [(1)], Olivia De Murtas [(2)], Gianfranco Diretto [(2)], Chiara Santi [(2)], Loretta Bacchetta [(2)], Francesca Mariani [(1)]

[(1)] CNR, IBCN, Rome, Italy
[(2)] ENEA Casaccia,Rome, Italy, Bioprocess and bioproducts laboratory, Rome, Italy

OC. 14 - BETALAINS, PHENOLS AND ANTIOXIDANT CAPACITY IN CACTUS PEAR (OPUNTIA FICUS-INDICA (L.) MILL.) FRUITS AND CLADODES FROM APULIA (SOUTH ITALY) GENOTYPES

Federica Blando [(1)], Clara Albano [(2)], Carmine Negro [(3)], Gabriele Maiorano [(1)], Carmela Gerardi [(1)], Giovanni Mita [(1)], Antonio Miceli [(3)], Luigi De Bellis [(3)]

[(1)] Institute of Sciences of Food Production (ISPA), CNR, Lecce, Italy
[(2)] Institute of Sciences of Food Production (ISPA), CNR, Milan, Italy
[(3)] Department of Biological and Environmental Sciences and Technologies (DISTeBA), Salento University, Lecce, Italy

OC. 15 - NEUROPROTECTIVE ROLE OF NATURAL POLYPHENOLS FROM SAMBUCUS NIGRA

Stefania Moccia [(1)], Carmela Spagnuolo [(1)], Gian Luigi Russo [(1)]

[(1)] Institute of Food Sciences, National Research Council, Via Roma 64, Avellino, Italy

OC. 16 - EFFECT OF TOTAL APPLE POLYPHENOLS EXTRACT AS INHIBITORS OF AMYLOID PROTEIN AGGREGATION

Valeria Guarrasi [(1)], Cinzia Giacoma Rappa [(1)], Maria Assunta Costa [(1)], Fabio Librizzi [(1)], Marco Raimondo [(1)], Silvia Vilasi [(1)], Donatella Bulone [(1)], Pier Luigi San Biagio [(1)], Maria Antonietta Germanà [(2)]

[(1)] Consiglio Nazionale delle Ricerche, Istituto di Biofisica CNR sede secondaria di Palermo, Palermo, Italy
[(2)] Universita` degli Studi di Palermo, Dipartimento di Scienze Agrarie, Alimentari e Forestali, Palermo, Italy

OC. 17 - NEUROPROTECTIVE ACTIVITY OF CONJUGATED LINOLEIC ACID IN AN ANIMAL MODEL OF AUTO-IMMUNE DISEASE

Paolo Bergamo [(1)], Antonio Monaco [(2)], Ida Ferrandino [(2)], Ennio Cocca [(3)], Floriana Boscaino [(1)], Francesco Maurano [(1)], Mauro Rossi [(1)]

[(1)] Italian National Research Council (CNR), Institute of Food Sciences, Avellino, Italy
[(2)] University "Federico II" of Naples, Department of Biology, Naples, Italy
[(3)] Italian National Research Council (CNR), Institute of Biosciences and Bio-Resources, Naples, Italy

12.45-01.30 p.m.

ORAL COMMUNICATIONS
GUT MICROBIOTA AND METABOLISM

Chair: P. Brigidi (Italy)

OC. 18- DPPIV ACTIVITY OF THE GUT MICROBIOTA: A NEW TARGET FOR THE INTESTINAL HOMEOSTASIS?

Marta Olivares Sevilla [(1)], Audrey M. Neyrinck [(1)], Sarah Pötgens [(1)], Martin Beaumont [(1)], Laure B. Bindels [(1)], Nathalie M. Delzenne [(1)]

[(1)] Université catholique de Louvain, Louvain Drug Research Institute. Metabolism and Nutrition Research Group., Brussels, Belgium

OC. 19 - SUPPLEMENTATION WITH A FOODBORNE COMPLEX MICROBIAL COMMUNITY EXERTS PROTECTIVE ACTIVITY ON OBESITY-ASSOCIATED INFLAMMATION IN A MURINE MODEL

Chiara Devirgiliis [(1)], Marianna Roselli [(1)], Paola Zinno [(1)], Barbara Guantario [(1)], Alberto Finamore [(1)], Rita Rami [(1)], Giuditta Perozzi [(1)]

[(1)] CREA- Council for Agricultural Research and Economics, CREA-AN Food & Nutrition Research Centre, Roma, Italy

OC. 20 - EFFECTS ON CHOLESTEROL METABOLISM OF BIFIDOBACTERIA IN HUMAN INTESTINAL MICROFLORA

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OC. 21 - LIPOPOLYSACCHARIDE AND PROBIOTIC BACTERIA EFFECT ON RAT JEJUNUM VILLOUS EPITHELIUM BY TRANSMISSION ELECTRON MICROSCOPY

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OC. 22 - BIFIDOBACTERIUM ANIMALIS SSP. LACTIS 420 WITH OR WITHOUT POLYDEXTROSE CONTROLS BODY FAT MASS AND WAIST CIRCUMFERENCE IN OVERWEIGHT AND OBESE ADULTS — RANDOMIZED CONTROLLED CLINICAL TRIAL

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OC. 23 - SHAPE GUT MICROBIOTA, SHAPING OUR BODY COMPOSITION

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OC. 24 - INVESTIGATION OF COMPETITION BETWEEN STAPHYLOCOCCAL MEMBERS OF THE HUMAN NASAL MICROBIOTA AND MULTI-RESISTANT S. AUREUS

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PROCEEDINGS

MASSIMO CRESPI : A TRIBUTE IN MEMORIAM
A TEACHER WHO CONTRIBUTED TO THE DEVELOPMENT OF MODERM MEDICINE, SCIENCE AND HUMANITY.

I met Massimo Crespi in 1968 and I began, because of him, to be interested in digestive endoscopy. We organized the 2nd World Congress of Digestive Endoscopy in 1970 in Rome. From this date on we continued to work together in expanding this technique first in Italy and then ali over the world. He was a real Leader plenty of good ideas and I leamed a lot from him. We started to be involved in the ESGE very early and his brilliant ideas contributed enormously to give a rational setting to the OMEO (now WEO) in order to expand his concepts using digestive endoscopy in cancer prevention. He was a man with clear ideas in mind to help humanity by preventing some digestive diseases. He believed this is and at a very young age, he visited many underdeveloped countries in order to study the precancerous lesions of the esophagus, stomach, colon and recto. In the seventies and eighties he went to North Iran, China, Thailand, North (Somalia, Libya, Tunisia) and Central Africa making live demonstrations obtaining very important results reported in the Major G.I. Congresses in Europe, Asia and America.

Massimo was an extraordinary man a scientist Leader in bis field, loyal, sincere and international diplomat. As a colleague he was honest and an outstanding collaborator to work with.

He was involved in the most important Scientific Gastroenterology Associations being also one of the Founding Member of the UEGF (now UEG) of which he was Chairman for one year; President of the ESGE for 4 years; President of the OMEO (now WEO) for 2 years. Secretary General of the MTCC (Mediterranean Task Force for Cancer Control).

During 50 years of collaborating we became very good friends and it will be impossible to forget a person with his qualities. His “joie de vivre”, his love for Nature especially the sea with his precious boat made his life happy and satisfied.

May his soul rest in peace!
Alberto Montori

BIOACTIVE PEPTIDES IN DAIRY FOOD PRODUCTS

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Biologically active peptides can be produced from milk proteins through different pathways involving the action of indigenous enzymes, digestive enzymes, microbial enzymes from starter and non-starter cultures acting during milk secretion, milk storage, milk processing and milk digestion. Proteolytic activity in fresh raw milk is attributed to indigenous and microbial enzymes. Among the indigenous enzymes, milk contains at least two main proteinase systems, plasmin-plasminogen system and lysosomal enzymes, as well as possibly other proteolytic enzymes. These enzymes play a role in the liberation of bioactive peptides during milk secretion and storage. As an example, in goat milk a great number of peptides were found in goat milk incubated up to seven days without any protease inhibitors [1]; plasmin was shown to play a major role in hydrolysis of casein and high numbers of peptides derived from the hydrolysis of β-casein. Almost 90% of the peptides identified shared structural homology with previously described bioactive peptides in caprine and bovine milk and dairy products showing encrypted sequences of bioactive peptides able to exert ACE-inhibitory activity [2,3], antihypertensive activity [3,4] and antioxidant activity [5].
The supplementation of cheeses with probiotic bacteria represents the aggregation of added value to a product that already has benefits inherent in its composition and the development of probiotic cheese is a current topic in the scientific literature and represents a trend for the dairy industry. The increase of survival and delivery of probiotic at controlled rates in cheese matrix has been improved by applying new technologies such as immobilized cell technology [6] and microencapsulation methods [7,8]. Cheeses could offer certain advantages as delivery system of live probiotics to the gastrointestinal tract having higher pH than fermented milk and high fat content that may protect the organisms during passage through the gastrointestinal tract [9]. Gastrointestinal enzymes (pepsin, pancreatin, and trypsin) are not able to degrade allergens that reach the intestine unaltered where they elicit an immune response; some studies have indicated that probiotic therapy offers great potential for the control of the allergenic inflammation associated to food allergies [10] First research on the production of functional cheeses from ewe milk was performed on PDO Canestrato pugliese cheese using *Bifidobacterium bifidum* and *Bifidobacterium longum* [11] as adjunct starter. Further studies tested the effectiveness of the incorporation of fresh cells of probiotic bacteria as *Lactobacillus acidophilus*, *Bifidobacterium lactis*, and *Bifidobacterium longum* into traditional lamb rennet paste for Pecorino cheese production. In latest studies cells of *B. longum* and *B. lactis* and L. acidophilus were encapsulated in alginate microspheres and added in the rennet paste used for cheesemaking [12]. Depending on the type of dairy products the level of peptides naturally formed in the matrix varies along with the equilibrium between the liberation and the further hydrolysis during ripening. However, the bioactive peptides have been characterized in a wide variety of dairy products distinguished on the basis of time of ripening in fresh, short and long ripened cheese and on the basis of the technological process as fermented cheese, pasta filata cheese, and cooked cheese.
In functional Scamorza cheese made from ovine milk, containing a mix of *B. longum* and *B. lactis* and *L. acidophilus* specific peptides deriving from microbial enzymes were found in cheese at fifteen days of ripening. Several fragments were identified which shared structural homology with previously reported peptides with ACE-inhibitory activity, antimicrobial activity, antihypertensive activity, immunomodulating activity. Specific peptides deriving from microbial enzymes may be regarded as tracing fragments and may represent a tool to verify the presence and activity of probiotic cultures in cheese. In functional Scamorza cheese fragments were identified deriving from β-galactosidase and from endonuclease associated to *B. longum*, or deriving from enzymes yielded by *Lactobacillus acidophilus* [13].

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Table 1. Peptides identified in the fraction collected by RP-HPLC of the soluble nitrogen fractions of Scamorza cheeses1 (adapted from Albenzio et al., 2015).

Fraction	Origin	Protein fragment	Sequence
S- BB Peak 1	β-CN	f210-220	VLGPVRGPFPI
	αs1-CN	f1-23	RPKHPIKHQGLPQEVLNENLLRF
S- BB Peak 2	β-galactosidase Bifidobacterium longum	f462-471	KAGAESGGMEMS
S- BB Peak 3	κ-CN	f78-90	SAEPTVHSTPTTE
	αs1-CN	f1-6	RPKHPI
	Endonuclease B. longum	f15-28	ETHLEAQPTVASAQ
S- LA Peak 1	Glycerophosphodiester phosphodiesterase family protein Lactobacillus acidophilus	f79-97	LALLFSILVLFIMPM
S- LA Peak 2	ATP-dependent metallic peptidase HflB Lactobacillus acidophilus	f512-518	RGGGAGEEE

1S-BB= Scamorza cheese containing the mix of Bifidobacterium longum BL-46 and Bifidobacterium lactis BB-12; S-LA= Scamorza cheese containing Lactobacillus acidophilus LA-5.

SESSION: MICROBIOTA AND GUT BRAIN AXIS: THE EU MYNEWGUT PROJECT

MECHANISMS UNDERLYING HOST-MICROBIOTA CROSSTALK: INSIGHTS FROM PRECLINICAL STUDIES

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Introduction

The goal of the European union funded MyNewGut consortium is to decipher the interactions between diet, gut microbiota and health in the context of obesity and behaviour disorders. To reach this objective, epidemiological and interventional studies in humans are conducted together with preclinical trials that aim to identify mechanisms underlying the cross talk between the gut microbiota and health. New data have been obtained in preclinical models of obesity and *ex vivo*, that will help to understand the role of the gut microbiota on inflammation "at distance of the gut", one of the key component of the development of pathologies associated with obesity, like non-alcoholic steato-hepatitis, or insulin-resistance.

In this context, bacterial components produced in the gut are studied as potential key intermediates between the microbiota and host organs, namely the liver. Among potentially harmful components, lipopolysaccharides (LPS), one of the main structural components of the gram negative bacteria, have been shown to translocate out of the gut in certain feeding conditions, in preclinical models of obesity, but also in humans (Cani et al., 2007). This "metabolic endotoxemia" may contribute to a low tone inflammation that drives metabolic alterations at distance of the gut. On the other hand, bacterial metabolites produced from nutrients fermentation may exert interesting physiological effects for the host. Most of previous work has been focused on nondigested carbohydrates derived bacterial metabolites such as short chain fatty acids (acetate, propionate and butyrate). In contrast, far less is known about the effects of metabolites produced from amino acid (AA) by the microbiota (Portune et al., 2016). Notably, aromatic AA degradation by the gut microbiota leads to the production of a wide variety of compounds. For instance, tyrosine, tryptophan and phenylalanine degradation produces, respectively, *p*-cresol, indole, phenylacetate and benzoate (*Figure 1*).

Interestingly, derivatives of these bacterial metabolites are present in host systemic circulation and their presence is strictly dependent on the gut microbiota, as shown by metabolomics studies in germ-free *versus* conventional mice (Wikoff et al., 2009). The implication of these observations is that bacterial metabolites derived from aromatic AA are absorbed through the intestinal mucosa and transported until the liver through the portal circulation (*Figure 1*). However, the effects of these metabolites on the liver are not known while they might be important regulators of the gut - liver axis.

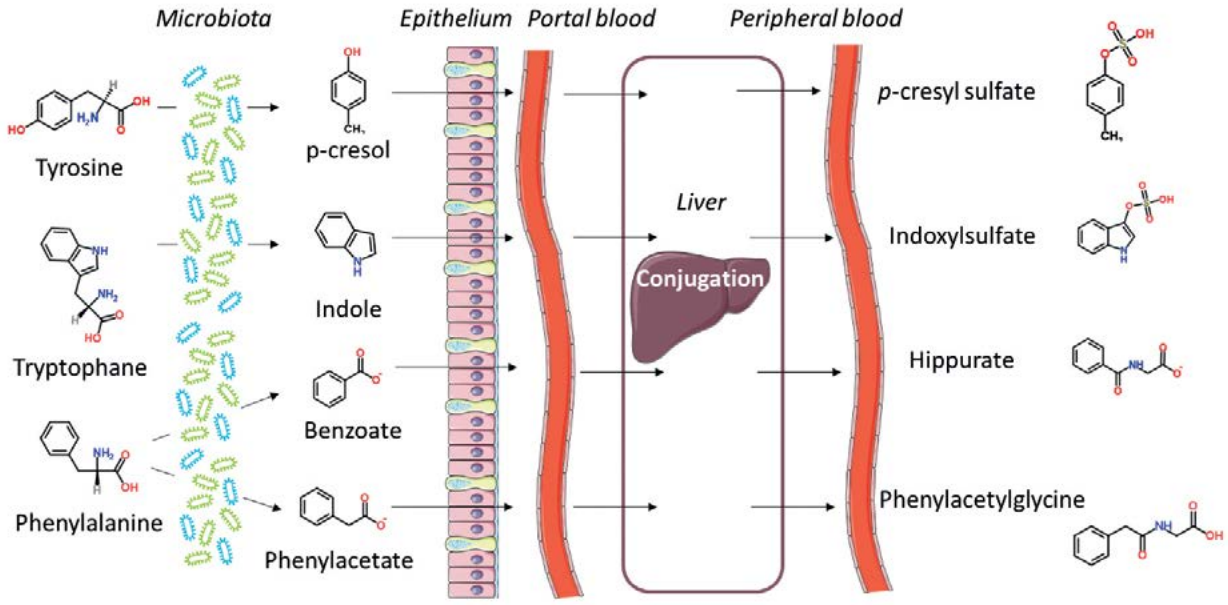

Figure 1: Production of "host-microbiota" co-metabolites from aromatic amino acids.

Innovative methodologies to evaluate the effect of microbiota-derived metabolites on liver inflammation

One of the aims of the experimental work performed in the context of the MyNewGut project was to evaluate the effects of metabolites produced by the gut microbiota on liver inflammation, with emphasis on the role of compounds derived from microbial metabolism of aromatic AA.

The original model of mouse precision cut liver slices (PCLS) is used since it presents the advantage of maintaining the tissue architecture with all cell types (hepatocytes, Kupffer cells, endothelial cells and stellate cells) together with their interactions (Neyrinck et al., 2004). Briefly, after liver excision, tissue cores are prepared (diameter 5 mm) and sliced (thickness 250 µM). PCLS are cultured in the presence of the bacterial metabolites of interest and the incubation of the PCLS is done in the absence or presence of LPS to mimic the endotoxin influx coming from the gut, notably when barrier

function is impaired. After treatment, enzymatic activities (lactate dehydrogenase, amino-transferases) in the culture medium are measured for cytotoxicity assessment. This model also allows to evaluate the effect of metabolites and LPS on gene expression after mRNA extraction and quantification by qPCR of the expression of key markers of inflammation or metabolism.

Our first data show that, as expected, LPS (100 ng/ml) induces the expression of pro-inflammatory genes (MCP1, TNF-α, iNOS, IL-6, IL-1β) in liver slices within 2 to 20 hours of incubation, without cytotoxic effects. In this model, no effect of short chain fatty acids (acetate, propionate and butyrate) were observed. In contrast, our preliminary data suggest that the aromatic AA-derived bacterial metabolites indole and p-cresol are able to counteract the LPS-induced increase in pro-inflammatory genes mRNA levels while no effects are observed for benzoate and phenylacetate.
To evaluate the implication of Kupffer cells in the modulation of liver inflammation in response to gut bacteria-derived components, PCLS are also prepared from mice treated with clodronate liposome (10 mg/kg), a treatment known to deplete the resident macrophages in the liver (Van Rooijen and Sanders, 1996). Indeed, our data show that clodronate liposome injection efficiently deplete Kupffer cells in the liver after 48 hours as demonstrated by the strong reduction of the mRNA levels of the macrophage marker F4/80. Further experiments will be performed to evaluate the relevance of anti-inflammatory effects of indole and p-cresol in vivo and to unravel the underlying mechanisms.

Conclusion

PCLS is a promising ex vivo model to study the influence of the gut microbiota on liver inflammation. Indeed, our experiments with PCLS allowed us to identify metabolites produced by the microbiota from aromatic AA, namely p-cresol and indole, which are able to counteract LPS-induced expression of pro-inflammatory genes in a process partly mediated by Kupffer cells. The regulation of the production to these metabolites, notably through dietary interventions targeting the gut microbiota, might therefore be a promising strategy in the management of liver inflammation.

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ARCHAEA: A FORGOTTEN ISLAND FOR DESIGNING NEXT GENERATION PROBIOTICS

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Archaea is one of the three domains of life,[1] whose members are unicellular microorganisms distinct from bacterial and eukaryotic cells with regard to evolution coupled with some distinct cellular/molecular processes that they exhibit. To date, no pathogens have been reported within the *Archaea*, neither in strains from humans nor from any other animal or plants. Naturally-occuring archaea in the human gut represent a mean of 1.5% of total gut microbes,[2] most of them being hydrogenotrophs performing a metabolism found exclusively in some archaea, the methanogenesis.
In 2011, the Stanley Hazen laboratory (Cleveland Clinic, USA) identified trimethylamine N-oxide (TMAO) in plasma which was predictive in the higher quartile for a 2.5-fold increase of Major Adverse Cardiac Events (MACE) at two years.[3] TMAO is directly involved in the development of atherosclerosis, acting in the intestine (alteration of the cholesterol reverse transport through biliary acids), in the liver (modification of the quantity and composition of the biliary acids pool) and in arteria (inflammation, differentiation of macrophages into cholesterol containing foam cells) as shown initially by *in vitro* studies and animal models (ApoE$^{-/-}$ mice). Plasma TMAO results exclusively from the liver oxidation by human flavine monoxygenase 3 (FMO3) of a gut-generated compound called trimethylamine (TMA). TMA, the molecule which gives rotten fish their unpleasant odour, is not found in human diet, but is generated from various nutrients of a broad range of alimentary sources (phosphatidylcholine, choline, TMAO itself, L-carnitine) by some unrelated gut bacteria, and through various enzymatic pathways. Lowering plasma trimethylamine oxide (pTMAO) levels is one of the goals for the prevention of CVD risks. One proposition is to bioremediate TMA *in situ* in the human gut into an inert compound, thereby limiting its absorption and its further conversion into deleterious plasma TMAO: however, no safe gut bacterial representative was found suitable to eliminate this methylated compound.
This could be however achieved by some archaea.[4] While one archaeal species is mainly detected in humans (*Methanobrevibacter smithii*), molecular analysis of archaeal and methanogenesis markers revealed also a novel methanogenic lineage thriving in the human gut.5 Mainly known from genomic studies, some members of this lineage (*Methanomassiliicoccales*) possess the genetic capability to use TMA for their methanogenesis.6,7 This metabolic process necessitates the encoding behaviour of an atypical proteogenic amino-acid, pyrrolysine (Pyl), also called the 22nd amino-acid, which is incorporated in nascent proteins using a specific *amber*-suppressor Pyl-tRNA, thus allowing the recoding of this usual stop codon and continuation of translation.8
The presentation will explain in more details these specific features encountered exclusively in these archaea with results obtained recently.7 It will show, through the examples of cardiovascular disease and trimethylaminuria, that an innovative way of next generation probiotic design is applied, considering that (i) the microorganisms that fit this use are directly selected from human gut microbial inhabitants, and (ii), that their selection is based on the knowledge of the mechanisms underlying the pathology, being therefore first rationally selected using simple *in-vitro* tests (here, capability of TMA fermentation). In any case, further experiments are needed to address the question that some *Methanomassiliicoccales* may be efficiently and safely used in human pathologies. This provides however the first example that an archaeon can be a potential candidate for probiotic use. Consequently, representatives of the domain *Archaea* which has been neglected so far for this topics are proposed for the development of pharmabiotic / probiotic applications (Archaebiotics)4 through their distinctive properties.

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IMPACT OF DIETARY FAT AND PROBIOTIC ADMINISTRATION ON ZEBRAFISH (DANIO RERIO) CORE MICROBIOTA: EFFECTS ON GLUCOSE AND LIPID METABOLISM

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Gut microbiota plays an important role in maintaining the integrity of the intestinal tract and is also involved in the regulation of several host metabolic pathways. In addition, the ability of probiotics to stimulate immune system is well documented and recently it has been demonstrated that the gut microbiota play a role in the control of host's energy metabolism, body weight, fat distribution and insulin sensitivity [1,2,3]. In addition, several studies demonstrated the ability of probiotics (e.g. *Lactobacilli* and *Bifidobacteria*) to preserve the equilibrium of the intestinal microflora by shifting the gut microbiota community towards those beneficial species [4,5].

The study presented here, by using a multidisciplinary approach, evaluated the effects of *Lactobacillus rhamnosus* (IMC 501®), administration on gut microbiota and its capability to change lipid and glucose metabolism, and appetite control, during the early stage of larval development using zebrafish as model.

The evaluation of the impact of *L. rhamnosus* on gut microbiota composition in zebrafish larvae was conducted by metagenomic analysis of bacterial V1-2 and V3 region. Transcriptomic analysis, focusing on a wide network of genes which regulate lipid and glucose metabolism as well as appetite control, were also performed. We also explore the effects of microbiota related-changes induced by the probiotic at the intestinal epithelium level by ultrastructural analysis. Additionally, we investigated variation on total content of cholesterol, triglyceride, fatty acids and glucose after probiotic administration, as well as feed intake and body weight.

Metagenomics results found that the administration of *L. rhamnosus* shifted gut microbiota community by elevating the abundance of reads belonging to *Lactobacillus* spp. and *Streptococcus thermophilus.*

Microbiota changes were concomitant with a transcriptional down-regulation of lipogenic genes such as *fit2, agpat4, dgat2, hnf4α* and up-regulation of *mgll* gene (Fig. 1A). These results were coupled with a decrease of total body cholesterol and triglyceride content and an increase of fatty acid levels in probiotic treated zebrafish larvae (Fig. 2 A-D).

With regard to glucose metabolism, microbiota changes after probiotic supplementation up-regulated *nucb2a*, *insulin* and *glp-1* gene expression and down-regulated *goat* gene expression with a concomitant reduction of total glucose content in probiotic treated zebrafish larvae (Fig. 1A, 1B).

In addition, results showed a significant increase of anorexigenic transcripts such as leptin and mc4r in probiotic treated zebrafish larvae and a significant decrease of the expression level of orexigenic gene *npy* and *cb1* concomitant with a reduction of feed intake in treated larvae (Fig. 1A, 1C).

On the contrary, recently Gioacchini et al. [6] showed a significant increase of the cb1 and cb2 expression level associated with a significant decrease of *faah* and *mgll* after the administration of a mix of probiotic (VSL#3produced before January 31, 2016; VSL Pharmaceuticals, Inc., Gaithersburg, MD, USA), showing the ability of these mix of probiotics to act on endocannabinoid system (ECS) and, regulate appetite. A beneficial effect of *L. rhamnosus* treatment and further microbiota-changes was also evident at gut morphological level; an increase in microvilli and enterocyte lengths and a decrease in lipid droplets size in the intestinal epithelium were observed. These changes resulted in increased zebrafish larval growth.

Additional study from our laboratory aimed at exploring the effects of dietary lipid content with or without probiotic *L. rhamnosus* on adult zebrafish gut microbiota community, lipid metabolism, appetite control and gut ultrastructure.

As reported in Falcinelli et al. (2017, in press) metagenomics analysis performed on adult zebrafish fed a high, medium, low fat diet (HFD, MFD, LFD, respectively) with or without *L. rhamnosus* supplementation (P) showed clear effects on gut microbiota composition.

In HFD, MFD, LFD (with and without the probiotic) the core microbiota at genus level comprised 17 genera, however, the gut of fish fed a HFD and MFD shared *Janibacter* and *Anaeroccocus* genus, which were not present in the gut of fish fed a LFD (Fig. 3A).

The administration of the probiotic increased the abundance of *Mesorhizobium* in MFD-P, *Gordonia*, in LFD-P and HFD-P, while *Oxalobacteraceae* was typical of MFD-P and HFD-P (Fig. 3A).

In addition, our results found that total cholesterol and triglyceride content and body weight increase as fat content is increased in the diet, however, as demonstrated in larvae, supplementation of the probiotic significantly decreased the total cholesterol and TAG in zebrafish fed a HFD and MFD (Fig. 3B-C). Moreover, the presence of probiotic in the diet attenuated weight gain in fish fed with high and medium fat diets, but not in the low fat group.

Similar to our findings in larvae, the addition of *L. rhamnosus* in the diets of adult zebrafish induced transcriptional reduction of orexigen encoding mRNAs including *npy* and upregulation of anorexigenic genes nucb2a and glp-1. Additionally, Western Blot results showed that NUCB2 peptide was significantly lower in HFD-C compared to MFD-C and LFD-C. The supplementation of the probiotic significantly decreased NUCB2 in HFD-P and MFD-P fed fish compared their respective control diet (HFD and MFD). In the gut, the immunoreaction results showed higher number of positive cells to nesfatin-1 in HFD compared to MFD. Particularly, the supplementation of probiotic highlighted a significant decrease of immunoreactive cells to nesfatin-1 in HFD. Additionally, Western Blot results showed that HFD fed zebrafish contained significantly decreased preproghrelin protein levels compared to MFD and LFD. The supplementation of the probiotic did not affect preproghrelin protein in HFD and MFD, however, preproghrelin protein was significantly increased in the LFD-P. Increased abundance of ghrelin immunopositive cells were found in the gut of LFD group compared to HFD group. However, the supplementation of the probiotic did not exert any effect on ghrelin.

Regarding lipid metabolism, as reported in larvae, probiotic treatment down-regulated genes involved in lipogenesis such as *hnf4α*, *fit2*, and *npcl1a1*, and up-regulated those genes involved in lipolysis such as *mgll*.

Finally, ultrastructure analysis showed that the intestine of adult zebrafish fed a HFD-P, MFD-P and LFD-P diets, similarly to their respective controls, showed an intact intestinal epithelium, well organized microvilli and no sign of epithelium damage (data not showed).

In conclusion, in both larvae and adult zebrafish the administration of probiotic and/or different dietary fat content was associated with different gut microbiota composition. The shifted microbiota in turn modulated a set of genes involved in lipid and glucose metabolism and appetite control coupled with decreasing in cholesterol, triglycerides, short chain fatty acids and glucose levels. Overall, our results suggest positive effects for probiotics in energy homeostasis and has potential in modulating metabolic diseases.

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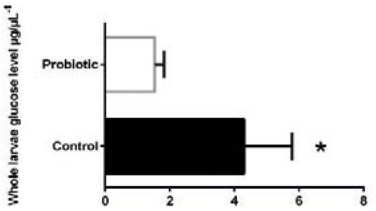
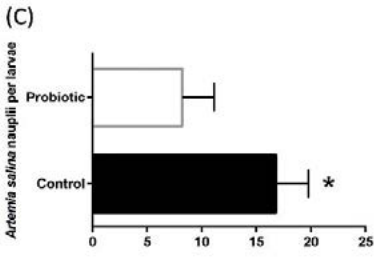
(A)

	PROBIOTIC GROUP (Respect to control)
LIPID METABOLISM GENES	
fit2	⬇
agpat4	⬇
dgat2	⬇
hnf4α	⬇
mgll	⬆
GLUCOSE METABOLISM GENES	
nucb2	⬆
insulin	⬆
Glp-1	⬆
goat	⬇
APPETITE CONTROL GENES	
leptin	⬆
mc4r	⬆
CB1	⬇
NPY	⬇

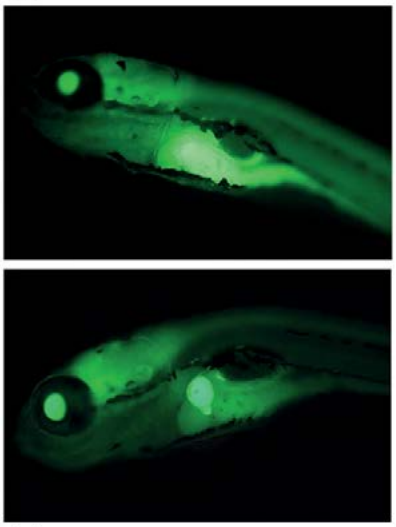
(B)



(C)



(A)



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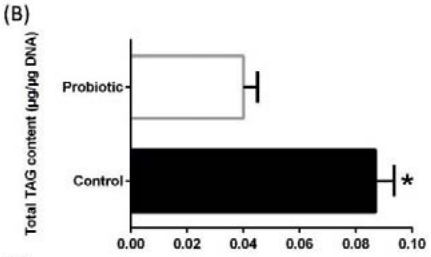
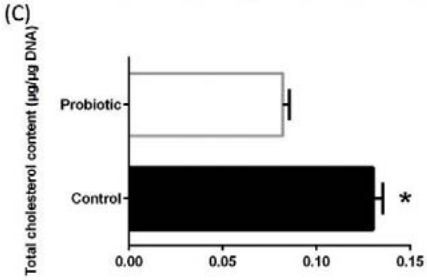


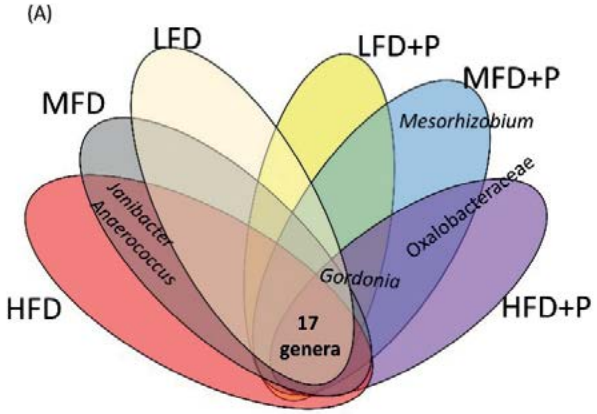
(C)



(D)

	CONTROL	PROBIOTIC
GALLBLADDER Fluorescence intensity (a.u.)	348.3 ± 45	539.7±50 *

(A)



(B)

TAG CONTENT (µg /µ DNA)	CONTROL	PROBIOTIC
HFD	92.5 ± 0.8	73.5 ± 0.4 *
MFD	83.7 ± 3.8	66.5 ± 4.9 µg *
LFD	67.6 ± 0.3	66.1 ± 0.3

(C)

CHOLESTEROL CONTENT (µg /µ DNA)	CONTROL	PROBIOTIC
HFD	6.2 ± 0.4	3.1 ± 0.2 *
MFD	7.6 ± 5.5	5.5 ± 0.3 *
LFD	5.3 ± 0.4	4.9 ± 1.1

STRESS AND IMMUNE FUNCTION: THERE IS A ROLE FOR THE GUT MICROBIOTA?

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There is a functional communication between the gastrointestinal (GI) and central nervous system (CNS). This communication is bidirectional and it involves anatomical connections like the vagus nerve and humoral components including the immune system and the hypothalamus- pituitary-adrenal (HPA) axis. There is increasing evidence suggesting that another key player in this interaction is the intestinal microbiota (1).
Physical and psychological stress is known to affect not only the immune system, but also hormonal and gastrointestinal homeostasis. Immune mechanisms are regulated by the hypothalamic pituitary-adrenal axis as well as by neuronal influences via sympathetic, parasympathetic and peptidergic/sensory innervation of peripheral tissues.
Immune and neuroendocrine systems have been shown to exert integrated responses to environmental signals and the relationship between stress and immune function has been demonstrated in many contexts, including proliferative response to mitogens and cellular activity (2).
Stress conditions can lead to an imbalance between pro- and anti-inflammatory cytokines or to an uncontrolled production of cytokines. Disregulation of innate and adaptive intestinal immune responses directed against the bacterial flora including a breakdown of oral tolerance to environmental antigens and commensals are involved in several pathogenetic mechanisms (3).
Moreover, the integrity of intestinal ecosystem could be affected by several external factors, including antibiotic use, radiation, altered GIT peristalsis, dietary changes, psychological and physical stress. Studies conducted either in humans and in animal models demonstrated that psychological stress could directly affect microflora composition and sometimes with long lasting effects, with a marked decrease in Lactobacilli specie. Stress-induced gastrointestinal changes lead to the establishment of an intestinal environment less conducive to Lactobacilli survival, adherence, and replication (4).
Prolonged psychological stress also results in a significant reduction in the production of mucin and a decreased presence of acidic mucopolysaccharides on the mucosal surface, which facilitates intestinal colonization by pathogenic organisms (5, 6).
A balanced intestinal microflora is important not only for the maintenance of intestinal homeostasis, but also for the regulation of immune system functionality and with direct effects on gut-brain axis.
In the light of the interactions between CNS and intestine, the use of probiotics and prebiotics can be useful to improve the intestinal homeostasis and reach the onset of dysbiosis associated with physical and psychological stress conditions.
We conducted a study in order to evaluate how a probiotic product can be effective in preventing the possible alterations of the immune response associated with psychological stress condition. In particular, the effects of a probiotics product was tested on healthy adult volunteers self reporting a psychological stress condition. Specific stress markers such as SAA, cortisol and CgA, immunological parameters, such as sIgA, NK activity, IL8, IL10, TNF-α level in faeces, and the composition of the intestinal microbiota were evaluated. The probiotic product was composed by *Lactobacillus acidophilus* LA-5® DSM13241(1x109CFU), *Bifidobacterium animalis subsp. lactis* BB-12® DSM15954 (1x109CFU), *Lactobacillus paracasei* CRL431® ATCC55544 (0,04x109 CFU), *Bacillus coagulans* BC513 (0,04x109 CFU), vitamin B1, B2, B5, B6, B12 and niacin.
The administration of product to the enrolled volunteers did not exert a direct effect on stress salivary markers and NK activity, but in the probiotic group we found a reduction in abdominal pain and an increase in fecal IgA and IL10 levels. Moreover, probiotic product induced a moderate increase in *Bifidobacterium* and *Lactobacillus* spp., as expected, and in *Faecalibacterium* spp., decreasing the amount of *Dialister* spp. and *Escherichia – Shigella* populations.
In conclusion, product administration was effective toward mucosal barrier protection supporting the number of SCFA producers and decreasing the load of potentially detrimental bacteria, inducing lower intestinal inflammation and reduction of abdominal discomfort.
These are promising results for the possible use of probiotic supplementation not only for the restoring of intestinal homeostasis , but also to support the immune system functionality in stressed subjects

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PROBIOTIC, PREBIOTICS AND NEW FOODS, NUTRACEUTICALS AND BOTANICALS FOR NUTRITION AND HUMAN AND MICROBIOTICA HEALTH. ROME, SEPTEMBER 10/12, 2017, UNIVERSITÀ URBANIANA

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Abstract

For many years scientist focused your attention on melatonin (N-acetyl-5-metoxytryptamine) as one of the hormone of the diffuse neuroendocrine system. Moreover, at the beginning (1965-1980), the main source of this hormone has been considered to be the Pineal gland or *Epiphysis cerebri.* Today (2017) it is well known that melatonin is widely distributed in all organ systems of the animal kingdom. In the last 25 years it has been demonstrated that melatonin has been identified in most all biological systems and for that reason has to be considered an ubiquiteous substance. The hormone has been detected in extrapineal organ such as retina, Harderian gland, gut mucosa, cerebellum, airway epithelium, liver, kidney, adrenals, thymus, thyroid, pancreas, ovary, carotid body, placenta and endometrium. It has been also localized in non-endocrine organs such as mast cells, natural killer cells, eosinophilic leukocytes, platelets, endothelial cells and bone marrow cells. Among many functions, in animal kingdom melatonin synchronizes circadian and circannual rhythms, have potential benefits of treatment of insomnia, as well as neurodegenerative disorders and cancer, play an important neuroimmunoregulatory role and act as a hydroxyl radical scavenger and antioxidant. Moreover, melatonin has been identified in all major non-metazoan taxa, including bacteria, dinoflagellates, euglenoids, trypanosomides, fungi, rhodophyceans, pheophyceans, chlorophyceans, angiosperme, edible fruits, medicinal plants and in almost all animal kingdom from lampray, amphibia, reptilia, aves, fishes and mammalian. Currently, despite the vast abundance of melatonin data, little is known about its functions in plant kingdom. The complexity of our understanding on the physiological role of melatonin in lower phyla, plants, invertebrates, vertebrates, including humans, is still a subject of debate. My communication is aimed at summarizing what is known and not known about the presence and role of melatonin in the plant kingdom and is also an attempt to answer the provocative question: is melatonin an evolutionary molecule?

CHANGES OF VAGINAL MICROBIOTA AND HIGH ESTROGEN LEVELS IN CONTROLLED OVARIAN STIMULATION (COS)

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Abstract

Goal: To assess the changes in the vaginal microbial flora of women before and after hormone treatment for controlled ovarian stimulation (COH) during *in vitro* fertilization (IVF).

Background: The influence of pathogenic vaginal microorganisms on implantation rates after IVF cycles is controversial.

Patients and Methods: A cohort of 108 infertile patients, unable to conceive naturally for at least [1] year, were prospectively enrolled in this study at the Siena University Hospital. For each IVF patient, one vaginal swab was collected before and the second after the gonadotrophins treatment, at the oocyte pick up. Changes in the vaginal microflora were assessed by using culture based techniques and quantification of diamines.

Results: In this study we showed a significant increase in vaginal bacterial pathogens and in diamine content after hormone administration. Moreover patients having positive swabs and high diamine contents showed a significantly decrease in the implantation rate.

Conclusions: The significant shift in the vaginal flora observed after gonadotrophins administration suggests a link between the changes in the flora after hormonal treatment and pregnancy rates in IVF.

Introduction

In vitro fertilization is the widely offered to treat couple infertility. This procedure requires exogenous gonadotrophins administration to induce multiple oocytes development in a single cycle[1]. The vaginal flora has long been considered to be indirectly controlled by estrogens. For instance, throughout the menstrual cycle, during pregnancy and as a woman passes from reproductive years into menopause, hormonal and various other physiological changes occur that are invariably accompanied by alterations in the microbial flora. In about 30% of women of child-bearing age, the normal vaginal flora, mainly composed of *Lactobacillus* spp., decreases, to be replaced by other microorganisms. This vaginal flora disorder is known as Bacterial vaginosis (BV)[2,3,4]. The influence of pathogenic vaginal microorganisms on implantation rates after IVF cycles is controversial[5]. In this study we aimed to assess if there is a significant shift in the vaginal flora of women undergoing hormone treatment towards pathogenic bacteria which could create a condition like bacterial vaginosis.

Material and Methods

A cohort of 108 infertile patients, 90% Caucasians, undergoing COS for IVF treatment at the Center for Couple Infertility (Siena University Hospital), were prospectively enrolled in this study. All patients have been unable to conceive naturally for at least 1 year before entering the study. All samples were taken with prior informed consent; the study was completed in 12 months. Two vaginal swabs were collected for each IVF patients: the first at the screening visit, one months before hormone therapy, and the second one after the hormone therapy, at the oocyte pick up. Changes in the vaginal microflora were assessed by using culture based techniques (Blood agar, Columbia CNA, Gardenerella, Mannitol Salt, Mc Conkey, Sabouraud, Rogosa and Scheadler agar plates) and quantification of diamines which can be used as biomarkers of the bacterial catabolic activity.

Results

In this study we showed that 35.3 % of women tested were positive for bacterial pathogens and 64.7% had no pathogens before hormone administration whereas, after the treatment, women with bacterial pathogens rose to 56.9% when compared to 43.1% without pathogens (Odds Ratio OR=2.61 CI 1.49-4.57 p=0.0007). The diamine content in the vaginal fluids significantly increased in vaginal swabs of treated women in comparison to the same patients before hormonal stimulation (Fig. 1). Finally, the implantation rate results to be significantly decreased in patients having positive swabs and high diamine contents (Fig. 2).

Discussion

Our results show that there is a significant shift in the vaginal flora of women after hormonal treatment for COS towards pathogenic bacteria which could create a condition like BV. We hypothesize that there could be a link between the changes in the flora after hormone treatment and very low pregnancy rates in IVF. If these results will be confirmed in a larger cohort of patients, it may be suggested that women undergoing IVF should be treated to avoid abnormal vaginal microbiota development.

