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EXPO MILANO 2015

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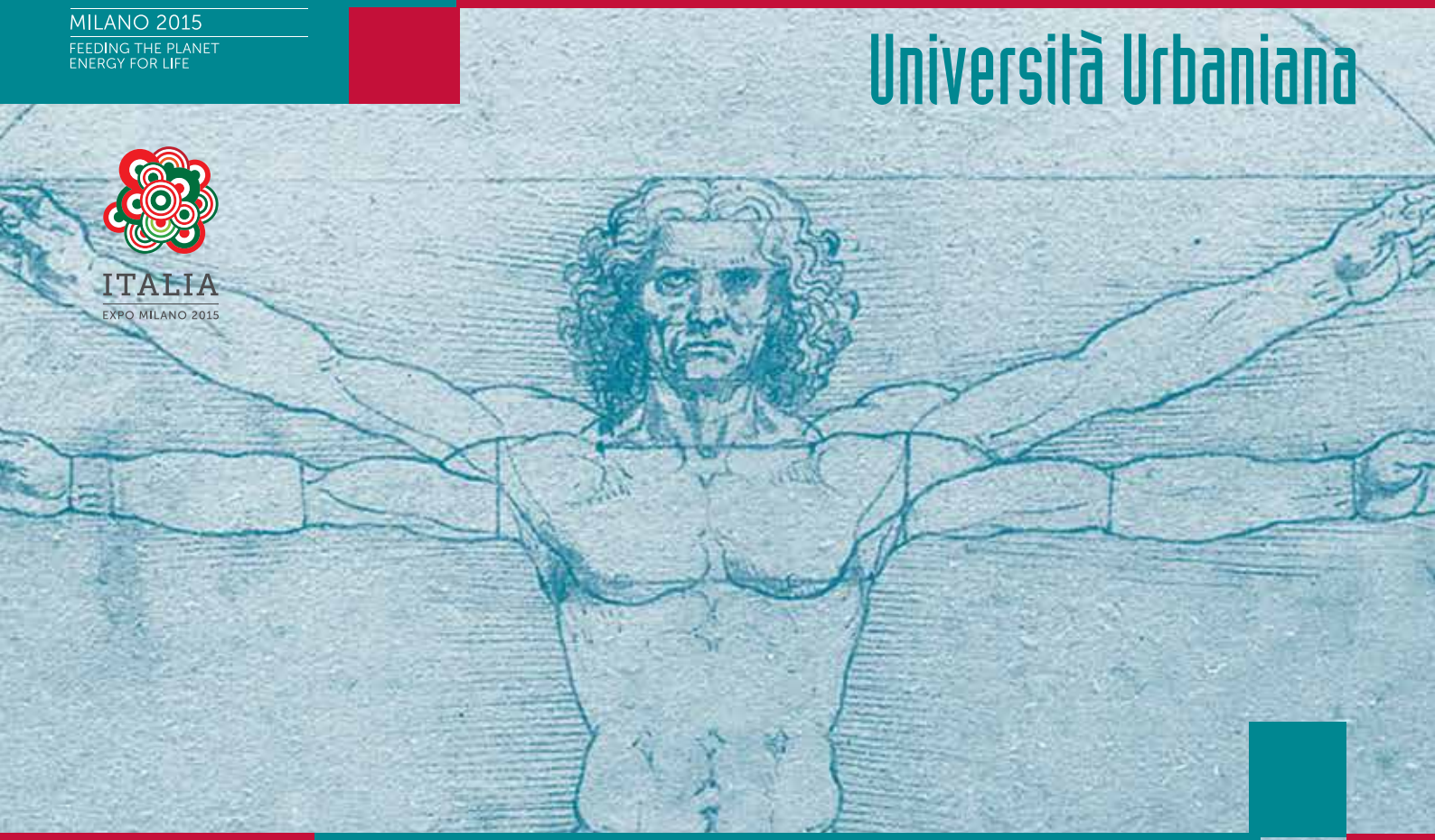
EXPO MILANO 2015

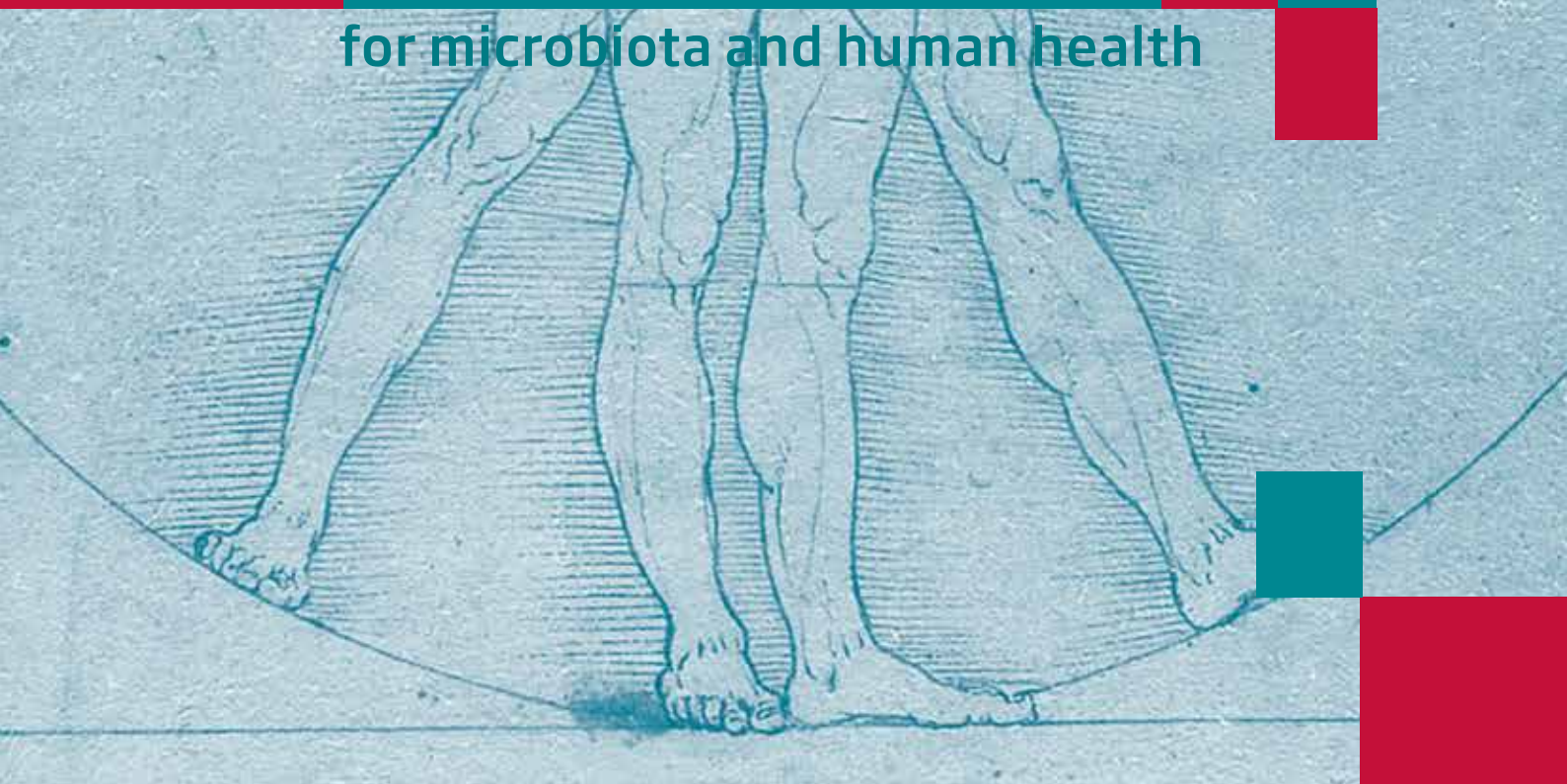
Rome - September 13/15, 2015

Università Urbaniana



8TH PROBIOTICS, PREBIOTICS & NEW FOODS

for microbiota and human health



SCIENTIFIC ORGANISERS

L. Capurso (Italy)

L. Morelli (Italy)

INTERNATIONAL SCIENTIFIC COMMITTEE

G. Barbara (Italy)

P. Brigidi (Italy)

G. Delle Fave (Italy)

J. Dorè (France)

V. Fogliano (The Netherlands)

A. Gasbarrini (Italy)

F. Guarner (Spain)

M. Rescigno (Italy)

K. Tuohy (Italy)

PEDIATRIC DAY

A. Guarino (Italy)

SCIENTIFIC SECRETARIAT

G. Capurso (Italy)

M. Elli (Italy)

UNDER THE PATRONAGE OF

SIGE, Società Italiana di Gastroenterologia

MTCC, Mediterranean Task Force for Cancer Control

PROBIOSTUDIO
ITALIAN ACADEMY FOR THE STUDY OF HUMAN MICROBIOTA

NUTRITION FOUNDATION OF ITALY

SINut
Società Italiana di Nutraceutica

ASSOCIAZIONE GINECOLOGI UNIVERSITARI ITALIANI
AGUI
1992

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COLDIRETTI

THE PEDIATRIC DAY IS UNDER THE PATRONAGE OF

ESPGHAN, European Society for Paediatric Gastroenterology, Hepatology and Nutrition

INDEX

09.00-11.30 a.m. **JOINT MEETING SIGE & MTCC**
Chair: A. Montori (Italy)
Moderators: A. Gasbarrini (Italy), M. Crespi (Italy)

Alcoholic Liver Disease: role of microbiota
M. Antonelli (Italy)

Probiotics in peptic ulcer
Z. Sharaiha (Jordan)

Probiotics and liver diseases
A. N. Elzouki (Qatar)

Mucosal adhesion and anti-inflammatory effects of Lactobacillus GG
C. Pagnini (Italy)

The role of Vitamin D in colorectal carcinogenesis
S. Manxhuka-Kerliu (Kosovo)

A functional tomato-based product for prostate cancer prevention
S. Iacobelli (Italy)

Gastrointestinal health, nutraceuticals and cancer
A. Saggioro (Italy)

11.30 a.m.-01.00 p.m. **LECTURES**
Chairs: B. Annibale (Italy), M. Del Piano (Italy)

Probiotics history
G. Gasbarrini (Italy)

Probiotics and diverticular disease: evidence based?
B. Annibale (Italy)

The emerging role of gut microbiota in autism pathogenesis: a new hope for effective prevention and treatment
E. Grossi (Italy)

Probiotics for Africa: a progress report
L. Mogna (Italy)

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

02.00-03.30 p.m. **OPENING CEREMONY**
L. Capurso (Italy)
R. Marabelli - Ministero della Salute (Italy)
C. Lambert - IPA Europe
V. Savarino - SIGE (Italy)
A. Guarino - ESPGHAN

03.30-04.00 p.m. **OPENING LECTURE**
Chair: B. Scarpa (Italy)

A system view on microbiota and health
B. van Ommen (The Netherlands)

04.00-06.00 p.m. **GUT MICROBIOTA**
Chairs: F. Guarner (Spain), J. Dorè (France)

Clinical relevance of enterotypes
J. Dorè (France)

Integrated meta-omic profiling to unveil microbiota patterns
L. Putignani (Italy)

Quest for causality: the case of Akkermansia
C. Belzer (The Netherlands)

Celiac disease and gut microbiota
Y. Sanz (Spain)

Gut microbiota and obesity
A. M. Castellazzi (Italy)

06.00-07.30 p.m. **GUT MICROBIOTA, ANTIBIOTICS AND PROBIOTICS**
Chairs: A. Gasbarrini (Italy), G. Ippolito (Italy)

Introduction
G. Ippolito (Italy)

Gut microbiota and antibiotics
C. Scarpignato (Italy)

Gut microbiota diabetes and insulin resistance
A. Everard (Belgium)

Gut microbiota antimicrobials and obesity
E. Murphy (Ireland)

Fecal trasplant
A. Gasbarrini (Italy)

WELCOME COCKTAIL

SIGE-GUT MICROBIOTA STUDY GROUP UPTODATE MEETING
Coordinators: A. Gasbarrini (Italy), G. Capurso (Italy)

03.00-04.00 p.m.

SESSION 1
GUT MICROBIOTA COMPOSITION
Moderators and discussants: M. Cicala (Italy), D. Festi (Italy)

Bacteriome
V. Iebba (Italy)

Virome
S. Petta (Italy)

Mycome
L. Putignani (Italy)

04.00-05.00 p.m.

SESSION 2
GI RELATED DISORDERS
Moderators and discussants: C. Ciacci (Italy), L. Biancone (Italy)

Celiac disease
G. Losurdo (Italy)

Gut microbiota and inflammatory bowel
F. Zorzi (Italy)

Colon cancer
C. Fazio (Italy)

05.00-06.00 p.m.

SESSION 3
LIVER AND PANCREAS
Moderators and discussants: G. Delle Fave (Italy), D. Alvaro (Italy)

Pancreas
M. Signoretti (Italy)

Gut microbiota in “liver disease”
F. Ponziani (Italy)

Biliary tract
M. C. Bragazzi (Italy)

06.00-07.00 p.m.

SESSION 4
NOVELTY ON MICROBIOTA MODULATION
Moderators and discussants: G. Cammarota (Italy), E. Corazziari (Italy)

Rifaximin
F. Ponziani (Italy)

Inulin
L. Laterza (Italy)

Microbial transplantation
G. Ianiro (Italy)

08.30-10.30 a.m.

NEW FOODS

Chair: V. Fogliano (The Netherlands)

Discrete chemical and physical dietary fiber structures and their potential role in favoring gut bacteria
B. R. Hamaker (USA)

An advanced *in vitro* technology platform to study the mechanism of action of pre- and probiotics in the gastrointestinal tract
M. Marzorati (Belgium)

Functional food from broccoli: the glucosinolate story
R. Verkerk (The Netherlands)

Bitter taste and satiety: a new concept to design effective food components
P. Vitaglione (Italy)

Functional food activating PPAR gamma in the treatment of lactose intolerance
P. Desreumaux (France)

Novel food formula suitable for 3D printing
C. Severini (Italy)

10.30-12.00 a.m.