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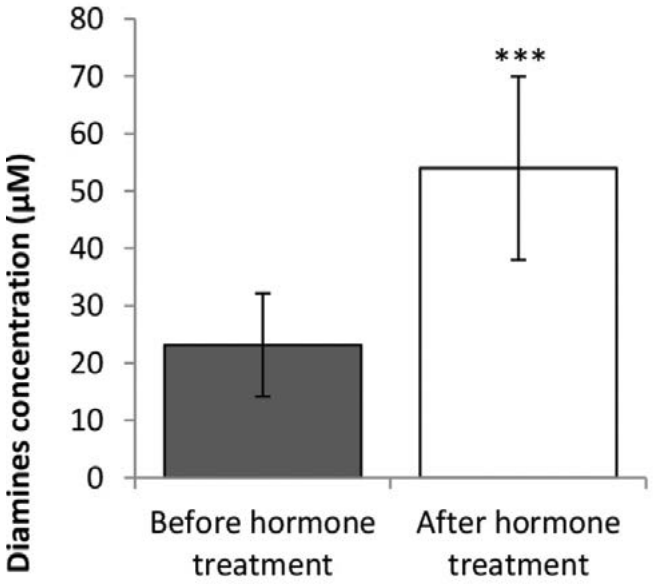

Figure 1: Concentration of diamines in the vaginal fluids of 108 women, before and after hormone treatment during in vitro fertilization. ***P<0.001

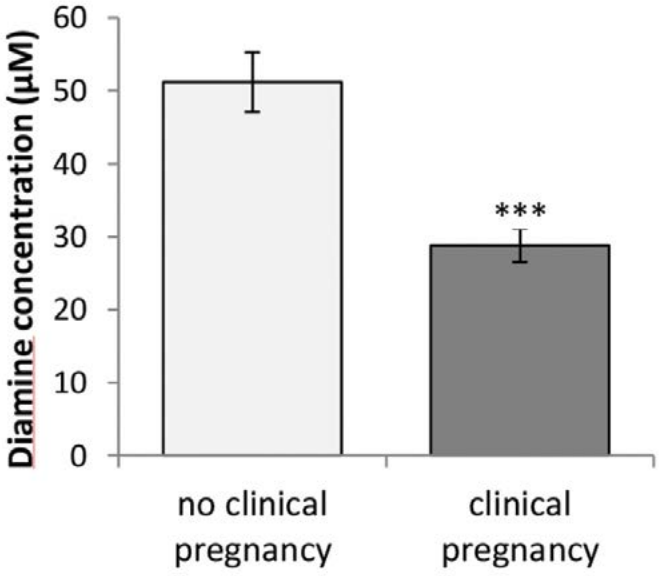

Figure 2. Concentration of diamines in the vaginal fluids of 108 women as a function of clinical pregnancies. ***P<0.001

POTENTIAL ROLE OF GUT MICROBIOTA IN ALS PATHOGENESIS AND POSSIBLE NOVEL THERAPEUTIC STRATEGIES

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Amyotrophic Lateral Sclerosis (ALS) is a progressive, fatal neurodegenerative disease that primarily affects the motor system and presents with progressive muscle weakness. Most patients survive for only 2-5 years after disease onset, often due to failure of the respiratory muscles. Currently there is no intervention that can significantly change the course of the disease. It is now recognised that ALS involves also non-motor systems. In about 50% of cases, the degenerative process extends to the frontal and anterior temporal lobe, giving rise to a variable degree of executive dysfunction language impairments or behavioural changes. Approximately 10% of affected individuals develop frontotemporal dementia.
ALS is a familial disease in about 10% of patients, with the remaining 90% developing sporadic ALS. Toxic exposures, combined with genetic susceptibility, may trigger motorneuron degeneration explained via the gene-time-environment hypothesis (Al-Chalabi A;2013). Various cellular disturbances have been described in ALS, such as alterations in RNA proccessing, protein metabolism abnormalities, increased oxidative stress, defects in axonal transport, synaptic disorders and altered motor neuron environment (Robberecht 2013), which are all associated to motor neuron degeneration. Evidence of an immuno-inflammatory component in ALS pathogenesis is compelling (Zhao W, 2013). A pathological hallmark of the neuroinflammation is prominent microglia activation at involved sites. T-regulatory lymphocytes (Tregs) are important immunomodulatory cells that regulate the balance between activation and suppression of the immune response.
Recent pre-clinical studies suggest that dysfunction of gastrointestinal tract may play a role in Amyotrophic Lateral Sclerosis (ALS) pathogenesis through a modification of the gut microbiota brain axis. Infact, the importance of an organ called "microbiota", an ecological community of commensal, symbiotic and pathogenic microorganisms that share our bodies is emerging in the scientific literature (Baquero, 2012). These intestinal bacteria exert a bidirectional interaction between the gut and the Central Nervous System (CNS) (Cryan, 2012) with an important role in some neurological diseases such as autism (de Theije, 2011), multiple sclerosis (Berer, 2011) or Parkinson disease (PD). In detail, in PD Scheperjans et al., in 2012, demonstrated a clear differences between fecal microbiota in PD patients and control subjects with a 78% lower abundance of Prevotellaceae in the fecis of PD patients (Scheperjans, 2012). No detailed studies describe microbiota in Alzheimer disease and in Amyotrophic Lateral Sclerosis.
Gastrointestinal motor dysfunction can occur in ALS, both as delayed gastric emptying and as delayed colonic transit times. In 2015, Wu et al. showed in G93A transgenic mice as a model of human ALS a damaged intestinal structure and an enhanced intestinal permeability. In the same model abnormal intestinal microbiome was demonstrated. Zang et al., in 2017, confirmed these results and demonstrated for the first time that the ALS-causing SOD1 mutation that leads to protein aggregate formation in neurons and skeletal muscle indeed forms aggregates also in the intestine of ALS mice and human intestinal epithelial cells. These results suggest that SOD1G93A plays an essential role in pathophysiologic alterations also in the intestine. The same authors treated the G93A transgenic mice with 2% butyrate, a natural bacterial product, able to restore the intestinal microbial homeostasis showing an improvement of survival and weight loss.
A hypothesis is that intestinal bacteria can represent an epigenetic entity that interacts with environmental factors (Stilling, 2014) in determining pathogenic influence on the CNS. Maybe there are different mechanisms by which these bacteria can affect the CNS but in particular their autoimmune role seems interesting, consisting of an alteration in circulating levels of inflammatory cytokines that can damage the CNS. This mechanism could be important in the pathogenesis of ALS; infact, the role of neuroinflammation through microglial activation and reactive astrocytosis is well demonstrated in the genesis and evolution of ALS (Boillee, 2006a, b).
Another mechanism by which the intestinal bacteria could enter in the ALS pathogenesis is the production of neurotoxins. Some strains of Clostridium produce neurotoxins that selectively affect the motor system. The most famous are the ones causing botulism or tetanus, but other strains such as Clostridium baratii and Clostridium butyricum produce neurotoxins, of which the effect on the nervous system is still unclear. We can assume that a colonization of ALS patients with certain species of bacteria that produce neurotoxins on the motor neuron can influence the disease in susceptible patients. An alteration in the intestinal bacterial flora as an external trigger could explain the rare cases of ALS in spouses (Corcia, 2003) or in some clusters (Sabel, 2003).
The use of probiotic microorganisms, typically lactobacilli and bifidobacteria, showed significant benefits not only in some gastrointestinal diseases but also in some behavioral disorders such as anxiety, depression and chronic fatigue syndrome. It is believed that these effects are related to a reduction of inflammatory cytokines (Cryan, 2011) and oxidative stress (Innis, 2007) and a modification of nutritional status (Cryan, 2011).
With these assumptions, our study is the first focused on microbiota analysis in ALS patients.

Aim

Our principal aim was to study the microbiota composition in ALS patients and verify the correlation with the phenotypes. Moreover we have examined the influence of a treatment with medical device acting on the gastrointestinal barrier on the microbiota composition and the progression of the disease.

Methods

We enrolled 50 ALS patients and 50 healthy controls, matched for sex, age and origin. Patients and controls with gastrointestinal, autoimmune or inflammatory disease were excluded. Faecal samples were used for total genomic DNA extraction using the QIAamp DNA Stool Mini Kit (Qiagen, West Sussex, UK), according to the manufacturer's instructions. Two PCR-DGGE analyses were performed to investigate total eubacteria and yeasts populations. They target the V2-V3 region of 16S rDNA and the D1 region of 26S rDNA, for Eubacteria and yeasts, respectively.
The bacteria belonging to *Clostridium* sensu stricto (*C. baratii, C. hystoliticum, C. butyricum, C. prefringens, C. botulinum and C. tetani*), *Enterobacteriaceae, E. Coli, Bifidobacterium, Lactobacillus* and yeast were dosed using qPCR approach targeted on 16S rRNA gene.

Patients were also randomized to double-blind treatment either to the Medical Device or placebo for six months and assessed by ALS-FRS-R, FVC (%) and BMI. The fecal DNA, was also collected at 3 and 6 months after the baseline.

Results

PCR-DGGE analysis showed a clear cluster division between the bacterial profiles of ALS patients and the healthy subjects, mainly based on the presence/absence of bands in the profiles. For the yeast, the profiles was much more simpler with respect to that of Eubacteria and no unequivocal association with the presence or absence of disease could be evidenced. With a qPCR analysis we observed a lower DNA concentration in patients compared with controls, with a low abundance of *Clostridium* and yeast, and a high abundance of E. Coli and Enterobacteria. The analysis of the effects of bacterial concentrations and treatment on clinical assessment is currently in progress.

Conclusions

Our preliminary results confirm the hypothesis that microbiota is modified in ALS patients and it could contribute to the pathogenesis of the disease. Microbiota-based treatment approaches can represent a new therapeutic target.

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FUNCTIONAL SPAGHETTI SUPPLEMENTED WITH SUPERCRITICAL CO2 PUMPKIN OIL ENCAPSULATED IN α-CYCLODEXTRINS

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Dry pasta is a staple food of the traditional Italian cuisine now renowned worldwide. It is conventionally made with refined durum wheat semolina resulting, thus, deprived of most bioactives occurring in the embryo and in the outer layers of the kernel. Nevertheless, coming widely and frequently in our diet, pasta is perfect to deliver nutraceuticals and, as such, has been the object of several supplementation strategies to improve its health promoting value. Fruits and vegetables, including agri-food industry products and by-products, are valuable sources of bio-functional ingredients suitable for recovery and incorporation into pasta. To obtain high quality active compounds with enhanced bioaccessibility and bioavailability, the selection of an appropriate extraction techniques is crucial. Supercritical carbon dioxide (SC-CO2) is a green, sustainable system to efficiently extract solvent free, food grade nutraceuticals from a range of edible plant materials. Despite that, only few reports concerning pasta supplemetation with SC-CO2 extracts have been published till now [1]. Recently, we have described a procedure to obtain an antioxidant enriched oil from a dehydrated powdery matrix constituted by a mixture [1:1 by dry weight (dw)] of pumpkin (Cucurbita moschata Duch., cultivar Lunga di Napoli) pericarp (esocarp plus mesocarp) and seeds using SC-CO2. The oil resulted an interesting source of carotenoids, tocochromanols and unsaturated fatty acids [2,3]. Most of these lipophilic compounds are susceptible to oxidation and very reactive to light, oxygen, free radicals and/or high temperatures, limiting the use of the oil in the formulation of functional products due to low stability over time and/or during food processing.
α-Cyclodextrins (α-CDs) are biocompatible cyclic oligosaccharides, obtained by enzymatic conversion of starch, able to form inclusion complexes with lipophilic molecules (Fig. 1). They have been approved by many international food authorities as soluble dietary fibers, prebiotics and novel food ingredients. Encapsulation of SC-CO2 extracted pumpkin oil in α-CDs in a ratio 1:2 by weight has resulted in a protective effect on the main bioactive compounds over 6 months storage, indicating the potential application of the oil/α-CD complexes to pasta supplementation [4].
The aim of this study was to explore the feasibility of producing enriched pasta by adding the SC-CO2 extracted pumpkin oil in free form or encapsulated into α-CDs. Specifically, semolina from the durum wheat cultivar Vertola was used to produce four types of pasta: (i) control spaghetti (S-CTRL); (ii) spaghetti supplemented with 2.66% α-CDs (S-α-CD); (iii) spaghetti supplemented with 1.33% SC-CO2 extracted pumpkin oil (S-Oil) and (iv) spaghetti supplemented with 3.99% pumpkin oil/α-CD inclusion complexes (S-Oil/α-CD) (Fig. 2). Raw pasta was assayed for total carotenoids, total tocochromanols, fatty acid composition, total dietary fibers and resistant starch according to validated procedures [5, 6, 7, 8]. Textural quality was also evaluated, through sensory parameters by a trained panel of three experts as described by D'Egidio et al. [9].
The chemical composition of control and supplemented raw pasta is reported in table 1. As expected, no differences were evidenced between S-CTRL and S-α-CD samples with respect to lipophilic components, while S-Oil and S-Oil/α-CD spaghetti were significantly ($p<0.05$) enriched in total carotenoids, total tocochromanols, polyunsaturated (PUFA) and monounsaturated (MUFA) fatty acids compared to S-CTRL. In particular, total carotenoids and total tocochromanols were increased more than 8.2- and 25.4-fold, respectively. Statistically significant differences in total carotenoid amounts were found between S-Oil (2.06□0.07 mg/100g dw) and S-Oil/α-CD (2.34□0.16 mg/100g dw) samples, suggesting that α-CD complexation improves carotenoid stability during the phases (mixing, extrusion and drying) of pasta production. On the basis of the "Scientific opinion on dietary reference values for vitamin A and E" [10,11], 100 g of uncooked S-Oil and S-Oil/α-CD provide, respectively, 12 and 14% of the recommended daily allowance (RDA) for vitamin A (700 □g/die) and about 2% of RDA for vitamin E (15 mg/die) (Table 1). Furthermore, α-CD containing spaghetti showed higher percentages of total dietary fibers and resistant starch compared to S-CTRL; these differences resulted both statistically significant in the S-α-CD sample (+23% and +61%, respectively), while significance was restricted to resistant starch only in the S-Oil/α-CD sample (+56%) (Table 1).
With regard to the sensory evaluation of the textural properties of cooked pasta, all samples have been judged good for firmness (resistance to chewing by the teeth), while S-Oil and S-Oil/α-CD spaghetti were less performing than S-CTRL and S-α-CD in terms of stickiness (amount of material adhering to the pasta surface) and bulkiness (adhesion of pasta strands to each other). Anyway, a preference of S-Oil/α-CD over S-Oil pasta has been expressed by the panelists.
In conclusion, supplementation of semolina with SC-CO2 extracted pumpkin oil/α-CD complexes allows the preparation of quality spaghetti improved in terms of dietary fiber, resistant starch and lipophilic bioactives' contents, providing a substantial proportion of the RDA for E and A vitamins. It also increases the stability of some of the active components during pasta production and ameliorates the textural parameters of cooked spaghetti compared to those supplemented with free oil. Therefore, it is likely that S-Oil/α-CD spaghetti are readily accepted by consumers if their potential role in ameliorating human health and wellbeing is appropriately communicated.

Fig. 1. α-Cyclodextrins structure (a) and schematic representation of a simple inclusion complex (b).

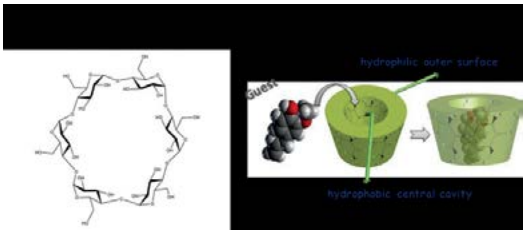

Fig. 2. Macroscopic appearance of control (S-CTRL) and supplemented raw pasta: spaghetti containing α-CDs (S-α-CD), spaghetti containing pumpkin oil (S-Oil) and spaghetti containing pumpkin oil/α-CD complexes (S-Oil/α-CD).

Table 1. Chemical composition of control (S-CTRL) and supplemented raw pasta: spaghetti containing α-CDs (S-α-CD), spaghetti containing pumpkin oil (S-Oil) and spaghetti containing pumpkin oil/α-CD complexes (S-Oil/α-CD). Values are expressed as mg/100 g dw or as relative percentage (%) and represent the mean ± standard deviation of three independent replicates (n = 3). Data were submitted to one-way analysis of variance (ANOVA), differences among groups were detected using multiple comparison procedures (Tukey post hoc test, □ < 0.05), different letters denote significant differences among samples.

	PASTA			
	S-CTRL	**S-α-CD**	**S-Oil**	**S-Oil/α-CD**
Total Carotenoids (mg/100g dw)	0.21±0.02^{a}	0.29±0.08^{a}	2.06±0.07b (%RDA VitA=12)	2.34±0.16^{c} (%RDA VitA=14)
Total Tocochromanols (mg/100g dw)	0.08±0.01^{a} (%RDA VitE=0.5)	0.14±0.03^{a} (%RDA VitE=0.9)	3.12±0.28b (%RDA VitE=2)	3.43±0.25^{b} (%RDA VitE=2)
Fatty acids composition (%)			64.77±0.90^{a}	
SFA	64.50±1.33^{a}	64.77±0.90^{a}	5.31±0.14^{a}	43.58±3.87^{b}
MUFA	5.38±0.98^{a}	5.31±0.14^{a}	29.92±0.54^{a}	13.85±2.00^{b}
PUFA	30.18±1.05^{a}	29.92±0.54^{a}	3.86±0.18^{b}	43.32±4.10^{b}
Total dietary fiber (%)	3.13±0.01^{a}	3.86±0.18^{b}	0.58±0.04^{b}	3.48±0.40^{a}
Resistant starch (%)	0.36±0.03^{a}	0.58±0.04^{b}	0.41±0.01^{a}	0.56±0.01^{b}

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MEDITERRANEAN DIET AND MICROBIOME

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Objective

Food sources have played a major role in guiding the evolution of *Homo sapiens*. The role of diet in influencing the composition of the gut microbiota is widely recognized (Albenberg and Wu, 2014; Power et al., 2014). However, until recently, not many studies have broadly and systematically considered the association between long-term dietary habits and gut microbiota (Wu et al., 2013; Wu et al., 2014). Access to foods is the first factor affecting the gut microbiota as it can vary according to how differently some populations eat (De Filippo et al., 2010). Specific patterns of the gut microbiota have been also linked to diet, clearly correlating a specific composition of the microbiota with animal fat and protein-based versus vegetable-based diets (Wu et al., 2011). In addition, several studies have shown an association between consumption of fiber and increased microbial richness (Claesson et al., 2012; Cotillard et al., 2013; Le Chatelier et al., 2013) and others have shown how a sudden dietary change induces changes in microbiota composition (David et al., 2014). Intestinal microbiome can be considered a useful biomarker of long-term consumption of healthy and unhealthy diets (Albenberg and Wu, 2014). Therefore, it is important to pose the question on whether and to what extent long-term dietary choices can impact on the composition of the microbiota and how this can influence the production of beneficial microbial metabolites. To investigate the potential effects of the Mediterranean diet (MD), in a cross-sectional survey we assessed the gut microbiota and metabolome in a cohort of healthy Italian subjects in relation to their habitual diets. In addition, an intervention study is on-going aiming to evaluate the effect of the consumption of a Mediterranean diet in subjects at cardiovascular risk and with unhealthy dietary and lifestyle.

Methods

Daily dietary information and gut microbiota and metabolome were assessed in a population of 153 healthy subjects following either omnivore, vegetarian or vegan diet and with different MD adherence rates. The microbial diversity was assessed by 16S rRNA gene sequencing, while metagenomes were retrieved for a selection of subjects within our cohort.

Results

Subjects could be stratified according to diet and microbiota. In addition, the consumption of fruit vegetables and legumes in individuals with the highest adherence to MD was clearly linked to higher levels of fecal short chain fatty acids, this happening even considering the omnivore population alone. The fiber consumption was also significantly associated to higher levels of fibre-degrading Firmicutes and *Prevotella* (De Filippis et al., 2016a). On the other hand, higher urinary trimethylamine oxide levels were detected in individuals with lower adherence to the MD. Furthermore, we used oligotyping of 16S rRNA gene sequencing data to exploit the diversity within *Prevotella* and *Bacteroides* genera and found considerable sub-genus diversity. We found that some *Prevotella* oligotypes are significantly associated with the plant-based diet but some are associated with animal-based nutrients, and the same applies to *Bacteroides*. Dissecting shotgun metagenomes from the same subjects we also investigated the possible presence of different P. copri strains and their functional potential, in order to further explore the presence of a diet-induced selection. High diversity within P. copri was highlighted: strains present in omnivore subjects were clearly different compared to those in vegetarians/vegans.

Conclusions

High-level consumption of plant foodstuffs consistent with a MD is associated with beneficial microbiome-related metabolomic profiles in healthy subjects. The study of sub-genus and strain level diversity highlight a current oversimplification of diet-dependent microbe-host associations and suggest that strain-level differences may occur following diet-dependent selection of the microbiota and this can have fundamental functional consequences correlated with possible health benefits.

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VALORIZATION OF THE NEGLECTED PRUNUS MAHALEB L., FRUITS AS A SOURCE OF FUNCTIONAL MOLECULES

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Abstract: Prunus mahaleb L. is a tree producing dark-red small stone fruits, not used for fresh consumption due to their astringent and sour taste. In this study a "mahaleb fruit concentrated extract" (*mfce*) was obtained and chemically characterized. The extract showed high anthocyanin, flavonol and coumarin content. *Mfce* was assayed for its biological activities and showed strong antioxidant capacity and anti-proliferative, pro-apoptotic, anti-inflammatory and anti-mutagenic effects when tested in vitro. This study demonstrated several healthful effects of *mfce* that may be of interest as natural food colorant and as source of molecules for the formulation of functional foods.

Key words: *Prunus mahaleb L.*, anthocyanins, antioxidant activity, pro-apoptotic effect, anti-inflammatory effect, anti-mutagenic property

Prunus mahaleb L. (*P. mahaleb*) is a deciduous tree native of Mediterranean region, Iran and Central Asia. The tree prefers warm and dry climate, well-drained soil and it is robust and resistant to several diseases. For these characteristics, mahaleb cherry tree is used as rootstock in grafting sweet cherry, especially in Italy and above all in Apulia Region. *P. mahaleb* produces highly pigmented small stone fruits that have astringent and sour taste, therefore not suitable for human consumption. In our previous studies[1] and in few others works[2,3], these fruits had been characterized revealing a high content in anthocyanins. Anthocyanins are flavonoids responsible for the bright red, blue and purple colors of several plant products4. Besides their potential in the food industry as natural dyes and nutraceutical component with added value, the consumption of fruit and vegetable containing anthocyanins, in combination with other flavonoids and ascorbic acid, has shown protection against several chronic and degenerative diseases[5-7]. In order to reevaluate *P. mahaleb* fruits as source of natural colorant and functional molecules for food formulations, a concentrate extract was obtained using only food grade solvents; successively the extract was chemically characterized and its biological activities were *in vitro* evaluated[8,9].

Mahaleb fruit concentrated extract (*mfce*): chemical characterization

A concentrated extract was obtained from *P. mahaleb* fruits using food-grade ethanol acidified with 1% citric acid 1M. After extraction, the majority of the solvent was evaporated to obtain a very concentrated extract with a high content of soluble solids (60 ± 1.47 °Brix), a pH value of 5.31 ± 0.21 and a density equal to 1.26 ± 0.03 Kg/L. Using this protocol, we obtained a product with high anthocyanin content and a good potential for industrial use because it is easily transportable and preservable. *Mfce* was chemically characterized by HPLC analysis (Tab. 1). Anthocyanins are the main polyphenol compounds in *mfce* and their concentration is higher than in red fruit juice concentrates (elderberry, blackcurrants, chokeberry, etc.)10. The HPLC identification and quantification of the individual anthocyanins in *mfce* shows that cyanindin 3-rutinoside is the most abundant (Tab.1). HPLC analysis of *mfce* indicates a rutin and quercetin-3-glucoside content higher than previously reported10 for blackcurrant juice concentrate. Moreover coumarin content in *mfce* is very high (1.089 ± 0.034 mg/g of lyophilized *mfce*) and because of its strong flavor and hepatotoxic activity in laboratory animals11, its quantification should be taken into account when suggesting *mfce* as a colorant or additive in food.

Healthful biological effects of *mfce*

It is known from literature that anthocyanins (in particular cyanidin) are molecules stimulating anti-inflammatory, antioxidant and antiproliferative activities, then they are implicated in protection against chronic and degenerative disease as cancer, atherosclerosis and diabetes7,12. With the aim of investigating the potential healthful biological effects of *mfce*, the extract was assayed in vitro for: 1) antioxidant activity; 2) anti-proliferative, gap junction intercellular communication (GJIC) modulation and pro-apoptotic effect in a breast cancer cell line (MCF-7); 3) anti-inflammatory properties in human vascular endothelial cells (HUVEC); 4) anti-mutagenic effect on yeast Saccharomyces cerevisiae strain D7. To test the antioxidant activity of *mfce*, three in vitro chemical assays were used: oxygen radical absorbance capacity (ORAC), trolox equivalent antioxidant capacity (TEAC) and Folin-Ciocalteu (F-C) assay. Table 1 show that mcfe has a strong antioxidant activity higher than the values reported for commercial fruit juice concentrate10,13. *Mfce* showed a dose-dependent antiproliferative effect with a significant decrease of cell viability in cells treated with 18.4 mg of extract/ml of culture medium. The same active concentration of *mfce* induced statistically significant time-dependent increase in GJIC in MCF-7 cells and consequently a pro-apoptotic effect of mcfe was demonstrated (Fig. 1). Anti-inflammatory activity was demonstrated when HUVEC were first treated with various concentration of *mfce* for 1 h and then stimulated with inflammatory trigger lipopolysaccharide (LPS) for 4-16 h. The results demonstrated that endothelial leukocyte adhesion molecules (VCAM, ICAM-1 and E-selectin) where induced by stimulation with LPS, but *mfce* reduced their expression in a concentration-dependent manner even at 1 mg of extract/ml of culture medium. These anti-inflammatory in vitro effects may be related to the strong free-radical scavenging activity and to the occurrence of bioactive phenols of *mfce*. In the end, the antimutagenic effects of *mfce* on S. cerevisiae cell cultures treated with 13 mM H2O2 were measured as mitotic gene conversion (GC) and reverse point mutation (PM). As shown in Fig. 2, treatment with H2O2 induced high level of GC, while in cell cultures pre-treated with *mfce*, GC values were significantly decreased. Similar results were obtained for PM values. It is known that H2O2 produces hydroxyl radicals14, and because *mfce* shows high antioxidant capacity, its anti-mutagenic activity could be due to scavenging activity against ROS. To demonstrate the effect of *mfce* on ROS concentration, intracellular redox state was measured using dichlorofluorescein-diacetate. This probe, once inside the cell, attacked by ROS, produces a fluorescent compound. The results of this test show that yeast cells have a basal level of metabolic oxidation that increased two fold when the cultures are treated with H_2O_2, whereas *mfce* pre-treatment induced a significant decrease in intracellular oxidation.

Conclusion

This study suggests that mahaleb fruits, not used for fresh consumption, may be processed to obtain a concentrated fruit extract with higher an

thocyanin content than other red berry fruit concentrate extracts already industrially used. Moreover *mfce* mediates several bioactivites (antioxidant, anti-inflammatory, antiproliferative, pro-apoptotic and antimutagenic) when tested *in vitro*. Further studies on the stability of *mfce* and animal and human trial should be designed to assess its potential application as food colorant and functional food.

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Table 1: Chemical characterization and antioxidant activity of mfce. (Reworked from Gerardi et al. 2015, and Gerardi et al. 2016)

Sugars	**mg/g* ± SD**	**Organic acids**	**mg/g* ± SD**	**Anthocyanins**	**mg/g* ± SD**	**Flavonols**	**mg/g* ± SD**	**Antioxidant activity**	
Glucose	359 ± 54	Tartaric acid	193 ± 18	Cy 3-glucoside	5.224 ± 0.101	Quercetin	0.067 ± 0.002	ORAC (mmolTE/ml ± SD)	0.450 ± 0.018
Fructose	205 ± 27	Malic acid	81 ± 7	Cy 3-rutinoside	9.104 ± 0.101	Quercetin 3-O-glucoside	0.933 ± 0.010		
Sorbitol	175 ± 35	Succinic acid	5 ± 0.5	Cy 3-xylosyl glucoside	2.924 ± 0.100			TEAC (mmolTE/ml ± SD)	0.446 ± 0.030
		Citric acid	158 ± 14			Rutin	0.691 ± 0.011		
		Ascorbic acid	0.094 ± 0.007	Cy 3-xylosyl rutinoside	5.152 ± 0.059			FC reducing capacity (mg GAE/ml ± SD)	22.734 ± 0.253

SD: Standard deviation (n=4); Cy: Cyanidin; TE: Trolox equivalent; GAE: Gallic acid equivalent; *: g of lyophilized *mfce*

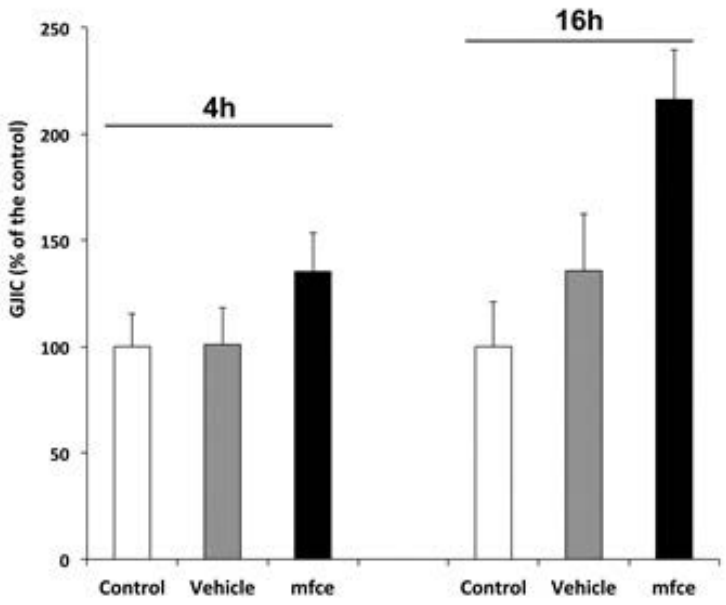

Figure 1. Effect of mfce on gap junction intercellular communications (GJIC) in MCF-7 treated for 4 h and 16 h. GJIC was calculated as percentage of the untreated control. Control, medium only; Vehicle, treated with vehicle only; mfce, treated with 18.4 mg/mL of P. mahaleb extract. The results are expressed as mean ± SD of three independent experiments, ** p<0.03. (From Gerardi et al., 2016).

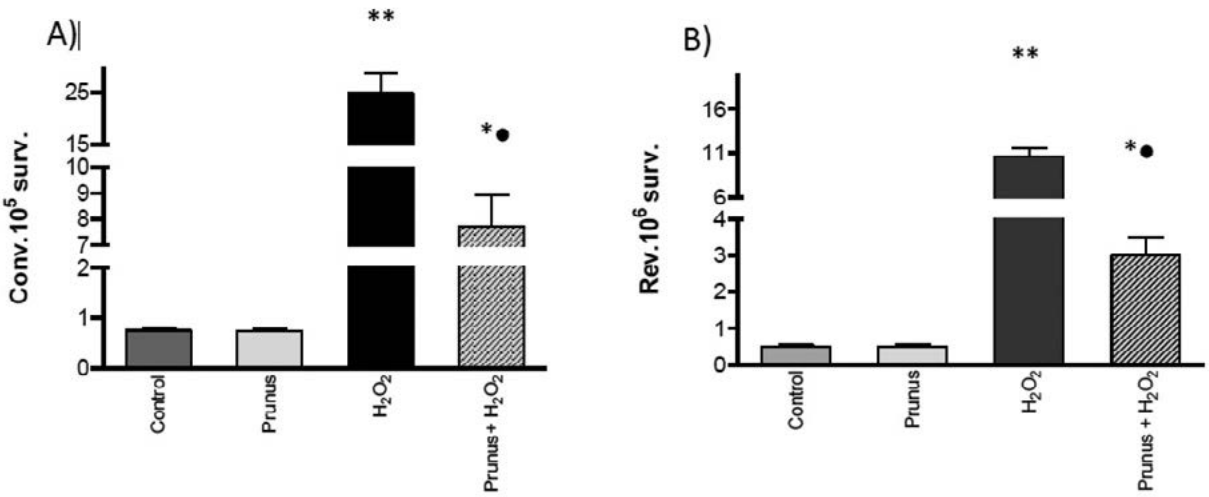

Figure 2: Effects of mfce (1 mg/mL) on: A) mitotic gene conversion and B) point reverse mutation in the D7 strain of S. cerevisiae. The results are derived from triplicate determinations and presented as means ± SD * significantly different from control and from mfce, with * p ≤ 0.01, ** p ≤ 0.001.● significantly different from H2O2 with p ≤ 0.001, according to one-way analysis of variance and Bonferroni post-test. (Reworked from Gerardi et al., 2016).

ARTICHOKE AND GINGER PROMOTE DIGESTIVE FUNCTION AND GASTROINTESTINAL MOTILITY

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Ginger (*Zingiber officinale*) and artichoke (Cynara cardunculus) are plants that have been cultivated and used for health purposes since ancient times. These plants have a substantial body of scientific data in support of their digestion-enhancing activities [1].

Ginger has long been used in Chinese and Indian traditional medicine to treat gastrointestinal disorders such as indigestion, flatulence, fever, nausea and vomiting and is also used for the treatment of nausea in pregnant women or after surgical interventions, as well as in the prevention and treatment of chemotherapy-induced nausea and vomiting. [2-6]

It has been suggested that the extract of Cynara cardunculus (ALE) can complement the effects of ginger, since the former is active on small bowel while the latter on stomach [7].

ALE increase bile flow and exert hepatoprotective [8], serum cholesterol lowering [9], and antioxidant and antispasmodic effects [10–12]. The bitter compounds of ALE and particularly cynaropicrin are responsible for the digestive beneficial effects [13, 14]. Holtmann et al. 7 showed that ALE were significantly better than placebo in reducing symptoms and improving the disease-specific quality of life in patients with functional dyspepsia.

Recently, two interesting clinical studies have been completed in order to verify the digestion-enhancing activities of the combination of ginger and artichoke extracts (namely PRODIGEST®).

The first clinical study, conducted with a randomized, cross-over design, shows that, the product significantly promotes gastric emptying in healthy volunteers after the consumption of a standardized meal without being associated with notable adverse effects [15]. Impaired gastric emptying is a well-recognized contributory factor to the pathophysiology of gastrointestinal conditions like functional dyspepsia [16] and nausea [17] andthe use of nutritional supplementation for the treatment of these conditions has been recently underlined [18]. However, the use of well-standardized, well-characterized and highly reproducible botanical extracts is recommended in order to obtain reliable clinical applications.[19-22]

The second study showed that the supplementation with ginger and artichoke extracts is efficacious in the short-term treatment of functional dyspepsia (FD) [23].

This effect appears to be statistically significant when compared to placebo. It is interesting to note that the efficacy appears quickly, that is, within 14 days, and afterwards it is maintained until the 28th day of intervention. Infact the results show the advantage of the supplementation, as compared to placebo, with a significant amelioration of 0.85 units on the MCA severity scale (of range 0–3) at 14 days. This result, adjusted for baseline symptoms and typologies of dyspepsia, persists until the end of the study (28th day). The percentage difference between the supplementation and the placebo groups approached 34%. This therapeutic gain is greater than what has been observed in previous studies with antisecretory and prokinetic drugs 24, as well as with artichoke extracts. [7]

Notheworthy, the supplemented group shows treatment efficacy in 86.2% of cases after 28 days of supplementation, with marked reduction of dyspepsia intensity (grades 2 and 3 of the considered scale) in 63.1% of the treated cases.Only 52.5% of subjects in the control group shows a positive effect and only 24.6% of the placebo treated subjects has a marked reduction of symptoms (grades 2 and 3).

Therefore PRODIGEST® is a good example on how to redesign well known extracts such as cynara and ginger to develop a product endowed with exhaustive clinical data supporting in parallel the functionality (gastric emptying) and the effectiveness (relief of discomforts).

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MOLECULAR MODELS OF PEPTIDES WITH NUTRACEUTICAL PROPERTIES

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Introduction

In the last decades, a great deal of research has been devoted to the study of natural compounds contained in plant cells that can both prevent the onset and development of several pathologies and delay cellular aging. The beneficial effects of fruits and vegetables are very likely due to many of their components such as vitamins, polyphenols, isotyocianates, etc. However a fundamental biological role can be played by peptides, small protein molecules.
The use of peptides in nutraceutical field could be hampered by their low bioavailability due to several reasons : a-digestion by proteolytic enzymes, b-quick elimination by the kidney, c-difficulty to penetrate the cell membranes.
Molecular models of peptides suitable to carry out nutraceutical activities are investigated.

Methods that can be utilized to elucidate the peptide structure

The peptides are extracted from different sources by water or water/ethanol. The extracts are fractionated by HPLC. Each fraction is then lyophilized, solubilized in methanol, and injected into ESI–MS using the flow injection analysis (FIA) mode. The ions shown by MS spectra of HPLC fractions are subjected to MS/MS analysis, and the results were scanned to cross over ions corresponding to peptide molecules. Examples of MS parameters are capillary temperature 220 °C, capillary voltage 10 V, spray voltage 4KV, collision energy from 17 to 40 keV.
Automatic Determination of Amino Acid Sequences Compatible with the Given Mass Spectral Data. For what concerns the study of peptide structure, the mass spectra analysis may be performed with a recently reported automatic combinatorial method that carries out the computation of all amino acid sequences compatible with a given molecular ion[1]. The possible sequences of these compounds are automatically obtained by considering fragment ion masses that are potential breakdown products. This result was obtained by developing a mathematical model of the problem and by searching for all possible sequences of given components satisfying certain constraints. The analysis does not rely on known protein database but on the computation of all amino acid sequences satisfying given mass spectral data. In some cases the information contained in the spectrum is sufficient to determine a unique sequence. Sometimes the spectrum does not contain enough information for an unequivocal determination of the sequence. In the latter case, all possible sequences that fit the spectrum are listed.

Susceptibility of peptides to digestion by protease

The affinity of the peptides to protease binding may be depending from amino acid sequences and chemical modifications of N- and/or C- terminal and/or amino acid lateral chains.
For example it has been reported that synthetic epidermal mitosis inhibiting peptapeptide (pyroGlu-Glu-Asp-Ser-Gly) is phosphorylated in vitro at serine level by protein kinase NII isolated from calf thymus chromatin. A serum enzyme, which rapidly cleaves the synthetic epidermal mitosis inhibiting pentapeptide, also hydrolyses the synthetic transcription inhibiting pentapeptide (pyroGlu-Ala-Glu-Ser-Asn). The phosphorylated forms of both pentapeptides are protected from the serum enzyme activity[2].

Excretion of peptides by kidney function

Low molecular weight peptides may be rapidly eliminated by kidney function. Natural peptides typically have poor absorption, distribution, metabolism, and excretion (ADME) properties with rapid clearance, short half-life, low permeability, and sometimes low solubility. Strategies have been developed to improve peptide drugability through enhancing permeability, reducing proteolysis and renal clearance, and prolonging half-life[3].

Cell penetrating peptides

Three main mechanisms are involved in the peptide internalization by cells.
1- the peptide posses a signal sequence that can interact with cell membrane. For example the N-terminal sequence Arg-Gly-Asp of the peptide lunasin isolated from cereals and active against cancer cells proliferation. The activity of lunasin inside the cell is due to the C-terminal sequence constituted by 8 aspartic acid residues[4].
2- the peptide is constituted by a quite hydrophobic structure. The hydrophobic or hydrophilic degree of a domain inside a protein may be calculated by the Kyte-Doolittle5 or Hopp-Woods6 scales respectively.

In a short sequence the hydrophobic (or hydrophilic) pattern can be strongly influenced by the charged N- and//or C-terminal.
For example the peptide isolated from pepsin-hydrolysed soybean globulin that exerts in vitro an hypocholesterolemic effect[7]:
+NH3-Ile-Ala-Val-Pro-Gly-Glu-Val-Ala-COO-
(4.5/1.8/4.2/-1.6/-0,4/-3.5/4.2/1.8) numbers in parenthesis represents the Kyte-Doolittle values of the amino acids above reported.

The positive hydrophobic values are prevalent but the presence of two charged terminal groups certainly affects the behavior of the peptide molecule. Interestingly several active peptides with blocked N- and/or C- terminals are reported[8,9].
Mitosis inhibitory peptides

Epidermal peptide	pyroGlu-Glu-Asp-Ser-Gly-OH
Liver inhibitor	pyroGlu-Gln-Ser-Gly-Asp-NH2
Melanocyte inhibitor	pyroGlu-Phe-Gly(NH2)
T-lymfocyte inhibitor	AcGlu-Ser-Gly(NH2)
Thymus deprimerone	pyroGlu-Ala-Glu-Ser-Asn

3- Binding of peptides with carriers such as phospholipids The isolation from wheat sprouts of peptides complexed with phospholipids has been described10. Total hydroalcoholic extract of wheat sprouts is treated with 90% cold acetone as a preliminary step direct to separate bioactive peptides from polyphenols. The addition of acetone causes the formation of a yellow buoyant gelatinous drop that prevailingly contains peptides and phospholipids.

Two molecular peptide-phospholipids complexes have been characterized[10].

PC(phosphatidylcholine) + NH2-Gly-His-Phe-OH	m/z 1117.87
LPC(lysophosphatidylcholine) + AcAsp-Ser-Val-Cys-Val-Asn-NH2	m/z 1196.00

In conclusion, the hypothesis that complexes of phospholipids with other molecules are present in fractions from wheat sprouts extract is supported by several direct evidences. The possible hypothesis that these complexes are native and not produced during the extraction procedure is more speculative; however, it is interestingly in agreement with the progressive loss of bioavailability of some active molecules following the steps of purification. This has been observed by many authors in extracts not only from vegetables but also from animal cells.
For this reason, the problem discussed in this article may represent one of the causes according to which many promising molecules active 'in vitro' on important molecular mechanisms (replication, transcription, translation, etc.) resulted almost completely inactive in cellular and animal tests. Of course, the peptides activity "in vivo" may be mediated also by specific mechanisms such as cell recognition through molecular receptor.

Conclusions

There is growing evidence that peptides isolated from vegetal and animal cells or produced by enzymatic digestion of proteins demonstrate nutraceutical properties. In particular peptides derived from lactoferrin, casein, cereals and animal food products have different biological effects on human health, such as antiproliferative, antimicrobial properties, blood pressure-lowering (ACE inhibitory) effects, cholesterol-lowering ability, antithrombotic and antioxidant activities, enhancement of mineral absorption/bioavailability, and opioid-like activities .

The nutraceutical properties of peptides may be quenched by several mechanisms such as rapid excretion, digestion by proteases, low ability to penetrate cell membranes. Strategies are being developed to improve peptide drugability through enhancing permeability, reducing proteolysis and renal clearance, and prolonging half-life3. Some peptide structural features are important, in particular chemical modifications of N- and C- terminals or of amino acids side groups may improve some parameters of peptide bioavailability.

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"LACTOFERRIN NUTRACEUTICAL PEPTIDES"

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Lactoferrin (Lf) (formerly known as lactotransferrin) is an iron-binding glycoprotein, belonging to the transferrin protein family, together with serum transferrin (sTf), melanotransferrin and the inhibitor of carbonic anhydrase. Lf is present in mammalian milk and it is produced and released by mucosal epithelial cells and neutrophils in most of the mammalian species, including humans [1-3]. Lf main function is the unspecific protection of the host towards infections and other diseases, belonging to the so-called natural immunity [4]. In birds there is a Lf homologous protein which is Ovotransferrin (Otrf), present in large quantity in hen's white egg [5]. Lf and Otrf are 80 kDa glycosylated proteins of about 700 aminoacids with high homology among species. Their three dimensional structure consists of a single polypeptide chain folded into two symmetrical lobes (N and C lobes), which are highly homologous with one another (33–41% homology) [6-7].

1. Lactoferrin peptides

An increasing number of functions have been associated with Lf peptides produced by proteolytic enzymes, and their importance is rising since they could be naturally produced in the human intestinal lumen after ingestion of human or bovine milk. In particular, three peptides displayed remarkable biological activities. They are amphiphilic molecules originated from the N-lobe of Lf, and their antimicrobial activity has been ascribed to their hydrophobicity, cationic charge, and secondary structure (Fig.1).

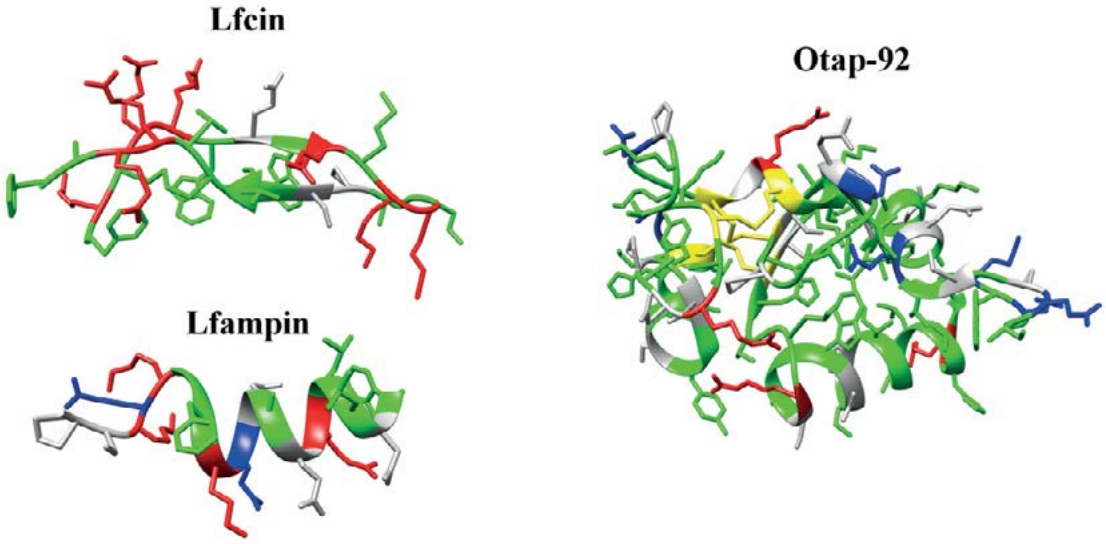

Figure 1: Structure of lactoferricin (Lfcin), lactoferrampin (Lfampin), and Otap-92. The colors of peptides indicate aminoacid properties: Green: hydrophobic; Blue: negatively charged; Red: positively charged; Gray: polar. In Otap-92 the disulphide bridges are shown in yellow The ribbon indicates the presence of secondary structure. The pictures were drawn by UCSF-Chimera package [8].

These three peptides were called Lf(1–11), Lfcin and lactoferrampin (Lfampin). In detail, Lf(1–11) is the oligopeptide that includes the first eleven aminoacidic residues of the Lf. The presence of the hydrophobic and hydrophilic residues lends to it a highly cationic nature. It has been demonstrated that Lf(1–11) interacts with the membrane of several bacteria. Lf(1–11) is active in vitro and in vivo against various bacteria and yeast although showing few synergistic effects with antibiotics. Such effective antimicrobial effect of LF1-11 was attributed to the first two arginines at the N- terminus of human lactoferrin [9]. Lfcin is an amphipathic, cationic peptide. Indeed it is the most studied anti-microbial peptide derived from milk proteins. It can be generated by the pepsin-mediated digestion of Lf (aminoacid residues 17–41). The peptide has an abundance of basic aminoacids including Lys and Arg, as well as hydrophobic residues like tryptophan and phenylalanine. Lfcin displays several biological activities including antiviral, antibacterial, antifungal and anti-inflammatory activities [10]. Lfampin comprises residues 268–284 in the N1 domain of Lf, and was found to be located in closed proximity to Lfcin. Lfampin exhibits broad antimicrobial action against several Gram-positive and Gram-negative bacteria, yeast and parasites [11]. Lf- derived peptides showed often a considerably higher antimicrobial activity than the native protein with broad antibacterial spectrum at lower concentration. However, their activities are not limited to the bacterial properties but several protective activities against infections have been found in Lf-derived peptides. These activities are summarized in Table I.

Table I: anti-infective activities of lactoferrin peptides. For further details and a complete list of references, see [9, 12].

Activity		Peptide
Antibacterial	Gram positive	Lf(1-11), Lfcin, Lfampin
	Gram negative	Lf(1-11), Lfcin, Lfampin
Antiviral		Lf(1-11), Lfcin, Lfampin
Antifungal		Lf(1-11), Lfcin, Lfampin
Antiparasitic		Lfcin, Lfampin

Together with the antimicrobial activity of Lf, other biological activities of Lf peptides have been identified. Among these, the most important one is the anticancer activities of Lfcin as shown in Table II.

Table II: anticancer activities of Lfcin derivative peptides. For further details and a complete list of references [12-13]

Cancer type	Mechanism of anticancer action
Breast	LFcinB-CLICK and the chimeras composed of hLF11 and LFcinB1 in breast cancer cell line (MDA-MB-231).
Colon	Lfcin causes arrest in the at S phase through downregulation of cyclin E1 in CaCO2 cells.
Gastric	Lfcin induces apoptosis human gastric cancer cell line AGS.
Head, neck, and oral	Pepsin-digested-lactoferrin peptides induce apoptosis via JNK/SAPK activation in squamous cell carcinoma cell line SAS.
Leukemia	LfcinB6 (RRWQWR) induces citoxicity via caspase-mediated and cathepsin B-mediated mechanism in T-leukemia cells. Lfcin kills T-leukemia cells by triggering the mitochondrial pathway of apoptosis and through the generation of reactive oxygen species. LF11-322 (PFWRIRIRR-NH2), peptide fragment derived from human lactoferricin, induces necrosis in leukemia cells (MEL and HL-60 leukemia cells). LFcinB-CLICK (FKCRRWQWRMKKLGAPSITCVRRAF) and the chimeras composed of hLF11 and LFcinB1 (GRRRRSVQWCA-P-RRW-QWR-NH2) in Leukaemia cells (Jurkat).
Lung	Lfcin inhibits VEGF expression and induces apoptosis on non-small cell lung cancer H460.
NCS	Lfcin inhibits tumor growth and induces apoptosis through activation of caspases in neuroblastoma cells and in vivo.

The mechanism(s) responsible for the anticancer activity of Lfcin is not known, althought bLFcin6 (a six amino acid peptide derived from bovine lactoferricin) is able: i) to be rapidly internalized into HeLa cells, ii) to form stable electrostatic complexes with siRNA and iii) to deliver siRNA into cells, resulting in significant knockout activity at both the mRNA and protein levels, similarly to that mediated by TAT [14]. However, several studies suggest that exogenous treatment with Lf and its derivatives can efficiently inhibit the growth of tumors and reduces susceptibility to cancer alone or combined with other therapeutic agents. Clinical trials involving the use of this protein in cancer therapy are ongoing and the relatively low cytotoxicity of Lf and its derivatives peptides as compared with known anticancer drugs, along with the lack of data about the mechanisms of action, is likely to encourage the clinical use of Lf in cancer treatment [15].

2. Ovotransferrin Peptides

Otrf is the Lf homologous protein present in avians and it can reach high concentration in hen's white egg. Fig. 1, panel B shows antibacterial peptides deriving from proteolytic digestion of Otrf.OTAP-92 is a peptide of 9.9 kDa, consisting of 92 aminoacidic residues (Leu109-Asp200) showing strong sequence similarity with defensins. The antibacterial action of OTAP-92 may be due to its relatively high alkalinity and to the cysteine array [16]. Otrf fragments: DQKDEYELL (hOtrf219-27) and KDLLFK (hOtrf269-301 and hOtrf633-638) are capable of blocking Marek's disease virus infection in chicken embryo fibroblasts (CEF), even though the infection blocking efficiency of the isolated peptides is lower than that of the intact protein [17-18]. Interestingly, from an evolutionary point of view, these two Otrf peptides share sequence homology with two protein fragments, derived from human and bovine lactoferrin, known to be effective against Herpes simplex Virus (HSV-1) [17]. Others Otrf derived peptides shows several biological activities reported in table III.

Table III. Physiological and pharmacological activities of Ovotransferrin's peptides. For further details and a complete list of references, see [19-22]

OTRF PEPTIDES	ACTIVITIES	MECHANISMS
Otrf peptide OTAP-92	ANTIMICROBIAL	Bacterial Membrane damage
Otrf peptides: 219–227; 269–301; 633–638.	ANTIVIRAL	Viral adsorption inhibition
Reduced autocleaved Otrf (rac-Otrf)	ANTICANCER	Apoptosis induction
	ANTIOXIDANT	Autocleaved Otrf aspreservative to prevent □-carotene discoloration
Otrf Peptides (IRW or IQW)	ANTINFLAMMATORY	AttenuateTNF-□-induced inflammatory responses
Otrf peptide (KVREGT)	ANTIHYPERTENSIVE	Angiotensin I-Converting Enzyme.
Otrf Peptides: DLLFKDSAIMLK FFSASCVPGATIE	ANTIOXIDANT	Catechin conjugation
Ohters Otrf peptides	ANTIOXIDANT	Synergistic antioxidant effects with vitamin C, epigallocatechin gallate (EGCG), and caffeic acid.

Conclusions

Lf is one of the most studied nutraceutical protein showing considerable anti-infective activities as well as noticeable potentiality in preventing the different stages of cancer including initiation, promotion, and progression. Most of these protective activities of Lf are present, sometimes to a large extent, in peptides deriving from the proteolytic digestion of the whole protein. It is worth noting that Lf peptides generated by proteolytic digestion of Lf from bovine milk in intestinal lumen may pass the intestinal barrier and possibly, they could have systemic effects. The same applies for Otrf and Otrf's peptides from hen's white egg, whose defensive properties may be of importance for human wellness.As a conclusion, the data here summarized strongly support the use of bovine milk and egg white (preferably raw or cooked at low temperature) and its derivatives as dietary additives in normal and pathological human conditions.

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FOCUS ON GUT MICROBIOTA AND PROBIOTICS IN INFLAMMATORY BOWEL DISEASE

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Introduction

Crohn's Disease (CD) and Ulcerative Colitis (UC), commonly known as Inflammatory Bowel Diseases (IBD), are characterized by chronic gastrointestinal inflammation. IBD etiology is not fully known, but several mechanisms are involved in IBD pathogenesis: genetic and environmental factors, dysregulation of the intestinal barrier function, and over-activation of the mucosal immune system (Danese and Fiocchi, 2006). A specific role of gut microbiota has been shown: dysbiosis is associated with IBD, with a reduction in symbiont species (Kaur et al. 2011), particularly in *Firmicutes* phila (Sokol et al. 2006). The prolonged exposure of gastrointestinal mucosa to microbial products has several consequences, e.g. impaired mucosal tolerance and antimicrobial immunity, increased intestinal permeability and abnormal responsiveness of T cells to microbial signals (Rapozo et al. World J Gastroenterol, 2017). According to the World Health Organization's (WHO) probiotics are "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2001).

Evidences for gut-microbiota restoration therapies in IBD

Several probiotics and/or gut-microbiota restoring intervention are now available for IBD patients.
Available data shows a potential role of antibiotics in inducing remission both in Crohn's disease and in ulcerative colitis, but further trials are needed to better evaluate the role of different molecules (Khan et al. 2011).
Fecal transplantation has also been evaluated in ulcerative colitis patients, with promising results (Moayyedi, 2016).
Several probiotics have been evaluated in IBD patients, due to heterogeneity of available data (Shen et al. 2014).
According to the European Crohn's and Colitis Organization (ECCO) guidelines, the use of some probiotic products can be considered appropriate (evidence level 1b or 2b): E. coli Nissle has comparable efficacy than mesalazine in maintaining remission in ulcerative colitis patients (Dignass et al. 2012). Moreover, a commercially available association of eight different bacterial strains has shown efficacy in preventing puochitis in UC patients after surgery (Van Assche et al. 2013).

Conclusions

Due to the underlying dysbiosis, IBD patient can potentially benefit from probiotic therapy. Several clinical trials have been conducted and different microbial strains have been studied and evidence exists for some of these products.
The heterogeneity of study designs, microbial strains tested and the lack of microbial endpoint in these studies do not consent a global efficacy evaluation. Only probiotics with well documented efficacy should be prescribed in IBD patients.

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PLANT PROBIOTICS FOR THE PRODUCTION OF HEALTHY FOODS

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The safe production of high-quality food is an important societal issue, boosted by many epidemiological studies reporting that consumption of *"functional foods"*, in particular fresh fruits and vegetables, plays a key role in the prevention of chronic diseases and in decreasing the risk of mortality from certain types of cancers and cardiovascular diseases. The protective action of plant-based food has been primarily attributed to secondary metabolites known as phytochemicals, that are dietary plant bioactive compounds beneficially modulating human metabolism. Phytochemicals may reduce or prevent oxidative damages, modulate detoxifying enzymes and hormone metabolism, stimulate the immune system, and show antibacterial and antiviral activity (Johnson, 2007). The most important phytochemicals are represented by polyphenols, produced by a wide range of plant species, glucosinolates, mainly occurring in cruciferous plants, and carotenoids (Duthie, 2000). The content and composition of phytochemicals may be modulated by a range of factors, including plant genotype, ripening stage, harvest season, industrial processing, soil quality and health, use of pesticides and chemical fertilizers, and agronomic management (conventional versus organic) (Giovannetti et al., 2013). For example, a comprehensive study, aimed at establishing an antioxidant capacity database in USA, provided different values for apple fruits, depending on the variety: the total antioxidant capacity per serving (138 g = 1 fruit) ranged from 3,578 to 5,381 in Fuji and Granny Smith varieties, respectively (Wo et al., 2004; Giovannetti, 2014). Another long-term work revealed that mean levels of quercetin and kaempferol were 79% and 97% higher in organically grown tomatoes, compared with those produced by conventional agronomic tecnhiques (Mitchell et al., 2007).
Recent studies showed that the quality and levels of phytochemicals may be affected also by plant probiotics, i.e. the beneficial soil microorganisms which establish mutualistic symbioses with the root systems of about 80 % of plants (arbuscular mycorrhizal fungi, AMF) and their associated bacteria (Giovannetti et al., 2013; Battini et al., 2016a, b). AMF absorb and translocate soil mineral nutrients - mainly P, N, S, K, Ca, Fe, Cu and Zn - to host roots, enhancing plant growth and biomass production, and increase crop tolerance of biotic and abiotic stresses (Smith and Read, 2008). In addition, they modify secondary metabolism, both in food and in medicinal plants, that increase their content in anthocyanins, carotenoids, phenolics, lycopene, as well as total antioxidant activity and antiradical power. Accordingly, AMF represent an efficient tool to enhance plant biosynthesis of phytochemicals with health-promoting activity (Giovannetti et al., 2013).
Interesting findings were reported for globe artichoke flower heads, which are a dietary source of bioactive polyphenols, in particular hydroxycinnamates and flavonoids, and of high-quality inulin: when inoculated with two different AMF probiotics, they increased their polyphenolic content and antioxidant activity, compared with control plants. The two AMF species showed differential activity, opening up the way to studies aimed at selecting the most efficient fungal symbionts (Ceccarelli et al., 2010). Another work reported that tomato fruits with higher contents of mineral nutrients and healthy compounds were obtained after mycorrhizal inoculation with the AMF species *Rhizoglomus irregulare*. The mycorrhizal symbiont enhanced the uptake of calcium (+15%), potassium (+11%), phosphorus (+60%) and zinc (+28%), compared with traditionally grown tomatoes, and the levels of lycopene (+18.5%), a carotenoid well known as a potent antioxidant, which is currently considered a promising pharmacological agent in cancer prevention on account of its antiproliferative effects and inhibitory action on the human estrogen receptors (Giovannetti et al., 2012). As the lipophilic fraction of the extracts of mycorrhizal tomatoes significantly increased their anti-oestrogenic power, the authors suggested that tomato fruits produced by mycorrhizal plants could antagonise *in vivo* the oestrogen-like activity possibly elicited by several environmental/industrial xenobiotics to which humans are exposed through the food chain.
AMF probiotics and their associated bacteria have been shown to enhance the production of the health-promoting phytochemicals anthocyanins and rosmarinic acid in the red-leaved basil cultivar Dark Opal grown under commercial growth conditions (Battini et al., 2016b). Genetic studies on the biochemical pathways leading to the production of rosmarinic acid, a bioactive compound with antioxidant and anti-inflammatory properties, showed that genes encoding key enzymes of such pathways were differentially expressed in sweet basil inoculated with probiotic AMF or bacteria (Battini et al., 2016a).
Further studies will focus on the selection of the best performing plant probiotics - AMF and their associated bacteria - able to enhance the biosynthesis of secondary metabolites with health-promoting activity, to be exploited as sustainable biotechnological tool for the production of safe and healthy plant foods.

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BACTEROIDES FRAGILIS

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The colonic microbiota is a complex and densely populated ecosystem, whose metabolic activity influences the nutritional status and health of the host through diverse mechanisms (4). The microbiota of adults is dominated by five phyla, more than 80% of the species belonging to just two of them, Firmicutes and Bacteroidetes. The genus *Bacteroides*, is an anaerobic, bile-resistant, non-spore forming gram negative rod that accounts for up to 20-50% of the total gut microbiota in humans. In the gut, these microorganisms generally maintain a complex and beneficial relationship with the host; however, when escape this location, they can act as significant clinical pathogens, displaying a high rate of associated mortality. The ability of *Bacteroides* to behave as beneficial or harmful microorganism is largely linked to its capacity to adapt and thrive in different environments. *Bacteroides* species have the ability to sense and adapt to nutrient availability, being among the nutritionally most versatile microorganisms in the human gut. These bacteria also have multiple cellular pump systems to expel outside and release from the cell toxic compounds, and they can interact with the host immune system, being by this way able to control other competing microorganisms (12).
Bacteroides fragilis is the most frequent species of the genus at the mucosal surface, although it accounts for only 0.5% of the human colonic microbiota. It contributes to the development and maturation of the host immune system. Recent research also demonstrated the amelioration of autism spectrum disorder in mice treated with *B. fragilis* as probiotic (6). In contrast, *B. fragilis* is also the most frequently isolated anaerobic pathogen and it is abundant in systemic infections, whereby it is considered as an opportunistic pathogen. Paradoxically, and for the reasons indicated above, it is also regarded as a beneficial microorganism, being included in the group of potential new generation probiotics.
Short chain fatty acids (SCFA: acetate, propionate, and butyrate) and organic acids (lactate, formate, succinate) are formed as catabolic end-products from the microbial fermentation in the colon of undigested dietary carbohydrates (generally polysaccharides), and to a lesser proportion of proteins and amino acids that escape digestion. SCFA play important roles in the host; they strongly influence the physiology in the colon, they serve as energy sources to host cells and to the intestinal microbiota, and strongly influence host metabolism through diverse signaling mechanisms (9). In particular, propionate acts by controlling hepatic gluconeogenesis and inhibits competitively the lipogenesis and cholesterogenesis promoted by acetate, contributing to the reduction of hepatic and visceral fat (2, 3). Both, propionate and butyrate inhibit lipolysis and lipogenesis and increase the insulin-mediated glucose uptake in the adipose tissue (5).
Plasma circulating amino acids in the host seem to contribute to glucose homeostasis, related with the stimulation of insulin and glucagon secretion; branched chain and aromatic amino acids have been related with increased risk of diabetes and insulin resistance, and it has been suggested that the altered functionality of the intestinal microbiota promotes the higher levels of these types of amino acids found in diabetes and insulin resistance (7).
Bacteroides is the main propionate producer in the human colon, the levels of this microorganism being correlated with fecal concentration of propionate (1). The succinate/propionate pathway is the only metabolic way of propionate production from hexoses in this microorganism. It is known that the amount and proportion of SCFA and organic acids formed in cultures of *Bacteroides* (acetate, succinate, lactate, and propionate) is variable and the production of propionate is generally favored at long generation times, with complex carbohydrates, and under carbon source limitation.
Therefore, we have studied the metabolism of *B. fragilis* growing in media with different combinations of carbohydrates and nitrogen sources. Catabolic end-products formed in a medium containing organic nitrogen sources (peptone and yeast extract) was higher than in medium with an inorganic nitrogen source (ammonium sulfate) (8, 10). Acetate accounted for 30-54% of the total products formed in any condition, constituting an important way for obtaining energy by this bacterium. Notably, an inverse correlation was found between the production of propionate/succinate and lactate. Thus, lactate formation was favored in the absence of organic nitrogen sources and when rapid fermentable carbohydrates were present. Conversely, propionate formation preferentially occurred in the presence of organic nitrogen sources and complex carbohydrates (8, 10). These metabolic changes occurring in *B. fragilis* probably reflect a preferential use of the glucolytic pathway and acetate formation for obtaining energy (and keeping redox balance) in the presence of rapidly fermentable carbohydrates. However, in the presence of complex/slowly fermentable carbohydrates and when amino acids are available, the carbon skeletons of these last compounds could enter the catabolic route at the level of pyruvate; in such conditions the propionate/succinate pathway seems to be potentiated, serving as a way for energy obtaining whilst helping the cell to restore the redox balance (8).
Our proteomics and gene expression analyses support the hypothesis of the activation of amino acids catabolism and enhancement of the succinate/propionate pathway in *B. fragilis* grown in the presence of amino acids when only complex and slowly fermentable carbohydrates are present (8). Therefore, the preferential metabolic route for energy production and redox maintaining, and the final metabolic products formed by *B. fragilis*, may be largely dependent on carbohydrates and nitrogen sources available for this microorganism. These results strongly suggest the possibility of regulating the metabolism of *Bacteroides* by controlling dietary carbohydrate/protein balance. Moreover, when we analyzed the amino acids present in cultures of *B. fragilis* added with different carbohydrates, we found a decrease in the concentration of leucine, isoleucine and phenylalanine after incubation in any condition, whereas valine and tyrosine showed much less increases in a medium with complex carbohydrates as compared to glucose (8). This point to a potential capacity of *B. fragilis* for regulating branched chain and aromatic amino acids in its growth environment.
Considering the environmental conditions in the human large intestine, dietary fiber fermentation promotes a slight decrease of the luminal pH whereas high protein/amino acids fermentation favor a slight increase of pH. On the other hand, the acidic pH that generally occurs in the caecum under enough organic nitrogen conditions seems to favor butyrate producing species, whereas advancing through the colon to the rectum the pH increases, then favoring *Bacteroides* growth. Then, we hypothesize that the combination of dietary organic nitrogen sources with appropriate carbohydrates may be used to modify the metabolic activity of colonic *Bacteroides* populations by modulating the profile of organic acids formed and enhancing propionate formation in some parts of the large intestine while promoting changes towards healthier profiles of serum amino acids in the host (11).
The potential role that the functional control and metabolic reprogramming of *Bacteroides* through diet may play in the regulation of the host metabolism deserves more attention. It is essential to decipher: 1) the extent to what proteins and carbohydrates could affect the genus *Bacteroides* and other relevant microorganisms in the gut 2) whether changes in SCFA and organic acids profile induced by remodeling the metabolic activity of *Bacteroides* by diet have the capacity to potentiate other less nutritionally versatile beneficial microbes through cross-feeding or other interaction mechanisms.

Omics technologies, animal models and human intervention studies are necessary for deciphering how microbiota metabolism can be remodeled as a response to dietary interventions.
In the framework of the current European Union regulations, it is very unlikely that *B. fragilis* will be allowed to use as probiotic, and even less in food. However, the potential to shift the metabolism of commensal *B. fragilis* and other members of the genus *Bacteroides* present in the human gut through specific combinations of dietary carbohydrates/proteins merits further attention as a way to restore the metabolic balance of the microbiota and to promote durable host's health effects.

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BIFIDOBACTERIA

BIFIDOBACTERIUM BIFIDUM-HOST INTERACTION AS A PARADIGM OF THE HOLOBIONT VISION OF THE HUMAN BEING

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Abstract

An enormous number of microorganisms, mostly bacteria, constantly colonizes and forms complex communities, called microbiotas, at various sites within the human body. For this reason, humans are considered holobionts, i.e. super-organism, defined as the host organism and all its symbiotic microbiotas. All animals and plants harbor abundant and diverse microorganisms and it is now clear that these microbial symbionts affect the fitness of the holobiont. Nonetheless, the understanding of the mechanisms of interaction between the host and the single microbial organisms constituting the holobiont is still limited, particularly at molecular level.

In this work, we propose *Bifidobacterium bifidum* as a model symbiont of the human holobiont. *B. bifidum* is a species commonly associated to healthy infants, which can be found also in the colon of healthy adults. In *silico* analyses of the genome of this bacterium revealed that it can be considered an example of the human-microbe co-evolution. In fact, the genome of *B. bifidum* strains contains a plethora (more than 9 % of the total predicted proteins) of putative proteins involved in the transport and metabolism of host and diet derived carbohydrates, including mucin glycans and human milk oligosaccharides. Furthermore, *B. bifidum* encodes at least three putative loci involved in the adhesion to intestinal cells, included genes for pilus-like structures, which could support bifidobacteria intestinal colonization and their cross-talk with the host or other resident bacteria. In addition, we identified in *B. bifidum* the presence of outer surface proteins directly implicated in the modulation of the host immune response (1-3).

Representative strains of the species *B. bifidum* have been used in trials in humans and mice, which demonstrated *in vivo* the *in vitro*- and computationally-predicted abilities of this bacterium to interact with the host by modulating the intestinal microbial ecosystem and influencing host's gene expression. Particularly, *B. bifidum* intake modulated in healthy adults the fecal concentrations of butyrate and the relative abundance of dominant intestinal bacterial families such as *Prevotellaceae* and *Ruminococcaceae* (4). Furthermore, we observed that *B. bifidum* may influence the microbiota of mice in different intestinal districts; specifically, *B. bifidum* determined a significant reduction of the total bacterial load in the ileum, while induced a significant increase of the abundance of several Bacteroidales OTUs in the cecum and colon. The evident modification of the intestinal microbial ecosystem plausibly contributed to the observed changes in the expression of several genes in the mouse intestinal mucosa. In fact, *B. bifidum* induced the expression of the anti-inflammatory cytokine IL-10 in the cecum and, particularly, in the ileum, whereas it significantly reduced the transcription in the colon of the cyclooxygenase COX-2, an enzyme which has been associated to intestinal inflammation and colorectal cancer. Moreover, *B. bifidum* stimulated gene expression changes in the host at both the transcriptional and miRNA level in a time-dependent manner, by modulating miR-148a expression, which plays an important role in the regulation of immune response (5).

The in vivo study in the murine model also revealed that *B. bifidum* modulated the expression of genes involved in intestinal motility and permeability. In fact, we found in the colon the overexpression of the transporter involved in the re-uptake of serotonin (SERT) and the downregulation of the tryptophan hydroxylase TPH1, which is the key enzyme in the synthesis of serotonin. Interestingly, we found that, in the colon, *B. bifidum* exerted an opposite effect on the expression of SERT and THP1. In addition, the expression of zonulin, which is a protein that promotes the permeability of tight junctions, was significantly downregulated by *B. bifidum* in the cecum.

In conclusion, the genome of *B. bifidum* reflects its adaptation to the human gastrointestinal tract. The species *B. bifidum* has been shaped by the exclusive co-evolution among this bacterium, the human host and other commensal organisms, giving rise to the comprehensive process of superorganism (holobiont) evolution. Such microorganism contributes actively to the maintenance of host physiologic homeostasis (i.e. health) supplying a rationale for its use as probiotic and effective tool for the cure/treatment of pathologic conditions.

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OBESITY - GUT MICROBIOTA IMPACTS ON NON-COMMUNIABLE DIESEASES

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Gut microbiota- guidance to the regulatory systems of the body

The traditional defensive conception of microbes as pathogens is shifting towards a concept of host-microbe dialogue. In fact, humans and microbes engage bidirectional exchange of endocrine, immune and neural signals with targets in metabolic, immune, humoral and neural pathways (Kau et al. 2011; Moloney et al. 2014). Disturbances in the gut microbiota are an important element in the development of non-communicable diseases, such as allergic, autoimmune and inflammatory diseases, as well as obesity. One attractive theory to the emergence of non-communicable diseases involves the rapid adaptive competence of the microbiome towards environmental exposures, deviant from that of the host genome. The healthy microbiota is rich, stable and resistant, but concomitantly also resilient to change caused by external exposures (Levy et al. 2017). These characteristics challenge all attempts to define the healthy versus aberrant gut microbiota composition, expressed as dysbiotic; the adaptations to an altered environment involve dynamic modifications in the microbiota composition and activity. Thus it may be preferable to compare the clinical, metabolic, immunologic and neurologic consequences of the compositional development of defined microbiota profiles, not simply cross-sectional microbiota profiles.

Compositional development of gut microbiota: origins and timing

The risk of consequences of dysbiosis, an imbalance in the taxonomic composition of the gut microbiota, abating health culminates at an early age, when the host's immune, metabolic and microbiological constitutions lend themselves to long-term and even permanent adjustments by environmental exposures (Rautava et al. 2012). Such conditions may be generated already during the perinatal period. The mother provides the first inoculum in microbial colonization, possible already *in utero*. An infant's probability of being colonized by bifidobacteria, which typify the microbiota of a healthy breast-fed infant, is low if the mother has a higher body mass index and weight-gain during pregnancy and delivers by caesarean section. After birth, the sources of environmental exposure directly shaping the risk of disease are mainly associated with breast-feeding, and more specifically, the breast milk composition. Human milk is rich in bioactive compounds including health-promoting microbes, their optimal growth factors, human milk oligosaccharides (HMOs). HMOs are a class of over 200 known origosaccharides, which regulate the gut colonization process of the infant and inactivate pathogens therein. However, the microbes in breast milk also vary according to the mode of delivery and the mother's health and weight gain during pregnancy (Cabrera-Rubio et al. 2012). Antibiotic exposure in early life has been shown to result in relatively long-term perturbations of the gut microbiota. Consistently with the proposed role of the gut microbiota in the pathogenesis of non-communical disease, early antibiotic exposure has also been associated with the development of inflammatory bowel disease, asthma and obesity in both epidemiological and experimental studies. From the clinical point of view, being overweight or obese appears an intergenerational condition, transmissible via a vicious circle: obese children often become obese adults and maternal obesity over-nourishes the foetus, thereby programming adult size and health with a heightened risk of obesity later in life. More recent evidence provides the microbiota transfer from the mother to the neonate during pregnancy and delivery and via breast milk as one mechanisms of this phenomenon (Isolauri et al. 2016).

Gut microbiota: a way out to fight obesity?

Recent evidence from experimental studies indicates that the gut microbiota acts as a key regulator of body weight and energy metabolism. This is achieved by influencing the two main causes of obesity, energy acquisition and storage, and contributing to insulin resistance and the inflammatory state of obesity (Bäckhed et al. 2004; Cani et al. 2007). High-energy diet alters the microenvironment of the gut and delivers excess energy for the microbiota provoking dysbiosis by preferently selecting obesogenic microbiota (Turnbaugh et al. 2006; Kau et al. 2011). Altered microbiota composition, compositional richness and diversity increases energy harvest from the diet already rich in energy. The nutrients unabsorbed by the host are fermented in the large intestine to short-chain fatty acids, which further increase the energy efficiency of the diet by delivering extra calories to the host. Persisting dysbiosis contributes to the inflammatory immune milieu, which perpetuates microbiota composition yielding and consolidating a pro-inflammatory gut milieu and thereby weakening of the gut barrier function. The net results is a systemic inflammation causally related to insulin resistance and the chronic inflammatory state maintaing the obese phenotype and risk of metabolic disease. Taken together, the amount of energy extracted from the diet depends on the composition of the diet but also on the composition of gut microbiota. In point of fact, diets considered as source of the obesity epidemic, characterized by processed foods, caloric sugars, high protein and saturated fat compostion, have been shown to modify extensively the gut microbiota towards a proinflammatory profile. Converesely, replacing these energy nutrients, without targeting the microbiota, has failed to provide a solution to the obesity epidemic (Suez et al. 2014).

Conclusion

Non-communicable diseases, allergic and inflammatory conditions as well as obesity appear to represent both causes and consequences of altered compositional development of the gut microbiota. Unfortunately, most of the evidence thus far is derived from experimental studies, and specifically by the fact that, in terms of microbiota profiles, these provide contrasting data to the limited number of human studies. The best-characterized healthy microbiota is that of a healthy breast-fed infant, who also remains healthy long-term. Recent studies further indicate that the compositional development of the gut microbiota is a critical process, reflecting the age and the environment. In point of fact, an age-appropriate composition appears to be fundamental to health. Precocious maturation of the microbiota has been linked to overweight development (Kalliomäki et al. 2008; Dogra et al. 2015), while undernourished children exhibited a younger gut microbiota profile than expected for their chronological age, hampering the maturation of the gut microbiota and the gut barrier functions (Subramanian et al. 2014). Realization of the fundamental role of the microbiota in the development and maintenance of the vicious circle of obesity has led to the introduction of novel modes of intervention based on the consumption of beneficial live microorganisms as probiotics. Such an approach is facilitated by on-going studies combining probiotic and nutrient modified diets with a focus on adjusting the microbiota towards less obesogenic composition and activity. Indeed, the gold standard in efforts to ultimately resolve the link between host weight and the microbiome profile are well-designed randomized placebo-controlled human intervention studies.

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NUTRACEUTICAL APPROACH TO MENOPAUSAL COMPLIANTS

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Vasomotor menopausal symptoms impair women's daily quality of life. Appropriate treatment include an early administration of hormonal replacement therapy (HRT) (1). Customisation of the dose, routes of administration, types of combination, annual controls and a treatment duration less than 5 years are guarantees of a good risk/benefit ratio (2). Nevertheless, HRT use is usually restricted to moderate or severe symptoms, and is limited by contraindications and warnings such as mammary cancer or advanced menopausal age. Moreover, many women simply refuse HRT for a variety of reasons concerning fear of cancer and weight gain (3) and request a "natural" approach (4). In the US and Britain surveys show that 80% of peri and postmenopausal women are current or former users of dietary supplements; although a growing body of evidence suggests that such therapies could result in improved clinical outcomes, more research on efficacy and safety is needed.
In this scenario, nutraceuticals have a role in the management of symptomatic menopausal women. Nutraceuticals are foods, parts of foods, and botanicals that provide medical or health benefits, including the prevention and treatment of disease. The term "nutraceutical" combines two words – "nutrient" (a nourishing food component) and "pharmaceutical" (a medical drug). The name was coined in 1989 by Stephen DeFelice, founder and chairman of the Foundation for Innovation in Medicine, an American organization. The philosophy behind nutraceuticals is to focus on a medical system that yet originated from ancient China, was gradually imported to Japan since approximately 1500 years ago, and has been improved and refined by many excellent physicians especially since the 17th century (Kampo medicine). Kampo has a holistic therapeutic approach, as the mind and body are seen as one entity. The therapeutic aim is to relieve symptoms and to restore harmony in bodily functions. The treatment regime is based on symptoms. For the determination of the appropriate herbal prescription, the physician carries out a thorough investigation of the complaints and symptoms of the patient, including taking their temperature, examining sensation, weakness or sweating, symptoms which are not often primarily taken into account in conventional medicine (5). Herbal medicines may be used in symptomatic menopausal women, they include isoflavones, pollen estracts, cimicifuga, red clover, and others.

Different oligoelements have been combined with herbal medicines to enhance the clinical effects. It is reasonable to add soy isoflavones (SI) with lactic acid bacteria in the form of spores, resistant to the gastric and biliary secretion, to promote the action of bacterial glycosidase and to assure the bioavailability of SI (6). Magnolia has been shown to have tranquillizer and neurotrophic properties (7), but there are few reports in relation his activity on hot flashes, mood, and sleep symptoms (8). Furthermore, it is shown that agnus-castus increases melatonin release and interact with opioid receptors and can play a role on vasomotor symptoms and sleep diseases (9,10).

Soy isoflavones (SI) are natural substances as genistein and daidzein with agonist-antagonist oestrogen action that have been demonstrated to alleviate climacteric symptoms at the dose between 40 and 80 mg/day (11,12). SI exert elective stimulation of beta-oestrogen receptors (betaERs) with less affinity and lower potency than oestrogens (13), moreover stimulate the synthesis of SHBG (14), therefore, safety in long term use could be expected. In our experiences, a combination of nutraceuticals based on SI and Lactobacillus sporogenes administered for 1 year was safe for endometrium, mammary tissue and hepatic function, and effective on menopausal symptoms (15).
Besides hot flashes, the sleep disturbances negatively affect quality of life in postmenopausal women. In large series, sleep complaints were present in almost half percentage of menopausal women and frequently associated with hot flashes or mood disorders (16,17). We showed that agnus castus and magnolia, in combination with SI can effectively and safely be used in symptomatic postmenopausal women when quality of sleep is the most disturbing complaint. (18)
Preliminary data show that herbal remedy from pollen extracts (19) may alleviate menopausal complaints through a central effects by altering serotonergic mechanisms involved in regulating sleep (20), but without any oestrogenic action (21). In a six-month prospective, observational study two alternative treatments for symptomatic menopausal women (>3 hot flushes, Kupperman score >20 and <30, sleep disorders score >5) were compared: pollen herbal extract and formulations with SI. The treatment efficacy was evaluated at baseline and every three months by: a) daily number and severity of hot-flushes recorded on a self-compilation diary (0 = absent; 1=mild; 2=moderate; 3= severe; 4= very severe); b) Kupperman score, a numerical index that scores 11 menopausal symptoms: hot flushes, paraesthesia, insomnia, nervousness, melancholia, vertigo, weakness, arthralgia or myalgia, headache, palpitations, and formication. Each symptom is rated from 0 to 3 according to severity and symptoms (where 0 = no symptoms and 3 = most severe), weighted and the total sum calculated. For vasomotor symptoms rated as mild (score 15-20), moderate (20-35), severe (>35); The maximum score is 51 points c) the Pittsburg Sleep Quality Index (PSQI) test for the evaluation of sleep disorders with a score from 0 to 21, higher scores reflecting more severe sleep diseases. The component scores consist of subjective sleep quality, sleep latency (i.e., how long it takes to fall asleep), sleep duration, habitual sleep efficiency (i.e., the percentage of time in bed that one is asleep), sleep disturbances, use of sleeping medication, and daytime dysfunction. The results give numbers in seven categories: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. While vasomotor symptoms improved symmetrically in both groups, improvement of sleep global quality was more evident in the pollen treated group compared to isoflavones group.
In conclusion, treatments alternative to HRT can effectively be used in symptomatic menopausal women. Health care providers should discuss with their patients alternative approaches for relief of menopausal symptoms and assist them in managing these herbal remedy through an evidence-based approach.

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DIET AND PROBIOTICS IN CANCER: FROM PATHOGENESIS TO CANCER TREATMENT

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Unhealthy diet is associated with overweigh, obesity and endocrine dysregulations that are all included as predictive and prognostic indicators for several cancer types. Accordingly, elevated intake of carbohydrate and fat are associated with initiation of hyperglycaemia, hyperinsulinemia and accumulation of adipose tissue with concomitant release of IGFs, sexual hormones and inflammatory cytokines. In the context of chronic inflammation, the adipose tissue releases specific adipokines, as IGF-1, leptin, TNF-α and IL-6, to address signalling for survival and cell proliferation on those targets typically involved in cancer such as PI3K, MAPK, IKK/NF-κB and STAT3. Previous in vivo experiments showed how rats sensitive to diet-induced obesity, are prone to develop kidney cancer. The association between diet and inflammation has been recently confirmed by the identification of the Dietary Inflammatory Index (DII), which may be considered a prognostic factor of different tumors. Of note, chronic inflammation is associated with tumor development. Indeed, the nuclear transcription factor NF-κB, which regulate several pro-inflammatory cytokines, is overexpressed in several cancer types (Mantovani, 2010).
The wrong diet habits may be also associated with altered gut microbiota that promotes and enhances cancer development, especially that of the intestinal tract (Tjalsma, 2012). The mechanisms, supporting this hypothesis may include the production of toxic metabolites that can lead to a chronic inflammatory condition, the metabolism of dietary heterocyclic amines and the biosynthesis of genotoxic compounds interfering with cell cycle control or causing a direct DNA damage (Candela et al, 2011). It is also known that life style habits, including diet, may induce epigenetic modifications that in turn may be responsible of tumor development. Therefore, the introduction of correct lifestyles for cancer treatment is mandatory. On this regard, a recent clinical trial was designed from our group of research to understand the efficacy of lifestyle changes in a Mediterranean population of breast cancer (BC) survivors. In particular, a lifestyle modification (low glycaemic index diet and exercise) within normal vitamin D levels was planned on disease-free survival. Such clinical study may be useful to understand if microRNAs in BC are modulated by dietary and other lifestyle aspects (Augustin LS et al, 2017).
Along with correct diet habits, gut microbiota equilibrium may have a significant impact for the prevention and treatment of cancer. Lactobacillus rhamnosus GG (LGG) is one of the first probiotics studied in cancer and used in experimental designs (Goldini et al, 1996). It is well characterized and it is known to have anti-inflammatory effects by gut microbiota restoration (Khailova et al, 2016). Accordingly, treatment with LGG in animal models may reduce the risk of colon cancer through the modulation of the gut microbiota and the downregulation of pro-inflammatory molecules (Gamallat et al, 2016). LGG was first isolated in the late '80 from fecal sample of healthy adult and its whole-genome sequence has been registered (Morita, 2009). It matches the selection criteria for probiotics, including high adhesion in vitro, survival trough gastrointestinal tract (gastric acid, bile) and tendency to form colonies with a good persistence in the gut (Gorbach, 1996). Gut microbiota alterations can be also caused by different anticancer approaches. On this matter, LGG was revealed to be useful and safe (Readman et al, 2014). Therefore, it is clear that LGG administration in cancer patients may improve their quality of life and enhance the compliance to treatments.

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PROBIOTICS IN CLINICAL PRACTICE: CONSOLIDATED INDICATIONS - ACUTE GASTROENTERITIS

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Introduction

Acute gastroenteritis (AGE) is a leading cause of morbidity and mortality in children worldwide.
In children living in developed areas, AGE is a common and relatively mild illness but represents a major cause of consultations in general practice and emergency and is the second leading cause of hospital admission in children with major impact on the quality of life of infected children and their families on health-associated costs. AGE is generally defined as a decrease in the consistency of stools (loose or liquid) and/or an increase in the frequency of evacuations (typically >3 in 24 hours), with or without fever or vomiting and usually lasting no more than 7 days [1].
Probiotics are traditionally defined as microorganisms that have a beneficial effect in the prevention and treatment of specific pathologic conditions. There are many mechanisms by which probiotics enhance intestinal health, including stimulation of immunity, competition for limited nutrients or inhibition of epithelial invasion and production of antimicrobial substances.
AGE is probably the main, certainly the original field of application for probiotics. A large number of data have been obtained since the paper that firstly provided evidence for efficacy of Lactobacillus rhamnosus GG (LGG) in the treatment of AGE [2].
In the last years, an increasing number of RCTs have been published on this issue in various settings and with different outcomes. However, the data available are progressively merging in providing compelling indications that probiotic administration is effective in preventing and treating AGE in children.

Prevention of acute gastroenteritis

Since probiotics are usually seen as safe, functional foods that may be added to infant formulas and other foods and administered for long period, the prevention of acute intestinal infections in healthy and at-risk children has been one of the natural fields of application.
Almost all prevention studies are performed with probiotic strains added to a milk-based feeding. The first trials focusing on this objective documented a reduction in incidence or severity of acute diarrheal disease [3]. However, further studies provide evidence of a very modest effect of some probiotic strains (LGG, B. *lactis*, alone or in combination with *Str. thermophilus*, and *L. reuteri, L. rhamnosus* -not GG- and *L. acidophilus*) on the prevention of community-acquired diarrhea, and some authors questioned the clinical relevance of these results although statistically significant [4, 5]. More recently, Gutierrez-Castrellon and colleagues demonstrated that daily administration of the probiotic strain *Lactobacillus reuteri* DSM 17938 to healthy children may reduce episodes of acute diarrhea. This prophylactic administration of probiotics conferred a cost-saving benefit to the families and health-care system [6].
These contradictory results may in part be explained by problems of concentration and viability of the probiotics in feeding. European and United States Pediatric Societies agree on the paucity of evidence in support of the use of probiotic-enriched infant formula. However, despite European Society of Pediatric Gastroenterology and Nutrition (ESPGHAN), describe data of efficacy as insufficiently convincing [7], the American Academy of Pediatrics considered beneficial the use of probiotic in special circumstances, such as children in long-term health care facilities or care centers [8].
A recent metanalysis found no significant reduction in the risk of nosocomial diarrhea, rotavirus diarrhea or diarrhea of any origin with *L. reuteri* DSM 17938 administration in hospitalized children [9].