PREBIOTIC MICROBIOTA MODULATION FOR IMPROVED HUMAN HEALTH

Chair: K. M. Tuohy (Italy)

Ageing, immunity and influence of the gut microbiota
P. Yaqoob (UK)

Modulation of the gut-brain axis with prebiotics for improved brain function
P. W. J. Burnet (UK)

How prebiotics help the gut microbiota to modulate liver and adipose tissue metabolism
N. Delzenne (Belgium)

Prebiotic microbiota modulation reducing the risk of metabolic syndrome
F. Fava (Italy)

The role of prebiotic milk oligosaccharides in host microbial interactions
S. Ross (Ireland)

Ketogenic diet and microbiota
A. Tagliabue (Italy)

12.00 a.m.-01.00 p.m. **LECTURES**
Chairs: G. Torre (Italy), M. Koch (Italy)

Mediterranean diet and gut microbiota
E. Roda (Italy)

Vitamin D and gut microbiota. Immune and anti-tumoral activity
M. L. Brandi (Italy)

The role of vitamin D in allergic disease in children
M. Miraglia del Giudice (Italy)

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

02.00-03.00 p.m. **LACTOBACILLUS GG IN CLINICAL PRACTICE**
Chair: L. Capurso (Italy)

The epigenetic effects of LGG in children with food allergy
R. Berni Canani (Italy)

The use of Lactobacillus rhamnosus GG in Pediatrics: evidence from the literature
S. Cucchiara (Italy)

The role of scientific evidence in the choice of probiotics: the LGG Case
A. Gasbarrini (Italy)

03.00-04.30 p.m. **MICROBIOTA IMMUNE SYSTEM AND BILE ACIDS**
Chairs: M. Rescigno (Italy), P. Nisticò (Italy)

Role of immune cells in microbiota handling
K. McCoy (Switzerland)

Microbiota and bile acids: the gut-liver axis
A. Moschetta (Italy)

Microbiota and barrier defence
M. Rescigno (Italy)

04.30-06.00 p.m. **MICROBIOME, DIET AND CO-EVOLUTION**
Chair: P. Brigidi (Italy)

Gut microbiome of the Hadza hunter-gatherer
A. G. Henry (Germany)

Metagenome sequencing of the hunter-gatherer gut microbiota
M. Candela (Italy)

The effect of diet globalization on gut microbiota
C. De Filippo (Italy)

Characterization of microbiota of non-western populations
D. Cavalieri (Italy)

06.00-07.30 p.m. **MICROBIOTA AND FUNCTIONAL GASTROINTESTINAL DISORDERS**
Chairs: G. Barbara (Italy), V. Stanghellini (Italy)

Introduction
V. Stanghellini (Italy)

Microbiota-brain axis from animal models to patients
S. M. Collins (Canada)

Microbiota epithelial interactions relevance for IBS
A. Gasbarrini (Italy)

Probiotics and FGID
F. Guarner (Spain)

Concluding remarks
G. Barbara (Italy)

09.00-10.00 a.m. **MICROBIOTA AND SKIN**
Chairs: M. Picardo (Italy), A. Cristaudo (Italy)

Microbioma skin gut axis
L. Drago (Italy)

Skin microbioma and acne
M. Ottaviani (Italy)

Skin microbioma and atopic dermatitis
A. Cristaudo (Italy)

10.00-11.00 a.m. **LECTURES**
Chairs: M. Anti (Italy), P.G. Natali (Italy)

Gut microbiota and liver
M. Koch (Italy)

Gut microbiota and cirrhosis: role of probiotics
R. K. Dhiman (India)

Laparoscopic Bariatric Surgery: evidence and unmet needs
P. Gentileschi (Italy)

11.00 a.m.-01.00 p.m. **VAGINAL MICROBIOTA AND WOMAN'S HEALTH**
Chiars: R. Di Iorio (Italy), F. Facchinetti (Italy)

Vaginal microbiota and woman's age
F. De Seta (Italy)

Genes and nutrition in metabolic syndrome
C. Zadro (Italy)

Microbiota changes in obese women
F. Facchinetti (Italy)

Prevention of maternogenic preeclampsia by high dose multiple strains probiotics supplementation
E. Ferrazzi (Italy)

Probiotics in the treatment of Candida vulvo-vaginitis and bacterial vaginosis
F. Vicariotto (Italy)

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

PEDIATRIC DAY

9.00-10.30 a.m **NASH AND OBESITY**
Chair: B. Koletzko (Germany)

Dysbiosis and pathophysiology
E. Isolauri (Finland)

Clinical data in children (RCT and metanalysis)
V. Nobili (Italy)

Indications and recommendations by societies and institutions
U. Baumann (Germany)

Developments: where are we, what needs to be done
B. Koletzko (Germany)

10.30-11.00 a.m. **Break**

11.00 a.m.-12.30 p.m. **FOOD ALLERGY**
Chair: J. Vanderhoof (USA)

Dysbiosis and pathophysiology
R. Berni Canani (Italy)

Clinical data in children (RCT and metanalysis)
A. Fiocchi (Italy)

Indications and recommendations by societies and institutions
S. Koletzko (Germany)

12.30-01.00 p.m. **LECTURE**
The use of probiotics in necrotizing enterocolitis
W. Mihatsch (Germany)

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

02.00-04.00 p.m. **FUNCTIONAL INTESTINAL DISORDERS**
Chair: Y. Vandenplas (Belgium)

Dysbiosis and pathophysiology
G. Barbara (Italy)

Clinical data in children (RCT and metanalysis)
- Colicky infants
  H. Szajewska (Poland)
- IBS
  A. Staiano (Italy)
- Constipation
  M. M. Tabbers (The Netherlands)

Indications and recommendations by societies and institutions
R. Francavilla (taly)

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

04.30-05.00 p.m. Interactive session and presentation of the ESPGHAN algorithms
Y. Vandenplas (Belgium)

05.00-06.00 p.m. **POSTER SESSION OR NEW STUDIES: A BRIEF SESSION TO PRESENT AND DISCUSS NEW DATA**
PROPOSALS NEEDED

08.30-10.30 a.m. **GUT MICROBIOTA AND IBD**
Chair: R. Caprilli (Italy)
Moderators: F. Pallone (Italy), M. Fantini (Italy)

The good and the bad guys of the intestinal microbiota
H. Sokol (France)

Western diet and IBD susceptibility
N. Barnich (France)

Instability of the gut microbiota in IBD
F. Guarner (Spain)

Use of food-grade bacteria recombinant for protease inhibitor to treat intestinal inflammation
N. Vergnolle (France)

Fecal transplant in UC
C. Y. Ponsioen (The Netherlands)

10.30-11.30 a.m. **LECTURES**
Chair: G. Delle Fave (Italy)

Redox regulation of gut, microbiota interactions
G. Rotilio (Italy)

B. clausii in immunity: evidences from preclinical to clinical
L. Morelli (Italy)

Radiotheray and gut microbiota
G. Arcangeli (Italy)

11.30 a.m.-01.00 p.m. **NUTRITION AND CANCER**
Chair: P. Marchetti (Italy)

Gut microbioma and gastrointestinal cancer: *les liasions dangereuses*
N. Tozun (Turkey)

Nutritional derivatives and cancer prevention
A. Albini (Italy)

Role of nutrition in cancer treatment
D. Rasio (Italy)

ORAL COMMUNICATIONS

08.30-10.00 a.m

PROBIOTICS 1

Moderator: C. Severi (Italy)

OC. 1 - BIFIDOBACTERIUM ANIMALIS SUBSP LACTIS CNCM-I2494 RESTORES TIGHT JUNCTION PROTEINS LEVELS IN A CHRONIC LOW-GRADE COLONIC INFLAMMATION MOUSE MODEL

Rebeca Martin [1], Laure Laval [1], Florian Chain [1], Sylvie Miquel [1], Jane M Natividad [1], Claire Cherbuy [1], Harry Sokol [1], Elena F Verdu [2], Johan van Hylckama Vlieg [3], Luis G Bermudez-Humaran [1], Tamara Smokvina [3], Philippe Langella [1]

[1] INRA, MICALIS INSTITUTE, Jouy en Josas, France
[2] Farcombe Digestive Disease Institute, McMaster University, Hamilton, Canada
[3] Danone Nutricia Research, Danone Nutricia Research, Palaiseau, France

OC. 2 - INTESTINAL MICROBIOTA IS INVOLVED IN GENETIC INSTABILITY, INFLAMMATION, LONGEVITY AND LATENCY OF LYMPHOMA IN ATM DEFICIENT MICE.

Robert Schiestl [1]

[1] Departments of Pathology, Environmental Health Sciences and Radiation Oncology, UCLA, Los Angeles, United States

OC. 3 - A DUAL-ENVIRONMENT CO-CULTURE SYSTEM TO BETTER EVALUATE EFFECTS OF FOOD INGREDIENTS ON INTESTINAL BARRIER INTEGRITY IN PHYSIOLOGICALLY RELEVANT CONDITIONS

Rachel Anderson [1], Nicole Roy [1]

[1] Food Nutrition & Health Team, AgResearch, Palmerston North, New Zealand

OC. 4 - DISCOVERY OF A CONJUGATIVE MEGAPLASMID IN BIFIDOBACTERIUM BREVE

Francesca Bottacini [1], Mary O'Connell Motherway [1], Eoghan Casey [1], Brian McDonnell [1], Jennifer Mahony [1], Marco Ventura [2], Douwe van Sinderen [1]

[2] University College Cork, University, Cork, Ireland [1], University of Parma, University, Parma, Italy

OC. 5 - EXPOSURE OF LACTOBACILLUS ACIDOPHILUS AND LACTOBACILLUS CASEI TO 2.4 GHZ ELECTROMAGNETIC RADIOFREQUENCY RADIATION ENHANCES THE GROWTH OF THESE PROBIOTIC BACTERIA

S Amanat [1], SMJ Mortazavi [2], F Shekoohi-Shooli [3], SM Mazloomi [1], F Sadeghi [3], S Nematollahi [4], M Haghani [3]

[1] Nutrition and Food Sciences Research Center, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran, Shiraz University of Medical Sciences, Shiraz, Iran
[2] Ionizing and Non-ionizing Radiation Protection Research Center (INIRPRC), Shiraz University of Medical Sciences, Shiraz
[3] Ionizing and Non-ionizing Radiation Protection Research Center (INIRPRC), Shiraz University of Medical Sciences, Shiraz, Iran
[4] Biostatistics Department, Shiraz University of Medical Sciences, Shiraz, Iran

OC. 6 - PROTECTIVE ACTIVITY OF LACTOBACILLUS RHAMNOSUS GG-DERIVED FACTORS ON PATHOGEN LIPOPOLYSACCHARIDE (LPS)-INDUCED DAMAGE OF HUMAN COLONIC SMOOTH MUSCLE CELLS

Alessia Cicenia [1], Floriana Santangelo [2], Loredana Gambardella [3], Valerio Iebba [2], Annunziata Scirocco [1], Massimo Marignani [4], Piero Chirletti [5], Lucia Pallotta [1], Marilia Carabotti [1], Enrico Corazziari [1], Serena Schippa [2], Carola Severi [1]
[1] Department of Internal Medicine and Medical Specialties, Sapienza University, Rome, Italy
[2] Department of Public Health and Infectious Diseases, Sapienza University, Rome, Italy
[3] Department of Medicine, Istituto Superiore di Sanità, Rome, Italy
[4] UOC Diseases of the digestive system and liver (S.Andrea Hospital), Sapienza University, Rome, Italy
[5] Department of Surgery, ''F. Durante'', Sapienza University, Rome, Italy

OC. 7 - PHYSICIAN PERCEPTIONS ON PROBIOTICS: RESULTS OF A MULTINATIONAL SURVEY

Christian Boggio Marzet [1], Annalisa Passariello [2], Roberto Berni Canani [2], Andras Arato [3], Serhat Bor [4], Ener Dinleyici [5], Uday Ghoshal [6], Francisco Guarner [7], Aldo Maruy [8], Ettair Said [9], Sohail Thobani [10], Lin Zhang [11]
[1] Pediatric Gastroenterology & Nutrition Section, Hospital Gral. de Agudos "Dr. I.Pirovano", CABA, Argentina
[2] Department of Traslational Medical Science, University of Naples Federico II, Naples, Italy
[3] Pediatric Gastroenterology, Semmelweis University, Budapest, Hungary
[4] Tıp Fakültesi Gastroenteroloji Kliniği, Ege Üniversitesi, Izmir, Turkey
[5] Department of Pediatrics, Eskisehir Osmangazi University, Eskisehir, Turkey
[6] Department of Gastroenterology, S.G.P.G.I, Lucknow, India
[7] Digestive System Research Unit, Vall d'Hebron Research Institute, Barcelona, Spain
[8] Gastroenterología Pediátrica, Hospital Nacional Cayetano Heredia, Lima, Peru
[9] Department of Pediatrics, Rabat University, Rabat, Morocco
[10] Department of Pediatric Gastroenterology, South City Hospital, Karachi, Pakistan
[11] Department of Pediatrics, 3rd Hospital of Hebei Medical University, Hebei, China

OC. 8 - THE ACTION OF DIFFERENT PROBIOTICS IN CORRECTING ACTIVITY OF INTESTINAL ENZYMES IN RATS AFTER ADMINISTRATION OF ANTIBACTERIAL AGENTS

Lyudmila Gromova [1], Elena Ermolenko [2], Yulia Dmitrieva [1], Anna Alekseeva [1], Yuri Borschev [2], Andrei Gruzdkov [1], Alexander Suvorov [2]
[1] I. P. Pavlov Institute of Physiology, RAS, St. Petersburg, Russian Federation
[2] Institute of Experimental Medicine, RAS, St. Petersburg, Russian Federation

10.00-11.30 a.m

PROBIOTICS 2

Moderator: G. Capurso (Italy)

OC. 9 - IMMUNOMODULATORY IN VITRO AND IN VIVO EFFECTS OF LACTOBACILLUS RHAMNOSUS AND ELDERBERRY EXTRACT ALONE AND IN COMBINATION

Stephan Maurel [1], Christine Libon [2], Sandrine Pourtau [2], Claire Issac [2], Laila Haddioui [3], Christophe Ripoll [1]
[1] Naturactive, Laboratoires Pierre Fabre, Castres, France
[2] Pierre Fabre Research Institute, Pierre Fabre R&D Center, Toulouse, France
[3] Fonderephar, Toulouse, France

OC. 10 - FEATURES OF THE PROBIOTIC ENTEROCOCCI INFLUENCE ON THE IMMUNE SYSTEM IN EXPERIMENTAL MODELS OF MULTIPLE SCLEROSIS AND INTESTINAL DYSBIOSIS

Elena Ermolenko [1], Irina Abdurasulova [1], Elena Tarasova [1], Galina Leontieva [1], Marina Kotyleva [1], Tatyana Kramskaya [1], Igor Kudryavtsev [1], Alexander Suvorov [1]
[1] Institute of Experimental medicine, University, Saint Petersburg, Russian Federation

OC. 11 - STUDIES ON THE IDENTIFICATION OF BIFIDOBACTERIA ISOLATED FROM HUMAN BREAST MILK OF INDIAN WOMEN

Shiva Prakash Myakala [1], Madhavi G [1], Nishanth Kumar S [1], Chathyushya K B [1], Sumalata G [1], Hemalatha R [1]
[1] *National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India*

OC. 12 - ELECTRON MICROSCOPIC INVESTIGATION OF PROBIOTIC BACTERIA INFLUENCE ON RAT INTESTINE MUCOSA IN DYSBIOSIS EXPERIMENTAL MODEL

Oksana Rybalchenko [1], Elena Ermolenko [2], Olga Orlova [1], Alexander Suvorov [2]
[1] *St. Petersburg State University, University, Saint Petersburg, Russian Federation*
[2] *Institute of experimental medicine, University, Saint Petersburg, Russian Federation*