Treatment of acute gastroenteritis

Treatment of AGE in children is currently the main indication for probiotics and has been discussed in several documents based on a huge mass of results. Interestingly the evidence obtained is consistent along the years and led to similar conclusions that may be applied to a similar extent at more or less universal setting. There is conclusive evidence that probiotics reduce the severity and duration of diarrhea in multiple conditions.
From a review of guidelines available worldwide, five produced either in developed or developing countries consider the use of probiotic as a therapeutic option in addition to rehydration [10]. The recommendation slightly varies among different guidelines according to the setting and the availability of products on the market, however, LGG and *Saccharomices boulardii* are the two most studied strains.
Two authoritative documents have been developed in 2014 by the ESPGHAN, one is a position paper that specifically address the use of probiotics in children with AGE [11] and the other is a more complete document on the overall management of AGE in children [1]. Those documents provide clear-cut recommendations. Evaluation was made specifically for individual strains or preparation and the recommendation was provided only if at least two distinct RCTs were available. Briefly, according to published data, administration of selected probiotics reduces the severity and duration of symptoms by approximately 24 hours (without substantial differences in efficacy among effective strains), and the risk of complications. A total of 3 strains were recommended for active treatment of gastroenteritis, in adjunct to oral rehydration therapy. LGG and *Saccharomyces boulardii* received a “strong recommendation” although the evidence in support is large and consistent, some methodological weaknesses partially affect this recommendation. *L. reuterii* received a “weak recommendation” with a very low quality of evidence”; this is mainly justified by the lack of data supporting the use of the new strain DSM 17938 developed after the elimination of the plasmid responsible of transporting of antibiotic resistance identified in the original strain *L. reuteri* ATCC 55730. However, after 2014 other RCTs have been published and in a recent metanalysis on 3 RCTs (n=256), *L. reuteri* administration reduced the duration of diarrhea (mean difference, MD -24.82 h, 95% CI -38.8 to -10.8) and increased the cure rate on day 1 and day 2. However, heterogeneity and wide confidence intervals call for caution in interpreting results [9].

Specifically for the treatment of AGE, strong evidence supported a dose-related effect, being high doses of LGG (> 10^{10} CFUs/day) more effective than low doses in reducing the duration of diarrhea [12].
Since the demonstration of a significant reduction of Rotavirus shedding in stools in children receiving *Lactobacillus rhamnosus* [2], and the following evidence of a dose-dependent effect [13], other documents reported that probiotics overall seems to have a stronger effect in Rotavirus-positive diarrhea rather than in other etiology. It should be considered that the spreading of Rotavirus immunization might change in part the current scenario.

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ROLE OF PROBIOTIC IN TREATMENT OF NONALCOHOLIC FATTY LIVER DISEASE (NAFD)

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Introduction

Non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (NAFLD/NASH) are presently the most common liver disease in developed and developing countries. It gradually became a common etiology for endstage liver disease and the most common liver disease among patients in liver transplant list during last two decades. This pandemic of NAFLD is the consequence of obesity pandemic and low physical activity across the world. It is also estimated that genetic susceptibility play an important role especially in progression from NAFLD to NASH. Recent studies suggest that up to 50% of NASH may be heritable. NAFLD/NASH is also a growing etiology of hepatocellular carcinoma (HCC).The incidence of HCC in western countries and Japan has recently tripled while the 5-year survival rate has remained below 12%.Presently there is no FDA approved drug for therapy of NAFLD/ NASH, therefore any new preventive measure or therapy for this disease is at top priority for future. Recent animal and human studies proposed that gut microbiota play an important role in pathogenesis of NAFLD/NASH and modulation of intestinal microbiota by probiotics may be a simple and effective strategy to treat this disease and even prevent it at community level. The gut microbiota consists of a complex of microorganism species, the concentration and type of which are mostly influenced by host genotype and nutrient availability. NAFLD have been shown to be associated with small intestinal bacterial overgrowth along with increased intestinal permeability and subsequent liver damage by exposure to intestine-derived bacterial products. Progression of NAFLD is mainly based on increased hepatic oxidative stress due to the increased production of ethanol, and lipopolysaccharides in the intestinal lumen, and subsequent release of inflammatory cytokines like TNF-α which appears to play a crucial role in both insulin resistance and liver inflammation. Several animal and few human studies have clearly shown that probiotic have immunomodulatory, anti-inflammatory and anti-fibrotic properties in subjects with NAFLD/ NASH .We aimed to investigate the efficacy of manipulation of enteric flora by addition of a synbiotic capsule as a novel adjunctive therapeutic strategy in addition to life style change in patients with NAFLD

Probiotic for treatment of NAFLD in human

Studies using VSL#3 (a synbiotic combination of pro/prebiotics) given to NAFLD/NASH patients for two to three months, improved liver enzyme levels, TNF-α and oxidative stress markers. Studies in Children also showed a significant decrease in liver aminotransferases with probiotic administration. In a recent double-blind, placebo-controlled study we used synbiotic supplementation (containing 200 million of seven strains of friendly bacteria plus fructo-oligosaccharide) in addition to life style modification to treat insulin resistance and improve the lipid profile in individuals with the metabolic syndrome1. After 28 weeks of treatment, the levels of fasting blood sugar and insulin resistance improved significantly with decline in TG and Total cholesterol level and increase in HDL cholesterol in treatment group[1]. In another[2] randomized placebo controlled trial we used the same synbiotic supplement in addition to life style change to treat Patients with NAFLD/NASH. At the end of the 28 weeks study, the ALT, AST, GGT, and HOMA-IR decreased in both groups; this reduction was significantly greater in the synbiotic group. In addition fasting high-sensitivity C-reactive protein concentrations, plasma TNF-α concentrations and concentration of peripheral blood mononuclear cell (PBMC) nuclear factor k-B (NF-kB) p65 decline significantly in treatment group. . These effects was seen beginning at week 14, and this trend was sustained until the end of the study. In this study we used elastography instead of liver biopsy and have shown that fibrosis Score decline in 95% of treatment groups compared with 36% of placebo group BMI and WHR decreased significantly in both groups ($P < 0.05$). None of the patients completing the study had any serious adverse events, which indicated tolerance to the treatment. Two minor adverse events were reported; one patient complained of moderate headaches and one of abdominal pain in the synbiotic and placebo groups.[2]

Conclusions

In conclusion, human studies have shown evidence that probiotic and synbiotic supplementation in addition to lifestyle modification is superior to lifestyle modification alone for the treatment of NAFLD/NASH, at least partially through attenuation of inflammatory markers in the body. Due to the high resilience of our intestinal microbiota that easily tends to return to baseline after perturbation, therapies aimed to modulate the gut microbiota are subject to early relapse to the initial dysbiosis state after stopping the intervention, unless long-term approaches are used. Although promising but further large scale long term clinical trial using different types of probiotic with longer term follow up are necessary before probiotic become a standard therapy for NAFLD/NASH.

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AN INNOVATIVE AND REMARKABLE ACTIVITY OF MICRONIZED CELLS OF THE PROBIOTIC STRAIN B. LACTIS BS01 ON MACROPHAGE ACTIVATION AND POLARIZATION: A NEW VACCINE STRATEGY

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Our body is constantly exposed to innumerable aggressions coming from both the external environment (microbes, bacteria and viruses) and from within (excess of mediators, free radicals, catabolism derivatives).
Related to that, one of the question central to microbiome research is why people in modern society, who are relatively free of infectious diseases, are so prone to inflammatory, autoimmune and allergic diseases. Since 1950, there has been a gradual lowering of infectious diseases and a specular increase of allergic and autoimmune diseases with epidemic incidence.
Irrespective of the proximity of these areas and the similarity of their climatic and geo-vegetative conditions, the occurrence of atopy and atopic diseases is many times lower in Russian Karelia than on the Finnish side of the border. Interestingly the economic gap between the areas is one of the deepest in the world and the living conditions in Russian Karelia are simple and still largely resemble those seen in Finland 50 years ago (rural life vs urbanized life) (1).
An early antibiotic exposure has several immunological effects, such as transient or persistent alterations in immunity, impaired tolerance to commensal microbiota, immunological defects and altered microbial control, impaired colonization resistance and emerging pathobionts and pathogens. The final clinical consequences in the adulthood are Inflammatory Bowel Diseases (IBD), asthma, obesity, type 1 diabetes, atopic dermatitis, and multiple sclerosis (2).
Inflammation is a reaction of the host to viral and bacterial infections with the physiological purpose of restoring tissue homeostasis. However, uncontrolled or unresolved inflammation can lead to tissue damage, giving rise to a plethora of chronic inflammatory diseases, including metabolic syndromes and autoimmunity pathologies with eventual loss of organ function. This justifies the increasing interest in studying inflammatory processes (3).
The monocytes/macrophages play a key role in the initiation and resolution of inflammation through different activation programs (4). Peripheral blood monocytes are an inhomogeneous population differing in their phenotypes and functions, and once differentiated into macrophages they can adopt a variety of different phenotypes dependent on changes in the tissue microenvironment, thus exhibiting a continuum of different functional steps (5).
In literature, there is increasing evidence that probiotic microorganisms may have immunostimulatory, anti-inflammatory and anti-oxidant properties and that these important activities result from the interaction of bacterial wall with the host cell membrane (6-8).
The aim of this research was to evaluate whether Micronized Cells (MCs) from selected biotherapeutic bacteria have the ability to modulate the polarization of monocyte/macrophage subpopulations to provide advantageously a first line of defense against infections.
The ability of MCs derived from the probiotic strain *Bifidobacterium animalis* subsp. *lactis* BS01 (LMG P-21384) to induce a differentiation in the monocyte/macrophage subpopulations and to act on the oxidative stress in an *in vitro* hyperhomocysteinemia model was investigated.
Micronized cells (MCs) were obtained from B. lactis BS01 strain using the Bioimmunizer extraction protocol (9). First of all, bacterial cells were lysed using micronization, then the cell wall extract was separated from the intact cells by centrifugation. The supernatant was recovered and further centrifuged in order to collect the extract. This component was precipitated using a saturated solution of ammonium sulphate, followed by an overnight rest at 4°C. A further centrifugation step was then applied, followed by 5 subsequent washings to remove the ammonium sulphate. The sample was then rehydrated in distilled water and lyophilized.
Peripheral blood mononuclear cells (PBMCs) of healthy subjects were isolated by standard internal protocol and monocytes were obtained by plate adhesion. Monocytes were stimulated with the probiotic strain and/or MCs (10mg/ml) for 24h and 5 days.
Monocyte/macrophage differentiation was evaluated by cytometry analysis of surface markers and the activity of the two subpopulations on oxidative stress was assessed in an *in vitro* oxidative stress model with a spectrophotometric test.
The statistical analysis of results was conducted using the t-test for paired data, with differences regarded as significant if the p-value was below 0.05.
The MCs have been shown to modulate significantly the two subpopulations of human monocytes/macrophages, both the "patrolling subpopulation" (CD16+) and the "inflammatory subpopulation" (CD16-), thus highlighting a strong immunostimulatory effect (Fig. 1 and 2).

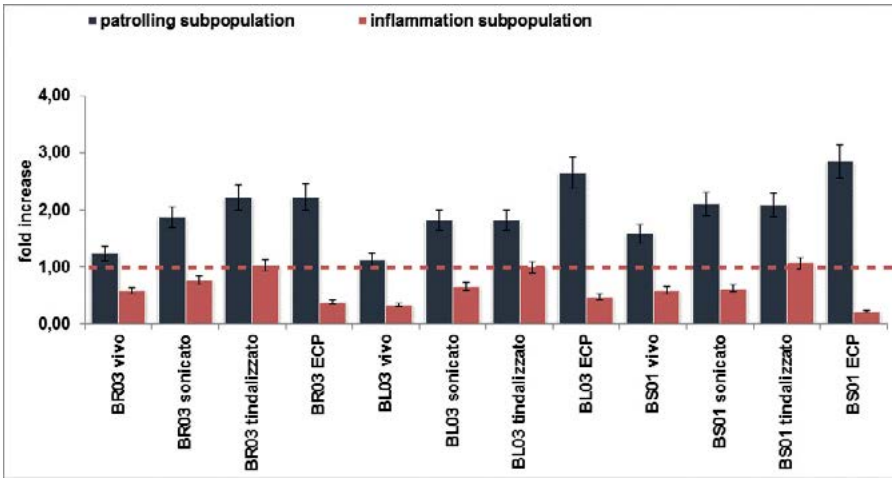

Figure 1. Modulation of the monocyte/macrophage population after 5 days stimulation. Flow cytometer evaluation of cell surface markers. ECP: Extract of Cell Wall; "tindalizzato": tyndallized; "sonicato": sonicated; "vivo": viable, intact cells. The dotted line indicates the baseline reference value.

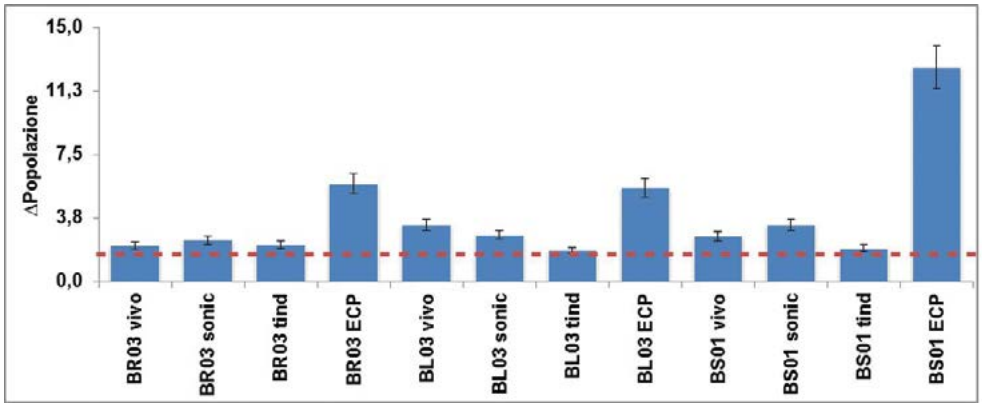

Figure 2. Activation ratio between the two monocyte/macrophage populations (CD16+/CD16-). ECP: Extract of Cell Wall; "tind": tyndallized; "sonic": sonicated; "vivo": viable, intact cells. The dotted line indicates the baseline reference value.

CD16+ cells come to the tissues constitutively and have different phenotypic characteristics than CD16- monocytes, which only move when the tissue is inflamed. In fact, the CD16- appear to be involved in the innate inflammatory response. In contrast, cells derived from the CD16+ population are involved in tissue homeostasis. By modulating the activity of CD16- and CD16+ subpopulations, it is therefore possible to regulate their function in inflammatory diseases. MCs, by stimulating the two subpopulations, are able to help counteract inflammation in the infected site as well as to activate the adaptive response against pathogenic bacteria, viruses and fungi. In addition, MCs were able to mitigate significantly the oxidative stress induced by homocysteine (Fig. 3).

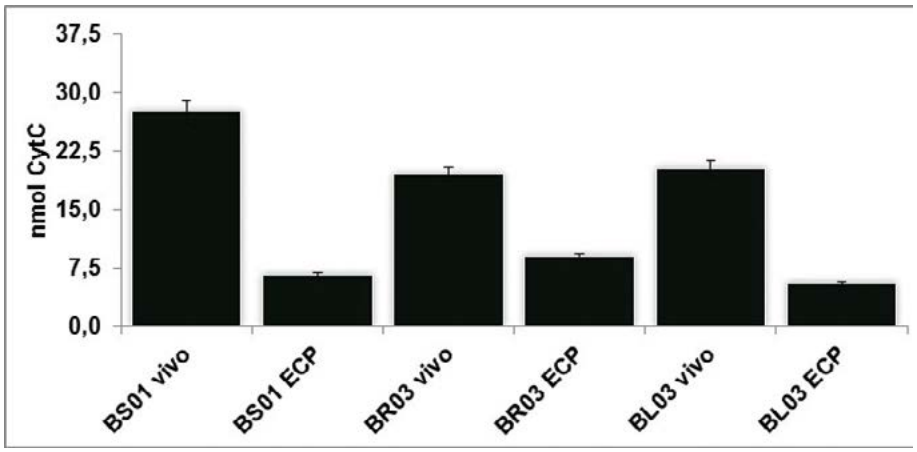

Figure 3. Evaluation of oxidative stress in a model of induced hyperhomocysteinemia (nanomoles of cytochrome C). ECP: Extract of Cell Wall; "vivo": viable, intact cells.

The demonstration that MCs can modify the oxidative stress induced in a hyperhomocysteinemia pattern further consolidates their function in inflammatory diseases. The effectiveness of MCs was even better if compared with BS01 viable cells.
Our findings suggest that MCs derived from the probiotic strain BS01 could be a possible therapy that could quickly and effectively prevent and/or cure viral, bacterial, fungal or protozoal diseases, as well as prevent and/or treat inflammatory processes triggered by external pathogenic agents.

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MICROBIAL COMPOSITION AND CELL VIABILITY OF A MULTI-STRAIN BACTERIA FORMULATION

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Introduction

A probiotic formula to be functional and reliable should: i) be taxonomically defined; ii) contain viable cells, iii) have a reproducible composition, iv) and ideally, should be controlled for probiotic molecular markers. Here we detail the consistency of the multispecies probiotic product VSL#3, which has been produced for the last 20 years and is marketed globally for threating inflammatory bowel disease, pouchitis and other intestinal diseases.
To show consistency in the quality, viability and composition of the multispecies probiotic product VSL#3 various batches of the multispecies probiotic VSL#3 were analyzed in detail and derived from productions in the USA and Italy. The product batches have been tested using a series of microbiological, phylogenetic and metagenetics methods. The microbiological analysis included plating on selective media, cell counting and viability analysis by Flow Cytometry (FCM) using fluorescent dyes that allowed high throughput separation and quantification of live, dead and damaged cells. A metagenetic approach, based on *16S rRNA* gene profiling, was used to define the bacterial community structure of different productions batches. In addition, *Lactobacillus helveticus* and *Lactobacillus acidophilus* S-layer proteins, which are known to exert anti-inflammatory effects by reducing the activation of NF-●B on the intestinal epithelial Caco-2 cell line, have been extracted, visualized on SDS-PAGE and identified by nLC-ESI-MS/MS analysis. Moreover, urease activity of *Streptococcus thermophilus*, known to exert positive effect on human health by competing with the undesired urease-positive bacteria of the human microbiota, was quantified using a spectrophotometric-, and flow cytometry-based assay.

Results and discussion

Taxonomy and viability of the multi-strain probiotic formulation
The different batches tested were all found to contain a common bacterial community structure based on the presence of the following species *Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus helveticus, Bifidobacterium breve* and *Bifidobacterium animalis* subsp*. lactis*. The stability of the batches was confirmed by FCM, and viable cells were always above the value of 2 x 1010 event/g. FCM analysis allowed to identify and quantify live, dead and damaged cell populations in the multi-strain probiotic formulation (Figure 1) without any information on the species distribution within each of those populations. However, it is quite relevant for the probiotic fate of the product, be able to address which is the viability of each species within a multi-strain formulation. This goal was achieved through the quantification of the relative abundance of each probiotic species using species-specific-qPCR assay performed on total DNA extracted from live and dead cell populations separated by FACS. Interestingly, the data obtained revealed that the species in the multi-strain formulation showed a species-specific viability level (Figure 1).

Putative probiotic molecular markers
The *L. helveticus* and *L. acidophilus* S-layer protein SlpA were detected in each VSL#3 batches tested, representing the majority of the surface proteins with a molecular weight ranging between 40 and 50 kDa, thus highlighting that this relevant immunomodulatory factors were not subjected to degradation during the preparation and the shelf-life of the multi-strain probiotic formulation. Likewise, urease activity peculiar of the species S. *thermophilus* was stable in all VSL#3 batches tested.

Conclusions

In conclusion, stability, molecular and taxonomic comparative analysis show that VSL#3 is reliably and reproducibly produced in different parts of the world. More importantly, the assessment and the quantification of putative probiotic molecular markers (S-layer proteins and urease) directly in the product, *i. e.* without a cultivation step, represents a first example of quality control in a probiotic product targeted to “probiotic-traits”, when quality controls are currently directed and limited to the evaluation of cell viability. In addition, the quantification of the viability by FCM combined with a cell sorting and a qPCR quantification of each bacterial species in the blend, represent a further new quality control step for a multi-strain probiotic product.

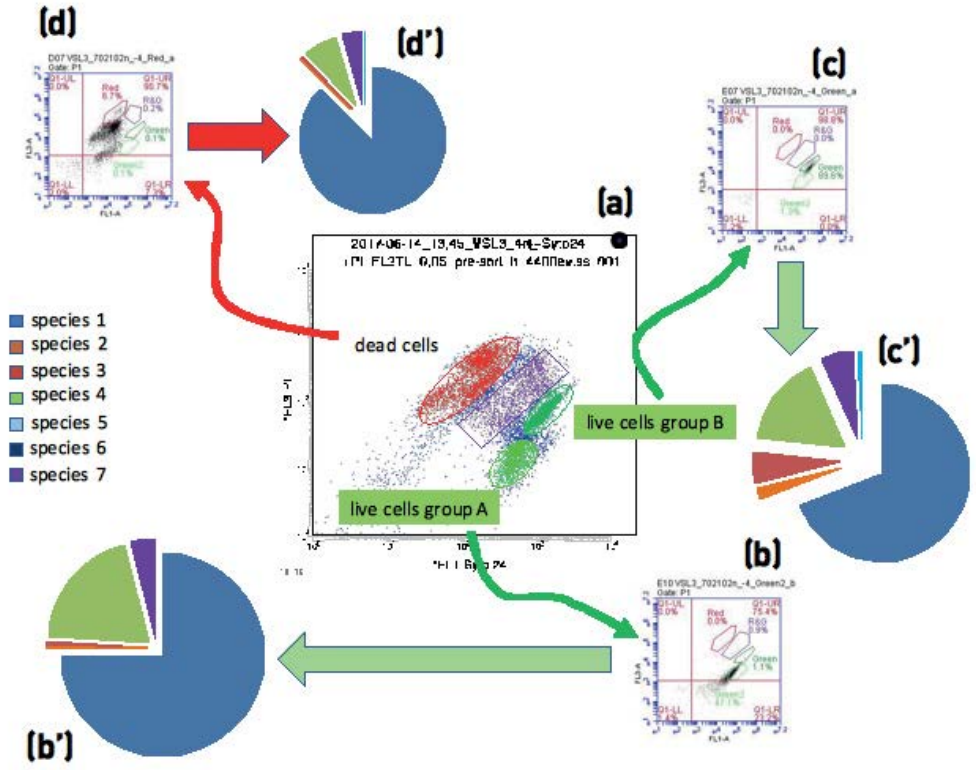

Figure 1. Quantification of live and dead cell population in the multi-strain probiotic formulation by FCM according to ISO19344-IDF232 (a). Post-sorting analysis by FCM of live and dead cell populations (b), (c) and (d). qPCR-based quantification of the relative abundance of the microbial species in the multi-strain probiotic formulation (b'), (c') and (d').

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GENDER MEDICINE: SEX AND MICROBIOTA INFLUENCES IN FUNCTIONAL GASTROINTESTINAL DISORDERS

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Functional gastrointestinal disorders (FGID) are disorders of gut-brain axis related to any combination of motility disturbance, visceral hypersensitivity, mucosal immune dysregulation, altered gut microbiota and central-enteric nervous dysregulation. Personality traits, psychological state, cognitive and coping skills influence the susceptibility of gut dysfunction. A biopsycholosocial approach is then need to manage the pathogenetic complexity of these disorders that result to be the product of the interactions of psycho-social factors and altered gut physiology via the gut-brain axis.

A female predominance has been reported in FGID and mostly in dyspepsia and irritable bowel syndrome (IBS). The IBS ratio women to men is up to 2-2.5:1. The biopsycholosocial model implies to consider separately sex and gender influences, sex referring to the biological make-up of the individual's reproductive anatomy whereas gender to an individual's lifestyle or personal identity. Several studies have focused on the influence of gender-related social factors on FGID, mainly dealing with differences in history of sexual, physical and emotional abuse in women. Studies on sex-related influences on the pathophysiology of FGID are more limited and mainly focused on IBS.

IBS is characterized by abdominal pain along with altered bowel functions. In clinical practice, a correlation between fluctuations in IBS symptoms and hormonal status is observed during menstrual cycle phases, pregnancy and menopause. Moreover, a significant correlation exists between IBS and dysmenorrhea and oral contraceptives influence gastrointestinal symptoms. No more differences in IBS ratio women to men are observed reaching the 60 years of age. Sex hormones have direct effects on the on the gut-brain axis and on the GI tract, estrogen, progesterone and androgen receptors being extensively distributed throughout the brain and the GI tract.

Sex hormones interact at multiple levels with many pathways involved in pain transmission, including primary afferents and central neuromodulator systems, and modulate stress responses through a direct effect on the hypothalamic-pituitary-adrenal axis. The clinical profile for patient-rated severity in IBS indicates an increase in ratio women to men in parallel to increase symptom severity index. In the gut, sex hormones affect intestinal barrier function and permeability, modulate enteric mucosal immune system and the neural-motor apparatus.

Finally, recent studies have demonstrated that gut microbiota is modulated by sex hormones and that gender bias may be exercised and/or reinforced by the commensal microbiota of the host, through regulation of sex hormone levels. Sexual maturation results to be the main determinant of the differences in commensal microbiome composition of male and female animals and recent data have demonstrated that puberty and pregnancy shape the intestinal microbiota in humans. Besides, microbes can increase or inhibit sex steroid signaling in mammalian hosts. Colonization by commensal microbes increases serum testosterone and confers protection against autoimmune diseases in male animals. Emerging data suggest that interactions between gut microbiota and altered immune function may play a role in the pathogenesis of IBS. At the moment, however, no data are available on sex-related microbiota differences in IBS patients, even if this could represent another important issue for Gender Medicine in Gastroenterology.

ORAL COMMUNICATIONS

EFFECTS ON CHOLESTEROL METABOLISM OF BIFIDOBACTERIA IN HUMAN INTESTINAL MICROFLORA

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INTRODUCTION AND AIM

Objective: In this study, it was aimed to reveal the effects on cholesterol metabolism of these bacteria by determine the effects of bile salt deconjugation and cholesterol assimilation rates of bifidobacteria in intestinal microflora.

METHODS

This study was used 16 fecal samples collected from feces of healthy children for isolation of bifidobacteria. For the characterization of the microorganisms, API tests and fructose 6-phosphate phosphoketolase test were done. Molecular analyses were assessed with PCR-amplified 16SrRNA gene sequences. Cholesterol elimination and sodium taurocholate deconjugation were determined by spectrophotometric methods.

RESULTS

The species primers and API tests were used to identify of *Bifidobacterium* strains. 8 isolates were identified as 6 strains of *B. longum* and 2 strains of *B. infantis*. In our study, all eight strains of *Bifidobacterium* sp. showed the different capacities for removing cholesterol from medium. The amounts of cholesterol removed by the cultures during the 48 h incubation ranged from 6% to 32%. Among *Bifidobacterium* species, the amounts of deconjugated sodium taurocholate was found as 1.38±0.2 mg/ml-2.17±0.5 mg/ml.

CONCLUSIONS

Disorders occurring cholesterol metabolism can lead to serious health problems ranging from cardiovascular diseases to other metabolism disease. The health benefits of probiotics, which have gained significant importance in recent years, have reached considerable dimensions. This importance is also emphasized by the microbiom-project. Furthermore, the discovery of superior probiotics, which are isolated from the human body and their use as a food additive may contribute to both the formation of a healthier intestinal flora and the prevention of certain diseases.

THE MOLECULAR IDENTIFICATION AND ISOLATION OF PROBIOTICS PRESENT IN SOME DAIRY PRODUCTS AND THE EFFECT OF AURICULARIA POLYTRICHA ON THESE PROBIOTICS

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OBJECTIVE

The aim of this study was to determine some probiotic microorganisms in milk products (kashar-cheese and butter), prebiotic effect of *Auricularia polytricha* which is a mushroom and has important medicinal and their antibiotic resistance of the isolated.

METHODS

The samples collected from kashar cheese and butter were used. For the characterization of the microorganisms, carbohydrate fermentation and catalase tests were done. Molecular analysis were assessed with PCR-amplified 16S rRNA gene sequences. Also, *A.polytricha* was extracted. The mushroom extracts added to MRS for evaluated prebiotic effect of mushroom. The isolates were grown in the prepared cultures 16-18 hours at 42°C, at the end of the incubation time bacterial cell concentration was determined spectrophotometrically. To antibiotic resistance, they were spread to MHA media and 8 antibiotics were placed onto petri. The zone size was measured.

RESULTS

In our studies, 2 different strains from three different commercial dairy products were obtained. *L. plantarum* and *E. faecalis*, which are important in terms of probiotics. The effect of *A. polytricha* aqueous extract on the proliferation of isolates were determined by measuring optical density and the extract showed proliferative effect only on *L. plantarum* strain. Antibiotic resistance studies indicated that *E. faecalis* strain were resistant to Tetracycline yet susceptible to Ampicillin.

CONCLUSION

When edible A.*polytricha* fungi, which have reduce LDL cholesterol, antitumoral and anticoagulant activity, are used prebiotically, creating a proliferative effect on probiotics which are important in terms of health. Supporting these probiotics with new prebiotics will also increase the positive contributions to health.

ORGANOLEPTIC & SENSORY EVALUATION OF A NOVEL FOOD PRODUCT FROM CARICA PAPAYA

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Carica papaya is a nutraceutical plant rich in vitamins, minerals, and phytochemicals, especially antioxidants. This exotic fruit native to the Indian Sub-continent, possesses numerous health benefits which are widely recognized and have been scientifically documented since time immemorial. In order to promote the inclusion of papaya in the diet, raw papaya chutney or also called as plastic chutney was organoleptically evaluated. A total of 55 urban Indian subjects of the age group 18-25 years were considered. The data collected was analysed statistically. This research endeavour is relevant because it aims to create awareness about the health benefits of raw papaya which is one of the nutrition pack end vegetable in adequate quantities with high bioavailability.

DISCOVERY OF NOVEL PROBIOTIC STRAINS WITH SUPERIOR PROPERTIES IN THE PRESENCE OF VIRAL GASTROENTERITIS AGENTS

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Hitit University, Molecular Biology and Genetics, Çorum, Turkey [1]

OBJECTIVE

In this study, it was aimed to investigate new probiotic strains with high probiotic-properties in the presence adenovirus, which has increases the morbidity and mortality rate among 0-5 year old children and has gastroenteritis development effect but not known as important as rotavirus.

METHODS

This study was used 21 fecal samples collected from children with gastroenteritis for isolation of bifidobacteria' strains as novel probiotic-bacteria with superior properties. ELISA used to determination of adenovirus in fecal samples. For the identification, API 20A and fructose-6-phosphate-phosphoketolase test were done. EPS-production, agglutination, acid and bile tolerance, bile salt deconjugation and competition with pathogenic-bacteria as adherence to the epithelial-surface were tested to determine the probiotic-properties. Study results compared with strains which isolated from 12 samples collected from healthy children's feces.

RESULTS

Six-isolates were identified from children's feces with gastroenteritis (4-*B. breve* and 2-*B. longum*) and 9 isolates were identified from healthy children's feces (6-*B. breve*, 2-*B. bifidum* and 1-*B. longum*). All strains were observed to have probiotic properties at different ratios. The probiotic-effects of bacteria isolated from children with gastroenteritis were found to be better than those of healthy.

CONCLUSION

The new microbiological studies emphasize the importance of the whole body flora, especially the intestinal flora. It may be possible to develop diets that can contribute to the treatment of gastroenteritis and other diseases by discovering probiotics that can sustain their survival even in the presence of pathogens and can bring out a number of probiotic properties, in the direction of the data obtained.

LIPOPOLYSACCHARIDE AND PROBIOTIC BACTERIA EFFECT ON RAT JEJUNUM VILLOUS EPITHELIUM BY TRANSMISSION ELECTRON MICROSCOPY

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TOPIC
Gut microbiota and metabolism

Introduction
Increased permeability of the intestinal mucosa is the main risk factor for the development of infection by bacterial translocation. The destruction of balance between reactivity and tolerance to microorganisms of the intestinal lumen-probiotic bacteria leads to the development of inflammation process.

AIM
Morphometric analysis of villous epithelium of rat jejunum ultrastructure under the influence of lactobacilli and LPS for determining the density of bacterial populations and evaluation the process of bacterial biofilms formation on the mucous membranes surface.

METHODS
Lactobacillus plantarum 8PA3 and LPS. Wistar rats were used in this study. The effect of probiotic bacteria and LPS on epithelial cells were carry out by removing segments of rat jejunum with subsequent incubation with cultures of bacteria, endotoxin and their complex.

RESULTS
Electron microscopy revealed the initial stages of attachment of bacterial biofilms formation on the rat's jejunum epithelium when lactobacilli and LPS were added to the lumen. The intercellular space and the absence of destructive changes in the area of tight contacts were detected in enterocytes under LPS influence. Effect of lactobacilli was in appearance of bacteria cells within membrane structures in the villous epithelium cytoplasm. Complex of LPS and lactobacilli influenced on enterocytes intercellular space were not detected.

CONCLUSIONS
The data indicate the nature of lactobacilli and LPS effects on the ultrastructure of enterocytes, which will allow to determine investigation the LPS role in the translocation of probiotic bacteria through the jejunum epithelium and the participation of LPS in the transport of microorganisms and macromolecules.

THE EFFECT OF DIETARY FIBRE SUPPLEMENTATION ON CLINICAL MANIFESTATIONS AND OESOPHAGEAL FUNCTION IN NON-EROSIVE REFLUX DISEASE PATIENTS WITH LOW DIETARY FIBRE INTAKE

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OBJECTIVE
Epidemiological data provide evidence of positive effect of fibre-enriched diet on GERD, but there is lack of interventional studies assessing the effect of dietary fibre on esophageal motility. Aim of the study was to investigate the effect of dietary fiber on symptoms and esophageal function testing parameters in non-erosive GERD (NERD) patients (NCT01882088).

METHODS
Thirty non-erosive NERD patients (18 men, 12 women, age (M±m) 34.7±9.28 yrs; BMI (M±m) 26.7±6.82 kg/m2) were examined using standard food frequency questionnaire and in case of <20 g/day dietary fibre intake underwent high-resolution esophageal manometry and 24-hours esophageal pH-impedance. The number of reflux symptoms per week and their intensity were studied using adapted to the Russian language GIS and GERD-Q questionnaires and Lickert scale. Repeated examinations were performed after 10 days of treatment consisted of standardized meal and dietary fibre supplement (Mucofalk® (Dr. Falk PharmaGmbH, Germany) 15.0 gram per day TID as an equivalent of 9.75 g of hydrophilic dietary fiber). No PPIs and prokinetics were allowed during the course of treatment, antacids were allowed when needed. The obtained data were analyzed using non-parametric statistics.

RESULTS
Dietary fibre supplementation was well tolerated, no significant adverse event was observed. Complete resolution of heartburn was achieved in 16 (53.3%) participants after the 10-day course of treatment. Significant decrease in heartburn frequency and intensity was found during the study (Table 1). Data of esophageal function testing are shown in the table 1.

CONCLUSIONS
Dietary supplementation with Mucofalk 15.0 gram per day TID is well tolerated by non-erosive GERD patients. Some of NERD patients with low dietary fibre consumption may benefit from dietary fibre supplementation for the reduction of symptoms' frequency and intensity. Larger studies are needed to confirm the obtained results.

Table 1 Results of 10 day-dietary fibre supplementation on GERD symptoms and esophageal function testing

	Before M±m	After M±m	P
GERD-Q	10.9±1.7	6.0±2.3	0.000003
Heartburn frequency (a week)	8.1±7.2	1.5±2.2	0.000004
Heartburn intensity (by Lickert scale)	4.6±2.5	0.7±0.9	0.000008
Number of gastroesophageal refluxes	67.9±17.7	42.4±13.5	0.000002
Number of acid refluxes	43.2±14.6	30.3±15.3	0.002
Number of weak acid refluxes	23.9±11.7	11.33±8.3	0.000016
High gastroesophageal refluxes	23.1±9.2	12.2±6.6	0.000004
% of time pH<4 at 5 cm above EGJ	5.5±4.8	5.5±7.6	0.2
Maximal duration of reflux, min	10.6±12.0	5.3±3.7	0.02
Mean lower esophageal sphincter (LES) resting pressure, mm Hg	18.0±10.85	29.9±15.5	0.000002
Minimal LES resting pressure, mm Hg	5.8±6.1	7.9±9.8	0.01

INVESTIGATION OF COMPETITION BETWEEN STAPHYLOCOCCAL MEMBERS OF THE HUMAN NASAL MICROBIOTA AND MULTI-RESISTANT S. AUREUS

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OBJECTIVE

The human body is inhabited by trillions of microbes that carry out a number of metabolic reactions that are necessary for human health and help preventing colonization by external pathogens. A typical example is how the nasal microbiota protects humans against carriage of multi-resistant *Staphylococcus aureus*. Here, we aim to develop an *in vitro* experimental system to investigate the competition between commensal and pathogenic staphylococci.

METHODS

First, we screened the nasal micorobiotas of Egyptian individuals by extracting DNA from nasal swabs then sequencing the V4 region of the 16S rRNA gene using Illumina MiSeq, and finally by bioinformatics analysis (QIIME software). Second, we conducted competition experiments between *S. aureus* and *S. epidermidis* in solid and liquid media.

RESULTS

We found that *Staphylococcus* was the most abundant genus in three of the analyzed samples. The microbial composition of three out of five samples was quite similar, while the other two had divergent microbial profiles. Interestingly, no geographical signal was detected, as the nasal samples from Egyptian individuals clustered with Human Microbiome Project nasal samples. In competition assays, no inhibition was observed on solid media, regardless of the method used. In liquid media, S. aureus failed to grow when *S. epidermidis* was cultured 12 hours earlier. On the other hand, whenever *S. aureus* was cultured first, S. epidermidis growth was inhibited. We also showed that the filtered supernatant of *S. epidermidis* inhibits *S. aureus* growth.

CONCLUSIONS

In conclusion, a mutual competition between the two *Staphylococcus* species was observed, and its specific mechanism is being investigated.

NEUROPROTECTIVE ACTIVITY OF CONJUGATED LINOLEIC ACID IN AN ANIMAL MODEL OF AUTO-IMMUNE DISEASE

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OBJECTIVE

MRL/MpJ-Faslpr (MRL/lpr) mouse is an animal model of neuropsychiatric lupus, in which autoimmune disease is accompanied by neurodegeneration. Conjugated Linoleic Acid (CLA) has been demonstrated to down-regulate autoimmune/oxidative stress signs in non-brain organs of MRL/lpr mouse through the activation of Nuclear factor-E2-related factor 2 (Nrf2) pathway and the downstream modulation of antioxidant/detoxifying enzymes (phase 2), however little is known about its neuroprotective ability.

METHODS

Blood/ brain RedOx status (Total Antioxidant Capacity; GSH/GSSG ratio; carbonylated protein, PC) and Nrf2 marker (phase 2 enzyme) were measured in brain cortex of young and old MRL/lpr mice (8-10 and 20-22 weeks of age, respectively) (n= 10/group) by combining enzymatic, immunoblotting and RT-PCR assays. Next, to evaluate neuroprotective ability of CLA, Neurodegenerative markers (Fluoro Jade B, FJB; Glial Fibrillar Acidic Protein, GFAP) were additionally evaluated on brain cortex sections prepared from old mice orally pretreated (for 5-weeks) with CLA (650 mg kg-1 body weight)(CLA+Old). Untreated old or BALB/c mice (n= 6/group) were used as diseased or healthy control, respectively.

RESULTS

Oxidative stress (in blood and brain), neurodegenerative signs and Nrf2 hyper-activation in old mice were significantly higher in old mice as compared to young animals. Notably, neuron degeneration and oxidative stress/Nrf2 markers were inhibited by CLA pretreatment and their levels were comparable with those measured in BALB/c mouse.

CONCLUSIONS

Our results indicate that Nrf2 hyper-activation accompanies the age-dependent neurodegeneration in MRL/lpr brain and both processes can be attenuated by dietary supplementation with CLA via the modulation of animal RedOx status.

PREBIOTIC POTENTIAL EVALUATION OF AGAVE FRUCTAN FRACTIONS USING AN EX VIVO SYSTEM: A COMPARISON BASED ON POLYMERIZATION DEGREE

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OBJECTIVE

To evaluate the prebiotic potential of Agave fructan fractions with different degree of polymerization (DP).

METHODS

Fructans from Agave tequilana var. Cenizo collected in Jalisco, Mexico, were evaluated. Two fractions: medium MDP (10-22 fructose units) and high HDP (22-60 fructose units) were studied. Potential prebiotic was assessed by the ARIS ex vivo system, a human digestive tract simulator with five reactors corresponding consecutively to stomach, small intestine, ascending colon, transverse colon and descending colon. ARIS was operated under controlled physiological conditions (pH, swallowing, residence time and temperature) to resemble in vivo conditions (Molly et al., 1993). Colon reactors were inoculated with fecal bacteria from 20 healthy adults. Fructan samples (1g/day) were added separately in the system and it was fed for 9 consecutive days. Four bacterial groups were evaluated; Lactobacillus, Bifidobacterium, Clostriduim and Salmonella. Bacterial counts were performed on days 0, 4 and 9.

RESULTS

Lactobacillus genus increased after MDP fructans administration on days 4 and 9. Aditionally Salmonella decreased (4.0X108 to 9.0X107CFU). In the case of HDP fructans, Bifidobacterium increased in the transverse colon (4.2X105 to 8X106CFU) and Clostridium genus decreased in the ascending colon at day 4 (7.2X107 to 4X106CFU). Furthermore, at day 9 there was an increase of Lactobacillus and Bifidobacterium, additionally Salmonella diminished in two colon sections

CONCLUSIONS

HDP Fructans showed higher prebiotic potential compared to MDP. Both fractions showed a better prebiotic effect and pathogen decrease at day 9 after administration compared to day 4.

SUPPLEMENTATION WITH A FOODBORNE COMPLEX MICROBIAL COMMUNITY EXERTS PROTECTIVE ACTIVITY ON OBESITY-ASSOCIATED INFLAMMATION IN A MURINE MODEL

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OBJECTIVE

Probiotic supplementation was suggested to counteract obesity-associated alterations, and fermented foods represent a natural source of live bacteria with probiotic features. The aim of this work was to evaluate the impact of a complex foodborne bacterial consortium on obesity-associated inflammation and gut microbiota composition.

METHODS

Mice fed a 45% high fat diet (HFD) were supplemented with $1x10^9$ CFU/day of either microbiota derived from «Mozzarella di Bufala Campana» (MBC), or the probiotic strain *Lactobacillus rhamnosus* GG (LGG). Serum metabolic parameters, white adipose tissue (WAT) inflammation and faecal microbiota composition were evaluated.

RESULTS

Reduced triglyceride and higher HDL-cholesterol levels were detected in MBC mice, accompanied by increased regulatory T and $CD4^+$ cells in WAT leukocyte subpopulations, as well as decreased macrophage and $CD8^+$ cell numbers associated with lower levels of pro-inflammatory cytokines and chemokines. Increased *Firmicutes/Bacteroidetes* ratio was observed in all mice groups following HFD, irrespective of bacterial supplementation.

CONCLUSIONS

The protective capacity of MBC microbiota toward HFD-induced fat accumulation and inflammation suggests a stronger effect of supplementation with a mixed microbial consortium vs single-strain probiotics. The immunomodulatory activity could be due to synergistic interactions within the microbial consortium, highlighting the important role of dietary microbes with yet uncharacterised probiotic effects.

NEUROPROTECTIVE ROLE OF NATURAL POLYPHENOLS FROM SAMBUCUS NIGRA

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OBJECTIVE

A growing body of evidence suggest that the intake of polyphenols may contribute to counteract the neuronal injuries that trigger neurodegeneration, slowing the progression of the disease. The objective of this study was to demonstrate the potential neuroprotective effect of polyphenols derived from Sambucus nigra berries in a cellular model resembling Alzheimer's disease, the most common form of dementia characterized by accumulation of amyloid plaques, neurofibrillary tangles, neuroinflammation and oxidative stresses.

MATERIALS AND METHODS

To reproduce the neurodegenerative process, we used differentiated human neuroblastoma cell line, IMR-32, inducing neuronal damage by two specific toxic agents (beta amyloid and hydrogen peroxide). We verified the neuroprotective potential of Sambucus nigra methanolic extract measuring cell viability and evaluating its ability to reduce oxidative stress and apoptotis induced by neurotoxic agents.

RESULTS

The methanolic extract derived from elderberry Sambucus nigra, possessing elevated polyphenolic content and antioxidant capacity, was able to protect the differentiated IMR-32 cells, in a dose dependent manner, against apoptosis induced by both beta amyloid and hydrogen peroxide. Moreover, it reduced, through an "adaptive response", the oxidative stress induced by neurotoxic injury.

CONCLUSIONS

These results represent an initial indication that Sambucus nigra extract may find a space in future pre-clinical and clinical studies addressed to prove the preventive and/or therapeutic potential of this natural extract against neurodegenerative disease.

NEUROTROPHINS' MODULATION BY OLIVE POLYPHENOLS

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OBJECTIVE

Polyphenols are probably the most known and investigated molecules of nutritional interest as micronutrients present in abundance in our diet. Some of the most important food sources of polyphenols in the Mediterranean diet are olives and olive oil. A growing body of evidence from animal models to clinical studies indicates that polyphenol compounds may have neuroprotective effects in several pathologies of the nervous system through the control of oxidative stress, inflammation, apoptosis and mitochondrial dysfunction.

METHODS

Based on the most recent scientific literature, dietary intake of polyphenols attenuates oxidative stress and reduces risk for related neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, stroke, multiple sclerosis and Huntington's disease. Also at the peripheral level, they act as antioxidant, defending tissues against oxidative damage and scavenging free radicals.

RESULTS

Recent findings in animal models and humans show that polyphenols may have a role in regulating neurotrophins levels, in particular nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), suggesting that polyphenols may also induce their protective effects through the potentiation of neurotrophins action. NGF and BDNF, primarily known as biological mediators stimulating neuron growth, proliferation, survival and differentiation are recently studied also as metabotrophic factors, acting on glucose and energy metabolism, pancreatic beta cells and cardiovascular homeostasis.

CONCLUSIONS

In this context, a better understanding of the effects of polyphenols on neurotrophins and their receptors (TrkA, TrkB, p75NTR) could certainly generate interest for drug discovery and also for the potential dietary prevention of several neurological and cardiometabolic diseases.

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THERAPEUTIC PROSPECTS OF LACTOBACILLUS RHAMNOSUS GG (LGG) POSTBIOTICS ON INFECTIVE DAMAGE OF HUMAN COLONIC MUSCLE

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Recent studies have demonstrated the ability of secretory products (postbiotics) of Lactobacillus rhamnosus GG (LGG), grown under aerobic conditions, to protect human colonic smooth muscle cells (SMCs) from lipopolysaccharide (LPS)-induced oxidative damage. The supernatant effects depended on the culture phase, the higher protection observed after 24 hours. The aim of study is to verify if postbiotic LGG activity was preserved in experimental conditions similar to in vivo human colon milieu.

LGG (ATCC53103 strain) was cultured in MRS medium in aerobic, anaerobic and microaerophilic conditions and supernatants recovered after 12, 24, 48 hours. Primary human colonic SMCs cultures were exposed to purified LPS of a pathogenic strain of E. coli O111: B4 (1µg /ml) for 24 hours, in the presence and absence of supernatants. Postbiotics effects were evaluated on morphofunctional alterations and cellular antioxidant capacity. Data are expressed as mean±SE ($p<0.05$ significant).

The higher protective effects were observed with supernatants recovered from anaerobic culture after 12-hours. In the presence of 12 and 24h-supernatants, LPS-induced cell shortening was inhibited by 81.56%±7.27 and 83.39%±6.49 while the impaired Ach-induced contraction by 48.79±5.19 and 39.6±17.04 respectively. The LPS-impaired antioxidant capacity was recovered slightly by the 12h-supernatants but increased 5 times with 24h-ones. The protective effects of supernatants collected from aerobic and microaerophilic cultures were less evident.

In conclusion, LGG maximal postbiotic activity is expressed in anaerobic conditions similar to the in vivo colonic milieu. Future translation studies will validate the possible use of LGG and relative postbiotics in the therapy of post-infective gastrointestinal disorders.

DPPIV ACTIVITY OF THE GUT MICROBIOTA: A NEW TARGET FOR THE INTESTINAL HOMEOSTASIS?

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OBJECTIVE

The pharmacological inhibition of the DPPIV activity has been proposed to preserve the active form of gut peptides. Interestingly, some bacteria also express DPPIV-like activity. Our objective was to evaluate if vildagliptin (DPPIV inhibitor) targets the DPPIV-like activity of the gut microbiota and impacts its composition and/or functionality.

METHODS

Twenty-seven C57BL/6 mice were fed with control diet, high fat diet (HFD) or HFD + vildagliptin during 8 weeks with vildagliptin firstly administered for 2 weeks and then co-administrated with HFD for 6 weeks.

RESULTS

Vildagliptin significantly reduced the DPPIV activity in the cecal content and liver. Illumina 16S rRNA sequencing revealed that vildagliptin caused changes in the gut microbiota composition consisting in decreases in *Oscillibacter* and increases in *P. goldsteinii*. Besides, an increase in *L. johnsonii/gasseri* was observed (qPCR analysis). The *in vitro* exposure of *Oscillibacter* to vildagliptin inhibited its growth, however the mechanism does not imply the DPPIV inhibition. In the ileum, HFD reduced the crypts depth and expression (mRNA) of antimicrobial peptides but these effects were counteracted by vildagliptin. In the liver, vildagliptin reduced the expression of immune cell markers and cytokines. The *ex vivo* exposure of liver slices showed that these reductions were not related to a direct effect of vildagliptin.

CONCLUSION

Our study innovates in considering the DPPIV activity of the gut microbiota as a target of DPPIV inhibitors. We propose that vildagliptin, in addition to systemic effects, also improves the intestinal homeostasis. Futher studies are needed to unravel its interest to tackle gut dysfunctions.

BETALAINS, PHENOLS AND ANTIOXIDANT CAPACITY IN CACTUS PEAR (OPUNTIA FICUS-INDICA (L.) MILL.) FRUITS AND CLADODES FROM APULIA (SOUTH ITALY) GENOTYPES

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OBJECTIVE

Betanin, phenolics, and ascorbic acid content and antioxidant capacity (by TEAC and ORAC assays) were investigated in two differently colored cactus pear (Opuntia ficus-indica (L.) Mill) fruit varieties, 'Rossa' (purple) and 'Gialla' (orange), from Apulia (South Italy). Moreover, recent investigations have been carried out on phenolic compounds of cactus pear cladodes (purple fruit variety).

METHODS

Cactus pear fruit and cladode were extracted by aqueous methanol. Betanin quantification in cactus pear fruit extracts was done by HPLC using Amaranthus retroflexus inflorescence purified extract as a standard (because of the lack of commercial standard with high purity and the presence of two isomers in cactus pear fruit extract).

RESULTS

'Rossa' variety showed very high betanin level, less carbohydrates (glucose and fructose), but more phenolics, ascorbic acid, TEAC and ORAC than 'Gialla'. The contribution of betacyanin in 'Gialla' variety fruit was scarce (3.6 mg/100g FW). Phenolics were differently expressed in 'Rossa' (89 mg GAE/100 g FW), in respect to 'Gialla' variety (69 mg GAE/100 g FW), and this can account for the greater contribution to TEAC and ORAC values.

Interestingly, the phenolic content of cladode extract was much higher than in the fruits (500 mg GAE/100 g FW) and consequently the antioxidant capacity was greater.

CONCLUSIONS

These findings confirm the potential of cactus pear, particularly the purple variety from Apulia, as an important source of dietary antioxidant components which may exert beneficial effects on consumer's health (for fruit) or an economic source of antioxidant compounds (for cladode).

EFFECT OF MATRIX MODIFICATION ON DRYING AND SYMBIOTIC SYNERGY OF MICRO-SPHERES CONTAINING PROBIOTICS

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OBJECTIVE

Microencapsulation serves as a novel approach for sustaining gut health with association of probiotics. Recent interventions on probiotics involoves matrix modification and selection criteria which helps in elucidating the intrinsic and extrinsic factors that play an important role in shelf life of any microcapsules containing probiotics. The present study was undertaken to encapsulate indigenous and to investigate the symbiotic importance of starch, malto-dextrin and sodium alginate as coating material effecting shelf life and targeted release.

METHODS

Microcapsules were developed in solution of calcium chloride (0.1M). For matrix modification starch (0.75g), maltodextrin(0.38g) and sodium alginate(1.5g) was gelatinized until complete mixing. Developed matrix was mixed with 5% harvested cell biomass of probiotics and encapsulation was done using a syringe. Microcapsules were dried using Freeze drying, vaccum drying and conventional drying methods. Probiotic potential was evaluated as per ICMR guidelines 2011. Physical characteristics were studied through x-ray diffraction and optical microscopy.

RESULTS

The symbiotic association of probiotics with modified coating material elucidated, change in degree of crystallinity attained after drying. The study showed the importance of drying techniques on intrinsic/extrinsic environment of probiotics which is deeply governed by the chemical interaction and the partial amorphous/crystalline nature of coating material thus effecting shelf life. Freeze dried microcapsules were found having good probiotics potential in comparison to vaccum dried and conventional dried microcapsules. Gelatinization and chemical interaction of developed microcapsules can effect shelf life of the product thus effecting the symbiotic synergy of microcapsules.

CONCLUSION

Probiotic potential was maintained better in freeze drying than vaccum and conventional drying. This may have significant future applications for development of new formulations and products.

KEYWORDS

microencapsulation, drying, probiotics, symbiotic, synergistic

CORYLUS AVELLANA IMMUNOMODULATORY EFFECT ON HUMAN MONOCYTE-DERIVED-MACROPHAGES

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OBJECTIVE

Different studies described the health benefits of *Corylus avellana* and hazelnut bioproducts showing its antioxidative, hypocholesterolemic, cardioprotective and anti-inflammatory properties. Besides, no data exist on the hazelnut ability to activate human macrophages vs infection of *Staphylococcus aureus*, one of the most nosocomial-associated bacterium. The present study aimed to clarify whether *C.avellana* extract is able to potentiate human innate response vs *S.aureus* infection.

METHODS

We performed the transcriptional analysis, by q-rt RT-PCR, of a group of 29 genes associated to inflammation, innate immune response and iron metabolism, of monocyte-derived-macrophages (MDM) of healthy donors (a) only treated with *C.avellana* extract, (b) only infected with S.aureus and (c) treated - infected vs control MDMs, with three different doses of extract.
We also measured the S.aureus intracellular bacterial growth by CFU determination. The *C.avellana* extract composition was analysed by Metabolomic analysis.

RESULTS

MDMs treatment with *C.avellana* extract improved the containment of intracellular *S.aureus* replication and this reduction was coupled with the induction of genes involved in inflammation checkpoints (as CD39 and AdaR2b), while the inflammatory signalling induced by S. aureus was regulated by repressing pro-inflammatory cytokines (as TNF-alfa and IL-6).
The initial inflammatory status of donors' PBMCs (evaluated by q-rt for TNF-alfa and IL-6) affected their MDM response to the D1-D3 doses of extract.

CONCLUSIONS

C.avellana extract stimulated human macrophage response to a bacterium epidemiologically relevant, due to the diffusion of its antibiotic resistant strain (MRSA), and might suggest the development of this nut extract as an anti-inflammatory and immune regulatory coadjutant.

EX VIVO AND NUTRITIONAL EVALUATION OF A SYNBIOTIC ON THE IMPACT OF GUT MICROBIOTA AND BODY COMPOSITION IN OBESE SUBJECTS: STUDY REFERENCED IN BLOOD SIBLING

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OBJECTIVE

To evaluate the effect of a synbiotic on gut microbiota (GM) and body composition (BC) obese subjects.

METHODS

The symbiotic contained agave fructans and four bacteria; *Lactobacillus Paracasei*, L. gasseri, L. plantarum and L. rhamnosus.
This study was performed in two sections; ex vivo evaluation (EE) and nutritional intervention (NI).
Faecal samples (FS) of ten couples of human blood siblings: one obese (study population) body mass index ≥30kg/m2, and one non-obese (control) body mass index ≥18.5-≤24.9kg/m2, were collected.
The EE was conducted in a system that simulates human digestive tract, it consists of five reactors adapted to the conditions of: stomach, small intestine, ascending colon, transverse and descending. It was inoculated with obese microbiota and administered the synbiotic 9 consecutive days. Nutrient bioavailability and four bacterial groups (BG): *Lactobacillus, Bifidobacterium*, *Salmonella* and *Clostridium*, were monitored.
The NI consisted in administration the synbiotic to the obese subjects during three months, to evaluated BC was measured: waist, hip, weight and adipose folds of biceps and triceps. Was analysed GM composition pre and post synbiotic administration.

RESULTS

Post synbiotic administration in obese population and EE system. Was observed significant increase of *Lactobacillus* and modified levels of the other BG.
The NI showed reduction of weight, waist and hip measurements.
The bioavailability of carbohydrates and lipids decreased, while protein bioavailability increased.

CONCLUSIONS

The synbiotic showed significant changes in GM modulation, could be related to the decrease in weight, waist and hip of obese subjects.

NEW PROBIOTIC WHEY PROTEIN FORTIFIED BEVERAGE ENRICHED WITH BIFIDOGENIC FIBERS

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In this work a new whey protein-based beverage fermented with bifidobacteria and enriched with dietary prebiotic fibers was developed. Assayed strains, belonging to *Bifidobacterium animalis* subsp. *lactis*, *B. breve* and *B. pseudocatenulatum* species, showed cell counts significantly higher ($P<0.05$) when Whey-Based Medium (WBM), containing 10 g L^{-1} of lactose, was supplemented with 20 g L^{-1} instead of 10 g L^{-1} of whey proteins.
Based on these results, WBM20 was enriched with 10 g L^{-1} of inulin or resistant starch. After 48h of fermentation at 37°C only two among tested strains, displayed a faster and higher growth (up to 8 log cfu mL^{-1}) in comparison with WBM20 containing only lactose. These fermented whey-based beverages did not show significant reduction in total protein content and prebiotic fiber concentrations.
After 30 days of cold storage, bifidobacteria viability showed an average decrease of 3 log cfu mL^{-1} and a significant reduction in the concentration of small peptides and amino acids was found. As concerns prebiotic fibers, the concentration of inulin halved, whereas that of resistant starch remained unchanged. The sensory analysis always attributed average higher score to the whey protein-based beverage containing resistant starch.

EFFECT OF TOTAL APPLE POLYPHENOLS EXTRACT AS INHIBITORS OF AMYLOID PROTEIN AGGREGATION

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OBJECTIVE

Recent studies have reported that some polyphenols are capable to redirect the aggregation of amyloidogenic peptides, such as the β-amyloid peptide involved in Alzheimer's disease and α-synuclein involved in Parkinson's syndrome, leading to the formation of non toxic amorphous aggregates.
The purpose of our work is to investigate the protective molecular mechanisms exerted by the total polyphenolic pool extracted from apple fruits on the amyloid aggregation process by using as a model system of amyloid forming protein that is κ-casein from bovine milk.

METHODS

Polyphenols extracted from apple cultivar 'Fuji' grown in Sicily (Italy) were used. Biophysical techniques as Fluorescence Spectroscopy, Light Scattering and Atomic Force Microscopy, and bioinformatics tools were used to investigate the effects exerted by apple polyphenols on the aggregation process of κ-casein.

RESULTS

We found that polyphenols extracted by the apple peel of the cultivar 'Fuji', inhibited fibril formation in the κ-casein in a dose-dependent way. In particular, peel polyphenolic extract significantly reduced the protein aggregation rate, and the κ-casein aggregated species formed in the presence of polyphenols were different both in number and in morphology.

CONCLUSIONS

The study suggests to investigate the action of natural polyphenols as potential therapeutic agents for amyloidosis, supporting the use of small molecules inhibiting amyloid aggregation, that is one of the most modern strategies searched for the treatment of neurodegenerative disease.

EFFECT OF "IFLORA" AS A PROBIOTIC ON PENTYLENETETRAZOL-INDUCED SEIZURES IN RATS

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Founding member of Iranian society of probiotics and functional foods and Faculty member of IAU, Microbiology department, Science faculty, Arak branch, Arak, Iran [1] - IAU, Microbiology department, science faculty, Arak branch, Arak, Iran [2] - IAU, Biology department, science faculty, Tehran central bracnh, Tehran, Iran [3]

% of N source	0.1	0.2	0.3	0.4
$(NH_4)_2SO_4$	1.89	3.78	5.67	7.56
$(NH_4)Cl$	0.76	1.52	2.28	3.04

NOVEL STRATEGIES THAT ENHANCE THE BIOAVAILABILITY OF PROBIOTICS FOR THERAPEUTIC USE DURING INFLAMMATORY BOWEL DISEASE

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OBJECTIVE

While probiotics are a multi-million dollar enterprise, traditional probiotics are not robust or bioavailable during inflammatory bowel disease (IBD) due to poor colonization, as well as the highly oxidized conditions found within the inflamed gut. Natural probiotics need to be optimized for full bioavailability and efficacy. We have designed novel next-generation probiotics that colonize and survive the IBD gut. We hypothesize that our novel designer probiotics will result in better efficacy of probiotic therapy against IBD.

METHODS

The probiotic strain *Escherichia coli* Nissle 1917 was genetically modified to carry the ttr operon allowing it to utilize tetrathionate to survive oxidative stress. Lactobacillus reuteri DSM 20016 was modified to express N-acetylglucosamine binding protein A to enhance adhesion. C57BL/6 mice (n=10) were given either of the two designer probiotics or the unmodified parent strains via oral gavage and then challenged with 3.5% DSS via drinking water for 7 days to induce DSS-induced murine colitis. Intestinal immune responses were analysed.

RESULTS

Designer probiotics were more efficacious during colitis compared to the unmodified parent strains showing improved morbidity, reduced histopathology, and increased protective responses including butyric acid levels, Reg 3 gamma and Muc2 gene expression.

CONCLUSIONS

Novel designer probiotics are more protective against murine colitis. This research could result in improved probiotics for therapeutic use in IBD patients.

BIFIDOBACTERIUM ANIMALIS SSP. LACTIS 420 WITH OR WITHOUT POLYDEXTROSE CONTROLS BODY FAT MASS AND WAIST CIRCUMFERENCE IN OVERWEIGHT AND OBESE ADULTS - RANDOMIZED CONTROLLED CLINICAL TRIAL

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OBJECTIVE

Gut microbiota and host energy metabolism are interconnected, but causal evidence proving gut microbiota could affect body fat mass is scarce. Preclinical studies have shown that *Bifidobacterium animalis* ssp. lactis 420 (B420) can improve metabolic health and reduce fat mass. To investigate the effect of B420 on obesity-related parameters in humans, we performed a randomized, controlled, double-blinded clinical trial with B420 and/or Litesse®Ultra polydextrose (LU) (Clinicaltrials.gov NCT01978691).

METHODS

In total, 225 healthy participants (BMI 28-34.9) maintaining their habitual diet and exercise routines, were randomized into four groups for 6 months of parallel treatment: 1) Placebo; 2) LU, 12 g/d; 3) B420, 10^{10} CFU/d; 4) LU+B420, 12 g + 10^{10} CFU/d. Body composition (DXA) and anthropometric measurements were taken at 0, 2, 4 and 6 months of intervention, and after 1-month-follow-up period.

RESULTS

Per Protocol population included 134 subjects and improvement in weight management was noted for B420 and LU+B420 treatments. For LU+B420, body fat mass change was -4.5% (-1.4 kg, P=0.02 vs. Placebo), while LU and B420 alone had no effect. However, a post-hoc factorial analysis showed a significant decrease for B420 (–4.0%, P=0.002, vs. Placebo). Changes in fat mass were most pronounced in the abdominal region with similar changes in waist circumference.

CONCLUSIONS

His study, the largest and most strictly controlled clinical trial to date, demonstrates that both B420 and LU+B420 show benefits for controlling body fat mass, waist circumference and food intake compared to placebo.

SHAPE GUT MICROBIOTA, SHAPING OUR BODY COMPOSITION

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OBJECTIVE

To evaluate the effects of a dysbiotic corrector on intestinal microbiota and body composition in obese patients.

METHODS

A double-blind study with 99 obese patients (BMI >27 kg/m2) without a diagnosis of chronicle degenerative diseases was performed. Study consisted in the consumption of a dysbiotic corrector provided by Grupo Mabiosis S.A. de C.V. for three months without changing their lifestyles and eating habits. There were two groups randomly selected, group 1 (GP1) that consumed the product, and group 2 (GP2) treated with a placebo. Changes in intestinal microbiota composition were evaluated following four bacterial groups (*Lactobacillus spp, Bifidobacterium spp, Clostridium spp, Salmoneloides*) using selective growth mediums. Anthropometric measurements like weight (W), waist circumference (WC) and hip circumference (HC), were taken according to ISAK's (International Society for the Advancement of Kinanthropometry) protocol. Intake evaluation (24 hours reminder) was applied to know eating habits and a blood chemistry of 35 elements for knowing the metabolic status.

RESULTS

There was no difference between GP1 and GP2 in anthropometric parameters, although in GP1 differences were found in patients metabolically stables (MS) in W, WC, HC (-1.12 kg, -4.48 cm, -3.55cm) and a reduction in energy intake (kcal) (-304 total kcal) (-91.3 kcal from carbohydrates, -29 kcal from proteins and -207 kcal from lipids)), additionally a decrease in *Salmoneloides* (3.84x107 to 9.64x105 UFC/g) was observed between the initial data and the end of the study.

CONCLUSIONS

This dysbiotic corrector employed, could be used as a support treatment to treat metabolically stable obese patients.

EXPOSURE OF LACTOBACILLUS BULGARICUS AND LACTOBACILLUS CASEI TO 2.4 GHZ WI-FI RADIOFREQUENCY RADIATION ENHANCES THE GROWTH OF THESE PROBIOTIC BACTERIA

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OBJECTIVE

Probiotic products must contain more than 10 million living probiotic microorganisms per gram. Over the past several years, our lab has focused on the health effects of exposure to different sources of electromagnetic fields. Furthermore, we have recently explored physical methods for converting drug-resistant bacteria to drug-sensitive. The main goal of this study was to assess the bioeffects of short term exposure of Lactobacillus acidophilus and Lactobacillus casei to 2.4 GHz radiofrequency (RF) radiation emitted from a common Wi-Fi router on the proliferation of these probiotic bacteria.

METHODS

Pure culture strains of Lactobacillus acidophilus and Lactobacillus casei obtained from (Chris- Hansen Denmark). Samples were exposed to electromagnetic radiofrequency radiation (EMRR) emitted from a 2.4 GHz Wi-Fi router for 15, 30, 45 and 60 minutes at a distance of 5 cm from the router antenna. The control samples were sham-exposed to EMRR. All samples were grown in MRS broth at 37 °C for 18 hours. Cell counts were enumerated after 72 hours of incubation on MRS agar. The method of counting colony forming units (CFU) was used to assess the proliferation of bacteria.

RESULTS

The growth of Lactobacillus acidophilus in samples exposed to EMRR for 15, 30, and 45 minutes showed statistically significant increases (P=0.003, P=0.002, P=0.001, respectively) compared to those of sham-exposed bacteria. In this experiment, there was no difference between the growth in samples exposed to EMRR for 60 minutes and sham-exposed bacteria. On the other hand, in a similar pattern, while there was no difference for samples exposed/sham-exposed to EMRR for 15 and 45 min, the growth of Lactobacillus casei in samples exposed to EMRR for 30, and 60 minutes showed statistically significant increases (P=0.006 and P=0.004, respectively) compared to those of sham-exposed bacteria.

CONCLUSIONS

This study showed that short term exposure of Lactobacillus acidophilus and Lactobacillus casei to 2.4 GHz radiofrequency (RF) radiation emitted from a common Wi-Fi router significantly increases the proliferation of these probiotic bacteria. Further research in this field can open new horizons in probiotic food industry through stimulation of bacterial growth.

KEYWORDS

Radiofrequency (RF), Non-Ionizing Radiation, Probiotic Bacteria, Wi-Fi

OBTAINING EFFECTS OF AN ANTI-CANCER FORMULATION BASED ON A PROTEIN SOLUBLE HYDROLYSATE (PSH) OBTAINED FROM THE PROBIOTIC BACTERIA L. FERMENTUM DSM32448.

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OBJECTIVE

This study aimed to evaluate the effects of an anti-cancer formulation based on a protein soluble hydrolysate (PSH) obtained from the probiotic bacteria *L. fermentum* DSM32448.

METHODS

For PSH obtaining, actives cultures of DSM32448 were ultrasonicated, soluble proteins presents in the supernatant were analyzed by Bradford, SDS-PAGE electrophoresis and dried by lyophilization, then sieved and dissolved in water. The effects of the formulation were studied in a model of cell permeability quantifying the trans-epithelial electric resistance (TEER) in Caco-2 cells, in the stimulation of anti-inflammatory cytokines produced by human peripheral blood mononuclear cells, and in a murine carcinogenesis Azoximetane/dextran-sulphate (AOM/DSS) model, along to the DSM32448 application.

RESULTS

When 13 days-old cells were stimulated with 100 ng/mL *lipopolysaccharide* (LPS); TEER decreased by 27% between 18 and 24 hours of constant stimulus, however when the cells were stimulated with 150 ug/ml of PSH and re-stimulated with 100 ng/mL of LPS during 48 hours, the product of DSM32448 increases TEER values to 222% ($p<0.05$). DSM32448 induces the production of IL-10 by human peripheral blood mononuclear cells (up to 978±169 pg/ml). Microbiologically, was observed a potential prebiotic effect of PSH in the recounts of DSM32448 in murine faeces. Histologically, the induced model shown an acceptable carcinogenesis induction.

CONCLUSIONS

We observed a significant increase in TEER values in cells stimulated with PSH and induction of production of IL-10 by DSM32448, which directly increases the permeability of epithelial mucosa, preventing epithelial inflammation and colorectal carcinogenesis. Moreover was observed a potential prebiotic effect of PSH.

POSTERS

GENERAL APPROACH FOR CONSTRUCTING DIRECTED MULTISTRAIN PROBIOTIC METABOLITE FORMULAS

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OBJECTIVE

Multistrain probiotics are adaptive to extended contingents of individuals. The aim was to propose an approach for constructing formulas of mixed probiotic metabolites.

METHODS

Our method of strains ranging according to the superiority of each parameter (physicochemical, biochemical or biological) was applied. Acilact, its ingredient strains (*L.casei* K3III24, *L.helveticus* NK1 and 100ash); bifidobacterial probiotic strains (*Bifidobacterium longum* MS-42, *B.bifidum* No1 and *B.gallinarum* GB) were from G.N.Gabrichevsky Research Institute collection of microorganisms.

RESULTS

1. Probiotic sources were ordered into 4-code sequences (ranging Acilact, K3III24, NK1 and 100ash) according to decrease of each key 34 parameters (acidic and alkaline proteins, biosurfactants, other non-protein exopolymeric compounds, oxidoreductase/ proteinase/ lectin systems, aminoacid type production, pigments, antimicrobial activities, others) to establish multiprobiotic advantages as well as the most strain contributor. Three-step-algorithm was developed for constructing lactobacillar metabolite formulas. Results supported advantages of Acilact and its ingredients and served the basis for new variant formulas of Acilact. Biological activities coupled to glycoconjugate-type lectin systems were taken into consideration. 2. The approach was extended from 4-codes to 7-codes by addition of cases of metabolites of MS-42, No1 and GB. Algorithm was developed for constructing lactobacilli-bifidobacteria mixed metabolite formulas according to advantage contributions of genera. The argued expected new relationships between strains were formulated and predicted.

CONCLUSIONS

Proposed approach and algorithms open prospects for constructing new probiotic multistrain metabolite formulas of different directions depending on the final goals.

PROBIOTIC LECTIN SYSTEMS RECOGNIZING GLYCOCONJUGATE PATTERNS

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OBJECTIVE

Probiotic bacterial lectin systems (LS) bind to glycoconjugates (GC). The aim was to evaluate pattern specificities of LS.

METHODS

Protein fractions (27-220 kD, pI 4-8) of *B.bifidum* 791, *B.longum* B379M, X, *B.pseudocatenulatum* OV-2, *L.amylovorus* BT 24/88, *L.helveticus* NK1 (No 1-6) were separated using isoelectrofocusing in polyacrylamide (PAA) gel, electroblotting on membrane. LS were stained using biotinylated Adi-, Fs-, GalNAc- or MDP- (Sugar-PAA-b; www.lectinity.com), streptavidin-peroxidase and substrate. Chemiluminescence was registered using BioChemi System (UVP).

RESULTS

1. *Properties of LS:* mosaics in protein massifs; visualization as Adi> Fs>> GalNAc; mutual completeness of Adi-LS and GalNAc-LS (staining the same components); mutual completeness of Adi-LS and Fs-LS (staining more acidic and less acidic components, respectively); weak differences between lactobacilli and bifidobacteria Fs-LS (maximally for GalNAc-LS); minor components preferentially in more acidic (for Adi) or less acidic (for Fs) region; 1-7 majors (1 for lactobacilli [No5 for Fs] or bifidonacteria [No1 for GalNAc], maximally for lactobacilli [No6 for Adi]). 2. *Expression of LS.* LS of lactobacilli: No6 (GalNAc> Adi>> Fs); No5 (LS length: Adi> Fs>>GalNAc [trace], maximal major: Fs> Adi>> GalNAc [absence]). LS of bifidobacteria: No2 (Adi> Fs>> GalNAc [absence]), No3 (Adi> Fs> GalNAc [trace]), No1 (Fs> GalNAc), No4 (Fs [trace]). 3. *Simultaneous recognition of Fs and MDP.* MDP-LS expressed in lactobacilli: No6 (maior – around pI4 [absence of components of MDP recognition in Fs-LS], component expressed in a less extent – around pI5 [components of MDP recognition in Fs-LS of lactobacilli and bifidobacteria]); No5: both pI-components (similar to No6) equally expressed.

CONCLUSIONS

Probiotic LS recognize patterns of GC, feel internal glycoside residues. Probiotic LS may serve important participants of mucosal organ functioning.

ANTIBACTERIAL ACTIVITY OF FRAGARIA VESCA ELLAGITANNIN-ENRICHED FRACTION ON HELICOBACTER PYLORI CLINICAL ISOLATES

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Helicobacter pylori (Hp) infects human stomach mucosa and is associated with diseases of the upper gastrointestinal tract. The effectiveness of most commonly recommended Hp eradication treatments has declined to unacceptably low levels. Searching for new or additional therapeutic agents is necessary to overcome treatment failures.

This study aims to evaluate the activity of one *Fragaria vesca* (Fv) ellagitannin-enriched fraction (FELGFv) on Hp clinical isolates with different susceptibility to antibiotics.

Hp isolates were obtained from gastric biopsies. Minimum inhibitory concentrations of metronidazole (MTZ), amoxicillin (AMC), clarithromycin (CL), tetracycline (TET) and levofloxacin (LVX) were determined by E-Test. FELGFv was prepared from Fv leave alcoholic extract by fractioning on a Sephadex LH-20 column eluted with aqueous methanol and acetone 70%.

Hp isolates susceptibility to FELGFv was determined by disc diffusion. Fifteen µl of concentrations 15, 10, 7.5, 5 and 2.5 mg/ml were applied into discs on blood agar seeded with a 3 McFarland Hp suspension (microaerophilic atmosphere, 37ºC/72 h). Susceptibility was considered when inhibition zone diameter (IZD) was ≥15 mm.

Twelve isolates presented full susceptibility to AMC and TET, 50% were resistant to MTZ and CL and 33% were LVX resistant.

FELGFv concentrations 15, 10 and 7.5 mg/ml inhibited all the isolates and 5 mg/ml had effect in 67%, whereas 2.5 mg/ml had no activity.

It is known that ellagitannins protect gastric mucosa against induced lesions in mice, and this study report for the first time the activity of a Fv ellagitannin-enriched fraction for Hp isolates independently of the different patterns of susceptibility.

SUSCEPTIBILITY OF HELICOBACTER PYLORI CLINICAL ISOLATES TO FRAGARIA VESCA AND AGRIMONIA EUPATORIA L EXTRACTS

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In Portugal, and worldwide, there is a great prevalence of *Helicobacter pylori* (Hp) infections with high resistance to conventional therapies. *Fragaria vesca* (Fv) and *Agrimonia eupatoria* L (Ag) have been used for the treatment of several diseases, including gastric disorders.

Aim: evaluation of susceptibility of Fv and Ag extracts on four Hp clinical isolates.

Hp isolates were obtained from gastric biopsies. Minimum inhibitory concentrations of metronidazole (MTZ), amoxicillin (AMC), clarithromycin (CL), and levofloxacin (LVX) were determined by E-Test.

Extracts preparation: a tincture from aerial parts of Ag was prepared by adding ethanol 45% to the powdered plant; pulverized Fv leaves were treated with dichloromethane and extracted with ethanol 100% and 50%. Alcoholic extracts were filtered, concentrated and freeze-dried.

Hp susceptibility to extracts was determined by disc diffusion. Fifteen µl of concentrations: 200, 100, 50 and 10 mg/mL of each extract were applied into discs on blood agar seeded with a 3 McFarland Hp suspension (microaerophilic atmosphere, 37ºC/72 h). Susceptibility was considered when inhibition zone diameter (IZD) was ≥15 mm.

Three Hp isolates were susceptible to the antibiotics used, and one was resistant to MTZ, CL and LVX. Extracts concentrations 200, 100 and 50 mg/mL presented activity wherein Fv (IZD: 21-36 mm) showed higher susceptibility than Ag (IZD: 15-27 mm). Hp resistant and susceptible to antibiotics had a similar behavior to extracts.

FV and Ag extracts inhibited *in vitro* growth of Hp and could constitute promising bases for further investigation in discovery of new natural anti-Hp drugs.

CHANGES IN BALANCE OF T-HELPER SUBSETS IN THE GALT OF RATS UNDER CHRONIC SOCIAL STRESS AND MODULATION OF THE INTESTINAL MICROFLORA

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OBJECTIVES

It was investigated the influence of chronic social stress and modulation of the intestinal microflora composition on the ratio of T-helper subsets in GALT.

METHODS

Researchers have been conducted on 70 rats (female) of Wistar line, which were divided on 7 experimental groups: control rats (group 1); rats, which were modeled CSS1 by means of three weeks social isolation and prolong psychoemotional influence (group2); rats, which having CSS 2 modeling by means of keeping animals in over populated cages with every day change of grouping (group 3); rats with CSS1 and CSS2, which were made the modeling of intestinal microflora by means of administrations of aminoglycosed antibiotic *kanamycin* (group 4 and 5, accordingly); rats with CSS1 and CSS2, which were made the modeling of intestinal microflora by means of everyday administrations of *lactobacterine* (groups 6 and 7, accordingly). Using immunofluorescence method, has been investigated the influence of chronic social stress on ratio of T-helper subsets in the gut-associated lymphoid tissue of Wistar rats.

RESULTS

It was shown that the introduction of kanamycin to the stressed rats leads to the dominance of Th1- and Treg subsets, but modulation of the intestinal microflora lactobacterine reduces the ratio of $Tbet^{+}/Gata3^{+}$ - lymphocytes in the own lamina of mucous membrane of the fibres, increases in subepithelial zone and increases unidirectionally the ratio of Treg/Th17.

CONCLUSION

It has been established that CSS development was accompanied by an imbalance Tbet+/$Gata3^{+}$- and $Foxp3^{+}/Ror\gamma t^{+}$-cells, indicating the dominance of Th1- and Th17-differentiation and increasing levels of pro-inflammatory signaling in the gut.

KEYWORDS

chronic social stress, GALT, T-helper cells, Treg.

PRELIMINARY STUDY ON PROBIOTIC BACTERIA ROLE IN SIMVASTATIN ACTIVATION

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OBJECTIVE

Simvastatin is a lipid-regulating drug that is administered as a prodrug in a form of lactone (SVL) and hydrolyzed to its open acid form, simvastatin acid (SVA), chemically or enzymatically in human body. Due to emerging role of gut microflora and probiotic bacteria in drug metabolism, the aim of the study was to examine if selected probiotic bacteria may transform this drug into its active form.

METHODS

Our preliminary study was performed *in vitro*. The bacterial suspension of *Lactobacillus acidophilus* and *Bifidobacterium longum* (10^{8}/ml) was incubated with SVL solution (50 ug/ml) for 24 h. In order to access the intracellular, extracellular and total content of SVL and SVA after incubation, all samples were sonicated, centrifuged, processed and measured by LC-MS/MS.

RESULTS

After 24 h of incubation with selected probiotic bacteria, concentration of SVL has been decreased by 44% in total content (sum of extracellular and intracellular). Semiquantitative and qualitative analysis of incubation medium revealed a significant amount of SVA. Concentration of SVA after incubation period was much higher in extracellular content while SVL accumulated to a greater extent intracellularly.

CONCLUSIONS

Our preliminary results suggest that selected bacterial strains LA and BL transformed SVL into its active form SVA that remained mainly out of bacteria because of its more hydrophillic properties compared to SVL, a highly lipophilic compound that transport into bacteria. Further in vivo studies are needed in order to provide more detailed insight into the effect of probiotic bacteria into the SV therapeutic response.

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IN SILICO PREDICTION OF BILE ACIDS-DRUGS INTERACTIONS AT TRANSPORTER LEVEL OF LACTOBACILLUS ACIDOPHILUS NCFM

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OBJECTIVE

The effect of bile acids on drug transporters is a challenging topic due to consequences on efficacy and safety drug profiles. Up to date there has been no study on bile acids-drugs interactions at multidrug transporter level of probiotic bacteria. Accordingly, the purpose of this study was to use computational tools to assess the binding affinities of different bile acids towards bacterial multidrug transporters.

METHODS

Docking study, using SwissDock web-service, was carried out to estimate the binding affinities of three different bile acids: cholic acid (CA), 12-monoketocholic acid (MKC) and deoxycholic acid (DCA) to multidrug transporters in Lactobacillus acidophilus NCFM (LA). The list of multidrug transporters for LA was obtained from relational database.

RESULTS

The highest binding affinity, i.e. the lowest binding energy, for the majority of examined transporters (50%) was estimated for MKC. The most prominent effect of MKC was observed in the cases of LBA1821 from ATP family and LBA0753 from the family of secondary transporters. The second ranked bile acid was CA that gave the lowest docking results with 26.9% transporters. For 23.0% of studied transporters, DCA was estimated to have the highest affinity.

CONCLUSIONS

These findings might have a role in the prediction of bile acids and probiotics influence on drug pharmacokinetics. The greatest effect of MKC for the majority of studied transport proteins suggests that keto group has a significant influence on the interactions with membrane transporters. However, in order to confirm these results further *in vitro* and *in vivo* studies are highly recommended.

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EFFECT OF A PROBIOTIC MIXTURE ON ANXIETY AND DEPRESSIVE-LIKE BEHAVIORS IN RATS

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OBJECTIVE

This study aimed to assess the potential anxiolytic and antidepressant properties of a probiotic mixture in maternally deprived rats.

METHODS

Maternal deprivation was used to mimic anxiety- (Long Evans rats) and depression-like (Sprague Dawley rats) behaviors. Male pups were isolated from their mother and littermates 3 hours per day during 14 days from 1 or 2 days after birth. These rats at 6 weeks of age received either a probiotic mixture of four bacteria (1.10^9 UFC/day) that had previously been shown to reduce irritable bowel syndrome symptoms, or the vehicle for 9 weeks. Non deprived rats were used as controls. During the 9-week treatment, anxiety-like behaviors were evaluated in the novel object, light/dark box, elevated plus maze, and open-field tests and depression-like behaviors in the forced-swimming and sucrose-preference tests.

RESULTS

In maternally deprived Long Evans rats treated with probiotics (compared to vehicle-treated rats), there was a significant increase in the number of center entries and rearings in the open-field test and a significant increase in time spent exploring objects in the novel object test. In maternally deprived Sprague Dawley rats treated with probiotics, a significant decrease in the immobility time in the forced swimming-test was observed in comparison with vehicle-treated rats.

CONCLUSIONS

This study provides evidence that the probiotic mixture tested can beneficially affect anxiety and depression-like behaviors in rats exposed to maternal deprivation in early life. Further analyses are under progress to identify the mediators involved.

NEW GENERATION PROBIOTICS: GENOME VERSATILITY OF B. UNIFORMIS CECT 7771 FOR DIFFERENT CARBON SOURCES UTILIZATION

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OBJECTIVE

B. uniformis CECT 7771 positive effects on physiology and metabolic dysfunctions have been previously demonstrated. Our objectives were to study its genes implicated in carbohydrate metabolism, to elucidate how it exerts its beneficial effects in the host and find potential ways to improve its performance and making it more profitable for human health.

METHODS

Nanopore-based technology was used to sequence the genome of *B. uniformis* CECT 7771. We have performed in vitro kinetic studies of this strain and identified its genome expression patterns in presence of glucose, mucin, pectin, inulin, gum arabic and wheat bran extract using NGS. Genome expression patterns were confirmed by qPCR quantification using a selected set of genes of interest.

RESULTS

The growth in culture of this strain differed depending of the carbon source used, showing variability in fitness cost and genome expression patterns. *B. uniformis* CECT 7771 use of mucin *O*-glycans stimulate and promote the butanoate metabolism with no necessity of any dietary fiber supplementation. The use of pectin activated genes for butyrate and GABA production, central metabolites for the proper gut function.

CONCLUSIONS

B. uniformis CECT 7771, as a consequence of its mucin glycan utilization, could positively modulate the host immune response through concrete metabolic circuits activated by fermentation of host mucin *O*-glycans. In addition, it was found that pectin enhanced production of healthy metabolites by this strain, therefore, a combined administration of these would constitute a potential synbiotic product with effects in gut-brain axis disorders due to the production of GABA.

THE ROLE OF SMALL INTESTINAL BACTERIAL OVERGROWTH IN PATHOGENESIS OF LACTASE DEFICIENCY

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INTRODUCTION

Human microflora is a stable genetically determined system. The concentration of microorganisms in the small intestine fluctuates from 10^1 to10^4 CFU/ml of the intestinal content. Secondary lactase deficiency (SLD) is inability to digest lactose, the predominant sugar of milk. This inability results from decrease of lactase enzyme activity, which is produced in the small intestine.

Purpose: to define the influence of SIBO in patients with SLD in adult patients.