OC. 13 - ANTI-OBESITY POTENTIAL OF LACTOBACILLUS SALIVARIUS LPLM-01 IN A MURINE MODEL OF DIET-INDUCED OBESITY

Erica Castro [1], Joaquín Rojas-Fritz [2], Juan Pablo Mellado [3], María José Aguayo [3], Karen Pardo [3], Pamela Contreras [3], Sebastián Martínez [4], Margarita González [2], Rodrigo Bórquez [4], Jaime Cofré [3], Daniel Durán-Sandoval [2]
[1] *Faculty of Medicine, St. Sebastian University, Concepción, Chile*
[2] *Department of Clinical Biochemistry and Immunology, Faculty of Pharmacy., University of Concepción, Concepción, Chile*
[3] *Laboratory of Lactic Bacteria, University of Concepcion, Concepción, Chile*
[4] *Department of Chemical Engineering, Faculty of Engineering, University of Concepción, Concepción, Chile*

OC. 14 - EFFICACY OF PROBIOTICS IN PATIENTS WITH LACTOSE INTOLERANCE, A PRELIMINARY STUDY

Rachel Gingold-Belfer [1], Tsachi Tsadok Perets [1], Einav Shporn [1], Ido Blechman [1], Sigal Levi [2], Lea Pakanaev [1], Yaron Niv [1], Ram Dickman [1]
[1] *Gastroenterolgoy Department, Rabin medical Center, Petach Tikva, Israel*
[2] *Statistics Department, The academic College of Tel Aviv Jaffa, Tel Aviv, Israel*

OC. 15 - RESTORE AND MAINTAINING OF HUMAN GUT MICROBIOTA DURING THE ANTIBACTERIAL THERAPY

Svetlana Zakirova [1], Anastasia Koval [2]
[1] *Lomonosov Moscow State University, Lomonosov Moscow State University, Moscow, Russian Federation*
[2] *Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russian Federation*

OC. 16 - THE EVALUATION OF EMULSION TECHNIQUE FOR MICROENCAPSULATION OF LACTOBACILLUS PLANTARUM WITH ALGINATE-RESISTANT STARCH CAPSULES

Yahya Shafiei [1]
[1] *Department of Food Science and Technology, Khoy Branch, Islamic Azad University, Khoy, Iran*

OC. 17 - ANTIVIRAL ACTIVITY OF DIFERENT PROBIOTIC STRAINS IN VERO CELL LINE

Konstantin Ermolenko [1], Alexander Colobov [2], Anna Zakrevskaya [1], Lydia Kulyashova [1], Yulia Desheva [3], Elena Ermolenko [3]
[1] *Saint-Petersburg Pasteur Institute, University, Saint Petersburg, Russian Federation Institute of highly pure biopreparations, University, Saint Petersburg, Russian Federation*
[2] *Federal State Budgetary Scientific Institution "Institute of Experimental Medicine", University, Saint Petersburg, Russian Federation* [3]

OC. 18 - PROBIOTICS IMPROVE THE IRON ABSORPTION FROM A MEAL

Gunilla Önning [1], Michael Hoppe [2], Malin Björklund [3], Niklas Larsson [3], Gun-Britt Fransson [3], Lena Hulthén [2]
[1] Pure and Applied Biochemistry and Probi AB, Lund University, Lund, Sweden
[2] Department of Internal Medicine and Clinical Nutrition, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden
[3] Probi AB, -, Lund, Sweden

OC. 19 - AGING-RELATED CHANGES OF GUT MICROBIOTA COMPOSITION FROM NEW-BORN TO CENTENARIAN, CROSS-SECTIONAL STUDY

Toshitaka Odamaki [1],Kumiko Kato [1], Hirosuke Sugahara [1], Nanami Hashikura [1], Sachiko Takahashi [2], Jin-zhong Xiao [1], Fumiaki Abe [2], Ro Osawa [3]
[1] Next Generation Science Institute, Morinagamilk Industry, Zama, Japan
[2] Food Ingredients & Technology Institute, Morinagamilk Industry, Zama, Japan
[3] Department of Bioresource Science, Graduate School of Agricultural Science, Kobe University, Kobe, Japan

11.30a.m.-01.00 p.m.

NEW FOODS

Moderetor: K. M. Tuohy (Italy)

OC. 20 - DESIGN OF EXPERIMENT APPROACH FOR DEVELOPMENT OF OAT BASED FOOD PRODUCT FORTIFIED WITH PREBIOTIC (HONEY) AS POTENTIAL PROBIOTIC VEHICLE

Bijender Kumar [1], Mahak Gupta [1]
[1] University of Jammu, School of Biotechnology University of Jammu, Jammu, India

OC. 21 - CHOLESTEROL CONTENT OF LIGHVAN CHEESE: A NATURAL PROBIOTIC TRADITIONAL CHEESE

Morad Bahar [1], Ainaz Alizadeh [2]
[2] Faculty of veterinary medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[1] Department of Food Science and Technology, Tabriz Branch, Islamic Azad University, Tabriz, Iran

OC. 22 - THE USE OF COMPLEMENTARY AND ALTERNATIVE MEDICINE IS FREQUENT IN PATIENTS WITH PANCREATIC DISORDERS

Serena Stigliano [1], Matteo Piciucchi [1], Livia Archibugi [1], Giulia Zerboni [1], Gianfranco Delle Fave [1], Gabriele Capurso [1]
[1] La Sapienza University, sant'Andrea Hospital, rome, Italy

OC. 23 - CHEMICAL CHARACTERISTICS AND SURVIVAL OF PROBIOTICS IN CHEDDAR CHEESE FORTIFIED WITH PHENOLIC COMPOUNDS OF MANGO (MANGIFERA INDICA L.) KERNEL OIL

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OC. 24 - THE PERSPECTIVE USE OF NOVEL STRAINS LACTOCOCCUS LACTIS SSP. LACTIS FOR FOOD

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OC. 25 - PHYSICOCHEMICAL PROPERTIES OF FUNCTIONAL SCAMORZA CHEESE FROM OVINE MILK

Marzia Albenzio [1], Antonella Santillo [2], Mariangela Caroprese [3], Rosaria Marino [2], Antonella Della Malva [4], Lucia Figliola [2], Agostino Sevi [2]
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OC. 26 - EFFECT OF HYDROPINIC TREATMENT WITH CALCIUM BICARBONATE WATER PLUS L. REUTERI ON OROCAECAL TRANSIT IN PATIENTS SUFFERING FROM CHRONIC CONSTIPATION

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OC. 27 - EFFECT OF HYDROPINIC TREATMENT WITH CALCIUM CARBONATE WATER PLUS L. REUTERI ON GASTRIC EMPTYING IN DYSPEPSIA

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OC. 28 - DOES PARTIALLY HYDROLYSED GUAR GUM HAVE A ROLE TO PLAY IN THE TREATMENT OF IRRITABLE BOWEL SYNDROME: A SYSTEMATIC REVIEW

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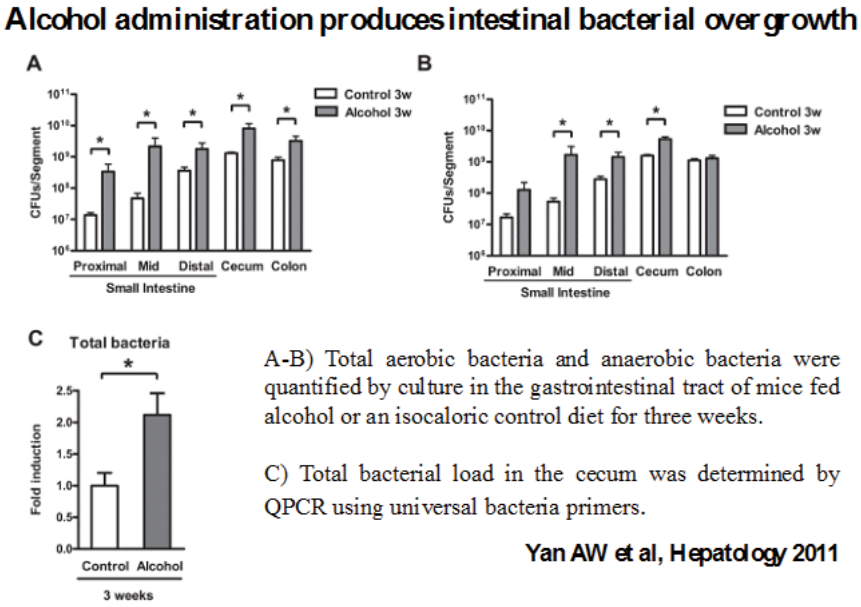
PROCEEDINGS

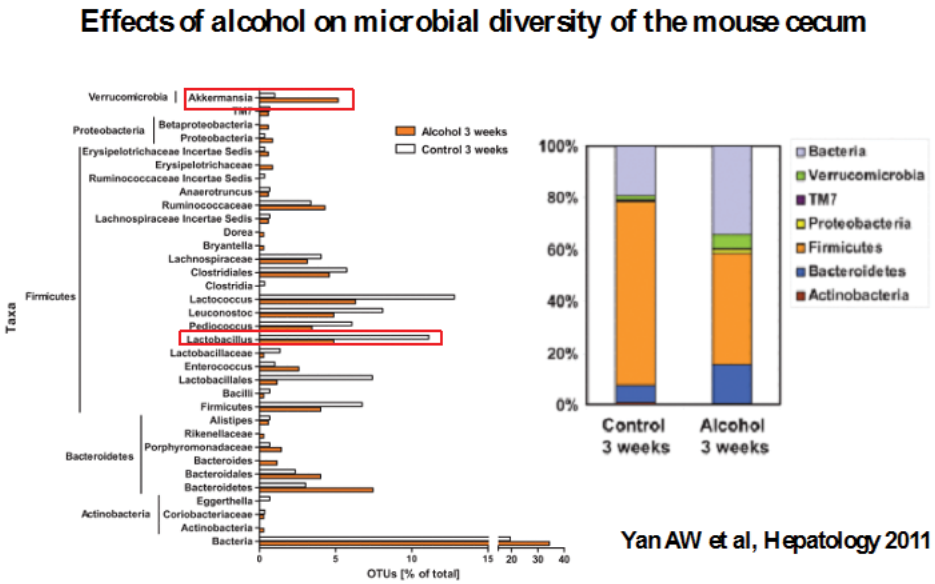
ALCOHOLIC LIVER DISEASE: ROLE OF GUT MICROBIOTA

Mariangela Antonelli, MD

Alcohol over-consumption, which means consumption of up to 2 drinks per day for men and up to1 drink per day for women, leads to an easier development of alcohol-related pathologies such as cardiopathy, pancreatitis, infections and, above all, liver disease and malnutrition.
As known from Bellentani's work in 1994, alcohol is the major cause of liver chirrosis in western countries: alcohol abuse alone works as an aetiological cause for 23% of the disease occurrences, while resulting in 36% of the occurrences if combined with HCV or HBV infection.
Environmental or host factors influence an individual's susceptibility to alcoholic liver disease. Efforts to define these "co-morbidity factors" are becoming a major focus for alcohol researchers and should help to clarify the mechanisms by which alcohol injures liver cells: among these, there have been recently enlisted gut microbiota.
Gut microbiota is a complex of different microbes present in the gastrointestinal lumen. It consists of about 10 to 14th microbial cells (about 10 times the number of somatic cells in the human body). Their collective genomas contains at least 100 times as many genes as our own genome, named microbiota. Over 80% of microbiota is formed by bacterial phyla named Bacteroides and Firmicutes. Some of these bacteria live in commensalism state while others could potentially become pathogens.
The exact role of microbiota is still unknown, although some functions such as: maintenance of barrier functions, development and modulation of immune system and immunological tolerance, vitamin synthesis, drug and toxic metabolism, behavioural conditioning, have already been demonstrated. These bacterial species and their concentrations vary along GI tract.
Variations related to age, dietary habits, geographical origin, stress levels and lifestyle habits – such as alcohol use – can induce modifications of gut microbiota.
Our research group's review onto the correlation between the use of alcohol and gut microbiota shows that alcohol abuse induces changes in the composition of gut microbiota and therefore that the modulation of gut microbiota seems to be a promising strategy to reduce alcohol-induced liver injury.
According to the 2011 work of Yan *et al*, intestinal bacterial overgrowth was observed in GI tract of mice fed with alcohol for 3 weeks compared with control mice fed with isocaloric liquid diet.



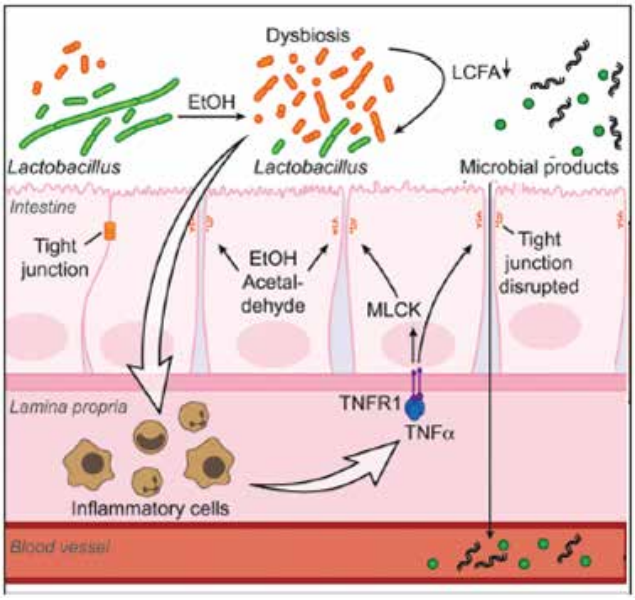
Significantly, a relative abundance of bacteroides and verrucomicrobia bacteria was observed in mice fed with alcohol, compared with a relative predominance of firmicutes bacteria in control mice. Alcohol-fed mice also presented an overgrowth of akkermansia muciniphila, a microbe able to degrate mucin: this change could promote bacterial translocation. Moreover, lactobacilli were depleted in alcohol-treated mice, which might explain the beneficial effect of probiotics in the prevention of increased gut permeability, endotoxinemia and liver injury reported in alcohol-fed mice.



But how does alcohol induce changes in gut microbiota? The first mechanism is the faecal stasis and bacterial overgrowth resulting in a higher number of luminal bacteria, derived from reduced GI motility by alcohol. Also hypochlorhydria due to alcohol consumption is associated with small intestine bacterial overgrowth. Another mechanism is represented by alcohol induced suppression of innate immune response and adaptive immune response, by GALT lymphoid cells depletion. Moreover, alcohol use in mice is able to suppress bactericidal protein expression regenerating islet derived (Reg)-3b and Reg 3g.