AIMS & METHODS

In this study, 386 patients (the mean age – 33.9±9.09; F/M 249/137) with postinfectious irritable bowel syndrome (IBS) were analyzed concerning lactase deficiency. All patients underwent intestinal endoscopy with biopsies from the mucosa of the descending duodenum in order to determine lactase deficiency. The biopsies were taken in order to determine lactase deficiency (normal, mild and severe) by means of lactose quick test (LQT). To diagnose small intestinal bacterial overgrowth (SIBO) all patients underwent lactulose breath test during 2 hours.

RESULTS

SLD was detected in 36.5% of patients with postinfectious IBS. Mild SLD was determined in 25.6% of patients, and severe SLD – in 10.9% of patients. The specific clinical symptoms of mild SLD were moderate flatulence with abdominal pain (80.7%); the majority of patients (73.7 %) had normal stool consistency, one time a day; the other patients had semi-liquid faeces, 2-3 times a day (26.3%). The clinical symptoms of severe SLD were diarrhea (stool44 times a day) in 85.7% of patients, abdominal pain and flatulence (90.5%). SLD in all cases was accompanied by SIBO (the average level of lactulose breath test was 80.3±28.3ppm, N≤20ppm). It turned out that the degree of lactase deficiency depends on the severity of SIBO in the lumen of the small intestine. Thus, when mild SLD average value SIBO was 72.4±25.1ppm, whereas severe SLD average indicators of SIBO achieved higher values, 99.3±26.9ppm (N≤20ppm). To establish the degree of dependence of SIBO in the small intestine and the degree of deficiency of lactase in the small intestine biopsies performed a statistical analysis of the results by calculating the Spearman rank correlation coefficient to study a statistically significant link between the various phenomena. In this study, an inverse correlation between the degree of lactase deficiency in patients with the SLD and the severity of SIBO in the small intestine, i.e. the higher the hydrogen concentration in the exhaled air, the less activity of the enzyme lactase in the small intestine biopsy specimens ($r = -0.49$, $p<0.001$).

CONCLUSION

SIBO in all cases was accompanied by SLD. Thus, the high frequency of the SLD associated with SIBO in the small intestine in patients postinfectious IBS can be explained by the growth of pathogenic microflora in the small intestine.

Disclosure of Interest: None declared

MICRO-STRUCTURAL CHARACTERIZATION OF POLYMERIC FILMS (FOOD MATRIX) ADDED WITH ESSENTIAL OIL FOR SUSTAINABLE PACKAGING DESIGN

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OBJECTIVE

The aim of this study was to determine the mechanical and microstructural properties of polymeric food matrix added with essential oil for sustainable packaging design.

METHODS

Alginate and chitosan films were prepared by mixing 1% w/v with distilled water and 1% v/v with acetic acid solution, respectively; 4 treatments were performed: alginate, alginate with essential cinnamon oil (ECO), chitosan and chitosan with ECO. The solutions were dried at 70° C for 12 h.

For microstructural parameters three techniques were used: Thickness (THK) was measured with Mitutoyo micrometer, Atomic Force Microscopy (AFM) was used to obtain roughness (Ra) in Multimode V Veeco and Infrared spectrophotometry of Fourier (FTIR) with Frontier Perkin Elemer was used to obtain functional groups; for mechanical properties Nanoindentation (NI) technique (NHT-TTX, CSM Instruments) was performed to obtain hardness (H) and elastic modulus (EM).

RESULTS

Alginate ECO films showed less thickness (14+1.6 mm) than chitosan ECO (39+1.9 mm); and alginate ECO film presented higher value of hardness but less in elastic modulus (37.5+3.2 MPa and 0.2+0.1 GPa, respectively). Chitosan ECO film was smoother and homogenous than alginate ECO film (14.3+4.8 nm and 29.1+11.2 nm, respectively). Finally, FTIR vibration bands for alginate ECO films were found in -C-H 2920.5 cm^{-1}, -OH 3200 cm^{-1}, -C=O 1600 cm^{-1} and for chitosan ECO films -O=C-NH2-NH2 1670 cm^{-1}, -N-H 1557 cm^{-1}.

CONCLUSIONS

It was possible to design a polymer food matrix added with cinnamon essential oil considered as thin films with microstructural and mechanical characteristics suitable for use as sustainable packing.

SELECTED MICROORGANISMS FROM AN ANTIBODY BINDING PERSPECTIVE IN PROFESSIONAL ATHLETES

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OBJECTIVE

The goal of this work was to elucidate similarities between microorganisms from the perspective of the steady state humoral immune system.

METHODS

The reactivity of serum IgG of 14 young, professional athletes was analyzed to 24 selected microorganisms using an in house ELISA. Serum IgM and IgA reactivity was also analyzed.

RESULTS

IgM was found to be the most cross reactive antibody class, r^2= 0.7 - 0.92, for very different bacteria such as *Lactobacillus* species and *E. coli*. High correlation in Gammaproteobacteria specific IgG correlated highly with the reactivity to LPS (from E. coli) (r^2= 0.77 for LPS vs. *P. aeruginosa* to r^2= 0.98 for LPS vs. E.coli).
The correlation analysis of IgA reactivity, was less uniform than IgG reactivity, and IgA seems to differentiate Gammaproteobacteria on the species level, and not on the class level which seems to be the case for IgG. The correlation was also analysed between total IgG and IgG subclasses specific for the same microorganism, and IgG2 was identified as the main subclass recognising different microorganisms, as well as recognising LPS.
Absence of or negative correlation was found between bacteria specific IgA and IgG in case of *Lactobacillus* and *Staphylococcus* geni, whereas correlation was positive for *Candida albicans*, *Enterococcus faecalis*, *Streptococcus* species and tested Gammaproteobacteria.

CONCLUSIONS

Outlined here is a simple experimental procedure and data analysis which yields functional significance, and while the chosen population of professional athletes represent a specific population this procedure gives an age related cross section of serum antibodies reactivity towards the chosen bacteria.

THE EFFECT OF LACTOBACILLI - CONTAINING CHOCOLATE ON STREPTOCOCCUS MUTANS AS A MAIN CAUSE OF TOOTH DECAY

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OBJECTIVE

Probiotics are live microorganisms that induce health effects on the host, because of safety and lack of side effects. Today, many attentions have been paid to produce probiotics products with specific functions such as control of infectious diseases. Therefore, research works regarding to potential use of probiotics for control of dental caries as a universal and very important infection may be express beneficial health and economic aspects.

METHODS

In the present study, firstly, two bacterial strains, *Lactobacillus acidophilus* PTCC 1643 and *Lactobacillus casei* PTCC 1608, were used for production of probiotic chocolates. After optimization of temperature conditions of bacteria, the effect of probiotic chocolate on *Streptococcus mutans* as a main cause of tooth decay was analyzed.

RESULTS

The inhibitory effect of *Lactobacillus strains* on *Streptococcus mutans* was revealed for both LAB strains and for probiotic chocolate. The chocolates containing: *Lactobacillus acidophilus* and *Lactobacillus casei* have more inhibitory effect against *Streptococcus mutans* in compare with Lactobacilli free chocolate.

CONCLUSIONS

The lactobacilli containing chocolate stored under optimum temperature conditions can be used as a vehicle of probiotic bacteria with potential beneficial effects against *Streptococcus mutans*.

THE INTENSITY OF BILIARY SECRETION AND BILE COMPOSITION IN RATS WITH MONOSODIUM-INDUCED OBESITY

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OBJECTIVE

To study the biliary excretion and the composition of bile and lipids in the case of visceral obesity, caused by the use of monosodium glutamate (MSG).

METHODS

We included 20 Wistar male rats and divided into two groups (n=10). Newborn rats of group 1 (control) received subcutaneously 8 µl/g saline. Group 2 received 3 to 4 mg/g MSG subcutaneously on the second, fourth, sixth, eighth, and tenth day of life. Within 4 month after birth, rats were on a standard diet. The concentration of taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCA), taurodeoxycholic acid (TDCA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA) were measured during 3-hours experiment in 6 thirty-seconds portions.

RESULTS

Introduction of MSG during the neonatal period leads to the NAFLD development in the 4-months old rats. In rats with metabolic syndrome (group 2) during 3-hours experiment the concentration of TCA increased in all 6 bile portions from 10,2% ($p<0,05$) in the 1 portion to 15,3% ($p<0,05$) in the 6 portion. The common level of TCDCA and TDCA in the bile of the same group was higher comparing with control. The concentration of CDCA and DCA also increased during experiment from 24,0% to 25,2% ($p<0,05$) in group with visceral obesity comparing to the control.

CONCLUSIONS

MSG is widely distributed and is naturally occurring in various food. MSG-obesity leads to the stimulation of the processes of conjugation of cholic acid with taurine but inhibit the conjugation of cholic acid with glycine in hepatocytes and decrease the biliary excretion.

EXOPOLYSACCHARIDE PRODUCTION FROM PROBIOTIC PEDIOCOCCUS ACIDILACTICI

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OBJECTIVE

In recent years. demand of natural polymers for various industrial applications has led to an increased attention in exopolysaccharide (EPS) production. Exopolysaccharides are long-chain polysaccharides containing branched repeating units of sugars or sugar derivatives such as glucose, fructose, mannose and galactose etc. which are secreted into their surrounding during growth.
Pediococcus sp. are considered probiotics, produce bacteriocins, are active against Listeria and other pathogens, and their use as costarter is recommended because they improve the flavor of fermented foods, mainly cheeses. In this study, the synthesis of exopolysaccharide (EPS) from a probiotic *Pediococcus acidilactici* were investigated.

METHODS

P. acidilactici strains were isolated from milk and identified by automatic riboprinter system. Exopolysaccharide (EPS) production by *P. acidilactici* was optimised using the response surface methodology (RSM). The strain was grown in M17 medium at pH 6.5, 2% glucose, fructose, sucrose and lactose were added to the medium to study the influence of and mono disaccharides on the growth and production of exopolysaccharides.

RESULTS

The isolate was identified as *P. acidilactici*. Maximum EPS production was 45,69 mg/L, and the optimised values of the three variables predicted for maximum EPS production included a temperature of 350C, peptone and glucose concentrations of %5 and %10 respectively.

CONCLUSIONS

It can be used successfully in the production of dairy products due to the fact that it is producing EPS. In this way, it can contribute significantly to the formation of Rheology properties of the products. In addition, additives such as gums or stabilizers need to be added as well as having the potential to be positive in health. In terms of bioeconomics, it can contribute to increasing quality.

THE STUDY EFFECT OF SOME AFLATOXIN B1 IEVELS ON NUTRITIONAL DIET, GROWTH FACTORS, HISTOPATHOLOGICAL CHANGES, BLOOD FACTORS AND CONGESTION OF NECROSIS ON ACIPENSER STELLATUS

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Acipenser stellatus is a kind of valuable caviarian fish in Caspian See. In this study, the effects of additing aflatoxin with different levels on growth factors, skin lesions of stellate sturgeon were investigated under controlled conditions over the course of a 40 days experiment. A total of 180 juvenile (10 g) were randomly allotted to 5 treatment groups including (control: without AFB1+ 100% BD, T1: 25 pbb AFB1+ 100% BD, T2: 50 pbb AFB1+ 100% BD, T3: 75 pbb AFB1 + 100% BD, T4: 100 pbb AFB1 + 100% BD). Each treatment group was devided into three replicates of 12 fish per replicate on 500 litre tank. At the end of experiment, our results showed that significant differences on FCR and GR between control group and treatments. There is no significant differences between groups on SGR. Also with regard to toxin concentration and time of exposure to AFB1 in experimental fish different degree of skin lesions were observed in different parts of body. Histopathological changes in liver, kidney, spleen and gills of fish fed different level AFB1 and very wide range from congestion to necrosis. Progressive fat deposition, hepatocyte degeneration and necrosis in liver, especially in concentration 75 and 100 AFB1/kg of diets after 40 days of feeding, emphasize the mentioned results. At the end of the 30 days exposure period, blood samples were taken from the control and experimental fish. Blood was assayed for selected haematological parameters (Haematocrit, Hemoglobin, red blood cell count, white blood cell count, total albumin, ALT and AST). The derived haematological indices of MCH, MCV and MCHC were calculated. Alterations in serum levels of ALT and AST are liver specified and the significant reduction in total protein and albumin content is observed with significant differences.

KEYWORDS

Acipenser stellatus, AFB1, skin lesions, Histopathological changes, Haemathological Parameters, SGR, FCR, GR, BD.

EX VIVO MURINE GUT MODEL FOR SCREENING OF ANTIBACTERIAL PROPERTIES OF PROBIOTIC STRAINS

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OBJECTIVE

Antibacterial properties of probiotic strains are generally evaluated using *in vitro* methods e.g. competitive adhesion assays which are typically followed with *in vivo* infection models. However, use of *in vivo* infection models may be restricted in some establishments. We hypothesized that the antimicrobial properties of probiotics can be tested using an *ex vivo* gut model with gut segments obtained from healthy animals. Therefore, we evaluated the antimicrobial properties of *Lactobacillus rhamnosus* against *Salmonella typhimurium* DT193 using an *ex vivo* murine gut model.

METHODS

Gut segments (ileum and colon) were harvested from 12 healthy BALB/c mice homozygous for the scid mutation immediately after animals' death and treated with an in-house developed protocol, in brief i) the tissues were inoculated either with 1mL of NaCl 0.9% (controls) or 1mL of *Lactobacillus rhamnosus*, the tissue ends secured and incubated (20min at 37oC in Maximum Recovery diluent (MRD)); ii) the tissues were emptied and flushed with PBS (1ml) and inoculated with *S. Typhimurium* DT193 (1ml) with ends secured and incubated (20 min at 37oC in MRD); iii) the tissues then were flushed with PBS (1ml) and individually macerated in MRD for 30 sec to obtain a homogenate followed by serial dilution and plating (in duplicate) onto MacConkey Agar No.3 (CM0115) for the recovery of *S. Typhimurium*. Standard inhibition assays were performed for comparison.

RESULTS

The results from gut segments agreed with the inhibition assays.

CONCLUSIONS

Thus an *ex vivo* murine gut model can be further developed for the screening of antibacterial properties of probiotic strains.

EFFICACY OF BIFIDOBACTERIUM ANIMALIS SSP. LACTIS HN019 ON CELLULAR IMMUNE FUNCTION IN HEALTHY ELDERLY: A SYSTEMATIC REVIEW AND META-ANALYSIS

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BACKGROUND

Elderly have an increased susceptibility to infections that is associated with a decline in cellular immune function. Limited clinical data suggests that probiotic bacteria could have potential for improving polymorphonuclear (PMN) cell phagocytic activity or natural killer (NK) cell tumoricidal activity in the elderly.

OBJECTIVE

To determine the efficacy of *Bifidobacterium animalis* ssp. lactis HN019 (HN019) supplementation on PMN cell phagocytic activity or NK cell tumoricidal activity in healthy elderly.
Methods: We searched various databases in June 2016 for studies reporting PMN cell phagocytosis activity or NK cell tumoricidal activity following HN019 consumption in healthy elderly. A random effects meta-analysis was conducted and the pooled standardized mean difference (SMD) and 95% confidence interval (CI) comparing HN019 to control was calculated for each outcome.

RESULTS

Out of 85 records that were reviewed, 4 studies were included in the final analysis. Subjects were healthy elderly with a median age between 60 and 70 years and intervention times ranged from 3 to 6 weeks during which the subjects consumed placebo or HN019 ($5x10^9$ to $3x10^{11}$ colony forming units (CFU)/ day). HN019 supplementation with all the doses was highly efficacious in increasing PMN phagocytic capacity with an SMD of 0.74 (95% CI: 0.38 to 1.11, $p<0.001$) and with doses ranging from $5x10^9$-10^{10} CFU/day moderately efficacious in increasing NK cell tumoricidal activity with an SMD of 0.43 (95% CI: 0.08 to 0.78, $p=0.02$).

CONCLUSION

Daily consumption of *B. lactis* HN019 enhances NK cell tumoricidal activity and PMN phagocytic capacity in healthy elderly adults.

THE EFFECT OF THE ORAL PROBIOTIC L.RHAMNOSUS ON BIOFILM PRODUCTION OF ORAL PATHOGENS

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OBJECTIVE

Many microorganisms colonize in the oral cavity. Smoking, systemic diseases, low pH and low salivary flow may result with pathogenic bacteria settling in this area. Oral pathogenic bacteria adhere to the tooth surface, resulting in biofilm-forming tooth decay. This may affects general health. Various chemicals and antibiotics are used to prevent tooth decay; and in recent years probiotic bacteria have brought to the agenda. In this study, first the probiotic properties of oral lactic acid bacteria were determined and then antibacterial and anti-biofilm activities of these bacteria against oral pathogenic bacteria were evaluated.

METHODS

Fourty oral lactic acid bacteria were tested. These bacteria were firstly tested for antibacterial activity by well and drop method. The probiotic properties (acid and even salt tolerance, pepsin resistance, auto-aggregation and coaggregation) of isolates with antibacterial activity were determined. Molecular characterization of oral lactic acid bacteria were performed using sequencing analysis of the 16S rRNA gene. Sequences were compared with NCBI GenBank entries using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST). The effect of selected isolates on biofilm formation of oral pathogens was determined by microtiter plate method.

RESULTS

Probiotic properties were determined for 9 oral LAB isolates. Eight isolates were identified as *L. rhamnosus* and 1 isolate was *L.plantarum*. Cell-free filtration of the two isolates selected among those 9 isolates blocked the biofilm formation of oral pathogens.

CONCLUSIONS

On the one hand prevention of oral pathogens by probiotic LAB is thought to be beneficial in preventing tooth decay. On the other hand it can be used to prepare various foods such as ice cream, yogurt cheese, etc., preventing tooth decay by preventing oral pathogens from becoming adherent to the tooth. In addition, consumption of probiotic foods will positively affect health, especially in children. However, there is a need for further in vivo studies for the definitive result.

BENEFICIAL EFFECTS OF A PROBIOTIC MIXTURE IN ADULT PATIENTS WITH ACUTE DIARRHEA

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OBJECTIVE

Probiotics have well-known beneficial effects on diarrhea. However, benefits have to be substantiated by experimental evidence because probiotic effects tend to be strain specific. We previously reported that a mixture of three strains of lactic acid bacteria and one strain of bifidobacteria (*B. longum* LA 101, *L. helveticus* LA 102, *L. lactis* LA 103 and *S. thermophilus* LA 104) was strongly antidiarrheic through the combination of antimotility and antisecretory properties in the castor oil-induced diarrhea model in rats. Observations were also in favor of an antinociceptive effect. The objective of the present study was to assess the benefits of the probiotic mixture in patients with acute diarrhea.

METHODS

This was a descriptive prospective observational study performed in adult patients with acute diarrhea (>3 soft stools/day or at a frequency considered abnormal by the subject) that took one dose of the mixture (30×10^9 bacteria) per day during 4 days. Patients had to complete a questionnaire before, during and after supplementation. The main objective was to describe the evolution of stool consistency (Bristol scale) over 5 days; stool number, abdominal pain, flatulence, bloating, tiredness, nausea, and vomiting were also assessed.

RESULTS

Data from 34 patients were analyzed. Normalization of stool consistency and frequency occurred after two days of supplementation. There was also a rapid decrease in abdominal pain (within two days) whereas bloating and tiredness symptoms disappeared more progressively. No side effects were reported.

CONCLUSIONS

These results suggest that the probiotic mixture tested could relieve effectively acute diarrhea and associated symptoms.

UTILIZING LACTIC ACID BACTERIA IN THE PRODUCTION OF LOW FODMAP CEREALS

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OBJECTIVE

The efficacy of a low FODMAP (Fermentable oligo-, di-, monosaccharides and polyols) diet has been shown in IBS sufferers; however a significant limitation to the diet is the spectrum of options of low FODMAP foods. Our aim is to use lactic acid producing bacteria in the process of producing low FODMAP alternatives to foods naturally high in these carbohydrates, cereals such as bread and pasta. These prebiotic sugars are often fermented by the gut microbiota. However they may produce gas and other byproducts which can cause luminal distention, pain and other symptoms associated with IBS sufferers. Strict adherence to a low FODMAP diet has been shown to significantly reduce these symptoms in 70% of sufferers.

METHODS

Initially a screen will be set up to look for Lactobacillus with the ability to digest FODMAP sugars. This includes 32 strains of Lactobacillus and all 11 FODMAP sugars. An MRS media without meat extract or glucose will be prepared, to which each FODMAP sugar can be added individually (a media supplemented with Mannitol, Raffinose, Fructose etc.). 200 µl of media will be filled into each well in 96 well plates. Each strain is to be grown in triplicate, anaerobically at 37 degrees for 48 hours. Readings are taken at 6, 9, 12, 24 and 48 hour time points.
After the fastest growing and most effective strains are determined via Optical Density, this screen will be repeated with a suitable amount of biological replicates.
These strains of interest will then be used in a micromatrix. This is a 24 well small scale faecal fermenter which can simulate the conditions of the gut within each well. The bacteria will be run in a batch culture with the sugars, and in separate pure culture runs. The micromatrix will provide details on the relative abundance of the bacteria and the remaining sugars after the 24 hour cycle.

EXPECTED RESULTS

The results from screening the sugars will provide knowledge of strains which are capable of digesting these sugars. After these strains have been identified, it will be possible to test them in a scenario more comparable to the gut using the micromatrix. From this we can see which strains are digesting the most sugar in batch culture and pure culture. This system will also give an idea if all the relevant sugars can be lowered to the Monash university 'low FODMAP' level during processing using the selected bacteria.

CONCLUSIONS

Strains which are effective in digesting these FODMAP sugars will have a variety of applications in the processing of low FODMAP foods, and may potentially be used in the production of these foods. These food products will provide necessary variety to the LFD(Low FORMAP Diet), as well as addressing some of the nutritional deficiencies associated with the diet such as vitamin B12, iron, zinc etc.

A CASE REPORT WITH (HEPATIC?) ENCEPHALOPATHY

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CLINICAL CASE

52-year-old obese female presented experiencing mental confusion, amnesia, tachyarrhythmia and diarrhea. Alcohol abuser.
Abdominal ultrasound exam, blood tests and a negative brain CT allowed the diagnosis of alcoholic cirrhosis, complicated with hepatic encephalopathy. We ascribed her diarrhea and part of her neurologic and psychiatric symptoms (Hepatol., 2017) to gluten consumption, despite she had been previously diagnosed with coeliac disease.
Treatment with branched-chain amino acids and rifaximin and a gluten free diet were started.
The patient was readmitted for loss of consciousness and tachyarrhythmia. Excluded major bleeding, we performed an ECG, which showed a Brugada syndrome. A defibrillator was inserted. A new episode of confusion and disorientation occurred a month later, accompanied by diarrhea; blood tests were normal, but she had restarted gluten consumption. Despite the gluten removal from the diet, the encephalopathy became stable.

CONCLUSION

In this case, we considered fecal microbiota transplant (FMT), basing on interesting data:
- FMT could play a role in hepatic encephalopathy: a 57-year-old cirrhotic man suffering from grade 1-2 hepatic encephalopathy had benefit from FMT. (Hepatology 2015) and there are data about the improvement after FMT in other alterations of gut microbiota. (Gut 2017)
- There is no evidence that microbiota modulation could play a role in neurologic symptoms related to gluten consumption in coeliac patients. (UE Gastro. J. 2014). However, FMT has been successfully performed in refractory celiac disease complicated with enteropathy associated T cell lymphoma, with full recovery of symptoms and intestinal mucosal villi (J Gastrointest Liver Dis 2016).

DOCKING BASED DETERMINATION OF BILE ACID BINDING SITE ON CLOSTRIDIUM DIFFICILE CspC GERMINANT RECEPTOR

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OBJECTIVE

Studies have shown that several bile acids interact with CspC protein, consequently either triggering or inhibiting *Clostridium difficile* spore germination. The primary aim of this study was the determination of the binding site on *C. difficile* CspC germination receptor for various bile acids and their conjugates. In order to characterize the binding site, interactions between bile acids and the receptor molecule were examined.

METHODS

For the purpose of locating and analyzing the bile acid binding site on the CspC protein of *C. difficile*, molecular docking simulations were conducted using AutoDock Vina. 25 different bile acids, as well as their tauro- and glyco-conjugates, were docked to the CspC receptor. After analysis of the preliminary results and defining potential key amino acids responsible for bile acid-CspC interactions, flexibile docking with AutoDock Vina was carried out.

RESULTS

Comparison of top scoring results of docking simulations confirmed that all of the studied bile acids bind to the unique binding site on the CspC receptor. Analysis of the binding geometries and the existing interactions in bile acid-CspC complexes, singled out 32 amino acids as potential determinants of the receptor binding site.

CONCLUSIONS

All investigated bile acids, including the ones with previously experimentally established stimulating/inhibiting effect on *C. difficile* spore germination, share the same binding site on CspC receptor. Present findings suggest that further investigation of the influence of secondary and semi-synthetic bile acids on the germination process may potentially lead to a discovery of novel therapeutic alternatives for treatment of *C. difficile* infections.

SMALL INTESTINE BACTERIAL OVERGROWTH CAUSES ARTERIAL HYPOTENSION IN CIRRHOSIS

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OBJECTIVE

Small intestine bacterial overgrowth (SIBO) and arterial hypotension is common in cirrhosis. We have a hypothesis that SIBO can provide arterial hypotension in cirrhosis. So the purpose of the study is to assess the relation between SIBO and arterial hypotension in cirrhosis.

METHODS

45 patients with cirrhosis were included in the study. All patients underwent the lactulose hydrogen breath test to assess SIBO. Systolic and diastolic blood pressure (SBP and DBP) and heart rate (HR) was determined using automatic sphygmomanometer, stroke volume (SV) was determined according echocardiography. Cardiac output (CO) = SV x HR, mean arterial pressure (MAP)=(SBP+2DBP)/3, and pulse pressure (PP) = SBP - DBP, systemic vascular resistance (SVR) = SAP/CO.

RESULTS

SIBO were detected in 21 patients (46,7%). SIBO was associated with falling SBP (109,0÷12,5 vs 124,5÷21,4; p=0.005), MAP (81,9÷9,5 vs 91,0÷14,1; p=0.017), PP (40,7÷8,7 vs 50,4÷15,6; p=0,015). DBP was decreased insignificantly (68,3÷9,1 vs 74,2÷12,3; p=0.083). Blood pressure falling was associated with SVR falling (15,8÷4,0 vs 21,2÷5,3; p=0,0004), growth of SV (73,8÷17,0 vs 62,9÷15,6; p=0,030) and CO (5,47÷1,34 vs 4,49÷1,04; p=0.008). HR was increased insignificantly (74,7÷11,3 vs 72,3÷10,7; p=0,481).

CONCLUSIONS

SIBO in cirrhosis leads to arterial hypotension through SVR falling. As a compensatory mechanism SV and CO are increased.

EVALUATION OF STIMULATORY EFFECTS OF THE JABUTICABA (MYRCIARIA CAULIFLORA) EXTRACT ON PROBIOTICS

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OBJECTIVE

The phenolic compounds from the jabuticaba skin (*Myrciaria cauliflora*), due to its antioxidant action, could slow oxidative stress of the probiotics. The objective was to evaluate the growth of different probiotics strains in the presence of jabuticaba extract, verifying their stimulant effect.

METHODS

Extract concentrations of 2, 4, 6, 8 and 10% were dispensed in MRS broth. The *L. acidophilus* LA3, *L. paracasei* BGP-1 and *Bifidobacterium animalis* subsp. *lactis* BLC-1 cultures were inoculated and incubated at 37°C for 18h, under anaerobiosis conditions. After incubation, the cultures were diluted in sodium citrate 2% and propagated in MRS agar plates then incubated at 37°C from 72h. Distilled water was used as negative control. All tests were performed in triplicate and the stimulatory effects of jabuticaba extracts were measured by comparing the probiotic colony numbers in the presence of extracts from those obtained from controls.

RESULTS

The results of analysis showed that the number of probiotic colonies obtained from *L. acidophilus* LA3 culture increases as the extract concentration increases. However, for the *L. paracasei* BGP-1 culture it is observed that the number of probiotic colonies remains constant, regardless of the extract concentration. Finally, for the *Bifidobacterium animalis* subsp. *lactis* BLC-1 culture noted that for 2 and 4% extract concentration, the number of probiotic colonies remains constant, then this number increases again and remains constant up to 10% of extract concentration.

CONCLUSIONS

It was concluded that the jabuticaba skin extract did not show a deleterious effect, but increased the viability of probiotics.

IS IT POSSIBLE TO ELIMINATE SOURCE OF CAMPYLOBACTERIOSIS BY USING PROBIOTICS?

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OBJECTIVE

To evaluate how the application of *Lactobacillus fermentum* CCM 7514 influences expression of genes related to infection with *Campylobacter jejuni* CCM 6169 in chickens.

METHODS

One day old chickens were divided into 4 groups/20 birds per group: control (C), *Lc. fermentum* CCM 7514 (LC), *C. jejuni* CCM 6191 (CJ), and *Lc. fermentum* CCM 7514 + *C. jejuni* CCM 6191 (LCCJ). *Lc. fermentum* was administered at $1x10^9$ CFU daily per os to LC and LCCJ groups during first 7 days of life. Chickens of groups CJ and LCCJ were infected on day 4 with *C. jejuni* CCM 6169 at $1x10^8$ CFU per os. Samples of caecum were collected 24 hours after end of *Lc. fermentum* application. Relative expression of selected cytokines was measured using RT^2 Profiler PCR Array.

RESULTS

In groups with applied lactobacilli was recorded generally lower expression of inflammatory cytokines and chemokines when compared to CJ group. The highest expression in CJ was observed at IL-15, IL-16, IL-8L1, IL13RA1, IFNAR2, TGFB2 and TNFSF10. On the contrary in mixed group was the most pronounced expression of CCR7, CSF1, IL-10 beta receptor, IL-2RG, IL-6ST, IL-7R and TNFSF13B. Similarly in group LC was recorded reduced level of immune response.

CONCLUSIONS

Application of *Lc. fermentum* had two possible mechanisms. One could be the reduction of colonization of caecum by campylobacter, second is the direct interaction with immunocompetent cells. Simultaneously, higher expression of pro-inflammatory cytokines in CJ group showed that infection with *C. jejuni* is not so harmless in chickens as thought previously.

EFFECT OF MANGANESE-SATURATED LACTOFERRIN ON THE POPULATION NUMBERS OF PROBIOTIC BACTERIA

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OBJECTIVE

The aim of our study was to elucidate impact of various lactoferrin forms differing in iron and manganese saturation status on growth of probiotic bacteria in vitro. Lactoferrin is an iron-binding protein found in abundance in mammalian colostrum and milk. Its ability to bind iron required for growth of pathogenic bacteria has been implicated in development of proper microflora in the neonatal gut. On the other hand, probiotic bacteria of Lactobacillus genus have different growth requirements since manganese commonly replace iron in the active sites of their proteins. It could be effective in promoting growth of manganese-dependent Lactobacillus strain.

METHODS

Bovine native lactoferrin was purchased and modified to obtain: iron-depleted (apolactoferrin), iron-saturated (hololactoferrin) and manganese-saturated lactoferrin (MnLf). We tested reference strains: Lactobacillus plantarum ATCC® 14431™, Lactobacillus rhamnosus ATCC ® 53103™ as well as strains from the oral probiotic formula: L. plantarum PL02 and L. rhamnosus KL53A. Strains were inoculated in minimal medium devoid of iron and manganese. Four lactoferrin forms (native Lf, apolactoferrin, hololactoferrin, MnLf) were then added at concentrations of 0.6, 5 and 40 mg/ml to the culture.
Culture was maintained for 24 hours in 37°C. Optical density was measured every 30 minutes. Additionally population numbers were determined by quantitative plating of the cultures on MRS Agar plates.
Furthermore, aspired media were assayed for iron and manganese content with ICP-OES technique to determine the concentration changes of these metals in the culture medium.

RESULTS

Manganese-saturated lactoferrin did significantly increase growth of all the tested probiotic strains in all three tested concentrations as can be deduced from optical density measurements. This result was confirmed with quantitative plating of the cultures: MnLf at the highest concentration (40 mg/ml) had a significant impact on the population numbers of all tested Lactobacillus strains. Such effects were not observed for native, iron-depleted or iron-saturated form of the protein. Further, the increase in the population number of probiotic bacteria did correlate with the decrease of manganese content in the broth after 24 hours of culture which points to the metal utilization by Lactobacillli.

CONCLUSIONS

Our results have shown that form of lactoferrin saturated with manganese have a positive impact on the population numbers of tested Lactobacillus strains. This is in agreement with the dependence of these probiotic bacteria on the manganese which commonly replaces iron in the active sites of their proteins. Observed differences in the probiotic bacteria growth coincide with the decrease of the manganese concentration in the culture broth which further supports the hypothesis that Mn is utilized by Lactobacillus. It is plausible that lactoferrin saturated with manganese could serve as a prebiotic for manganese-dependent probiotic bacteria of Lactobacillus genus by locally increasing Mn concentration and – as such – might be used to modulate the intestinal microflora, especially in the neonates.
This study was financed by the National Science Centre (Poland, project number: 2013/09/B/NZ6/00849).

THE IMPACT OF TROFIC EFFECTS OF MULTI STRAIN PROBIOTIC PREPARATION ON PARACELLULAR PERMEABILITY

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OBJECTIVE

The aim of the present study was to elucidate the mechanism of action of VSL#3 to reduce intestinal permeability, and its effect in modulating the activity on tight junctions, focusing on the effects of secreted bioactive factors.

METHODS

Two different lots of VSL#3 (Manufacturer: Nutrilinea Srl, Gallarate (VA) - Italy) were used. HT29 cell line was treated with a conditioning media (CM) prepared using 1g of probiotic formula grown in culture medium (free of serum and antibiotics) at 37°C for 48 hours without shaking and in anaerobic conditions.
The effects of probiotic on proliferations of cells were evaluated by cell growth curve by ECIS (Electric Cell-Substrate Impedance Sensing) and MTT assay.
The effect on apoptosis will be analyzed by cytometry using double staining with Annexin V-FITC and Propidium Iodide, as well as it's well established. Furthermore, the expression of tight junctions, in particular claudin-2, occludin and ZO-1, was be investigated by western blot analysis.

RESULTS

The multistrain probiotic formula increased HT29 cell proliferation, together with a decrease of 90%of apoptotic cells in presence of CM. The expression of tight junction proteins increase in 24 hours for claudin and ZO-1, and after 48 hours of treatment in case of occludin.

CONCLUSIONS

These preliminary data in vitro could be able to explain how VSL#3 works at intestinal mucosa level, in particular by secretion of factors that enhances barrier integrity. The proliferative stimuli and the increase of expression of tight junction proteins are consistent with an effect on mucosa regeneration and re-epithelization.

IDENTIFICATION OF PROBIOTICS STRAINS PROMOTING A TYPE I IMMUNE RESPONSE

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OBJECTIVE

Approximatively 10 millions of Italians suffer from allergic rhinitis (AR). The allergic response is a type II immune response. The onset of allergic diseases and the composition of gut microbiota are closely linked as microorganisms can influence immune cells activation. Recent studies showed the immunomodulatory activity of probiotics. Dietary supplementation with probiotics able to promote the activation of a type I anti-allergic response would be beneficial for individuals affected by AR. The aim of this study was to evaluate the type of immune response promoted by different probiotic strains.

METHODS

Probiotics strains, deposited at International Culture Collection, were incubated with human monocytes from healthy donors. Monocytes stimulated with LPS+IFNgamma and IL-4 were used respectively as M1 and M2 polarized control macrophages. Gene expression (M1 genes: CXCL9, CXCL10, IL-1beta, IL-12, TNFalpha; M2 genes: Arginasel, CCL17, CCL22, IL-10, TGFbeta) was measured by Real-Time PCR and cytokines release by ELISA.

RESULTS

We tested 6 probiotic strains: *L.plantarum* P17630, *L.paracasei* I1687, *L.paracasei* I1688, *L.salivarius* I1794, *L.gasseri* P17632, *L.acidophilus* P18806. Monocytes incubated with L.plantarum P17630 and L.paracasei I1688 selectively up-regulated genes associated to a type I response (M1 genes) and expressed low levels of genes associated to a type II response (M2 genes).

CONCLUSIONS

All strains showed immunomodulatory activity, and, in particular, *L.plantarum* P17630 and *L.paracasei* I1688 demonstrated the ability to skew the activation of monocytes towards an anti-allergic response. Results suggest the use of these specific probiotic strains in the treatment of allergic diseases such as AR.

ANTIFUNGAL ACTIVITY OF EXTRACTS PRODUCED BY LACTOBACILLUS FERMENTUM STRAINS AND ANALYSIS OF CANDIDA ALBICANS YEAST/MOLD (Y/M) SWITCHING

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OBJECTIVE

Candidiasis is one of the most common vaginal infections in sexually active women and many products are commercially available for its treatment. Although side effects due to antifungal systemic drug are reduced by the use of the newer azole derivatives, the increased resistance in some strains are turning to look for new therapeutic approaches and/or active molecules of different nature. The aim of this work was the evaluation of antimycotic activity of extracts produced by of two *Lactobacillus fermentum strains*, which have already demonstrated antibacterial activity.

METHODS

For this purpose, eight strains of *Candida albicans* (clinical isolates) have been investigated. Moreover, the effect on *Candida* yeast/mold (Y/M) switching has also been investigated.

RESULTS

Our results showed that all strains were susceptible to supernatants at the MIC ranges of 25-0.025% V/V after 24/48h incubation at 35°C. Furthermore, Y/M switching has been inhibited.

CONCLUSIONS

This remarkable activity against *Candida albicans* strains suggesting that *Lactobacillus fermentum* derivatives may offer a new strategic approach to fight fungal pathogens and as well as to reduce the risk of relapse. However, clinical studies, based on suitable pharmaceutical formulations, could demonstrate this *in vivo* activity.

APPLICATION OF DIETARY FIBER IN THE CORRECTION OF ESTROGEN METABOLISM DISORDERS

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OBJECTIVE

After hepatic transformation estrogen metabolites are excreted in bile. In the gut they undergo fermentation by microbiota and later partake in enterohepatic circulation. Dietary fiber is used to correct dyslipidemias and hyperglycemia, hence it may also effect other metabolic pathways. In this pilot study we tested, whether soluble, gel-forming, nonfermented fiber supplement affects estrogen metabolism.

METHODS

We included 43 women with established diagnosis of disorders, associated with changes in estrogen metabolism (uterine fibroids, endometrial hyperplasia, endometriosis, mastopathy) and assessed urinary levels of 16-alfa-hydroxyestrone, 4-hydroxyestrone and 2-hydroxyestrone at baseline and after 3 months, during which women received 6,5 g of soluble, gel-forming, nonfermented fiber as a dietary supplement every day(psyllium).

RESULTS

At baseline every woman had increased levels of highly-active estrogen metabolites (mean 16-alfa-hydroxyestrone - 14,3±2,5 mmol/mol creatinine, 4-hydroxyestrone - 3,1±0,6 mmol/mol creatinine) as well as decreased levels of low-active 2-hydroxyestrone - 9,3±3,1 mmol/mol creatinine. After 3 months of dietary supplement intake 16-alfa-hydroxyestrone decreased by 10,2±5,2%, 4-hydroxyestrone decreased by 11,9±3,7% and 2-hydroxyestrone increased by 13,6±4,1%.

CONCLUSIONS

Soluble, gel-forming, nonfermented fiber (psyllium) improves the profile of estrogen metabolites, probably by changing the mechanism of their fermentation by gut flora and rates of elimination. We suggest further controlled studies.

DEVELOPMENT OF PROBIOTIC-ENRICHED DRIED MURTA BERRY BY VACUUM IMPREGNATION AND EFFECTS OF DIFFERENT DRYING METHODS ON BIOACTIVE COMPOUNDS, ANTIOXIDANT ACTIVITY AND VIABILITY OF PROBIOTIC BACTERIA

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OBJECTIVE

The aim of this research was to study the *Lactobacillus casei* vacuum impregnation on murta berry and evaluate the effect of different drying processes on bioactive compounds, antioxidant capacity of impregnated murta, and determine the survival of these strains.

METHODS

The murta (*Ugni molinae Turcz*) berry was impregnated with commercial juice with an initial loading of L. casei of 10^7-10^8 CFU/ml by using different impregnation conditions (Pressure: 50, 150 and 300 mbar; Time: 5, 10 and 15 min). Impregnated samples were dehydrated by the vacuum-drying and freeze-drying processes. The antioxidant capacity (AC) was determined by ORAC and DPPH methods. Total polyphenols (TPC) and total flavonoids (TFC) content were determined by spectrophotometry method. Subsequently, the number of living cells was determined by traditional microbiology method.

RESULTS

The counts of viable microorganisms were performed on the impregnated murta berry, obtaining results of 10^6-10^7 CFU/g. The microorganism counts of the impregnated and dehydrated murta by vacuum drying and freeze-dried were 10^5-10^6 and 10^6 CFU/g, respectively. Lyophilized murta showed the highest percentages of AC retention with 77.7 and 79.5% for DPPH and ORAC, as well as the lowest loss percentages for CPT and CFT with 23 and 31%, respectively.

CONCLUSIONS

In conclusion, a functional food based on dried-impregnated murta berry with a high content of *L. casei* was developed, allowing the synergy of the antioxidant properties of the murta berry and the stimulation of the immune system by the probiotic bacteria included into berry

COMMUNITY SHIFT OF WATER MICROBIOME IN AN ALGAL-BACTERIAL BASED PHOTO-BIOREACTOR FOR WASTEWATER TREATMENT

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OBJECTIVE

Nowadays, due to worldwide water shortage, water utilities are forced to re-evaluate the value of treated wastewater. Consequently, wastewater treatment plants need to conduct biomonitoring. In this study, the microbiome of coking-wastewater treatment system was extensively biomonitored. The change in the relative abundance of pathogenic and beneficial bacteria was explored.

METHODS

Coking-wastewater from Egypt was treated in photo-bioreactor (operated for 154 days) with working volume of nine liters using algal-bacterial system. Biological toxicity assays (cytotoxicity, phytotoxicity and *Artemia-toxicity*) were used for assessment of human and environment safety risks. The microbiome of coking-wastewater treatment system was explored using Illumina-MiSeq sequencing.

RESULTS

Coking-wastewater was detoxified in terms of cytotoxicity, phytotoxicity and *Artemia*-toxicity. 1286870 effective sequences of 16S-rRNA gene were generated from 14 samples, collected from different influents and effluents. Significant difference in the microbial diversity between influent and effluent samples was recorded. Four phyla dominated in influent samples; *Proteobacteria* (77%), *Firmicutes* (11%), *Bacteroidetes* (5%) and *Deferribacteres* (3%) compared to only two in effluent samples; *Proteobacteria* (66%) and *Bacteroidetes* (26%). Co-culture of microalgae caused community shift in the core genera of effluent samples with significant increase in the relative abundance of some genera as *Agrobacterium* and *Ochrobactrum* (plant probiotics) and *Pedobacter* (antifungal and probiotic activities). On the other hand, co-culture of microalgae eradicated pathogenic bacteria such as *Arcobacter* and *Legionella* in treated effluent.

CONCLUSIONS

The superiority of algal-bacterial systems for coking-wastewater treatment was confirmed as co-culture of microalgae eradicated pathogenic bacteria with significant increase in the relative abundance of some probiotic bacteria in the treated effluent.

SCREENING OF ACTINOMYCETES ANTAGONISTIC TO PATHOGENIC VIBRIO SPP. FROM SEDIMENTS OF THE CASPIAN SEA

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OBJECTIVE

Actinomycetes showed many interesting activities in water, such as production of antimicrobial agents and degradation of starch and casein. vibriosis is a major disease problem in shrimp aquaculture, causing high mortality and severe economic loss in all producing countries. In order to develop the marine actinomycetes into probiotics to control vibriosis in aquaculture, the actinomycetes were isolated from the marine sediment the Caspian Sea, and their activities against *Vibrio* spp. Have been surveyed in this study.

METHODS

A total of 22 strains of actinomycetes were isolated from Caspian Sea sediments. Preliminary screening was done using cross-streak method against the pathogenic *Vibrio* spp. Strains. The most potent strains T1, T5, T11, and T19 were used to extract the antibacterial substances. The antibacterial activities were performed using Kirby-Bauer disk diffusion method. Potent actinomycetes were screened for amylase and protease activities.

RESULTS

All of the 22 isolates were active against at least one of the test organisms. Compounds extracted from strains T11, had a strong synergistic activity against *Vibrio* spp. All of potent strains were isolated from the marine sediments of Caspian Sea, belonged to the genus Streptomyces.

CONCLUSIONS

The results showed that marine actinomycetes of Caspian Sea sediments could be a promising source for biocontrol agents in aquaculture.

KEYWORDS

Marine actinomycetes, probiotics, Caspian Sea, *Vibrio*

EFFECT OF BIFIDOBACTERIUM LONGUM BB536 PLUS LACTOFERRIN IN THE TREATMENT OF IRRITABLE BOWEL SYNDROME (IBS). A DOUBLE BLIND CLINICAL TRIAL

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BACKGROUND
Bifidobacteria in microbiota of IBS patients is decreased. Lactoferrin acts as prebiotic for bifidobacteria and has antinflammatory, antioxidant activity.

AIM
An exploratory study to evaluate in IBS patients the effectiveness of Bifidobacterium Longum BB 536 + Lactoferrin on abdominal pain, bloating and the global IBS symptomatology.

PROTOCOL
Rome III IBS patients (age 18-65 yrs) after registering their symptoms for 1 basal run-in week on a first daily diary card, were randomly and blindly allocated to treatment with active drug, BL-BB536 3 x 10^9 UFC+-Lactoferrin 50 mg or identical placebo for 2 weeks. During the treatment period patients registered symptoms on a second daily diary card. IBS-SSS was reported at the end of the run in period and of the two weeks of treatment. Self-perceived intensity of abdominal pain and bloating was reported on a visual analogue scale (VAS) during the week of the run in period and the last 10 days of the treatment.

RESULTS
Thirty four outpatients completed the study trial (18, F15 mean age 44, in the BB 536+Lactoferrin arm and 16, F11 mean age 46, in the placebo arm). In comparison to basal run-in period the IBS-SS score reduction was 50 ($p<0.007$) after BL-BB536+Lac and 30 (n.s.) after placebo. Compared to placebo, pain and bloating VAS was significantly reduced after BL-BB536+Lac ($P<0.007$; $p<0.03$).

CONCLUSIONS
IBS patients benefit –mainly for abdominal pain severity- of two week treatment with Bifidobacterium Longum BB536 enriched with Lactoferrin.
Tomada et al 1986; Takeda et al 2009; Gill et al 2001

CATECHIN-RICH FOOD AND GUT IN REDOX MODULATION

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BACKGROUND AND OBJECTIVE
The gut microbiota is altered toward a less beneficial composition in overweight adults and this change can be accompanied by metabolic syndrome and inflammation. The latter can be influenced by catechin-rich foods and beverage. We aimed to review the effects of tea and chocolate on the gut microbiota.

METHODS
We performed a systematic search on Medline, Embase, Cochrane, ProQuest, Google scholar and researchgate databases for literature with the search terms: cocoa OR chocolate OR tea OR catechins AND microbiota OR microbiome.

RESULTS
The reviewed studies suggest that the gut microbiota is both a target and a player, being involved in polyphenol metabolism, in the interactions occurring between probiotics and prebiotics and polyphenols. In particular, the addition of catechin to a FOS diet inhibited Firmicutes and enhanced Bacteroidetes. Besides, catechin supplement controlled the body weight (BW), up-regulated serum leptin, increase more soluble carbohydrates, decrease soluble polysaccharides in feces, and inhibited or activated some specific genera. On the other hand, few studies investigated the effect of cocoa and tea on gut microbiota in humans.

CONCLUSIONS
In conclusion, the beneficial effects of tea and chocolate on gut microbiota of hosts needs to be further explored.

STUDY OF THE IMPACT OF A CHRONIC CO-EXPOSURE OF A PESTICIDE AND A PREBIOTIC ON THE INTESTINAL MICROBIOTA USING TWO IN VITRO MODELS

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OBJECTIVE

Preliminary studies have shown that chronic exposure to low doses of chlorpyrifos (CPF) causes intestinal imbalance in vitro. The aim of this study is to evaluate the preventive potential of a prebiotic (inulin) in co-exposure with the CPF on intestinal dysbiosis, bacterial translocation and integrity of the intestinal mucosa. For this we used two in vitro systems: the SHIME® (simulator of the human microbial intestinal ecosystem) and caco-2/TC7 intestinal cell cultures.

METHODS

The SHIME® was exposed to a daily dose of 3.5 mg of CPF, combined with 10 g of inulin for 30 days. The samples were collected at day 0 (baseline), d15 and d30 to determine the microbial environment. Caco-2/TC7 cell line was then exposed to samples from the colic fermenters (d0, CPF ± inulin at d15 and d30) to measure the impact on the epithelial barrier integrity.

RESULTS

Contrary to our previous results with CPF alone, prebiotic supplementation seems to compensate CPF-induced dysbiosis, particularly on the potentially pathogenic microflora with a short term increase (d15: $p < 0.001$) followed by recovery at d30 (NS vs J0). The opposite trend is observed for the beneficial flora. Inulin co-exposure also beneficially influences the fermentation profile, with higher production of short chain fatty acids (propionic and butyric acid).

Preliminary cell culture data indicate that CPF-induced inflammatory signal is inhibited by inulin co-exposure.

CONCLUSIONS

The CPF/inulin co-exposure therefore has a positive impact on bacterial profile and metabolism compared to CPF alone. Further analyses are underway regarding bacterial translocation and mucosal barrier integrity.

ESCHERICHIA COLI NISSLE 1917 MODULATE GUT MICROBIOTA COMPOSITION IN ULCERATIVE COLITIS PATIENTS

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OBJECTIVE

The aim of the present study was to evaluate the effect of administration of *E.Coli Nissle* 1917 (EcN) in the qualitative and quantitative composition of the gut microbiota.

METHODS

Five patients affected by Ulcerative Colitis (UC) were treated with EcN. Fecal samples were collected before starting the treatment (T0), after 10 days from the beginning of the therapy (T1) and one month following the start of treatment (T2). Genomic DNA was isolated from fecal samples. The V1-V3 region of 16S rRNA locus was amplified. Reads were analyzed grouped into operational taxonomic units The α and β diversity and the Kruskal Wallis test were performed.

RESULTS

The T test on good's coverage index revealed an increase of the gut microbiota wellness at T1 time point versus T0. Phylum taxonomic post hoc analysis revealed a decrease of Firmicutes at T1 versus T0. At family taxonomic level the post hoc analysis revealed that Clostridiaceae relative abundance showed at T1 median value higher than the T0 and T2. At genus level, the T test confirms the variability in T0 and T2 conditions, with significantly differences for many phyla.

CONCLUSIONS

EcN treatment leads to an improvement of the qualitative composition of the gut microbiota in patients affected by UC. These effects are stronger at the end of treatment in terms of wealth, with a stable variability between the genera after 1 month of treatment.

What will be the effect of this gut microbiota modulation on the history of UC?

ANTIMICROBIAL EFFECTS OF BIFIDOBACTERIUM LONGUM BB536 AGAINST GRAM-NEGATIVE AND GRAM-POSITIVE STRAINS

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Objective

The recent developing resistance to different antibiotics increases the attention on the potentially antimicrobial metabolites produced by safety probiotic strains. The aim of the present study was to analyze the in vitro inhibitory effects of the metabolites produced by the probiotic strain of *Bifidobacterium longum* BB536 against reference strains.

Methods

The inhibitory activity assay was performed by agar diffusion and broth dilution method using the supernatant obtained from probiotic strain *B. longum* BB536 against *Escherichia coli* ATCC25922, *Escherichia coli* ATCC35218, *Klebsiella pneumoniae* ATCC700603, *Pseudomonas aeruginosa* ATCC27853, *Streptococcus agalactiae* DSMZ2134, *Enterococcus faecalis* ATCC29212 *Staphylococcus aureus* ATCC29213. The supernatant was obtained from brothcolture of *B. longum* BB536 at 37°C in anaerobic conditions using different incubation times and medium composition. The agar diffusion assay was performed depositing a cells free supernatant, obtained by centrifugation and filtration, in wells set up in Mueller Hinton agar plates, where pathogens were spread. For the broth dilution method, an inoculum of each pathogen was inoculated in each single well containing the supernatant of *B. longum* BB536 diluted in Mueller Hinton broth using serial twofold dilutions.

RESULTS

The supernatant obtained from the probiotic strain *B. longum* BB536 showed inhibitory activity against all tested strains. In particular, a good inhibitory activity was observed against Escherichia coli ATCC35218, *Klebsiella pneumoniae* ATCC700603, *Pseudomonas aeruginosa* ATCC27853 and *Streptococcus agalactiae* DSMZ2134.

CONCLUSIONS

This preliminary study shows a good inhibitory activity of *B. longum* BB536 against both Gram-negative and Gram-positive strains. This antibacterial activity should be related to the production of acetic acid.

LACTOBACILLUS STRAINS ATTENUATE THE SALMONELLA ENTERICA SEROVAR TYPHIMURIUM MEDIATED PRO-INFLAMMATORY RESPONSE IN MICE MODELS: A PROBIOTIC PERSPECTIVE TOWARDS SALMONELLA MITIGATION IN LIVESTOCK

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OBJECTIVE

To screen the *Salmonella* mitigation ability of probiotic strains in mice model.

METHODS

Identity of all LAB strains were ascertained at molecular level following genus-specific PCR and 16S rRNA sequencing. Lactobacilli were analyzed for their probiotic and safety attributes. *Salmonella* mitigation efficacy of test strains were evaluated in mice infected with a single dose of S. Typhimurium (108cfu). Following infection (4th day), probiotic dose of approx. 109cfu was administered till the 7th day following which mice were sacrificed and vital organs were analyzed.

RESULTS

Test probiotic strains displayed high tolerance to acid and bile stress; bile salt de-conjugation, aggregation, adhesion (26.04±5.83 for *L. rhamnosus* and 7.85±1.7 for *L. casei*), cell surface hydrophobicity and anti-microbial activity against *Salmonella* spp. following infection, mortality was observed in *Salmonella* alone group from 4th day onwards, however, those fed with probiotic could survive until 7th day. *In-vivo* results showed pronounced and notable inflammatory changes in the intestine, spleen and liver of infection group (*Salmonella* control) in contrary to the probiotic. Test probiotic strains alleviated the symptoms of *Salmonella* mediated inflammation in studied organs.

CONCLUSIONS

Findings from this trial indicates that *S. Typhimurium* based infections can be mitigated with probiotic intervention. Preventive and prophylactic studies are warranted for better understanding of probiotic efficacy. Probiotic treatment could not prevent mortality, however, delayed the same and hence may have possibility of exploring them as preventive therapy in livestock. Initial leads from this trial would help to plan control strategies for Salmonella mitigation in animal husbandry.

BACTERICIDAL ACTIVITY OF PROBIOTIC LACTIC ACID BACTERIA ISOLATED FROM ARMENIAN MATSONI

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OBJECTIVE

Great variety of ecological and geographical conditions of South Caucasian Republics promoted development of unique associations of lactic acid bacteria (LAB) in dairy product Matsoni, which differs from various Caucasian areas, but is very stable and characteristic for the region of origin. It is considered an analog of yogurt and prepared from milk of cow, sheep, goat, buffalo, or a mixture. The bacteriocin containing strains of *Enterococcus faecium* and *durans* species, isolated from various samples of matsun, made from milk of different domestic animals from rural households, investigated for their inhibitory activity against pathogenic bacteria belonging to different taxonomic groups.

METHODS

The genotyping by 16S rRNA sequencing for LAB were used. Cell free culture broth (CFC) broth was purified by the Gel filtration method on the Sephadex Superfine G 25 rezin. Antimicrobial activity was determined by spot-on-lawn method and expressed in arbitrary units (AU/ml).

RESULTS

It was shown, that bacteriocins of LAB from Matsoni of cow milk possess bactericidal or bacteriostatic activity against antibiotic resistant intestinal, spoilage and food-borne pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *E. coli* and *Salmonella*. Endemic strains of LAB, isolated from Matsoni made from donkey, buffalo and goat milk, shown broad spectrum of activity against food spoiling microorganisms, moulds and fungi, such as *Salmonella* sp., *Esherichia coli*, *Aspergillus* and *Penicillium* species.

CONCLUSION

Endemic strains of LAB are able to produce bacteriocins with high and different inhibitory activity against broad spectrum of microorganisms isolated from different sources and belong to different taxonomic group.

LACTOBACILLI AND LACTOFERRIN: NEW APPROACH FOR VAGINAL HEALTH

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OBJECTIVE

Healthy human vaginal microbiota is characterized by several microbial strains, mainly members of the genus Lactobacillus. During vaginal infections, the balance existing among those strains is altered leading to overgrowth of opportunistic or pathogen bacteria and causing bacterial vaginosis or fungal infection. The aim of the current study was to evaluate the in vitro effects of two lactobacillus strains (L. acidopohilus GLA-14 and L. rhamnosus HN001) alone or in combination with lactoferrin on cervix cell viability and their capability to form biofilms.

METHODS

HeLa cells were used as model of human cervix. They were cultured under standard laboratory conditions and treated with lactobacilli culture supernatants with or without lactoferrin. The effects on cell viability were assessed by colorimetric methods (by neutral red assay). For biofilm evaluation, lactobacilli were grown for 48 h and biofilm was measured by Hucker Crystal Violet dyeing using a microtiter plate reader. Both biofilm ultrastructure and lactobacilli/cells interactions were studied by scanning electron microscopy.

RESULTS

Results showed that supernatants from both Lactobacillus strains' cultures did not reduce cell viability. Moreover, it has been observed that both strains are able to grown in biofilm and exhibited aggregation and adherence properties to both abiotic (plastic) surfaces and cell layer. In the most cases, these properties are modulated and increased by lactoferrin demonstrating the beneficial activity of this combination.

CONCLUSIONS

The current study support our previous results about the ability of L. acidophilus GLA-14 and L. rhamnosus HN001 to prevent and manage genital tract infections in women.

EVALUATION OF PROBIOTIC ATTRIBUTES AND ANTI-INFLAMMATORY EFFICACY OF NEWLY ISOLATED WEISSELLA CIBARIA STRAINS ISOLATED FROM HUMAN INFANTS AND ADULTS

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OBJECTIVE

Present study was taken up to evaluate probiotic attributes of four newly isolated *Weissella* strains, two each from fermented dosa batter and human infant faecal sample and their role in alleviating LPS-induced pro-inflammatory stress in murine macrophages.

METHODS

Weissella strains were isolated and purified using MRS agar plates and cultured in MRS broth at 37°C for 24 h. Further species identification of isolated strains was done by 16S rRNA gene amplification. Probiotic attributes were assessed as per DBT-ICMR guidelines that include tolerance to gastric acid (low pH 2.5 and 3.0) and bile stress (0.2 and 0.4% bile), cholesterol lowering properties, cell surface properties (cell surface hydrophobicity, congo red binding, salt aggregation assay), adhesion to gastric mucin and intestinal Caco2 cells. Further, isolated strains were assessed for lowering of nitric oxide production by LPS stimulated RAW 264.7 cells. Pro-inflammatory cytokines were evaluated with commercially available ELISA kits.

RESULTS

Four *Weissella cibaria* strains were isolated two each from fermented dosa batter and human infant faecal sample. 16S rRNA gene sequencing suggested that the strains had 99% sequence homology with *Weissella cibaria*. Strains were tolerant to gastric conditions (pH 3.0) and bile (0.2 & 0.4%) salts, showed moderate cell surface hydrophobicity, cholesterol reduction upto 19% by strain 28 and adhesion to intestinal Caco2 cells (69 and 76% by strains 28 and 29) and gastric mucin (43 and 35% by strains 16 and 28). All the strains prevented LPS-induced nitric oxide and IL-6 production in murine macrophages whereas strain 28 alone prevented IL-1β production.

CONCLUSION

W. cibaria strain 28 as a candidate strain for future studies since it had good adhesion to Caco2 cells and gastric mucin and cholesterol reduction as well as prevention of LPS-induced pro-inflammatory stress in macrophages.

ASSOCIATIONS BETWEEN VITAMIN D, SYSTEMIC INFLAMMATION AND SYNBIOTIC SUPPLEMENTATION: A SECONDARY STUDY FROM A RANDOMIZED CLINICAL TRIAL

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OBJECTIVE

To evaluate the effects of a symbiotic substance on the vitamin D status of elderly, and the association with systemic inflammation.

METHODS

This is a secondary study from a randomized, double-blind controlled clinical trial, lasting 24 weeks, with 49 participants, randomly assigned in two groups; the S-group received a synbiotic substance, and the P-group received placebo. Participants were evaluated at the beginning and at the end of the study for body mass index, body fat percentage, and; plasma ratio between anti and pro-inflammatory cytokines (specifically IL-10 and IL-6), plasma markers of gut permeability (LPS, I-FABP and DAO), and plasma leels of vitamin D.

RESULTS

The P-group reduced vitamin D, while the S-group did not; the S-group presented a significant reduction in DAO, while the P-group did not. The interaction of time x treatment was not significant. When analyzing the regression models together, the inflammatory balance (IL-10/IL-6) and two markers of intestinal permeability (DAO and LPS) correlated with the final values of vitamin D. In addition, the variation in vitamin D throughout the study period, together with the I-FABP, explained the inflammatory balance.

CONCLUSIONS

we found a slight effect of synbiotic supplementation on vitamin D status in community-dwelling elderly, with a significant role of inflammation on this effect.

NHIBITORY ACTIVITY OF VAGINAL LACTOBACILLUS SPP. STRAINS BY BACTERIOCIN PRODUCTION

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OBJECTIVE

Probiotics represent a complementary and integrative therapy that is useful in the treatment and prevention of urogenital infections in women. Our study focused on characterization of *Lactobacillus spp* strains isolated from healthy vaginal ecosystems to determine their probiotic properties in order to restore and maintain vaginal health.

METHODS

22 *Lactobacillus spp*, isolated from 42 healthy women were screened by deferred antagonism test on M.R.S. Agar with 0.1% $CaCO_3$. 15 strains showing antagonistic activity, identified by tuf gene sequencing, were characterized for: i) the presence of bacteriocin-encoding genes by PCR, ii) the ability to produce H_2O_2 by Eschenbach method and ii) the antibiotic susceptibility profiles by E-test.

RESULTS

Out of 22 *Lactobacillus spp*, only 15 (7 *L.gasseri*; 5 *L.crispatus*, 1 *L. fermentum* and 1 *L.delbrueckii*, 1 *Lactobacillus spp*) showed a total inhibitory activity against *S.agalactiae*, *E.coli*, *K.pneumoniae*, *S aureus*, *E.faecalis* and *E.faecium*, representing the main vaginal pathogens. 13/15 strains inhibited *C.albicans* while only 3/15 showed a weak activity against *C.glabrata*. 6/15 strains resulted positive for *helveticin J* and *acidocin A*. 14/15 isolates produced hydrogen peroxide at different levels. The antibiotic susceptibility profiles showed full sensitivity to ampicillin, amoxicillin clavulanic, tetracyclines, chloramphenicol, erythromycin, rifampin.

CONCLUSIONS

In our study, we detected 15 candidate strains to develop a new vaginal formulation that may provide an alternative approach in the prevention and treatment of vaginal infections.

IMPACT OF THE TRADITIONAL ALPINE CHEESE MICROBIOTA ON METABOLIC RISK FACTORS

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OBJECTIVE

Metabolic syndrome is characterized by several cardio-metabolic risk factors including obesity, hypertension, insulin resistance, and is related to the risk of developing type 2 diabetes (T2D). The discovery of new molecules, which can be used as safe alternative to drugs and improve the glucose metabolism, will reduce the incidence of T2D. The aim of the study was to test the ability of alpine cheese-resident lactic acid bacteria to produce health-promoting metabolites, such as gamma-aminobutyric acid (GABA) and conjugated linoleic acids (CLA).

METHODS

Lactobacillus brevis FEM 1874, previously reported as GABA producer strain, was tested in vivo on mice suffering obesity and T2D. One hundred-eight lactic acid bacteria strains isolated from alpine cheese were screened for the ability to make linoleic acid bio-hydrogenation in MRS medium by a rapid spectrophotometric method. The best one was tested for its ability to produce CLA in humans by fecal fermentations through a human gastro-intestinal model.

One hundred-eight lactic acid bacteria strains isolated from Trentino alpine cheese were screened for the ability to make linoleic acid bio-hydrogenation in MRS medium by a rapid spectrophotometric method. The best one was tested for its ability to produce CLA in humans, performing fecal fermentations in a human gastro-intestinal model.

RESULTS

Lb. brevis FEM 1874 survived gastro-intestinal digestion and showed some physiological effect into the animals. *Pediococcus pentosaceous* St5m was the best CLA producing strain and produced 0.3 mg/mL CLA.

CONCLUSIONS

This study demonstrates that microbiota from traditional mountain products harbors great health-promoting properties and is able to produce bioactive metabolites. This would contribute to the development of new multifunctional foods, enriched with bio-functional molecules.

IMMUNO-MODULATORY EFFECTS OF MICROBIAL METABOLITES OF FLAVAN-3-OLS AND DIMERIC PROCYANIDINS

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Objective

The gut microbiota is recognised to impact on human immune function, but we still know little about the immuno-modulating abilities of specific metabolites derived from microbiota biotransformation of dietary components. The present research aimed to evaluate the potential of microbially-derived metabolites of flavan-3-ols and dimeric procyanidins on human immune function.

METHODS

Peripheral blood mononuclear cells from healthy donors were subjected to stimulation with LPS in presence or absence of different microbial polyphenol metabolites. After 5 days, T cell proliferation was measured by means of CFSE staining. *IL6* and *IL1B* gene expression was measured after 4 hours of stimulation by real-time PCR.

RESULTS

The results showed the immuno-modulatory potential of flavon-3-ols and procyanidins bacterial metabolites. Hydroxyphenylacetic acid was able to decrease inflammatory gene expression induced by LPS. 3,4-dihydroxyphenylpropionic acid also presented a marked anti-inflammatory capabilities, reducing *IL6* and *IL1B* gene expression and LPS-primed T cell response.

CONCLUSIONS

Those preliminary data indicate that small phenolic acids produced by the gut microbiota upon biotransformation of complex plant polyphenols display different capabilities to regulate the inflammatory response. This has important implications for measuring the metabolic output of the gut microbiome and for identifying the molecular basis of how foods rich in polyphenols actually mediate their purported health effects in humans.

BIO-DECONTAMINATION AND EXTENDING SHELF-LIFE OF MEAT AND FISH PRE-PROCESSED FOODS WITH BACTERIOPHAGES

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OBJECTIVE

The aim of this research was to test the means and methods of phage-mediated bio-processing, allowing to eliminate pathogenic microorganisms from the surface of pre-processed food products in large food-producing facilities, and to extend their shelf-life.

METHODS

Raw sausages from a Moscow meat-processing plant and rainbow trout from an aquaculture facility in Karelia were selected as specimens for the test. Decontamination of specimens was carried out with original bacteriophage cocktails, fully pheno- and genotype-mapped.

RESULTS

The application of a novel method of bio-decontamination allowed eliminating E.coli in 50 kilograms of minced meat within 24 hours, whereas the meat not processed with phages was rejected by the plant's control lab. A thirty-second bacteriophage decontamination of 15 kilograms of freshly-caught rainbow trout shows that bacterial contamination of aquatic organisms can be delayed by 5 days.

CONCLUSIONS

Using the novel method of decontamination of pre-processed foods – phage-mediated bio-processing, would allow to keep the initial eco-purity, nutrition value and palatability of the products intact, as well as to extend their shelf life compared to the existing norms and standards.

ABILITY OF A PROPIONATE PRODUCING CONSORTIUM TO PROMOTE RECOVERY FROM ANTIBIOTIC-ASSOCIATED GUT DYSBIOSIS

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Metabolic syndrome can result from obesity that is a growing public health concern. Propionate is a major fermentation product in our gut with several health benefits towards energy homeostasis. For instance, propionate is able to stimulate satiety-inducing hormones leading to lower energy intake and thus weight loss. In this study, the effect of a consortium of Lactobacillus plantarum, Bacteroides thetaiotaomicron, Ruminococcus obeum, Coprococcus catus, Bacteroides vulgatus, Akkermansia muciniphila, Veillonella parvula was studied for its potential to increase propionate concentrations in vitro. The mucosal simulator of the human intestinal microbial ecosystem (M-SHIME) was used to assess the total short chain fatty acid (SCFAs) and propionate production, as indicators of gut microbial functionality. Clindamycin was used to trigger dysbiosis, and the addition of the propionate-producing community aimed at raising the propionate levels in the in vitro system. SCFA was measured using gas chromatography method. Moreover, the effect of the consortium was assessed in the epithelial barrier function, membrane potential and mitochondrial activity in a triple co-culture model of Caco2, Ht29-MTX and HepG2 cells. Addition of the microbial consortium promoted recovery of the propionate levels following the supplementation with clindamycin, in comparison with controls. This consortium also showed a positive effect on epithelial barrier function, membrane potential and mitochondrial activity compared to the controls when it metabolites were not added on the cells. Our results indicate that, for a successful outcome, the next generation of "smart" probiotics must be designed taking into account the metabolic interactions among the members of the probiotic community.

NEW APPROACH IN THE ACNE THERAPY: A SPECIFIC BACTERIOCIN ACTIVITY AND A TARGETED ANTI IL-8 PROPERTY IN JUST ONE PROBIOTIC STRAIN, THE L. SALIVARIUS LS03

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OBJECTIVE

Acne is the most common skin disease, causing significant psychosocial problems for those afflicted. Currently available agents for acne treatment, such as oral antibiotics have limited use. Thus, development of novel agents to treat this disease is needed. In the generation of inflammatory lesions, proliferation of *Propionibacterium acnes* in the obstructed follicles is believed to be critical.

The aim of this research was to assess the effectiveness of *Lactobacillus salivarius* LS03 in exerting an antibacterial action especially against P. acnes and an anti-inflammatory effect by inhibiting *P. acnes*-induced IL-8 release.

METHODS

The ability of the LS03 strain to inhibit *P. acnes* growth was evaluated using a plate inhibition assay.
The Peripheral Blood Mononuclear Cells (PBMCs) were incubated with PHA (phytohemagglutinin) at 1 µg/ml for 1h and with probiotic strains for 24h to simulate an *in vitro* IL-8 release model. The quantification of IL-8 was performed by ELISA.

RESULTS

The strain *L. salivarius* LS03 exerted a surprising and superior inhibition capacity against the target pathogen strain. This inhibitory activity was attributable to the capacity of the LS03 to secrete very active bacteriocins against *P. acnes*.
Furthermore, the strain LS03 was able to significantly hinder the release of IL-8 secreted by PHA-activated PBMCs.

CONCLUSIONS

The use of probiotic strains with an incisive effect in inhibiting IL-8 is of fundamental importance to limit the pro-inflammatory action of the chemokine in the inflammation site.
Our results suggest that LS03 strain could represent an alternative treatment for antibiotic/anti-inflammatory therapy of acne vulgaris.

VARIABLE DISTRIBUTION PATTERNS OF FUCOSE UTILIZATION GENES AND PATHWAYS IN ENTEROBACTERIA AND THEIR POTENTIAL ROLE IN GUT MICROBIOME COMMUNICATION WITH PASSENGER BACTERIA

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OBJECTIVE

Fucose, a hexose deoxy sugar, is one of the components of the carbohydrate moiety of the mucosal glycoconjugates. Recently, fucose and rhamnose were suggested to play a major role in communication between external microbial pathogens, e.g., *Salmonella* and the human resident microbiota; however, the exact role and involvement of every member of the fucose utilization operon has not yet been elucidated.

METHODS

Comparative genomics and subsystems analysis of microbial genomes was used to investigate the conservation and variation among fucose utilization genes, operons, and regulons in *E. coli*, *Shigella*, and *Salmonella* and then compared with potential fucose utilization genes in other resident gut microbes.

RESULTS

A major pattern of distribution of these fucose utilization genes in *E. coli* was found; yet, a small proportion of genomes (5 out of 83 analyzed fully sequenced *E. coli*) have a pathway variant involving an ABC transporter system that replaces the fucose permease gene (*fucP*). To confirm comparative genomics and subsystems analysis, PCR screening showed that a minority of *E. coli* bacteria isolated from Egyptian individuals lack the *fucP* gene while the majority have that gene well conserved. The fucose isomerase-encoding gene, *fucI*, was used as a control for conserved gene and was detected in most, but not all isolates.

CONCLUSION

Enteropathogens are equipped with a large set of specific metabolic pathways to overcome nutritional limitations *in vivo. E.coli* was found to utilize fucose using two pathway variants, reflected by a genetic change found within the operon in different strains.

FLOW CYTOMETRY: EVOLUTION OF MICROBIOLOGICAL METHOD FOR PROBIOTIC ENUMERATION

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OBJECTIVE

Flow Cytometry assay is emerging as an alternative rapid method for microbial detection, enumeration, and population profiling. The use of FC not only permits the determination of viable cell counts but also allows for enumeration of damaged and dead cell subpopulations. Results are expressed as TFU (Total Fluorescent Units) and AFU (Active Fluorescent Units). In December 2015, the International Standard ISO 19344 - IDF 232 "Milk and milk products - Starter cultures, probiotics and fermented products - Quantification of lactic acid bacteria by flow cytometry" was published. This particular ISO can be applied universally and regardless of the species of interest. The purpose of this trial was to verify that the analytical method ISO 19344:2015 (E) – IDF 232:2015 (E) is valid and reliable for quantifying the concentration of the probiotic *Lactobacillus rhamnosus* GG (ATCC 53103) in a finished product formulation.

METHODS

Analytical method validation was conducted on three different industrial batches of *L. rhamnosus* GG according to USP39<1225>/ICH Q2R1 in term of: Accuracy, Precision (Repeatability), Intermediate precision (ruggedness), Specificity, Limit of quantification, Linearity, Range, Robustness.

RESULTS

The data obtained on the 3 batches of Finished Product have significantly demonstrated the validity and robustness of the cytofluorimetric analysis.

CONCLUSIONS

Based on the results obtained, the ISO 19344:2015 (E) – IDF 232:2015 (E) "Quantification of Lactic Acid Bacteria by Flow Cytometry" can be employed for the enumeration of *L. rhamnosus* GG in a finished product formulation.

EFFECT OF CYSTEINE AND LYSINE ON SOME TECHNICAL PROPERTIES OF YOGHURT STARTER CULTURE BACTERIA

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OBJECTIVE

The purpose of the present study is to introduce the application of certain materials in fermented media for enhancing the growth and acid production, reduction of fermentation time, optimizing the structure and yoghurt a well as reducing starter culture consumption which result in balancing the corresponding expenditures.

METHODS

By using local starter culture consisting *lactobacillus bulgaricus-ssp delberueky* and *Streptococcus thermophilusin* 50:50 ratio, four samples of set yoghurt were prepared in duplicated tests. Three of four samples were supplemented by L-lysine, L-cysteine and the mixture of the two and the remaining sample as a control. The factors were studied based on fermentation time, bacterial viability, acid forming, sensory evaluation and the amount of syneresis.

RESULTS

The results showed a significant effect on reducing fermentation time, increasing titratable acidity, optimized viability; besides, the end product had firmer structure and a lower syneresis. Probably lysine has been responsible for optimization of *Streptococcus thermophiles* growth. Accordingly, there was an increase in the number of *Streptococcus thermophilus* which later led to a stronger start for fermentation process. On the other hand, as a result of cysteine reduction property enhanced condition for lactobacillus bulgaricus led to a higher acid production, hence a stronger texture and lower wheying off.

CONCLUSIONS

According to faster acidification, increasing bacterial activity that speed up production of fermented dairy products and reduce the amounts of starter culture used, leads to a decrease in overall energy consumption in dairy industry, this media fortification is suggested for applying in industerial starter culture production.

PROBIOTIC EVALUATION OF TWO BACTERIA ISOLATED FROM TEJUINO IN A DIGESTIVE TRACT SIMULATOR (ARIS)

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OBJECTIVE

"Tejuino" is a traditional fermented drink made with nixtamalized corn in the west of Mexico. The aim of this study was to evaluate the metabolites production and the effect in the intestinal microbiota of *Leuconostoc citreum* and *Weisella cibaria* isolated from tejuino in a digestive tract simulator.

METHODS

Probiotics were identified by molecular and biochemical analysis as *L. citreum* and *W. cibaria.* The short chain fatty acids produced by bacteria strains were measured by HPLC. Probiotic potential of L. Citreum was determined in an Automatic and Robotic intestinal system (ARIS) using mexican healthy adult population microbiota. The effect of *Lactobacillus spp., Clostridium spp., Bifidobacterium spp.* and *Salmonella spp*. over the population dynamics was measured by colony count on selective presences of *L. citreum*.

RESULTS

W. cibaria produced more lactic acid concentration (210.2mM) than *L. citreum* (100.25mM). Both probiotics produced butyric acid (6mM) and acetic acid (14Mm). *W. cibaria* produced isobutyric acid (0.5mM) and *L. citreum* produced propionic acid (23.16 mM). Results obtained from ARIS showed that *L. citreum* may retard or reduce the growth pathogens. A reduction of *Salmonela spp.* ($1x10^7$ to $1x10^4$ CFU/mL, $P < 0,05$) was presented with a possible change in metabolism. In presence of *L. citreum*, microorganisms populations reached ($1x10^9$ UFC/ mL.) and were similar as the control (food digested without *L. citreum*).

CONCLUSION

L. citreum and *W. cibaria* isolated from tejuino can be considered as probiotics because the short chain fatty acids production as well as bactericidal effect against *Salmonella spp*. and microbiota regulation.

SAFETY OF A NEW SYNBIOTIC STARTER FORMULA

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OBJECTIVES

Breastfeeding is the best way to feed all infants, but not all infants can be (exclusively) breastfed. Infant formula is the second choice infant feeding.

METHODS

The safety of a new synbiotic infant formula, supplemented with Bifidobacterium lactis and fructo-oligosaccharides, was tested in 280 heatly term-born infants during 3 months.

RESULTS

The median age at inclusion was 0.89 months. The evolution of all anthropometric parameters was within normal range. Weight evolution was in accordance with the WHO growth charts for exclusive breastfed infants, on P50 for girls and on P25 for boys. Box-plots for weight-for-length and BMI-for-age z-scores evolved from - 1 at inclusion to 0 after 3 months. Regurgitation occurred in 63 % of all infants after one month being fed with the study formula, thus at a mean age of almost two months, decreasing to 41.6 % at the age of three months and 20 % at the age of four months. The monthy decrease is statistically significant. Daily regurgitation decreased from 10.9 % (1 month intervention) over 3.0 % (2 months intervention) to 0.7 % (3 months intervention). Only very few infants (~ 1 %) regurgitated significant volumes ($p<0.001$).

After one month intervention, 15.6 % of the infants presented daily crying episodes, decreasing to 3.7 and 0.7 after 2 and 3 months intervention (all $p < 0.001$). Nighttime crying decreased from 38 to 4 % during the three months intervention. The percent of infants with unsoothable crying after one month intervention (median age 1.89 month) was 26.3 % of the babies that cried, but only 16 % of the total number of infants with information available on crying incidence. After two and three months intervention, the incidence of unsoothable crying decreased to 5 and 0.01 %, respectively (median postnatal age 3.89 and 4.89 months) ($p<0.001$). The percent of infants that cried for three hours or more decreased from 10.5 % after one month to 2.2 % after two months. No infant cried during three hours or more after three months intervention (mean calendar age 4.89 months) ($p<0.001$). No serious adverse event related to the study product was reported. The incidence of constipation, regurgitation, infantile crying and colic was clinically and statistically significantly lower than the reported incidence in literature for a similar age.

CONCLUSION

The new synbiotic infant formula was safe and well tolerated. Functional gastro-intestinal manifestations were lower than reported in literature.

ALPHA LACTALBUMIN WITH FOS AND INULIN COMPLEX IN PEDIATRIC MIGRAINE PROPHYLAXIS

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METHODS

77 children (54 males) 6-10 years aged (mean 8.97 ± 1.93 years) with MwA. Monthly migraine frequency was assessed from daily headache diaries, intensity on a VAS (visual analogue scale), and disability linked to migraine attacks using PedMIDAS questionnaire. The Bristol Stool Chart was used to define the presence of stool troubles, as indirect sign of putative inflammatory intestinal condition. ESR and CPR were evaluated. All parameters were assessed at T0 and after 3 months of treatment with complex.

RESULTS

At baseline (T0) MwA children showed normal values of all inflammatory indexes examined (ESR: mean 0.63±0.48 mm; CPR: 0.29±0.14 mg/dL) and not significantly different at T1 (ESR: mean 0.57±0.33 mm, $p=0.506$; CPR: 0.33±0.12 mg/dL, $p=0.164$).

After 3 months of treatment (T1) MwA children showed a significant reduction in frequency (9.81±2.44 vs 6.18±1.73 attacks/month; $p<0.001$) with no significant reduction in severity (8.03±1.97 vs 7.91±2.15; $p=0.790$) and PedMIDAS score (26.52±11.43 vs 22.48±14.63; $p=0.162$). According to the Bristol Stool Test evaluation, at T1 MwA children showed a significant higher prevalence of type 2 stool (commonly considered as "normal stool") respect of T0 evaluation (64.28% vs 38.09%; $p=0.029$)

CONCLUSIONS

The present open trial study suggests the safety and potential efficacy for MoA pediatric brief prophylaxis of the Alpha Lactalbumin plus FOS and inulin complex.

EFFECT OF RED CLOVER (TRIFOLIUM PRATENSE) ON THE GROWTH AND FUNCTIONAL PROPERTIES OF TWO LACTOBACILLUS PARACASEI AND LACTOBACILLUS RHAMNOSUS PROBIOTIC STRAINS

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OBJECTIVE

Red clover is a natural phytoestrogen, rich of mineral salts, vitamins, polysaccharides, saponins, coumarin derivatives and flavonoids. The aim of our work was to evaluate the effect of the presence of red clover during the growth of two probiotic strains, *Lactobacillus paracasei* and *Lactobacillus rhamnosus*. In particular, the influence of the herb on microbial viability was evaluated; the polyphenol content and the antioxidant activity exhibited by the two strains and their capability to aggregate *in vitro* some pathogens was also evaluated.

METHODS

Two strains of *L. paracasei* and *L.rhamnosus* were grown in MRS broth with or without red clover (extracted in hot water and added to the medium) for 18 h at 37 degrees. The evaluation of total polyphenols was performed using the method of Singleton and Rossi (1965) with the Folin-Ciocalteau reagent and expressed as micrograms/ml. The free radical–scavenging activity of the heat killed cells of the two probiotics was measured with the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and expressed as percentage of the inhibition of the stable free radical DPPH (Brand-Williams et al.,1995); the aggregation test was performed using two pathogen strains (*B. cereus, E. coli*) following the method of Nazzaro et al. (2012).

RESULTS

The presence of red clover in the medium of growth not only positively affected the microbial growth but also increased at 100 % the concentration of total polyphenols present in the cells both of *L. paracasei* (from 75.38 micrograms/ml to 138.28 micrograms/ml, respectively) and *L.rhamnosus* (from 74.42 g/ml to 142.40 g/ml, respectively). The antioxidant power increased from 9.04 to 11.35 % in L.*paracasei*. Such effect was much more marked in *L.rhamnosus*, (from 8.09 % to 16.69 % in cells grown without and with red clover, respectively). Red clover did not positively meliorate the aggregative capability of the two lactobacilli but at least it reached to limit the decrease of their aggregative capability, after 3 hours of co-incubation with the two pathogens.

CONCLUSIONS

Red clover added during the growth of these two probiotics might thus meliorate some of their functional aspects, without negatively affect their viability, a fundamental prerequisite for probiotic microorganisms.

EFFECT OF NATIVE PROBIOTIC LACTOBACILLUS BREVIS ON IMMUNIZATION BY RECOMBINANT VPX PROTEIN AGAINST INFECTIOUS BURSAL DISEASE IN BROILER CHICKEN

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OBJECTIVE

Bursa of fabricius disease (Gumboro) is as immunosuppressive viral disease in young chickens. VPX is the precursor for capsids proteins, with antigenic properties which induce antibody responses. Probiotics and intestinal microflora play an important role in modulating of immune responses against infectious agents. The aim of this study was to investigate the effects of native Iranian probiotic bacteria, Lactobacillus brevis, as an drug & food supplements in the rate and feature of immune responses induced against of recombinant protein VPX

METHODS

Ninety one-day-old broiler chickens were randomly divided in 3 groups: 1: Negative control (with no vpx and probiotic) 2: Positive control (with recombinant protien vpx) 3:Trial 1(with recombinant protien vpx and probiotic).VPX was injected on days 17 and 26 intramuscularly and probiotic was orally gavaged from day 7 to 42. Chickens were orally challenged with 104.8EID 50 of very virulent infectious (strain SDH1) on day 35.Before challenging, the total antibody titers against the VPX protein were assayed with colorimetric method, ELISA with weekly interwal.

RESULTS

The titers of antibody was equivalent in all groups on early days but significantly decreased until day 25. In Group 1, the decreasing of antibody titers were continued until day 32, whereas in groups 2 and 3, the increasing of antibody titers were obvious. So that in group 3 titers of antibody were significantly increased in compared other groups and no mortality was observed. Injected virus caused 100% mortality in negative group mean while only 10% mortality was observed in group 2.

CONCLUSIONS

The results of this study showed that the use of probiotic improved immune responses by increasing the titer of antibody against Gumboro disease after vaccination.

ANTI-PROLIFERATION AND ANTI-PATHOGENIC EFFECTS OF LACTOBACILLUS SPP ISOLATED FROM HEALTHY HUMAN INDIVIDUALS

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OBJECTIVE

The microbiome consist of many different aerobic and anaerobic bacterial species that have an effective role in human digestive physiology and Lactic acid bacteria (LAB) are the main important or useful species of them. The aims of this study were to evaluation of attachment, anti-proliferation and anti pathogenic properties of these potentially probiotic strains.

METHODS

The strains were isolated from fecal samples of healthy individuals and identified by phenotypic and molecular methods. HT-29 cell line was used for investigation of the ability of the *Lactobacillus* adherence and inhibit the enteropathogen adhesion. The anti-proliferative activity of *Lactobacillus* isolates was assessed by XTT assay.

RESULTS

From 13 *Lactobacillus* strains, 5 (38.5%) strains were non-adhesive, 4 (30.7%) were adhesive and 4 (30.8%) isolates considered strongly adhesive. Among these 13 *Lactobacillus* species, one *L. Retir*e isolate shown the highest degree of inhibition of enteropathogen bacteria adhesion to HT-29 cells. XTT assay described that 3 different strains showed stronger anti-proliferative effect than the other and the maximum anti-proliferative effect was observed with *L. plantarum03*.

CONCLUSIONS

Lactobacilli have probiotic characteristics and health-promoting effects. Our results described that different *Lactobacillus* species isolated from fecal samples were capable for adhesion to HT-29 cells, antibacterial and anti-proliferative effect, but none of these *Lactobacillus* strains couldn't exhibited all of these valuable properties simultaneously. However, some of these strains could help to human health and immune system in strain specific manner by the competition or inhibition of enteropathogen bacteria and anti-proliferative effect on colon cancer cells.