Setting a comparison among the several studies onto alcohol effects on the gut microbiota makes it difficult to establish what exactly happens. However, despite the different numbers of enrolled patients, the different types of sample, and the different methods of research, it seems that alcohol consumption and abuse is likely to produce quantitative and qualitative alterations of gut microbiota.

Alcohol-induced changes on gut microbiota play an important role in the pathogenesis of ALD.



Chronic alcohol consumption leads bacterial overgrowth and dysbiosis, which may respectively induce an increased gut permeability and an intestinal mucosa inflammation.

Metabolic changes such as lower bacterial synthesis of long-chain fatty acids (LCFAs) result in smaller amounts of “good” bacteria.

Inflammatory cells of the intestinal lamina propria are activated and secrete tumor necrosis factor (TNF)-alpha which results in a disruption of tight junctions.

Alcohol and its metabolite acetaldehyde might contribute to a dysfunction of the gut barrier

Microbial products can therefore translocate from the intestinal lumen to the portal venous blood with a massive exposure of liver parenchima to LPS,

which stimulates innate immune receptors with activation of kupferr and hepatic stellate cells. This induces the release of proinflammatory mediators like ros, leukotrienes, chemokines and cytokines that contribute to liver damage.
In bacterial translocation akkermansia muciniphila plays a crucial role because it is strongly induced by alcohol consumption. As previously said, it has been found to degrade mucin.
By degrading the intestinal mucus, bacterial translocation might be facilitated.
Moreover, a significant increase in endotoxinemia was found after acute alcohol intoxication. The endotoxinemia seems to be correlated with haemodynamic derangement in cirrhotic portal hypertension and with levels of soluble TNFa receptors.
In the study of Bajaji *et al*, alcoholic cirrhotics had a significantly higher abundance of *Enterobacteriaceae* and *Halomonadaceae*, lower *Lachnospiraceae*, *Ruminococcaceae* and Clostridialies XIV, high endotoxin and lower CDR (cirrosis disbiosis ratio) despite statistically similar MELD score and BMI compared to those without alcoholic aetiology.
Pre-clinical studies indicate that pre-treatment with antibiotics or probiotics reduce sLPS endotoxin and to prevents ALD. In the clinical practice, the cornerstone of alcoholic liver disease treatment is achieving and maintaining long-term total alcohol abstinence. However, there is a subgroup of alcoholic liver disease patients (about 5–15%) that shows progression to fibrosis and cirrhosis despite total alcohol abstinence. Therapeutic modulation of gut microbiota in addition to total alcohol abstinence might be an adjunctive strategy for the treatment of alcoholic liver disease with the aim to prevent or delay hepatic damage.
At present, some preliminary human evidences indicate that antibiotics and probiotics are effective to reduce gam-negative bacteria population and to prevent alcohol-induced liver injury and progression of liver disease. Rifaximin has been used in ALD and approved for the long term treatment of hepatic encephalopathy. In fact, the treatment with rifaximin improves systemic haemodynamics and renal functions, cirrhosis-related thrombocitopenya and survival, reducing the risk of developing complications of portal hypertension. It can be hypothesised that the effect of rifaximin in patients with ALD are related to intestinal decontamination.
In addition to this, the use of probiotics can improve liver functions; according to Loguercio's study, the use of probiotics for 3 months in patients with ALD results in significantly reduced plasma levels of oxydative stress parameters, an improvement of liver function and of cytokine levels.
In conclusion the modulation of gut microbiota could be a good strategy to reduce alcohol inducedliver injury and to prevent the progression of disease. Further datas are needed to collect to draft conclusions.

PROBIOTICS IN H. PYLORI INFECTION

Z. Sharaiha M.D.

It was notuntill1982 when two Australian researchers Barry Marshal and Robin Warren discovered H. Pylori which was soon implicated as a cause of chronic gastritis ,peptic ulcer disease and malt lymphoma.
Now a days the H. Pylori eradication has dropped to approximately 70%.This decline is due to drug resistance ,compliance and adverse side effects like, nausea ,vomiting ,metallic taste and diarrhea.
Eradication therapy, certainly prevents peptic ulcer recurrence, and improves gastritis over a period of two years.
prompted by failure of eradication of H. Pylori in all cases ,researchers have looked for other options
Several studies showed that some stains of Lactobacillus strains have antagonistic activity on H. Pylori both in vitro and vivo in gneotoxenic mouse models .Again, fermented milk in human was found to improve eradication from 5% to 15 % .
Some probiotic strains, LA 302was found to stimulate production of IL10,hence modulate host immune system.
Lactobacillus plantarum 302,and lactobacillus salivarius LA302waere found to inhibit growth of h. pylori and as well prevents the adhesion of pathogen to gastric mucosa.
In conclusion; the addition of probiotic to standard triple therapy regimen, improves the eradication rate by 13%,and decreases the adverse side effects by 41 %.independent of age and dose of probiotics .Un expectedly, the compliance did not improve.
However ,economic evaluation of cost effectiveness of this therapy needs to be determined both in developed and developing countries where poor socio economic standard and poverty prevails.

PROBIOTICS AND LIVER DISEASE: WHERE ARE WE NOW AND WHERE ARE WE GOING?

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According to the currently adopted definition by the Food and Agriculture Organization and World Health Organization (FAO/WHO), "probiotics are: Live microorganisms, when administered in adequate amounts confer a health benefit on the host". Probiotics are used as effective biological factors for modulation of gut micro flora, and recently they are suggested as natural means for improving the liver function. The colonized gut flora in healthy individuals is affected by many physiological and environmental factors e.g., nutrition, illness, aging, and stress are the main parameters affecting the imbalance and impairment of the natural pattern of gut mechanism in healthy individuals. Therefore, probiotics are suggested as a beneficial agent to better balance the gut flora. Because of the functional link to the intestine, the liver is known as the first organ barrier against the gut-derived bacterial fractions or metabolites, which are persistently released in to the circulation. The kupffer cells as the liver macrophages particularly reduce the amount of bacterial phagocytosis and endotoxins.

Numbers of liver diseases in which probiotics may have a role in its management, these include: alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), hepatic encephalopathy, primary sclerosing cholangitis (PSC) and hepatocellular carcinoma (HCC).

Alcoholic steatohepatitis and severe ALD manifest in approximately 30% of heavy drinkers Alcohol consumption over a long period elevates the growth of gram negative bacteria and increases the amount of *bifidobacteria* and *lactobacilli*.

NAFLD, usually asymptomatic, is the most common liver disorder in western countries. It is defined as a chronic condition with more than 5%-10% of liver augmented by extra fat. The prevalence of NAFLD is rapidly rising, and is becoming a worldwide public health problem. Studies have confirmed that NAFLD is associated with body mass index (BMI), components of the metabolic syndrome, and insulin resistance. This disorder represents a spectrum of conditions ranging from fat accumulation alone (steatosis without inflammation) to non-alcoholic steatohepatitis (NASH) with macro vesicular steatosis in hepatocytes, associated with inflammation and fibrosis. NAFLD occurs among all ages, both the genders and various ethnic groups, and its prevalence is reported to be 14-30% of the general population. The prevalence of NAFLD is increasing as is the worldwide trend in obesity and Type II diabetes. Among the general adult population, the prevalence is estimated to be 20–30%, 15% and 30% in Western countries, Asian countries and USA. In Italy, the corresponding figure was estimated to be 12.5% and 23% among general population and obese adolescents respectively. The pathogenesis of NAFLD, and its development and progression to NASH remains to be determined. According to Day and James, the double "hit" theory can greatly explain the pathway and the mechanisms involved. At the "first hit", the development of simple liver steatosis occurs and is accompanied with systemic factors. Obesity and insulin resistance has been known to have a pivotal role in this phase. Such damage would result in increase in vulnerability to fatty liver changes to subsequent inflammatory products like bacterial lipopolysaccharide (LPS). The main factors contributing to initiation of the second hit are oxidative stress, subsequent lipid peroxidation, produced pro-inflammatory cytokines, such as tumor necrosis factor (TNF) α, and hormones secreted from adipose tissue. All these factors aggravate the transformation of NAFLD to NASH or even to cirrhosis. Currently, there is limited proven effective treatment for NASH. Since its underlying cause and its prognosis are not well understood, therapeutic modalities considered for patients with NAFLD has typically been focused on the management of associated conditions such as obesity, diabetes mellitus, and hyperlipidemia. The main treatment for NAFLD is lifestyle modification, including weight loss through a combination of decreased energy intake and increased energy expenditure. Recent studies have reported a possible impact of gut micro flora overgrowth on the development of NAFLD and NASH. Several microorganisms inhabit the human gut, and gut micro flora have a dual impact on the liver function. The fermentation products such as ethanol, ammoniac and acetaldehyde produced by gut microflora are metabolized in the liver. Moreover, lipopolysaccharide from gram negative gut micro flora is continuously released after bacterial death as endotoxin and transported via a toll-like receptor 4 (TLR-4)-dependent process into intestinal capillaries. This induces cytokines formation and secretion from liver. It is suggested that liver injury and fibrosis could be partly caused by exposure to bacterial products like LPS. TLR4-bearing stellate cells respond to LPS, producing inflammatory cytokines and chemokines, but also promoting fibrosis. Further research supports the role of TLR4 in promoting fibrosis. It has been shown that deficiency in myeloid differentiation factor-2 (MD-2), the coreceptor of TLR4, and TLR4 expression, may attenuate liver inflammation and fibrosis in mice affected by NASH. The relation between gut micro flora and NAFLD might be mostly because of the endogenous LPS. Gut micro flora have important role in the host physiological function and metabolism through the following mechanism: conversion of pro-carcinogen into less harmful substances, production of vitamins, degradation of bile acid and cholesterol, as well as facilitating nutrient digestion, especially fermentation of non-digestible carbohydrates.

Subclinical hepatic encephalopathy was first described in cirrhotic patients who by conventional neurological and mental status examination appeared normal but had abnormalities in psychometric tests. This condition is currently recognized as Minimal Hepatic Encephalopathy (MHE), it is a part of spectrum along hepatic encephalopathy characterized by abnormalities in psychometric and neurophysiological tests without overt clinical symptoms. Incidence of MHE ranges from 30% to 84% in patients with chronic liver disease. MHE is an under diagnosed problem, but its effect on daily activities could be profound as it impairs attention span and reaction time. It has been shown that MHE impairs fitness to drive. Similar observations in this subpopulation of cirrhotics confirm that MHE is a strong predictor for traffic violations and accidents. Cirrhotic patients with MHE have poor health related quality of life and impaired daily functioning, and employability, as confirmed by lower scores on the sickness impact profile (SIP). The major treatment modalities for MHE have been similar to that of overt hepatic encephalopathy: targeting ammonia production and absorption. A study reported significantly higher counts of *Escherichia-coli* and *staphylococcus* in the stool samples of patients with mild encephalopathy and cirrhosis than in healthy controls. As the gut microbiota play an important role in the generation of ammonia, its modulation using probiotics has been evaluated by several studies as a therapeutic option for MHE.

PSC is an autoimmune liver disease which involves bile ducts in and out of the liver. Cholestatic features of bile ducts are a result from progressive obliterative fibrosis. Although a close association between PSC and inflammatory bowel disease has been reported, however, its pathogenesis remains unknown. Immune and non-immune mechanisms are suggested for the pathogenesis of PSC. There is a substantial amount of evidence that the lymphocytes located in the gut play a critical role for emerging PSC. On the other hand, bacteria residing in the gut may be a part of the cause of PSC through non-immune routs. These microorganisms are able to release toxic compounds. Since the administration of antibiotics is an appropriate treatment for some patients with PSC, there is a possibility indicating the role of bacterial flora together with intestinal inflammation in the pathogenesis of PSC.

Few studies were performed to assess probiotic effects on toxicity of aflatoxin in liver dysfunction and HCC. Diminution of aflatoxin concentration was observed in fecal samples after the administration of *Lactobacillus rhamnosus* LC705. In another study, consumption of *Lactobacillus rhamnosus* LC705 together with *Propionibacterium freudenreichii subsp. shermanii* led to lower AFB-N7 guanine in urine samples when compared to a placebo. In recent in-vivo, gene expression changes induced by *Lactobacillus rhamnosus* was studied. GG consumption in rats exposed to aflatoxin. Concomitant with lowering of the c-myc, bcl2, cyclin D1 and rasp21 expression in treated rats compared to control group, the frequency of tumors in liver was alleviated.
A growing body of evidence suggests a relation between overgrowths of gut microbiota with pathogenesis of some chronic liver diseases (Figure 1). Various experimental studies and clinical trials revealed promising effects of probiotics in improving these diseases; however given the limited experience in this field, generalization of probiotics as treatment of such conditions needs more trials with large sample size and long-term follow up.

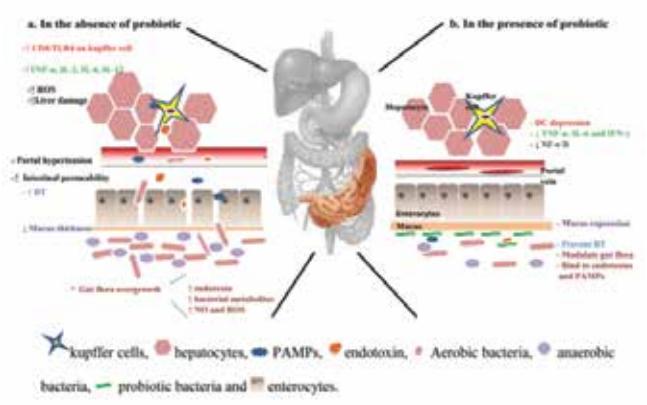

Figure 1. Mechanism of liver disease: a) in the absence of probiotics and b) in the presence of probiotics (source: reference 2).

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"TRANSITIONAL" MODELS FOR TRANSLATIONAL MEDICINE: AN EX VIVO ORGAN CULTURE TECHNIQUE TO EVALUATE PROBIOTIC UTILIZATION IN IBD

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The consistent technical and conceptual progress in the study of the microbiota have led novel impulse to the research for therapeutical application of probiotic bacteria in human pathologies, such as inflammatory bowel disease (IBD). Considering the heterogenous results of probiotics in clinical studies, the model of translational medicine may lead to a more specific and efficacious utilization of probiotic bacteria in IBD. In this regard, the selection and utilization of appropriate experimental models may drive the transition from pure in vitro systems to practical clinical application. We developed a simple and reproducible ex vivo organ culture method with potential utilization for the evaluation of probiotic bacteria efficacy in IBD patients.

Probiotics research: where are we now?