THE EFFECTS OF PROBIOTICS ON WOUND HEALING: THE REVIEW OF STUDIES IN IRAN

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OBJECTIVE

The skin is a complex organ which protects the body. The integrity of human skin is essential for protection of body against dehydration, bleeding and invasion of microorganisms. Therefore, wound healing is necessary for skin functions and there are many research for acceleration of wounds healing and reduction of their infection risks. Probiotics may be play an important role in this field.

METHOD

Scientific searches in electronic databases, especially Google, Scientific Information Database (SID), Google Scholar are done and the findings were evaluated and classified.

RESULTS

The main scientific findings in Iran about topical application of probiotic bacteria and its beneficial effects on wounds could be classified as follows:

1. Evaluation of antibacterial effect of probiotics such as kefir against infectious agents especially *Pseudomonas aeruginosa* on wound healing
2. Experimental studies including burn wounds and diabetic cutaneous wounds about beneficial effects of probiotic bacteria.
3. Formulation of topical components with *Lactobacillus* products

CONCLUSIONS

There are some findings indicating that topical application of probiotics and kefir accelerates the wound healing process and reduces wound area, inflammation, infection and stimulates immune responses. However further experiments and clinical trials would be necessary to elucidate the exact role of probiotic in would healing process.

CREATION OF NEW COMPLEX PROBIOTICS ON THE BASIS OF ENDEMIC LACTIC ACID BACTERIA

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OBJECTIVE

Recent investigation in the probiotic field has been expanded to include bacteria isolated from fermented dairy products beside those from intestinal origin. The selection of new strains, isolated from milk of different domestic animals, which would be promising as starter cultures and to investigation their antimicrobial and probiotic properties, phenomenon of antagonism and\or synergism between them is of interest.

METHODS

Genotyping by 16S rRNA sequencing for LAB were used. Cultivation was carried out in the whole milk, nutrient media on the basis of milk whey, in anaerobic conditions (without shaker, at 37oC, 48 hours).

RESULTS

More than 500 cultures of LAB were isolated from various samples of fermented dairy products from milk of different domestic animals. LAB strains were mainly presented by rod-shaped bacteria of *Enterococcus faecium* and *durans* species (60 %) and coccoid forms of *Lactobacillus jensenii, L.rhamnosus, L. casei, L. helveticus* and *L. casei* species (about 40 %). LAB strains from different domestic animals shown higher resistance to proteolytic enzymes and viability after influence of pH and bile, inhibited the growth of multidrug-resistant bacteria of different taxonomic groups. While LAB strains from cow milk possess higher antioxidant activity and resistance to antibiotics. The combined cultivation of those strains brings to increasing of antimicrobial activity and reducing of milk ripening time.

CONCLUSIONS

LAB can be used as basis for obtaining the new probiotics, products of functional nutrition with antimicrobial and health promoting effects, for prevention or treatment of different etiology infectional diseases as an alternative to antibiotic treatment.

SYNBIOTIC "OPEFERA" PREVENTS THE DEVELOPMENT OF EXPERIMENTAL OBESITY

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OBJECTIVE

The search of new non-toxic drugs for preventing the development of obesity is the most important challenge of modern science. The question about impact of probiotics and prebiotics on fat metabolism and obesity is being actively debated in the scientific literature. So the aim of the study was to investigate the effect of synbiotic "OPEFERA"(SO) on development of experimental obesity.

METHODS

The study was carried out on 36 white rats, that were divided into 6 groups (I-III – males, IV-VI – females). I and IV groups were intact control (4-month old). Newborn rats of groups II and III s.c. in volume 8 μl/g were administered a saline or monosodium glutamate (MSG) (4 mg/g) at 2-10 days of life. Since the age of 1 month, rats of III and V group had been injected with water, rats III and VI groups - SO (World Medicine) in a dose of $1{,}94\times10^9$ КОЕ+2,9 mg/inulin. Introduction had been performed with 2-week course for 3 months.

RESULTS

In male rats, there were more pronounced changes - body weight and visceral fat exceeded benchmarks in 3 and 5 ($p\leq0.001$) times, respectively. Body weight and visceral fat of female rats in group III was higher by 125% and 338% ($p\leq0.001$), respectively. It was established that under condition of obesity caused by the introduction of MSG, the level of adiponectin in serum decreased in male rats by 59% and 23% ($p\leq0.05$) in females compared with intact rats. The use of SO therapy led to recovery of adiponectin level: its concentration in serum grew in 1.9 times in males and in 1.4 times ($p\leq0.05$) in females which were treated with probiotic compared with rats injected with placebo.

CONCLUSIONS

Thus, the introduction of SO increased adiponectin levels in animals injected with MSG, that shows the effectiveness of probiotic therapy for the prevention of obesity.

THE EFFECT OF DIETS ENRICHED OF HOT AND COLD SPIN THISTLE OIL ON THE RATS KIDNEY

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OBJECTIVE

The basis of most chronic diseases is oxidative stress that mediates a wide variety of renal disorders. Phytosterols, fatty acids and phenolic compounds are important food components, and have antioxidant and anti-inflammatory effects. Thistle oil contains fatty linoleic acid and tocopherol, which have the ability to protect against peroxidation processes and inflammation. There is speculation that cold pressed oils much better effect on organism than hot streak, because they may lose their useful properties. The aim of our study was to investigate the morpho-functional state of rats kidneys with cold and hot spin thistle oils enriched diet.

METHODS

Research were carried out on 30 rats (140-160 g): 1 (control) - standard diet, 2 - enriched cold pressed thistle oil (10% of the total composition), 3 – hot streak thistle oil. In 14 days histological analysis of rats kidneys were conducted.

RESULTS

Cold pressed thistle oil caused increase the epitheliocytes nuclei area of the proximal and distal renal tubular and Bowman's capsule square and glomerular capillaries, which could indicated the improving of kidney function due to synthetic processes exposure in the cells and filtered. The area nuclei proximal and distal tubules were decreased under the diet enriched of hot extraction thistle oil, that could evidence of synthetic cell activity and filtering decreasing, namely deteriorating of kidney function. All the changes were confirmed by biochemical analysis.

CONCLUSIONS

So cold pressed thistle oil has better effect on the rat kidney state than hot pressed thistle oil, due to the antioxidant properties and oxidative stability of cold pressed oils.

GELATINE TANNATE AND TYNDALLIZED PROBIOTICS REDUCE COLITIS SEVERITY AND PROMOTE A FASTER RECOVERY IN DEXTRAN SODIUM SULPHATE (DSS) MODEL OF MURINE PROLONGED COLITIS

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Gelatin tannate together with a mixture of inactivated tyndallized probiotics including Lactobacilli, Bifidobacterium, Streptococcus are particularly indicated in the management of moderate and prolonged diarrhea. No information, however, exist from in vivo studies and no animal model has been used to confirm their efficacy or unravel specific mechanism of action.

OBJECTIVE

Aim of this study was to evaluate the therapeutic effect and mechanisms of action of the formulation containing gelatin tannate and tyndallized probiotics in the DSS model of murine prolonged colitis.

METHODS

C57BL/6 mice were exposed to three cycles of 2.5% DSS for 7 days in drinking water separated by 2 weeks of recovery with water alone (resting period). During each cycle, after 5 days of DSS administration, mice received the formulation containing gelatin tannate and tyndallized probiotics by gavage in 200 ul of drinking water (T group) for five consecutive days. Control mice received water only (CON group). Body weight, occult blood test and stool consistency were measured every day and used to calculate the Disease Activity Index (DAI) to assess severity of colitis. Survival was expressed as %. Mice were sacrificed 2 weeks after the third DSS cycle and colon length was measured.

RESULTS

No differences in DAI and body weight loss were found after the first cycle of DSS between T and CON. Conversely, T group showed a significantly reduced DAI ($p<0.005$) and body weight loss ($p<0.005$) during the second and third DSS cycle with also a significantly faster recovery of DAI and body weight during their respective resting periods compared to CON group ($p<0.0002$ and $p<0.005$, respectively). No differences in mortality were observed. At the sacrifice colon length was significantly higher in T group vs. CON group ($p<0.05$).

CONCLUSIONS

Taken together our preliminary observation suggest that the formulation containing gelatin tannate and tyndallized probiotics can decrease the clinical severity of colitis in mice. It seems also able to promote a faster recovery of intestinal homeostasis during prolonged colitis. Further analyses are required to better define the mechanisms of action underlying these findings.

EMERGING ROLE OF IL-33/ST2 LEVELS IN PREDICTING MUCOSAL RESPONSE TO ANTI-TNF THERAPY IN ULCERATIVE COLITIS

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OBJECTIVES

Tumor necrosis factor (TNF) inhibitors (anti-TNF) are considered to be effective in inducing mucosal healing in patients with moderate-to-severe Ulcerative Colitis (UC). The role of IL-33 and its receptor, ST2, in intestinal inflammation is incompletely understood, with both pro-inflammatory and regulatory properties described. Recent evidence has shown that anti-TNF is able to modulate the IL-33/ST2 axis in inflammatory conditions, probably through an interplay with gut microbiota. The aim of our study was to explore the potential role of the IL-33/ST2 axis in the mucosal healing process mediated by anti-TNF therapy in UC.

METHODS

Endoscopic MAYO score was calculated before the first anti-TNF infusion (T0) and after 6 weeks (T2). 24 UC patients (MAYO score at T0 $\geq$ 2), grouped into 12 responders with mucosal healing (MAYO score $\leq$ 1) and 12 non-responders to anti-TNF at T2 (MAYO score $\geq$ 2) were enrolled. 10 healthy controls undergoing routine colonoscopy for tumor screening were also enrolled. At each time point, serum samples were collected and ELISA performed to assess IL-33/ST2 protein levels. Intestinal biopsies were also taken from the rectum and IHC was done to evaluate mucosal IL-33/ST2 expression and localization.

RESULTS

IL-33 protein levels were significantly increased in responders vs. non-responders, both at T0 and T2. Among responders, IL-33 protein was slightly reduced at T2 vs. T0, while unchanged in non-responders. Interestingly, significantly higher levels of ST2 were found in responders vs. not responders at T0, while no differences between groups were found at T2. Among responders, ST2 levels were dramatically reduced at T2 vs. T0. No significant differences were found in non-responders at both time points. Healthy controls showed significantly lower levels of both IL-33 and ST2 compared with other groups. IHC confirmed these observations. In particular, IL-33 and ST2 staining was more intense within the inflamed and ulcerated mucosa of responders compared to non-responders at T0. After 6 weeks, ST2 staining was even more evident in responders, notably localized to the healed mucosa and in close proximity to areas of re-epithelialization. Little to no staining for both IL-33 and ST2 was present in healthy controls.

CONCLUSIONS

Our results suggest a possible role for IL-33/ST2 in predicting gut mucosal wound healing in patients with moderate-to-severe UC treated with anti-TNF. A possible interplay between IL-33/ST2 axis and gut microbiota could partially explain these results. Further studies are underway to determine mechanisms of action that support these findings.

MANIFESTATION OF PROBIOTIC MILK IN GUT MICROBIOTA AND IMMUNO-MODULATION OF PROTEIN ENERGY MALNUTRITION MURINE MODEL

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OBJECTIVE

Protein energy malnutrition is a potentially fatal body depletion disorder. The catastrophic effects of malnutrition include diarrhoea, malabsorption, increased intestinal permeability and alleviated immune response, thereby aggravating other pathological conditions. In order to overcome the global disease burden, probiotics could serve as a weapon in modulating the immune response and ameliorating gut dysbiosis conditions. The present study was, therefore, designed to evaluate the impact of feeding probiotic *Lactobacillus reuteri* LR6 strain as an adjuvant therapy to re-nutrition diet on the gut and systemic immunity using Protein Energy Malnutrition (PEM) murine model.

METHODS

A Protein Energy Malnourished (PEM) mice model was developed and evaluated for various changes in the body weight, organ weight, histological changes and the total protein content as compared to the control. Twenty-four mice were assigned to four groups (6 each/group). Group 1 was fed with protein,16%; energy, 437.2 calories; Group 2 with protein, 8%, energy, 380.88 calories; Group 3 with protein, 4%, energy, 343.2 calories and Group 4 with protein, 2%, energy=315.2 calories. The effect of probiotic milk was analysed after PEM model was developed. Using a metagenomic approach the differences between gut microbial communities obtained from malnourished and apparently healthy mice were analysed by administration of probiotic formulation. Body weight was taken until six weeks after which they were sacrificed. Weight of the visceral organs and their histological parameters were analysed. Faecal samples were analysed for the differences in microbial communities between the control diet, restricted diet and probiotic milk administered group through RT-PCR.

RESULTS

PEM mice model was developed successfully at protein, 2% and energy, 315.2 calories. The developed PEM murine model showed significant reduction in the body weight (>30%), visceral organs weight as well as histo-pathological parameters, decrease in total protein, albumin, immunoglobulin G (IgG), leptin levels and increase in ghrelin, C-reactive protein (CRP), and interleukin-1β (IL-1β) as compared to the control. Moreover, reduced and disintegrated villi, reduced number of goblet cells in the intestinal tissue as well as fibrosis and small germinal centers in the splenic tissue were observed in the malnourished group. Malnourished gut showed an abundance of enteric pathogens which are known to cause intestinal inflammation resulting in malabsorption of nutrients and lead to leaky gut conditions. Gut microbiota changes showed significant increase in the bacteroides count in malnourished group. Further, probiotic fermented milk (PFM) fed mice group showed significantly increased levels of total protein, albumin, IgG, leptin and decreased levels of ghrelin, CRP and IL-1β. Histological parameters showed up to 3-fold increase in the length of the villi, increased number of goblet and sIgA cells in the intestinal tissue as well as reduced inflammation with no fibrosis in the splenic tissue, besides enhanced percentage of phagocytic activity in PFM fed mice group. Colonization assay showed 2.5 log reduction in the translocation of pathogen in various organs (spleen, liver, small intestine) in PFM group.

CONCLUSIONS

Significant differences in morphological and anatomical parameters, gut microbiota as well as histopathology were observed between malnourished and control group. Administration of probiotic fermented milk as a dietary supplement during the re-nutrition process could be a good adjuvant in reversing the malnourished conditions and might serve as a natural therapy in restoration of the beneficial gut flora, rejuvenation of intestinal wall as well as enhanced mucosal immune response and thus could translate the immune status of the body from compromised to healthy one.

THE INFLUENCE OF PROBIOTICS ON GUT INFECTIONS

Lu Yuan [1] - Henk Busscher [1] - Henny van der Mei [1] - Brandon Peterson [1]
University Medical Center Groningen, University of Groningen, Department of Biomedical Engineering, Groningen, Netherlands [1]

OBJECTIVE

To investigate the influence of a probiotic and a pathogenic bacterial strain on intestinal epithelial tight junctions and morphology.

METHODS

Caco2BBe1 intestinal epithelial cells were cultured with DMEM high glucose growth medium. Transepithelial electrical resistance (TEER) was used to evaluate the tight junctions of these epithelial cells. *Bifidobacterium infantis* ATCC15697 (probioticum) and *Escherichia* coli HU734 (pathogen) were co-cultured with an intestinal epithelial cell layer and after 24 h. The influence of the bacteria on the tight junctions was measured with TEER. In addition, the villi structure of the intestinal epithelial cells was observed by contrast optical microscopy.

RESULTS

The *B. infantis* ATCC15697 did not influence the TEER value when co-cultured with intestinal epithelial cells. TEER values of the intestinal epithelial cell layers were significantly higher when they were exposed to the probiotic strain *B. infantis* ATCC15697 than when being exposed to the pathogenic E. coli HU734 after 24 h. The villi structure of the intestinal epithelial cells was not influenced by *B. infantis* ATCC15697 in contrast to co-culturing with E. coli HU734.

CONCLUSIONS

The probiotic strain *B. infantis* ATCC15697 could protect the tight junctions of intestinal epithelial cells as well as the intestinal villi structure.

POSITIVE CLINICAL OUTCOMES DERIVED FROM USING A MIXTURE OF SELECTED STRAINS DURING PREGNANCY

Anna Maria Parolari [1] - Raffaele Nigro [1]
Ospedale di Arco, Reparto di ginecologia e ostetricia, Trento, Italy [1]

OBJECTIVE

Enterococcus faecium L3 is one of the best studied colonizing probiotic strains and shows strong antagonistic activity against *Streptococcus agalactiae* due to the production of bacteriocins able to inhibit common gut and vaginal pathogens. Our aim was to highlight the role played by a L3-based probiotic formula when administered during pregnancy.

METHODS

We tested an L3-based probiotic formula on 127 pregnant women attending our gynaecological unit in 2015. We compared the study subjects with 279 pregnant women enrolled in the same year and with 892 other pregnant women who attended our gynaecological unit in 2013 and 2014.

RESULTS

Our findings demonstrate: (a) the safety profile of the product; (b) its ability to reduce gut disorders; (c) a 6% decrease in the incidence of streptococcal colonization; (d) an approximately 30% decline in episodes of premature rupture of membranes; (e) fewer caesarean sections during labour; and (f) a reduction in pathological umbilical cord blood pH.
Conclusions: Our results demonstrate that a probiotic treatment during pregnancy could have unexpected but favourable clinical results. Further randomized, double-blind, placebo controlled studies are now needed to confirm our preliminary findings.

A FOCUSED ROLE FOR PROBIOTIC STRAINS ON OXIDATIVE STRESS, MEMBRANE PERMEABILITY AND CYTOKINE MODULATION: AN IMPORTANT IMPLICATION IN PARKINSON'S DISEASE

Angela Amoruso [1] - Luca Mogna [1] - Marco Pane [1] - Cristoforo Comi [2] - Giovanni Mogna [3]
Biolab Research Srl, Research & Development, Novara, Italy [1] - University of Novara, Department of Neurology, Novara, Italy [2] - Probiotical SpA, Presidency, Novara, Italy [3]

OBJECTIVE

Parkinson's neurodegenerative disease (PD) is characterized by the slow and progressive loss of one or more functions of the nervous system. The bacteria present in the intestine could play a key role in the development of motor deficits typical of PD.
In addition, gastrointestinal symptoms, including constipation, often precede the motility disorders given by PD and a colon inflammation is well documented in these patients.
The present *in vitro* study was focused on the evaluation of the direct effects of probiotic bacterial strains on oxidative stress and on the release of cytokines involved in the pathology.

METHODS

40 PD patients were enrolled at different stages of the disease. The Peripheral Blood Mononuclear Cells (PBMCs) were isolated from whole blood. Several Lactobacilli and Bifidobacteria strains were used as stimuli. The modulation of the *in vitro* release of the major pro and anti-inflammatory cytokines by PBMCs was investigated, as well as the production of free oxygen radicals (ROS).

RESULTS

Most strains were able to inhibit the production of ROS by PBMCs. Furthermore, they significantly modulated oxidative stress in a strain-specific manner in an in vitro model of hyperhomocysteinemia.
Probiotic strains modulated considerably the release of Th1 and Th2 cytokines, showing a clearly anti-inflammatory activity.

CONCLUSIONS

Selected probiotics could play an important activity in Parkinson patients both in counteracting oxidative stress and in modulating the release of cytokines directly involved in the disease, suggesting that bacteriotherapy could represent a key choice to modify two crucial mechanisms involved in PD pathogenesis.

PROBIOTIC PRESCRIPTIONS IN CHILDREN WITH ACUTE GASTROENTERITIS: A SURVEY IN ITALIAN INPATIENT AND OUTPATIENT SETTINGS

Andrea Lo Vecchio [1] - Maria Donata Cambriglia [1] - Maria Cristina Fedele [2] - Antonietta Giannattasio [3] - Alfredo Guarino [1]

University of Naples Federico II, Department of Translational Medical Science, Naples, Italy [1] - University of Campania Luigi Vanvitelli, Department of Pediatrics, Naples, Italy [2] - University of Naples Federico II, Department of Translational Medical Science, Naples [3]

OBJECTIVES

Probiotic strains with proven efficacy are currently recommended by the guidelines as a first line treatment for children with acute gastroenteritis (AGE) in addition to oral rehydration solution. Although there is a global interest in the field, little is known about practice patterns in childhood. Aim of this study was to investigate pediatricians' prescriptions of probiotics for the management of AGE in Italian inpatient and outpatient settings.

METHODS

Data on prescriptions were collected through an online clinical reporting form in 31 Italian hospitals and a web-survey questionnaire for 185 pediatricians working in outpatient setting in different Italian regions and compared with the European standard recommendations.

RESULTS

A total of 1032 children with AGE and aged < 5 years were included in the study: 420 outpatients and 612 inpatients. Five-hundred and nine children (50.2%) received a prescription of probiotics during medical consultation, with a significant difference in prescription between outpatients (360/420, 85.7%) and inpatient (213/612, 34.8 %) setting ($p<0.0001$).
When prescriptions were compared to standard recommendations included in guidelines, a significant difference in prescription pattern emerged. Strongly recommended strains (*Lactobacillus GG* and *S. boulardii*) were used in 299/360 children seen in outpatient setting and in 53/213 hospitalized patients (83% vs 24.8%; $P<0.001$). Weakly recommended probiotics (*Lactobacillus reuteri*) were prescribed in 48/360 outpatients and in 104/213 inpatients (13% vs 48.8%; $P<0.001$). Not recommended probiotics were prescribed in only 13/360 outpatients and in 46/213 admitted children (4% vs 21.5%; $P<0.001$).

CONCLUSIONS

Primary-care pediatricians are more adherent to guidelines' recommendations. In contrast, inappropriate probiotic prescriptions were more common in hospital, where usually only one probiotic strain is available in the hospital drug code. Therefore, health care policies may drive physicians' prescriptions of probiotics.

FACULTY

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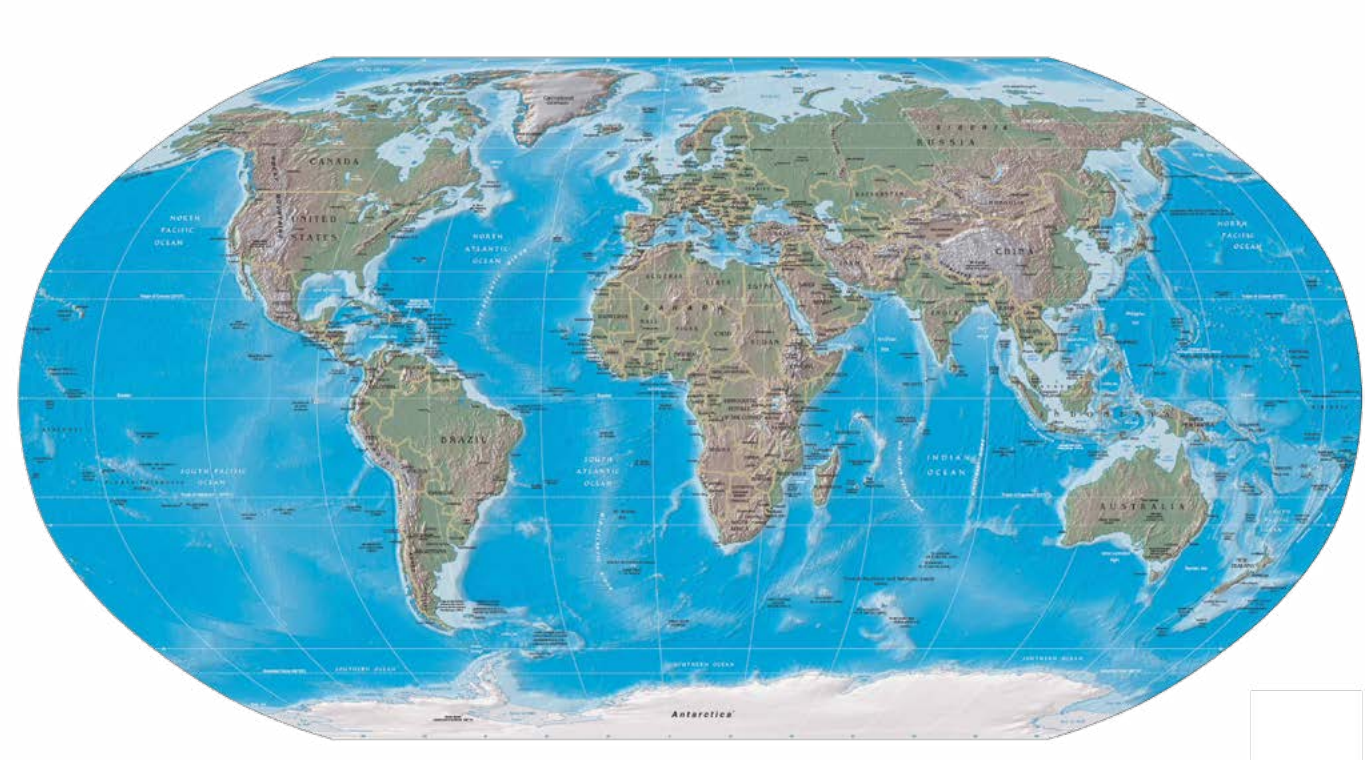

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Ukraine

GENERAL INFORMATION

DATES
September 10-12, 2017

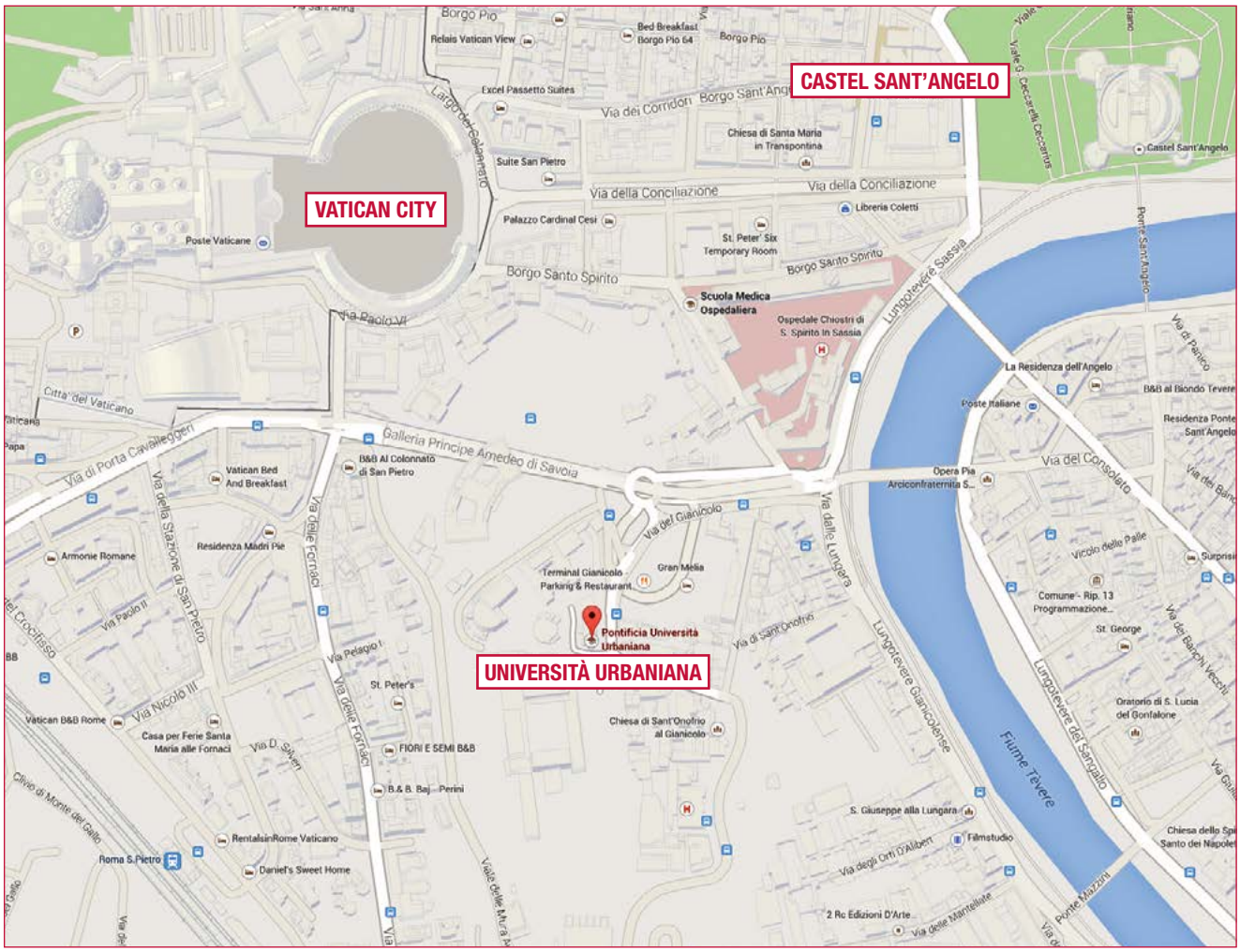
VENUE
Università Urbaniana, Terminal Gianicolo Via Urbano VIII, 16, 00165 Rome, Italy
Phone +39 06 69889611, Fax +39 06 69881871
www.urbaniana.edu

LANGUAGE
English will be the official language of the Meeting.

CLOTHING
Informal for all occasions.

CLIMATE
September in Rome is still hot but unpredictable.

BADGES
All participants exhibitors are kindly requested to wear their badges throughout the Meeting area in order to be admitted to the scientific sessions and to all the activities of the Meeting.



REGISTRATION FEES (22% VAT included)

Participants	€ 300,00
Biologists/Pharmacists	€ 150,00
Dieticians/Nutritionists	€ 150,00
Nurses	€ 100,00
Members Mediterranean Task Force for Cancer Control	€ 150,00
Members FederSalus	€ 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 abstracts
- Coffee corner and lunch
- Opening ceremony and welcome cocktail
- Certificate of attendance
- Italian CME certificate (to whom entitled)

Cancellation Policy

Written cancellation must be sent to the Organising Secretariat.
50% of the total amount will be refunded for cancellations received within July 31, 2017,
bank expenses excluded.
No refunds will be made after this date. Refunds will be made after the Meeting has been concluded.

BANKING AND EXCHANGE

The Italian monetary system is Euro. Foreign currency may be exchanged at banks during normal banking hours, at hotels, at airports and in exchange offices. 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 their accompanying persons), either during or as a result of the Meeting. Please check the validity of your own insurance.

CERTIFICATE OF ATTENDANCE

The certificate of attendance will be given to all registered participants at the Organising Secretariat desk at the end of the Meeting.

FOOD AND BEVERAGES

Lunch and coffee corner during breaks (as indicated in the programme) are included in the registration fee.

PARKING

Cars could be parked at terminal Gianicolo. Participants to the meeting will have a special rate. To obtain it, please ask at Organising Secretariat desk.

ABOUT ROME

Rome is the capital city of Italy and of the Lazio region, as well as the country's largest and most populous comune, with more than 2.7 million residents. The metropolitan area has a population of about 4 million. It is located in the central-western portion of the Italian peninsula, where the river Aniene joins the Tiber.
The Mayor of Rome is Virginia Raggi. 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, 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 thoroughly modern and cosmopolitan. As one of the few major European cities that escaped World War II relatively unscathed, central Rome remains essentially Renaissance and Baroque in character. The Historic Centre of Rome is listed by UNESCO as a World Heritage Site.

AIRPORT INFORMATION

Rome can easily be reached by plane and is served by two international airports.
Participants can fly into Rome via Leonardo da Vinci Airport, located in Fiumicino, 34 km from Rome's historic city centre or via Ciampino Airport, situated 15 km southeast of central Rome.

ACCESS TO ROME FROM THE AIRPORTS

Access from Leonardo da Vinci Airport:
The airport is served by the Leonardo Express train operated by Trenitalia, available at the airport terminal. The trip takes 30 minutes (no stops) to Termini Station in Rome - there are two such connections per hour. Alternatively, local trains leave once every 15 minutes, stopping at all train stations. You may have to change at Trastevere, Ostiense (Metro Piramide) or Tuscolana.
Cars rental are available in the airport terminal from all the usual companies.

Access from Ciampino Airport:
There is no rail transport at Ciampino Airport. The options are to take a bus to a rail station (either metro or regular train) or to take a bus or taxi all the way.

ALL THE WAY BY ROAD TRANSPORT

- Terravision runs a direct bus service to Termini. The price is € 5,00 c.a. one-way or € 10,00 c.a. return, taking 55 minutes (about 20 services a day). Despite timing buses to connect with flights, passengers on the return trip from Termini are asked to board the bus 2.5 hours before their flight's departure time. The last bus is at 23:00. Terravision also offers buses from Fiumicino airport to Termini, and a transfer bus between the two airports.

- Schiaffini also runs direct buses to Termini station for € 5,90 one-way, taking 45 minutes, but with far fewer departures than Terravision (see above). These buses are not mentioned on the airport website but they can be found on Schiaffini's own site.

- The fixed fare for a taxi ride to the city centre (inside the Aurelian Walls) is € 40,00, according to the official agreement between Roman taxi driver associations and Rome municipality. It is advisable to negotiate the total price including luggage supplements before boarding the taxi. Cars rental are available in the airport terminal from all the usual companies.

HOW TO GET TO THE MEETING VENUE

From Termini Rail Station:
By taxi - We recommend you to only use licensed taxis available outside the station.
Telephone number main taxis companies:
+39 06 3570 Radio Taxi
+39 06 5551 Samarcanda
+39 06 4994 La Capitale
By public transport - Arriving from Termini Railways Station - BUS 64 stop at Lgt. Sassia (S.Spirito Hospital) - 350 metres walking

From Leonardo da Vinci Airport/Ciampino Airport:
By taxi - We recommend you to only use licensed taxis available outside the airport.
Telephone number main taxis companies:
+39 06 3570 Radio Taxi
+39 06 5551 Samarcanda
+39 06 4994 La Capitale

TRANSPORTATION IN THE CITY

Rome has a very efficient transportation system that services the entire city, which includes the Metro network as well as buses, trains and taxis.

ORGANISING SECRETARIAT

Please do not hesitate to contact the Organising Secretariat if you require any additional information or assistance. Please address all correspondence to:

:e MEETING&CONSULTING

Via Michele Mercati, 33, 00197 Rome, Italy
Phone +39 06 80693320, Fax +39 06 3231136
E-mail: probiotics2017@emec-roma.com
Website: www.probiotics-prebiotics-newfood.com

SCIENTIFIC INFORMATION

ORGANISING SECRETARIAT DESK AT THE MEETING VENUE WILL BE OPEN AS FOLLOWS:

DAY	DATE	FROM	TO
Sunday	September 10	8.00 a.m.	8.00 p.m.
Monday	September 11	8.00 a.m.	7.00 p.m.
Tuesday	September 12	8.00 a.m.	2.00 p.m.

ORAL COMMUNICATIONS

Oral communications sessions are scheduled as follows:

September 10, AULA METCHNIKOFF from 10.30 a.m. to 11.00 a.m. - from 11.00 a.m. to 12.00 a.m.
September 12, AULA METCHNIKOFF from 12.00 a.m. to 12.45 a.m. - from 12.45 p.m. to 01.30 p.m.

POSTERS

Poster authors are kindly requested to hang the poster in the poster area from 10.30 a.m. on September 10 and remove it after 1.30 p.m. on September 12. 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:

• **"9th Probiotics, Prebiotics & New Foods, Nutraceuticals and Botanicals - for Nutrition & Human and Microbiota Health" per le seguenti categorie:**
Medico Chirurgo - discipline: Gastroenterologia; Medicina Interna; Pediatria; Ginecologia e Ostetricia; Microbiologia e Virologia; Medicina Generale (Medici di Famiglia); Pediatria (Pediatri di Libera Scelta).
Biologo
Dietista
Farmacista (ospedaliero - territoriale)
Infermiere
Infermiere pediatrico

Rif. n. 199514 - Crediti assegnati 14

Per aver diritto ai crediti ECM è necessario frequentare il 90% delle ore di formazione e superare il test di apprendimento.

Gli attestati riportanti i crediti ECM, dopo attenta verifica della partecipazione e dell'apprendimento, saranno inviati on-line dopo la chiusura dell'evento.

THE MEETING WAS MADE POSSIBLE THANKS TO THE CONTRIBUTION PROVIDED BY:

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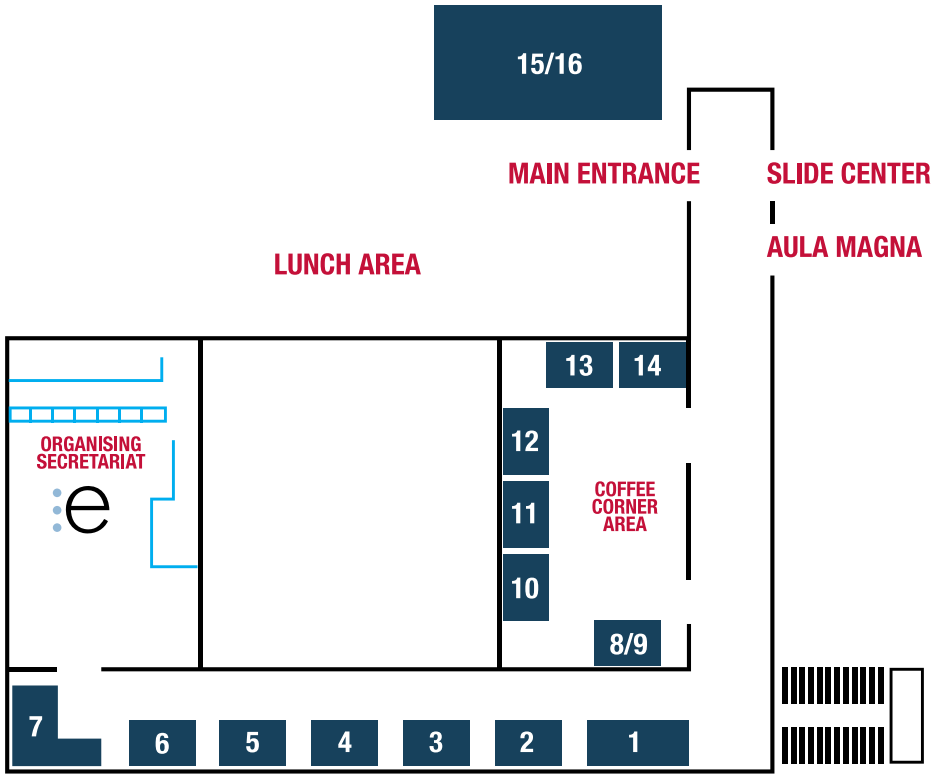
EXHIBITION AREA

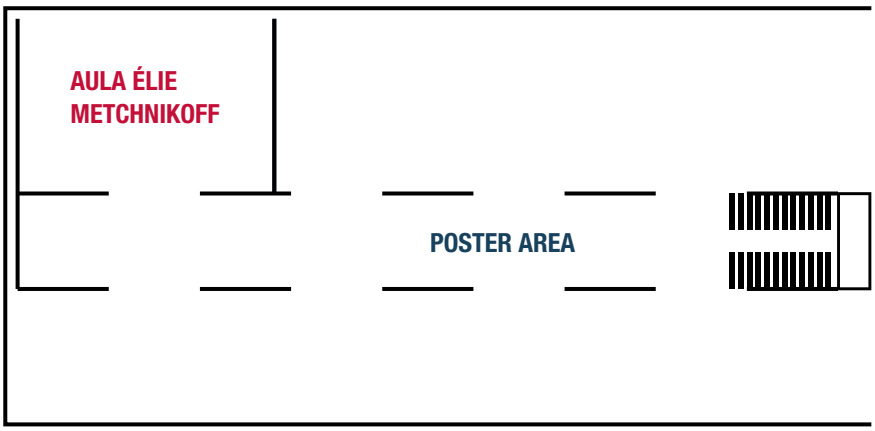
FLOOR PLAN - LEVEL I



EXHIBITORS

1) EVOLUTION HEALTH
2) PHARMEXTRACTA
3) NÓOS
4) S.I.I.T.
5) BMR GENOMICS
6) COREE
7) YAKULT
8) SANDOZ
9) SANDOZ
10) FARMACEUTICI PROCEMSA
11) ALFASIGMA
12) FERRING
13) PROBIOTICAL
14) PROBIOTICAL
15) DICOFARM
16) AG PHARMA

FLOOR PLAN - GROUND LEVEL



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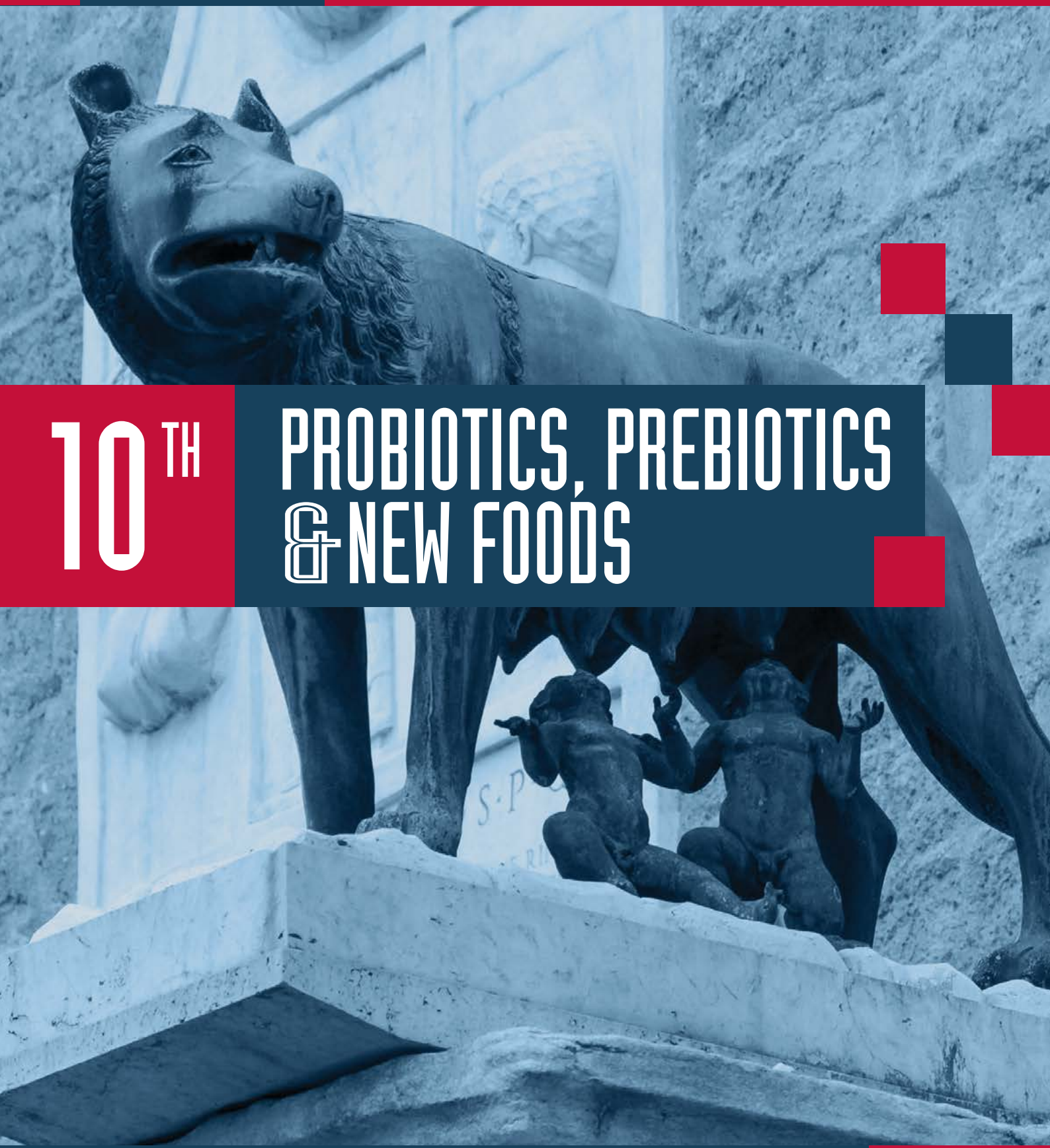

10TH PROBIOTICS, PREBIOTICS & NEW FOODS


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