Probiotic bacteria have been largely proposed in different pathologic conditions and their use have increseased in the last decades [1]. Nonetheless, solid evidence for their use in specific pathologic conditions are limited, and too often their utilization is much more driven by commercial suggestion than by scientific proofs. Recently, probiotic bacteria have been proposed in the setting of inflammatory bowel diseases (IBD) [2]. Indeed, the increased comprehention of the complex pathogenesis of the disease, togheter with the growing knowledge of the microbiota composition and functions, highly stimulated by the novel culture-independent gernomic-based techniques (i.e. next generation sequencing), have consistently pushed forward the idea of the influence of the microbiota in IBD onset and development [3]. In fact, experimental and clinical evidence that microflora alteration may not only be a consequence of inflammation, but may have an important causative role in IBD course, are mounting [4-5]. Those findings are stimulating the research on potential therapeutical application of microbiota manipulation, by reduction of potential pro-inflammatory bacterial species (by antibiotics), stimulation of endogenous bacteria with anti-inflammatory properties (by prebiotics), administration of specific bacteria with favourable profile (probiotics) or of their products (postbiotics), or, lately, with the transfer of the whole fecal echosystem from a healthy donor (fecal transplantation).

In IBD, the therapeutic application of probiotic bacteria have been so far more convincing in experimental models than in clinical setting [6]. The reason probably resides primarily in the complexity of human disease, that is not easily reproducible in experimental models. As a consequence, animal and experimental models may be at best representative of specific aspects of the human condition, which is characterized by a complex interplay of genetic and environmental factors, as well as by the involvement of different molecular pathways. In the simplification of an experimental model, many therapeutic approach may result efficacious but then not directly applicable in clinical setting. Moreover, even thou clinical studies of probiotics application in IBD are growing, they are still characterized by a consistent heterogeneity in the design, bacterial species used, dosages, time of treatment and end-points. In addition, even a relatively homogeneous disease, such as ulcerative colitis (comparing with Crohn's disease), may include a continuous spectrum of different condition ranging from isolated mild proctitis to extensive severe pancolitis. Therefore, for the design of future trial, the correct selection of patients would be as relevant as the correct selection of the probiotic bacteria to test, since a single bacterial species may be efficacious only in a disease with a specific pattern, in terms of extension, activity and severity of inflammation.

The investigation of probiotic bacteria in clinical settings has been influenced by the technical and conceptual revolution of the microbiota field. Nowadays, two different approaches are leading the research of possible clinical application of probiotics. From one side, the accurate analysis of microbiota alteration in pathologic vs. physiologic conditions is leading to the identification, and to the possible test, of bacterial species with a possible specific role in the prevention of the pathologic conditions investigated. The fascinating approach of the development of those novel probiotic species have several limitation, thou. In fact, different composition in microbiota between patients and normal controls may not necessarily imply a causative role of the bacteria for the pathologic condition. Moreover, since the putative bacteria have not been used as a supplement in humans before, the identified species would need a thorough in vitro and in vivo characterization in terms of safety, efficacy, and stability both in drug formulation and in the gastrointestinal tract. To the other side, the other branch of research is focusing on the in-deep characterization of the properties of probiotic bacteria already known and diffuse in the market, such as *Lactobacillus rhamnosus* GG (LGG), which represent the most studied species in this class of bacteria. In this setting, the aim of the research is to evaluate specific characteristic of the bacteria with a potential application in particular clinical situation, with the advantage of the major comprehension of the general characteristics and of the safety profile of the bacteria. In fact, since those bacteria are already in use from quite a long time, drug formulation have been already developed and the safety profile is generally favorable. Nonetheless, utilization of such products is often empirical and unspecific, and appropriate evaluation in specific clinical indication is warranted.

Whatever research approach is applied, the rational and scrupulous development of combined in vitro/in vivo studies appears crucial for the proposal and implementation of probiotic bacteria application as therapeutic option in specific pathologic condition, including IBD. In this setting of translational medicine, the role of appropriate "transitional" models (i.e. experimental models more representative of the in vivo situation than the classic in vitro systems, that may favor and guide the transition to a specific clinical application in human) could be of great relevance. In accord with that, we developed a simple, economic and reproducible ex vivo experimental model to evaluate the adhesion and the mucosal effect of bacterial species administration on normal and pathologic intestinal mucosa.

Ex vivo organ culture experimental model

A synthetic scheme of the procedure of the method is represented in Figure 1. Bioptic samples are collected during colonoscopy from normal (proximal and distal segment) and pathologic colon (i.e. adenomatous polyps, ulcerative colitis patients). Biopsies are then washed two times in fresh phosphate buffered saline (PBS) solution and weighed to avoid consistent difference among samples. For the evaluation of bacterial adhesion, bioptic specimens are put in a 2 ml Falcon tube with 200 µL of Roswell Park Memorial Institute (RPMI) media, with the addition of 20 µL (1:10) of a solution of different probiotic formulations in PBS (i.e. for the evaluation of LGG a final concentration of 6 x 106 CFU/200µL in the reaction tube was obtained). As a negative

control, biotic samples are put in RPMI media with 1:10 of PBS without any probiotic bacteria. The reaction is incubated at 37° for 2 hours, and then the bioptic samples are collected and washed two times in fresh PBS to remove non adherent bacteria, and finally put in RNA Later solution (Qiagen, Valencia, CA, USA) until further processing. Total DNA is extracted by Qiamp DNA mini kit (Qiagen, Valencia, CA, USA) according to manufacturer instruction. All the DNA samples are checked by spectrophotometer Gene Quant pro RNA/DNA calculator (Amersham, Pharmacia Biotech, New Jersey, USA) for concentration and purity, and the solutions are brought at the same concentration. Real-time (RT) PCR is performed with specific primers for the different probiotics species (already described in literature), and adherent probiotic bacteria are quantified in a semi-quantitative manner by a relative ratio to the lowest detected sample. PCR reactions are performed in a total volume of 20 µl in an iCycler iQ detection system (Bio-Rad Laboratories, Inc, Hercules, CA, USA), with 16 µl of SYBR Green PCR Supermix (Bio-Rad Laboratories, Inc, Hercules, CA, USA) and 4 µl of target DNA. For every set of primers, the original cycle conditions are generally maintained. House-keeping genes (β-actin and GADPH) were used for normalization of the values. After reactions, PCR products are run on a 2% agarose-gel with ethidium-bromide staining for visualization.

For the evaluation of mucosal effect of probiotic bacteria, we apply the same model to test the expression of pro- and anti-inflammatory cytokines, with few variations. In particular, bioptic samples are incubated for a longer time (6 hours) in order to evaluate variation in mRNA production after the incubation, and we add to the RPMI media with bioptic samples a 1:10 solution of probiotic conditioned media, prepared according to a procedure already described in literature [7], or PBS alone for negative controls. We set the incubation time to 6 h since longer time resulted in contamination of the reaction and degradation of RNA, with a consistent reduction of expression of the house-keeping genes. After incubation, total RNA is extracted by RNAeasy miniprep kit (Qiagen, Valencia, CA, USA), cDNA synthetized by GeneAmp RNA PCR kit (Applied Biosystems, Foster City, CA, USA), and mRNA for specific cytokines quantified by RT-PCR and normalized to house-keeping gene concentration, as already described above.

The present method have not been directly validated so far, but, for the evaluation of bacterial adhesion, indirect proof of correlation with the in vivo adhesion are available. In fact, we have previously demonstrated an in vivo reduction of concentration of total mucosal adherent bacteria in adenomatous polyps comparing with adjacent normal mucosa, together with an increment of production of anti-bacterial molecules, such as α-defensins. A similar reduction of bacterial concentration in polyp mucosa was observed in the ex vivo experimental model, when bioptic samples were incubated with a multistrain probiotic formulation [8]. Moreover, when we tested bacterial adhesion in the normal colon for the same probiotic mixture in our experimental model, different bacterial species adhered in a peculiar manner to proximal and distal colonic specimens. In particular, after incubation, a consistent adhesion of *S. thermophilus* and *B. infantis* was observed, while L. acidophilus was not detectable either in proximal and in distal colonic segments (unpublished data). Similar results were observed in vivo in a mouse model of spontaneous ileitis (i.e. SAMP1/YitFc), after 6 weeks of supplementation at high dose with the multiple probiotic formulation. In fact, after terminal ilea removal and extraction of total DNA, only *S. thermophilus* and *B. infantis*, but not L. *acidophilus*, were detectable at mucosal level, despite the latter bacteria was consistently detected in DNA extracted from feces of the mice [9].

The potential usefulness of the model for the evaluation of mucosal effect of the tested bacteria remains to be determined, and comparison with experimental and in vivo findings for cytokines expression after probiotic administration are currently ongoing. One could speculated that, since in the ex vivo model the bioptic samples are simply put in incubation in the tube with probiotic conditioned media, direct paracellular stimulation of the lamina propria compartment by probiotic media may occur. For that reason, the model may not be fully representative of the in vivo situation, where the epithelial cells represents the main interface between luminal content and sub-epithelial compartment. To address that point, comparison with the effect of probiotic on cells of the different intestinal compartments may help in the interpretation of data from the experimental model. In order to better mimic the in vivo situation, an experimental model in which surgical specimens are cultured and polarized have been recently described [10]. Comparing with the model described in the present paper, such a model offers the advantage of a more detailed study of the immune mucosal effect of different stimuli (i.e. different probiotic bacteria), in particular for the utilization of a cave cylinder which delimited the area of stimulation on the apical face of the mucosa. On the other side, such a model is basically more complex and necessitate surgical mucosal explants that are less available than bioptic speciments.

Conclusion

In the present paper, we have described an experimental method that is potentially applicable in every laboratory, that do not need complex procedures and with limited cost. The application and the potential limitation of this experimental model are still to be fully explored, but with that simple, economic and reproducible method the adhesion of different bacterial species to normal and pathologic colon may be tested. Since different probiotic species adhere in a peculiar way to normal and pathologic colon, as well as in different segments of the normal colon, such a method may be useful for the selection of bacteria for the treatment of specific pathologic situation. For instance, a probiotic bacteria with an elective adhesion to the distal colon may be particularly indicated in distal ulcerative colitis, whereas a species with a widespread adhesion to the whole colon may be more appropriate in pancolitis. Studies for the evaluation of this method for the effect of the bacteria on mucosal cytokines expression and production are on-going.
The correct application of translational medicine, with the appropriate utilization of specific experimental models for the selection of bacterial species with well described characteristics for specific clinical situation, will hopefully lead to a more tailored and efficacious utilization of probiotic bacteria in the field of IBD treatment.

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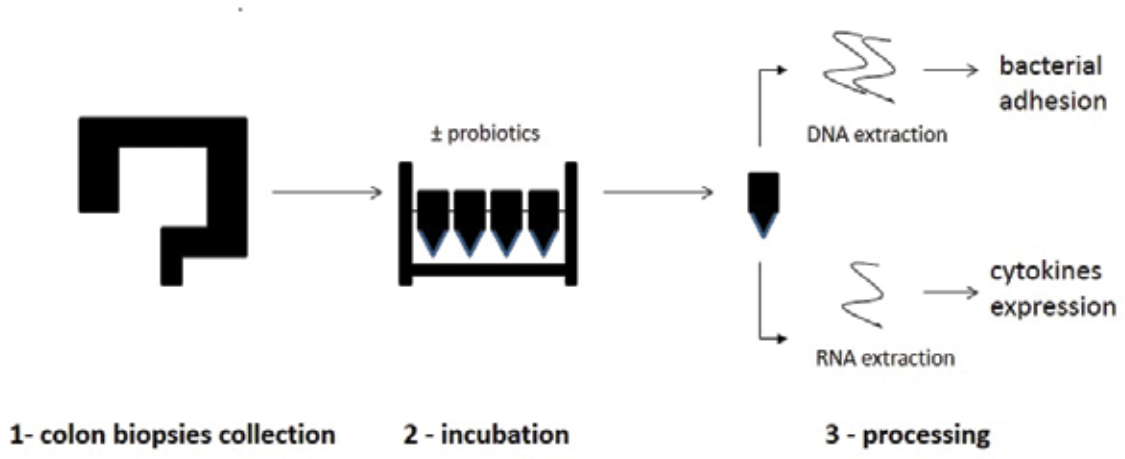

Figure legend
Figure 1. A schematic representation of the ex vivo organ culture technique. For a detailed description refer to the text.

THE ROLE OF VITAMIN D IN COLORECTAL CARCINOGENESIS

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Many investigations have been done regarding the correlation between cholecalciferol blood level and colorectal cancer. Initially, there were epidemiological studies that drew attention about the possible correlation between solar radiation and cholecalciferol blood level of a region's population and the incidence of colorectal cancer. In 1985 the first prospective study in this field was done, which showed that the risk of colorectal cancer disease was twice as higher in men who consumed insufficient amounts of vitamin D and calcium. Four years later, researchers compared vitamin D blood level with the risk of colorectal cancer and found that the risk increased by five times when cholecalciferol blood level was between 13.2 and 16.4 ng/mL. Our research included 125 patients, of whom 50 with colorectal cancer, 25 with colorectal polyp and 50 in the control group. Based on the high degree of morbidity and mortality of colorectal cancer, this study has enabled to achieve some important conclusions. It was found that the cholecalciferol blood level in patients with colorectal cancer is significantly lower, compared with the control group. Cholecalciferol blood level had a negative correlation with histological grade of the tumor, but did not correlate with tumor stage. Localization of tumor in the right or the left part of colon showed a correlation with the level of blood cholecalciferol as well. The average cholecalciferol blood level of patients with right colon adenocarcinoma resulted lower than in patients with left colon adenocarcinoma. The right location is associated with tumor development by the serrated pathway, advanced histological grade, phenotype of methylated islands CpG and microsatelite instability (in the molecular level), as well as a disposition to suffer mutations in important genes linked to cancer. Regarding histological grade, more than half of colorectal adenocarcinomas were second grade, followed by third grade adenocarcinomas. According to tumor stage, we found greater frequency of tumor detection in the second stage, then in the fourth and third stage, while the lowest frequency of detection was in the first stage carcinomas. The average age of patients diagnosed with colorectal cancer in our population was 60.74 years. In women, average age at the time of diagnosis was one year higher than in men. These results underline the female hormones' protective role against colorectal cancer. Also when analyzing the age groups involved, we confirmed that colorectal cancer frequency in young women was significantly lower than in young men. The average age of our patients with colorectal polyp, at the time of diagnosis was 56.44 years. Unlike patients with colorectal cancer, among female patients diagnosed with polyp, the average age is significantly younger than in male patients with this diagnosis. The majority of polyps found in the colon belonged to recto-sigmoid region. According to the histological type of polyps, tubular adenoma dominates, whereas dysplasia was significantly more common in villous adenoma, compared to tubular adenoma. The molecular basis of the idea that vitamin D has the potential to prevent cancer lies in its role as a nuclear transcription factor, which regulates cell growth, differentiation, apoptosis and many cellular mechanisms, with a key role for cancer development. In this study we investigated these processes through immunohistochemical markers PAK1 and Ki67, proven for their connection with the respective cellular processes. Cellular localization of PAK1 attributes to its functional activity. In CRC, nuclear PAK1 was associated with advanced stages. Patients with positive nuclear PAK1 had low vitamin D blood levels. The difference was not statistically significant, but the trend was positive. All patients with lymph node metastases had positive PAK1staining. According to our results, the expression of Ki67 (a marker of cellular proliferation), was highest in non-mucinous adenocarcinomas, average in mucinous adenocarcinomas, and low in colorectal polyps. However, the difference was not statistically significant. We also noticed that Ki67 expression was closely connected with increasing age. Ki67 expression correlated negatively with vitamin D blood levels: in patients with high Ki67 expression, vitamin D blood levels were less than half, compared to patients expressing low Ki67 staining. This part of our research highlights the importance of immunohistochemistry in making the decision regarding the treatment of patients with colorectal cancer, which should certainly be individual.

Key words: Cholecalciferol, colorectal carcinogenesis, PAK1, Ki67.

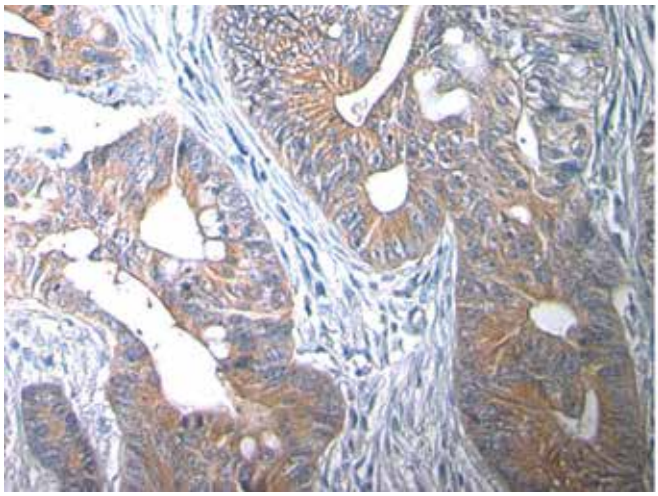

Figure 1. PAK1expression in non mucinous adenocarcinoma (cytoplasmic staining)

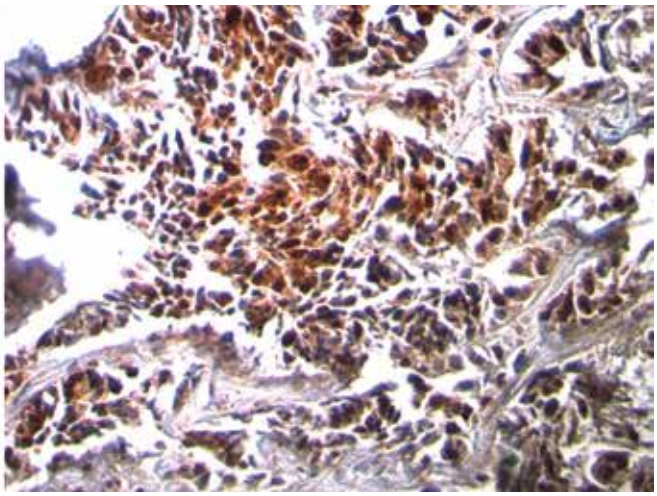

Figure 2. Ki67 expression

PROBIOTICS HISTORY

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The word probiotic (from the latin pro and the greek βιοσ literally meaning "for life") was introduced by the German scientist Werner Kollath in 1953 to designate "active substances that are essential for a healthy development of life". In 1965, this term was used by Lilly and Stillwell in a different context to represent "substances secreted by one organism which stimulate the growth of another". More specifically, Fuller in 1992 defined probiotics as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" (1).

The modern history of probiotics starts at the beginning of 1900s with the pioneering studies of the future Nobel laureate Elie Metchnikoff, an Ukraine scientist working at the Pasteur Institute in Paris. Louis Pasteur identified the microorganisms responsible for the process of fermentation, while Metchnikoff tried first to find out the possible effect of these microbes on human health. He associated the enhanced longevity of Bulgarian rural people to the regular consumption of fermented dairy products such as yogurt. He linked this to the *Bulgarian bacillus* which was discovered by a 27-year old Bulgarian physician Stamen Grigorov, and he later suggested that lactobacilli might counteract the putrefactive effects of gastrointestinal metabolism that contributed to illness and aging. Hippocrates, moreover, declared, 2000 years earlier, that *"death sits in the bowels*", and that "*bad digestion is the root of all evil*". Metchnikoff also claimed that toxins originated from bacterial putrefaction in the large intestine, and from there released into the circulation are the cause of aging. He also called *putrefying bacteria* those now recognized as proteolytic clostridia. Metchnikoff also stated that *"the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes*". This sentence describes in a clear way the "probiotic concept". Metchnikoff considered the lactobacilli as probiotics ("pro-bios", conducive to life of the host as opposed to antibiotic); probiotics could have a positive influence on health and prevent aging. The scientific hypothesis of Metchnikoff favoured the creation and development of the dairy industry in France, the first in Europe, thanks to the use of a fermented milk obtained from *Bacillus Bulgaricus*. In 2013 (2) an *expert consensus document* has been published on the *scope and appropriate use of the term probiotic: live microorganisms which when administered in adequate amounts confer a health benefit on the host.* Modern technology has also selected those strains producing a fermented milk with good organoleptic and nutritional qualities, more than other strains do . Yogurt and other foods made from fermented milk can be considered the first functional foods, according to a consensus document (3).

Nevertheless, the history of probiotics is as old as the human history (4), since it is closely related to the use of fermented food. It should be hypothesized that as farming started to replace hunting and gathering around 10.000 years ago, man began to produce fermented food and beverages. In fact during the neolitic period of the age of the stone, the domestication of animals occurred. Sumerians were the first people to settle and to develop animal husbandry.

In the ancient Indian Ayurvedic texts consumption of milk and dairy products is associated with a long and healthy life. Nevertheless, the first pictorial evidence on the practice of milking (whose technique has remained unchanged until the early '900 when they reached the first milking machines), emerged during excavations of the city of Ur - the ancient center of Mesopotamia, birthplace of Abraham - and dated to 3100 BC. A man sitting on a stool is depicted; he squeezes the long nipples of a cow and collects milk in a bucket. About nine centuries earlier, a farmer cut open his accounts in Sumerian cuneiform alphabet on clay tablets that are now preserved in the State Museum of the Middle East Berlin. He posted excellent returns in milk, butter and cheese from his rich herd of cattle. Are roughly contemporary both the seal exposed to the Museum of Natural History in Chicago in which a goat that, under the watchful eye of a goddess of fertility, offers her own milk to the pastor, and a polychrome Sumerian fresco by 2500 BC, kept in the museum of Baghdad; it shows large cows like aurochs with their young ; near them, large jars collect milk, while the cream poured into a churn from a *orciolino* becomes butter. In the so-called "*frieze of the dairy*" Sumerian priests carry the milking. The origin of fermented milk goes back to the ancient Egyptians and Phoenicians and Eastern cultures. The ancient Oriental peoples, Caucasians, Phrygian, Sarmatian and Macedonians nomadic shepherds kept milk from cows, sheep, goat, horse and camel in bottles made from the skin or from the stomach of the same animal where milk came into contact with the bacteria, probably the ancestors of the *acidophilus* and *bulgaricus* that today have become so famous.. Legend tells that one of these shepherd travelling in the hot sun of Turkish desert forgot milk in a goatskin bag for some time and whereupon found it transformed into a thick, creamy and tasty custard. This new product was referred to as "*yogurt*". Whatever the origin, the beneficial health properties and therapeutic use of yogurt and other fermented milks has been known since time immemorial, long before the existence of bacteria was recognized. For the Turks yogurt was the elixir of life. They believed that yogurt might give physical and inner wellbeing and prolong life span.

Yogurt was easily carried by nomads as it is easily storable and non-perishable; thus fermentation, from latin *fervere* (to boil), is the older form of food preservation. as well as a way to increase taste and digestibility of food.

Nobody knows exactly when man began to get fermented food and probably serendipitous contaminations in favorable environments played a major role. . The introduction of dairying was a major innovation in prehistoric agriculture, with milk and fermented product of milk such as *cheese* and *yogurt* being rapidly introduced as major components of diets of our ancestors.

Nearly every civilization has developed food fermentation of some type. Archeologist have found evidence for the production of a fermented beverage as early as 7000 BC in the Neolithic village of Jiahu in China and 5000 BC in Mesopotamia. In Asia fermented beverages were mainly made from rice, while in ancient Egypt and Mesopotamia were made from fruits (wine), from honey (mead) and from malted cereals (beer) (TAB.1).

Iconographic and written evidence from 3000 and 2000 BC indicated that Hindu, Egyptians, Greeks and Romans used *fermented milk products*, although its origins probably lie much earlier. Indeed they are mentioned in the early sacred books of Hinduism and in the Bible. In the Bible one of the first references is found in Genesis (18: 1-8) where it is said that Abraham offered to the Lord "veal, buns and sour milk."

According to legend the Prophet Muhammad gave as a gift the first *kefir* grains to the ancestors of the mountaineers of Caucasus. Kefir is a drink rich in lactic acid bacteria and probiotics from the fermentation of milk.
In Odyssey by Homer the Cyclops Polyphemus prepares the cheese in the cave.
Plinio in his Naturalis Historia says he tasted a thick and deliciously acid milk for which the barbarians were going crazy. He recommended the use of fermented milk for treating gastrointestinal infections.
The concept of *functional food* can be traced back even to Hippocrates IV century who wrote, "let the food be your medicine and medicine be your food". He supported the nutritional value of cheese which was given to Olympic athletes.
Marco Polo reported a comparable drink named *chemmisi* in China.
Tibetan nomads used fermented *yak milk*.
The *cheese* was neglected in the high middle age and recovered in the late middle age when it was mostly produced in monasteries.
In the XVI century, Suleiman the Magnificent sent a physician from his Turkish court to prescribe yogurt and successfully treat the severe diarrhea suffered by Francis I of France.
The health properties of these dairy products were a part of folklore until the concept of probiotics emerged, and the study of fermented milks and yogurt containing probiotic bacteria has become more systematic. Functional foods have thus developed as a food, or food ingredient, with positive effects on host health and/or well-being beyond their nutritional value, and fermented milk with probiotic bacteria has again become the prominent representative of this new category of food. Because otherwise, in the words of Hippocrates: *"Let food be your medicine and medicine be your food*", yet according to the ancient physicians, the animal milk is not appropriate food for man: Hippocrates and Galeno, while recognizing the high nutritional value, it only advised for medicinal use, emphasizing the many dangers in terms of food. These beliefs perhaps were based even on grounds of environment and climate: Mediterranean climate was not fit for consumption of a delicate product such as milk, at a time when instruments were missing for the hygienic control of the product and effective techniques for its conservation. The Romans were the first to use milk of cattle, which had been considered harmful, instead of milk of sheep. Marco Terenzio Varrone classified the different types of cheese.
The oldest cheese in the world was found on a mummy of 1615 BC in the chinese desert of Taklamakan; it was probably part of the food left in the grave.
In past centuries in Italy, at the time of threshing, women were used to bring workers a drink made with water, milk and lemon which produced milk coagulation. It was believed that this preparation would prevent intestinal infections in people who worked with struggling in the heat and dust. This costum continues even in our times in memory of the past.
In 17 century in some italian regions there was also a belief that other beverages prevented intestinal infection. We became aware of an ancient custom after having found an old cup named gamelio. Gamelio was a wedding gift (FIG. 2 a-b). It was used for washing after intercourse. The derived liquid washing was then drunk. This practice could represent a kind of germ planting.

A special form of probiotic therapy is *fecal bacteriotherapy or stool transplant or fecal microbiota transplantation* in which a bolus of washed suspended feces obtained from a healthy donor is directly infused into a patient's colon either as an enema or via endoscopy. Its use as therapy in humans was first reported by Eiseman et al., a team of surgeons from Colorado, who successfully treated four patients with fulminant pseudomembranous enterocolitis in the late 1950s (5). However, the first known account of fecal transplantation dates to a fourth-century Chinese handbook by the physician Ge Hong, who prescribed a human fecal suspension by mouth as a remedy for food poisoning or severe diarrhea. Later in the Ming dynasty, the influential XVI century Chinese physician used "*yellow soup,*" "*golden syrup*", and other remedies containing fresh, dried, or fermented stool to treat abdominal diseases.
Remarkably, the concept of transferring intestinal samples in veterinary practice was in use around the same time in Europe where the Italian anatomist Fabricius Aquapendente (1533-1619) described the practice known as *"transfaunation*" consisting in inoculating rumen fluid presumably into cows that had lost the capacity to ruminate. It is still used to treat ruminating animals, like cows and sheep, by feeding rumen of a healthy animal to another individual of the same species in order to colonize its gastrointestinal tract with normal bacteria. In addition, in the second part of last century, the fecal transplantation was extended to avian species in order to protect the chicken from infections, notably from Salmonella spp.
During World War II, German soldiers in Africa, upon observation of the native Bedouins, used fresh camel feces as treatment for dysentery. The dung consumption only worked if fresh because the active "ingredient" was later identified as *Bacillus subtilis*.
A recent editorial paper reports: Fecal Microbiota Transplantation-An old therapy *comes of age* (6). After the first report in the literature by Eiseman, fecal transplant has increased in popularity due to its efficacy and ease of use for the treatment of patients with *Clostridium difficile* infections. In January 2013, the New England Journal of Medicine (7) published the results of the first randomized controlled trial involving fecal transplant, comparing the therapy to treatment with vancomycin for patients with recurrent disease. The trial was ended early since it would be unethical to continue: fewer than a third of the patients given vancomycin recovered, compared with ninety-four per cent of those who underwent fecal transplants, the vast majority after a single treatment.
Historical perspectives provide a very meaningful context to the current state of the contemporary research on the intestinal microbiota and its manipulation to treat human diseases (8, 9, 10). Today the interaction of the gut flora with its host and mutual regulation has become one of the important topics of biomedical research even though its relevance and exact role require much more research.

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History and origins of some fermented foods

Food	*Approximate **year of introduction***	*Region*
Mushrooms	4000 BC	China
Soy sauce	3000 BC	China, Korea, Japan
Wine	3000 BC	North Africa, Europe
Fermented milk	3000 BC	Middle East
Cheese	2000 BC	Middle East
Beer	2000 BC	North Africa, China
Bread	1500 BC	Egypt, Europe
Fermented Meats	1500 BC	Middle East
Sourdough bread	1000 BC	Europe
Fish sauce	1000 BC	Southeast Asia, North Africa
Pickled vegetables	1000 BC	China, Europe
Tea	200 BC	China

TAB. 1

FIG 1 Cup called **amatoria** or **Gamelio** – Inside, the solar halo frame the woman.
XVII century Deruta old pottery, from G.Gasbarrini private collection.

PROBIOTICS AND DIVERTICULAR DISEASE: EVIDENCE-BASED?

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Summary

Diverticular disease is a common gastrointestinal condition. Clinical spectrum ranges from asymptomatic diverticulosis to symptomatic uncomplicated or complicated diverticular disease. Symptoms related to uncomplicated diverticular disease are not specific and may be indistinguishable from those of irritable bowel syndrome. Low-grade inflammation, altered intestinal microbiota, visceral hypersensitivity and abnormal colonic motility have been identified as factors potentially contributing to symptoms.
Probiotics may modify the gut microbial balance leading to health benefits. Probiotics, due to their anti-inflammatory effects and ability to maintain an adequate bacterial colonization in the colon, are promising treatment options for diverticular disease. This review focuses on the available evidence on the efficacy of prebiotics in uncomplicated diverticular disease.

Key words: Diverticular disease, probiotics, symptomatic uncomplicated diverticular disease

DIVERTICULAR DISEASE: EPIDEMIOLOGY AND CLINICAL PRESENTATION

Diverticular disease (DD) is an important gastrointestinal disease in terms of health-care costs in Western countries, with highest rates in the United States and Europe. All age groups can be affected and the prevalence increases with age (1). The term DD is used to denote a clinically significant and symptomatic condition or asymptomatic diverticulosis. In many individuals, colonic diverticula remain symptomless (diverticulosis), while about 20% of them develops symptoms, including recurrent abdominal pain or discomfort, bloating, changing in bowel habits (symptomatic diverticular disease). In contrast to the common belief that diverticulosis has a high rate of progression, only about 4% of patients develop acute diverticulitis (2).
In a significant proportion of DD patients symptoms resembling or overlapping those of irritable bowel syndrome (IBS) are present, making a differential diagnosis between the two conditions challenging (3). According to recent studies, beyond abdominal symptoms, symptomatic DD is associated with impaired quality of life, in particular vitality and emotional health, as DD may be experienced as a chronic illness characterized by ongoing abdominal symptoms and psychosocial distress (4). Patients with acute diverticulitis may be at risk for subsequent development of IBS, a condition for which the term post-diverticulitis IBS has been proposed, analogously to post-infectious IBS proposed some years ago (5). Some pathophysiological factors leading to symptom generation as low-grade inflammation, visceral hypersensitivity, abnormal colonic motility, and altered intestinal microbiota, are probably shared by both conditions, DD and IBS (6). As a potential key step in the pathogenesis of diverticular inflammation and abdominal symptoms in DD, alteration of the peri-diverticular bacterial flora has been suggested (6); therefore, probiotics may be an appealing treatment option for this condition (7).

PROBIOTICS: A PROMISING TREATMENT OPTION FOR DIVERTICULAR DISEASE?

Probiotics may modify the gut microbial balance leading to health benefits due to their anti-inflammatory effects and capability to enhance anti-infective defences by maintaining an adequate bacterial colonization in the gastrointestinal tract and by inhibiting colonic bacterial overgrowth and metabolism of pathogens (8). A recent meta-analysis showed that probiotics were effective treatments in IBS (9). Previous reviews on the use of probiotics in DD suggested a potential usefulness of this treatment in the management of DD (7, 10). In contrast, a recent consensus report stated with a 97% level of agreement that to date there is insufficient evidence to judge probiotics as effective in reducing symptoms in DD (11). A recent systematic review on the efficacy of probiotics treatment in diverticular disease in terms of remission of abdominal symptoms, retrieved eleven articles, which were performed over a period of 20 years mainly in Europe on an overall total number of about 760 patients with diverticular disease, with a slight female prevalence (55.1%), and an age range from 58 to 75 years (12). Table 1 shows the strains of probiotics used in these studies to treat abdominal symptoms related to DD. In many studies patients were treated with a single probiotic strain. The most frequently investigated probiotics were different strains of Lactobacilli, whereas Bifidobacteria or other probiotic strains were less frequently used. The interventions were very variable between studies, as the probiotics were administered together with drugs (antibiotics, anti-inflammatory agents as mesalamine or beclomethasone) and compared with the efficacy of the drug alone, or, in other studies, a probiotic treatment arm was used without any associated drug or compared with a high-fibre diet (Table 2). The variable nature and the relative poor quality of the available studies on the use of probiotics in diverticular disease make it difficult to evaluate the cumulative efficacy of these treatments. Only two of these studies were double-blinded randomized controlled trials. Moreover, five of the eleven studies were performed by the same authors. The follow-up periods in the single studies were very variable. Not only the probiotic strains employed as treatment were very different, but also the treatment protocols with regard to timing, dosage or combination with other drugs differed much. Pooling different studies using different strains may be a not suitable method to evaluate their efficacy, because specific strains of probiotics may have different effects in patients with DD. Moreover, the type of DD was not homogeneous between studies: some studies investigated patients with uncomplicated DD, other studies patients with acute diverticulitis in remission. Some studies investigated the reduction of abdominal symptoms, while other studies evaluated the maintenance of remission of abdominal symptoms. The clinical response to probiotics treatment may be potentially influenced by all these variables.

Conclusions

To date, the evidence on the efficacy of probiotics in DD still remains poor as high-quality studies are very few. At this point, available data do not allow to draw definite conclusions. Further work is needed to understand how probiotics can be employed in the management of patients with DD.

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The authors declare that there is no conflict of interest with regard to this work that they should disclose.

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Table 1. Probiotic strains employed in the treatment of diverticular disease

Probiotic strainS	References
Single strains	
Bifidobacterium infantis 35624	Stollman N, et al. J Clin Gastroenterol 2013
Escherichia coli strain Nissle	Fric P, et al. Eur J Gastroenterol Hepatol 2003
Lactobacillus (specific strain not reported)	Giaccari S, et al. Eur Rev Med Pharmacol Sci 1993
	Tursi A, et al. J Clin Gastroenterol 2006
Lactobacillus casei subsp. DG	Tursi A, et al. Hepatogastroenterology 2008
	Tursi A, et al. Aliment Pharmacol Ther 2013
Lactobacillus paracasei B21060	Lahner E, et al. World J Gastroenterol 2012
Lactobacillus paracasei subsp. paracasei F19	Annibale B, et al. Minerva Gastroenterol Dietol 2011
Mulitple strains	
Lactobacillus acidophilus, Lactobacillus helveticus, Bifidobacterium. subsp. 420 Streptococcus thermophilus DSM 24731, Bifidobacterium longum DSM 24736, Bifidobacterium breve DSM 24732, Bifidobacterium infantis DSM 24737, Lactobacillus acidophilus DSM 24735, Lactobacillus plantarum DSM 24730, Lactobacillus paracasei DSM 24733, Lactobacillus delbrueckii subsp. bulgaricus DSM 24734	Lamiki P, et al.. J Gastrointestin Liver Dis 2010. Tursi A, et al. J Clin Gastroenterol 2005 Tursi A, et al. Int J Colorectal 2007

THE EMERGING ROLE OF GUT MICROBIOTA IN AUTISM PATHOGENESIS: A NEW HOPE FOR EFFECTIVE PREVENTION AND TREATMENT

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Autism is a specific neurodevelopmental condition that typically displays qualitative socio-communicative impairment and restricted, stereotyped interests and activities (1). Although a large proportion of children with autism manifests abnormal development during the first year of life, 15-62% of them show a regression between eighteen and twenty-four months of age after a period of apparently typical development (2,3). Approximately 70% of individuals with autism present a variable degree of intellectual disability (4) and expressive/receptive language can be absent or very insufficient (5). Other problems, not exclusive of autism, are attention deficit and disturbed behaviors as etero-autolesivity. Thirty per cent of children manifest epileptic seizures by late childhood or adolescence and 10% of cases are associated with several genetic disorders as tuberous sclerosis, Angelman syndrome, phenylketonuria and fragile X syndrome (6). The etiopathogenesis of autism is not yet understood; the prevalence is undoubtedly rising and it is not clear if this increase is linked to the diagnostic improvement or to a greater susceptibility of the population to the disease. Many twins and family studies point out the importance of inherited predisposition to the disorder even though epidemiologic research suggest the strong contribution of prenatal and early postnatal environmental factors among which an abnormal intestinal flora. There is an increasing body of knowledge pointing out that gut flora influences a variety of social emotional, and anxiety-like behaviors, and contribute to brain development and function in animals(7-8) and humans (9). In a recent study carried out by Hsiao et al. these authors demonstrated that a particular model of autistic mouse displays behavioral symptoms relevant to ASD and other neurodevelopmental disorders (10-11), while also exhibiting defective GI integrity, dysbiosis of the commensal microbiota, and alterations in serum metabolites. The administration of a particular commensal (B. Fragilis) is able to reverse autistic symptons and metabolic derangement. These findings represent a major breakthrough in the microbiota hypothesis of ASD(12). In humans the possibility that autism is the consequence of an imperfect development of gut flora is supported by a number of observations. First, onset of the disease often follows antimicrobial therapy, for example, to treat ear infections that often are present in high frequency and persistency among young ASD patients. Second, GI symptoms are common at the onset of ASD and often persist. Finally, other antimicrobials may lead to a clear-cut response and relapse may occur when the antimicrobial is discontinued, which is demonstrated with, for example, the antimicrobials vancomycin and metronidazole. However, in higher doses, over a longer period of time (.6 d of treatment), vancomycin disrupts the anaerobic intestinal microflora and promotes colonisation by pathogens(13).

The literature about the role played by intestinal dysbiosis in autism is increasing and in the last ten years a number of studies have been published (14). All these studies have targeted fecal microbiota but two(ileal and cecal biopsies) using a wide range of techniques. All the studies are case-control comparative studies with a small-medium sample size, ranging from 15 to 58 autistic children and from 10 to 53 typically developing children. The age of the children has a wide range: from 3 to 16 years. As expected there is a strong inhomogeneity as regards the microbiological assay method employed. One group has employed FISH analysis with specific 16SrRna oligonucleotide probes; other bacterial tag encoded FLX amplicon pyrosequencing. Other studies have been carried out using real-time PCR assays on CFX 384TM detection system and only one using bacterial and yeast culture using traditional techniques.

Anyway only one study did not find any difference in microbiological pattern between autistic children and controls. In the others significant differences have been found in increase or decrease of specific bacterial population.

Table 1. Comparative studies of intestinal microbiological profile in autism

Authors	Ref.	Country	Study population	Microbiological assay method	Results
Parracho et al.	6	USA, 2005	58 ASDs children (3-16 years old) VERSUS 10 non-ASDs siblings (2-10 years old) and 10 unrelated healty childrens (3-12 years old)	FISH analysis performed in fecal samples with specific 16S rRNA oligonucleotide probes	Significantly increased incidence of *Clostridium histolyticum* in ASD group and intermediate non significant difference with sibling group
Finegold et al.	7	USA, 2010	33 ASDs children VERSUS 7 siblings and 8 unrelated healthy children	bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP) in fecal samples	Significantly increases in *Bacteroidetes* in fecal samples from ASDs children and increases of *Firmicutes* in control group
Adams et al.	8	USA, 2011	58 ASDs children (mean age 6.91 years) VERSUS 39 unrelated healthy children (mean age 7,7 years)	Bacterial and yeast culture using standard tecniques. Vitek 2 (GN , GP and YST system identification.	Significant lower level of species of *Bifidobacter* and higher level of *Lactobacillus* in ASD children. Similar levels of other bacteria and yeast
Wang et al	9	Australia, 2011	23 ASDs children (mean age 123 mo.) VERSUS 22 siblings (mean age 144 mo.) and 9 unrelated healthy children (mean age 114 mo.)	DNA extraction from fecal samples and qRNA analysis performed on a CFX 384TM real-time PCR detection system	Significant lower level of species of *Bifidobacterium spp.* in ASD children versus siblings and controls and lower level of *Akkermansia muciniphila* in ASD children versus controls only
Williams et al.	10	USA, 2011	15 ASDs children (mean age 4.5 years) VERSUS 7 unrelated healthy children (mean age 4.0 years)	DNA extraction from ileal and cecal biopsies and PCR assays (16S rRNA gene pyrosequencing analysis)	Significantly decreases in *Bacteroidetes* (with increases *Firmicutes/Bacteroidetes* ratio) and increases of *Betaproteobactera* was found in intestinal biopsy samples from ASDs children
Williams et al.	11	USA, 2012	23 ASDs children (3-10 years old) VERSUS 9 unrelated healthy children (3-10 years old)	DNA extraction from ileal and cecal biopsies and PCR assays (16S rRNA gene pyrosequencing analysis)	High level of *Sutterella* species was found in intestinal biopsy samples from ASDs children
Gondalia et al.	12	Australia, 2012	51 ASDs children VERSUS 53 healty control siblings	bacterial tag-encoded FLX amplicon pyrosequencing	No differencies between ASD and controls
DeAngelis et al.	13	Italy, 2013	20 ASDs children (10 autistic and 10 pervasive e developmental disorder not otherwise specified) (4-10 years old) VERSUS 10 healty control siblings (4-10 years old)	DNA and RNA extraction from fecal samples and 16S rDNA and 16S rRNA analysis	Compared with healthy controls median values of *Clostridium, Bacteroides, Prophyromonas and Prevotella, Pseudomonas, Aeromonas and Enterobacteria* were higher, instead *Enterococcus, Lactobacillus, Streptococcus, Lactococcus, Staphylococcus* were lower.
Wang et. Al	14	Australia, 2013	23 ASDs children (mean age 123 mo.) VERSUS 22 siblings (mean age 144 mo.) and 9 unrelated healthy children (mean age 114 mo.)	DNA extraction and qRNA analysis performed on a CFX 384TM real-time PCR detection system	Significant higher level of *Sutterella spp.* in ASD children versus siblings and controls

A part from this, the suggestive role of abnormal gut microbiota and the frequent presence of abnormal gut permeability in children with ASD has promoted clinical studies on probiotics.

In a double-blind, placebo-controlled study by Parracho et al.(15), Lactobacillus plantarum feeding of children with autism resulted in significant increased levels of the beneficial bacteria lactobacilli and enterococci, and a significant reduction of a cluster of Clostridium, compared with the placebo group. Through a 12-week study, the probiotic feeding resulted in reduced GI problems and, more importantly, in improved behaviour scores compared with baseline. In this respect, it is noteworthy that, during another double-blind, cross-over study, addressing the effects of the probiotic L. plantarum on autism failed during the changing of treatments in the cross-over period, because parents (who were blinded for the intervention) of children treated with the actual probiotics refused to make the switch, as they wanted their autistic children to continue their improvement(16). Noted improvements were decreased levels of clostridia bacteria in the stools and a positive effect on mood and general behaviour, as described by parents. Since this can only be considered as anecdotal evidence, further well-controlled studies are warranted. Another probiotic trial in autistic children was recently conducted by Kałuz˙ na-Czaplin´ska & Błaszczyk (17). Probiotic supplementation with L. acidophilus over 2 months led to a significant decrease in D-arabinitol and to a significant improvement in the ability to concentrate and carry out orders. D-Arabinitol is a metabolite of most pathogenic Candida species and its excretion in urine is elevated in autistic patients. Candida infections have been associated with autism previously. Unfortunately, these studies were not of sufficient methodological quality due to the absence of control groups, multiple treatments at once and/or small sample sizes.

In conclusion the studies on intestinal microbiological profile in autism are nevertheless in their infancy. There are many methodological issues to be resolved, like the standardization of microbiological assay methods, of sampling protocols and mathematical analysis of the results.

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DISCRETE CHEMICAL AND PHYSICAL DIETARY FIBER STRUCTURES AND THEIR POTENTIAL ROLE IN FAVORING GUT BACTERIA

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While much is known regarding dietary fibers and how they are utilized by the human gut microbiota, it is still difficult in most cases to use fibers to make predicted changes in the microbiota. Prebiotic oligosaccharides are known to favor certain bacterial groups, i.e. bifidobacteria and lactobacilli, yet a larger opportunity may exist to understand requirements and develop strategies to promote other bacterial groups with different kinds or blends of dietary fiber substrates. There is a vast array of dietary fiber structures in foods that arises from the many different fiber classes as well as variations in structure due to genotype, environment, and even within polysaccharide structures. Discrete chemical, and perhaps physical, structures appear to be aligned to individual strains that would make them able to compete in the broader complex gut environment. A global framework needs to be developed to better understand how dietary fibers can be used to obtain predicted changes in microbiota composition for improved health. The presentation will focus on the potential for dietary fibers to be used in such targeted ways for health benefit.

AN ADVANCED IN VITRO TECHNOLOGY PLATFORM TO STUDY THE MECHANISM OF ACTION OF PRE- AND PROBIOTICS IN THE GASTROINTESTINAL TRACT

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Introduction

The human gastrointestinal tract (GIT) is one of the major entry gates to the human body and is the first site of contact between ingested products and the host. Moreover, it is home to a large number of microorganisms – up to 10^{12}-10^{13} cells are present per gram of fecal matter in the distal colon – belonging to seven bacterial phyla of which Firmicutes, Bacteroides and Actinobacteria are the most abundant [1, 2]. This extremely complex community - which exceeds the number of cells from our body with a 10-fold - is considered to have multiple roles and key functions in influencing the host physiology (i.e. extraction of energy from indigestible compounds, stimulation of the gut immune system or synthesis of essential vitamins) [3]. On the other hand, in economic terms, certain microbial groups may also be seen as a liability contributing to a disease state, i.e. allergies, bowel inflammation and obesity [4].

Given the extensive genetic potential associated to this bacterial community, the intestinal ecosystem can be seen as a reservoir of metabolic functionalities ready to be exploited and modulated with the aim of improving the health of the host (defined as Gastrointestinal Resource Management). This modulation can be obtained with the use of prebiotics and probiotics or a combination of the two (synbiotics). In this respect, several approaches are available to study the efficacy of pre- and probiotics in the GIT. Human intervention trials are of course the golden standard to validate functional properties of products. However, human studies do not allow to easily perform mechanistic studies especially in areas of the GIT which are not easily accessible. Alternatively, laboratory animals may be used. However, some parameters, such as the microbial community composition, may not be fully representative for humans. The latter could be overcome by using human microbiota-associated animals. A final option is represented by well-designed *in vitro* simulation technologies.

The SHIME® technology platform

The better an *in vitro* system can simulate the real gut situation, the higher is the physiological significance of the obtained information. Among all the available systems, the Simulator of Human Intestinal Microbial Ecosystem (SHIME® - LabMET, Ghent University and ProDigest, Belgium) has already been proven to be a useful model for nutrition studies in terms of analysis of the gut microbial community composition and functionality [5, 6, 7, 8].

The SHIME® is a dynamic model of the GIT that simulates in sequence the stomach (acid conditions and pepsin digestion), small intestine (digestive processes including bile salts and pancreatic juices and absorption by means of dialysis) and the 3 regions of the large intestine, i.e. the ascending, transverse and descending colon (microbial processes). Careful control of the environmental parameters in these reactors allows to obtain complex and stable microbial communities which are highly similar in both structure and activity to the microbial community in the different regions of the human colon. In the so-called Mucosal-SHIME (M-SHIME®), the simulation of the ecology of the gut microbiota is improved incorporating a mucosal environment containing mucin-covered microcosms [9]. In this system, bacteria, which can adhere to the mucus layer, can colonize the microcosms and create a mucus-compartment in the reactor. By replacing 50% of the microcosms every 48h, the intestinal replacement of the mucus layer is simulated, allowing a continuous modeling of the mucosa compartment (Fig 1).

Fig. 1: TWINSHIME setup, consisting of two parallel SHIME systems. Each SHIME reactor contains 5 vessels simulating respectively the stomach, small intestine, ascending colon, transverse colon and descending colon. In each vessel of the M-SHIME (here the ascending colon is reported as an example) the mucosal compartment is formed by the addition of plastic microcosms (9 mm of diameter) coated with mucin type II-agar.

Finally, the interaction of bacteria with the gut wall and the resulting health effects related to changes in the gut-wall functioning can be studied by coupling the SHIME samples with a number of cell culture assays. The unique aspect of this approach relates to the fact that the complete environmental matrix - containing both the product and its direct metabolites - is evaluated in the test model and not only the isolated individual product. This allows to obtain a much more relevant view on the final health effects the tested product may have in the gut. Cell assays may vary according to the endpoint

that must be investigated. Co-culture models make use of both human intestinal epithelial-like cell lines, such as Caco-2 cells, in combination with immune cells (THP1 cells) [10]. This model mimics the intestinal mucosal interface, with immune cells lining the intestinal epithelia, thereby allowing at the same time to test for potential benefits in maintaining barrier integrity, and for anti-inflammatory properties of different compounds. In alternative, the recently developed Host-Microbiota Interaction (HMI™) module - a two-compartment reactor, which incorporates at the same time the presence of complex microbial communities originated from different areas of the SHIME (upper microbiota compartment) and of human cell lines (lower host compartment) can be used to perform long-term investigations (i.e. up to 48h) of the effect of specific products on the reciprocal host-microbiota adaptation [11]. Finally, by varying the cell lines used, it is also possible to assess the bioavailability of actives or the impact of a specific ingredient on satiety, liver function, cardiovascular health, etc…

Throughout the years, the SHIME® has been validated based on numerous in vitro, animal and human studies [12]. The model has been extensively used to study the metabolic fate and the mechanism of action of food compounds over a period of several weeks. A non-exhaustive list of studies performed includes: impact of various pre- and probiotics on the composition and activity of the gut microbiota; survival of probiotics in the upper GIT; digestibility of nutrients in the upper GIT; anti-inflammatory activity of non-digestible fibers; formulation effect on bioavailability of actives; targeted delivery of products in the GIT; anti-pathogenic activity of specific ingredients (i.e. anti-*Clostridium difficile*); assessment of community recovery after an antibiotic treatment; impact of the modification of specific ingredients in commercial blends; etc…

Conclusions

Well-designed *in vitro* technology platforms are a useful tool for the initial screening, selection, formulation and in-depth evaluation of the mode of action of food ingredients such as pre- and probiotics. They are therefore valuable in both the early development stages of innovative products and a necessary complementary tool for *in vivo* validation studies of the final efficacy and functional claims.

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NOVEL FOOD FORMULA SUITABLE FOR 3D PRINTING

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Introduction

Probiotics are live microorganisms that when ingested in adequate amount are able to confer health benefits on the host (FAO/WHO, 2001). Prebiotics are non-digestible carbohydrates which have a resistance to gastric acidity, are used for fermentation by gut bacteria and show ability to improve the activity of beneficial microorganisms (Rastall and Gibson, 2006). Probiotic microorganisms are commercially available as dried or freeze-dried form as well as are commonly consumed as food products (fermented or non-fermented) or as dietary supplements while the most important prebiotics, fructooligosaccharides and inulin, are commonly used in pure form or as ingredient in several food products such as yogurt. In Italy, the health authorities recommended that a probiotic food needs to have: a) minimum labeled concentration of 10^9 CFU of live microorganisms/daily dose, for each probiotics species guaranteed up to the "use by" date; b) each labeled probiotic species nomenclature conforms to the International Code of Nomenclature; c) absence of any pathogens (Ministry of Health, 2005). The commercial success of probiotic foods exponentially increased in the last 10 years and nowadays their market is about the 60-70% of the functional foods and beverages which have a global market of $ 176.7 billion (Kolozyn-Krajewska and Dolatowski, 2012; Hennessy, 2013). The majority of probiotic foods are dairy products but several vegetarian-based products have been recently developed on the basis of the increasing demand of peoples with lactose intolerance (Anekella and Orsat, 2013). Obviously, the use of mixtures of prebiotics and probiotics is of great interest for their synergistic effects but practical applications are still restricted to production of yogurt. However, the most important challenge remains the use of technologies able to obtain safe functional foods with desired sensorial properties without reducing the number of viable and active cells until the consumption.

Printing it is undoubtedly a technology that had a revolutionary impact on society, education and politic, across the world. In the last 15 years printing evolved from 2D to 3D systems following a layer-by-layer deposition of materials according to a computer designed structure. At first, this technology was used for application in rapid prototyping material (RP) materials to promote the development of several manufacturing industry. More recently, 3D printing was recognized as a very interesting tool in different fields such as architectural design, electrical components, jewelry, etc. Several 3D printing technologies are available, among that the most common is the direct ink-writing by which a filament of polilactic acid (PLA) is fused at high temperature and directly deposited by a dispensing system (Serra et al, 2014). Other 3D printing technologies are the powder-based inkjet 3DP, laser-assisted sintering, polymerization, fused deposition material (FDM), 3D microextrusion, etc. (Bose, et al., 2013; Murphy and Atala, 2014). A recently and pioneering application of 3D printing is in the field of tissue engineering. More specifically some authors have reported promising results in the use of 3D bioprinting of tissue and organs (Pfister et al., 2003; Bose et al, 2013; Serra et al., 2014; Murphy and Atala, 2014). For instance, osseous tissue, also known as bone, is a structure having in nature a fraction porosity of 50-90% (Bose et al., 2013) for which extracellular matrix and growth factors are of crucial importance. Under these considerations 3D printing has been used to produce biocompatible scaffolds which enable cell attachment and stimulate bone tissue formation in vivo (Bose et al. 2013). Since size and shape of pores as well as their internal connectivity are of great importance for transfer nutrients in the inner part of scaffolds favoring cell growth, 3D printing represents a tool for obtaining structure with desired microstructure properties.

Similarly, foods may be considered as porous materials composed from a void phase (pores) and a solid phase matrix with microstructure properties that significantly affect sensorial and nutritional attributes and microbial safety (Aguilera, 2005). However, no scientific information on the use of 3D printing technologies in food science is available. The use of 3D printing in food science could give light on the production of new series of foods with highly specific nutritional/functional properties obtained by depositing layer-by-layer desired amount of nutrients, probiotic bacteria (both in powder and in solution), prebiotics, vitamins, etc. On the other hand, food pastes prepared by mixing several ingredients with the aim to obtain specific sensorial and functional properties could be printed for obtaining foods with any 3D structure. Finally, modeling the microstructure of 3D printed food, several properties such as rehydration, heat transfer, mass transfer, crispness, etc., could be controlled improving both sensorial properties and their convenience during eating or cooking. In this paper the potential application of 3D printing technologies on the production of innovative food formula will be discussed, showing some first results on innovative food model systems.

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HOW PREBIOTICS HELP THE GUT MICROBIOTA TO MODULATE LIVER AND ADIPOSE TISSUE METABOLISM TO IMPROVE HUMAN HEALTH

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Dysbiosis: a component of metabolic alterations and sustemic diseases

In physiological conditions, we are living in symbiosis with the hundreds of bacteria that colonize our gut. This internal ecosystem, called the gut microbiota, regulates host energy metabolism, participates to the efficiency of the immune system, and regulates key gut functions. Several papers and reviews support the idea that a "dysbiosis" (inadequate changes of gut microbiota composition and/or activity related to host disease) characterizes overweight, obese or diabetic individuals, and drives alteration of gut barrier function, adiposity, steatosis, and cardiovascular risk (for review Delzenne et al 2015) . In our laboratory, we have recently shown, in experimental models of cancer cachexia, that the gut dysbiosis also plays a role in the occurrence of inflammation and muscle mass loss. Therefore, the gut microbiota modulation appears as an interesting tool in the prevention and/or treatment of the dysbiosis associated to obesity and metabolic disorders.

Prebiotics: modulators of the gut microbiota to improve host health

The concept of prebiotic has been elaborated in 1995, when it was shown for the first time that non digestible carbohydrates like fructans were able, by interacting with the gut microbiota, to change the gut microbial composition in favor of Bifidobacteria and to improve host health in different patho-physiological situations. This concept might now take into account the fact that a large panel of non digestible nutrients are able to modify the composition and/or the functions of the gut microbiota, and that even if the prebiotic compounds, like fructans, are often considered as Bifidogenic, they are able to change (increase or decrease) a wide range of bacteria, which could play a role in the management of host health (Bindels et al 2015a).

Prebiotics and steatosis

The first studies performed in animal models of obesity with inulin-type fructans allowed to point out an interesting effect on steatosis (triglyceride and cholesterol accumulation in the liver tissue). More recently, we have characterized the molecular events allowing fructans to counteract the steatosis induced by n-3 polyunsaturated fatty acids deficiency in mice (Pachikian et al 2012). Fructan administration changes gene expression in the liver tissue, by promoting peroxisome proliferator activated receptor alpha (driving fatty acid oxidation), and by blunting sterol response element binding protein 2, thereby decreasing cholesterol synthesis. The improvement of the steatosis by prebiotics might relate to the higher production of the intestinal hormone glucagon-like peptide 1, which is released in the portal vein, or to the release of metabolites issued from carbohydrate fermentation (propionate), or from fatty acids (conjugated linoleic acids) (Pachikian et al 2014; Druart et al 2015) .

Prebiotics and adiposity

In animal models of obesity, highly fermentable carbohydrates such as fructans, arabinoxylans, or glucans, are able to counteract several metabolic alterations linked to obesity, including hyperglycemia, or systemic inflammation (Neyrinck et al 2012). The mechanistic studies suggest that the changes in the gut microbiota occurring upon prebiotics can be related to an improvement of bacterial species implicated in the regulation of gut barrier and endocrine functions, with consequence on host energy homeostasis. In the white adipose tissue, prebiotic administration lessens adipocyte size, blunts peroxisome proliferator activated receptor gamma, and lessens inflammation. The molecular mechanism linking gut microbes n and adipose tissue homeostasis remains unknown, but several hypothesis have been proposed. Several human intervention studies with prebiotics support their interest in the control of glycemia, adiposity, and endotoxemia, which are related to changes in certain bacteria (increase in Bifidobacteria, in *Akkermansia muciniphila*, or F*aecalibacterium prausnitzii*) (Salazar et al 2014, for review Delzenne et al, 2013, 2015). Other studies are needed to evaluate the interest of food rich in prebiotics on obesity and related metabolic diseases.

Cancer cachexia, gut-liver and gut-adipose tissue axis : a role for prebiotic approaches

In experimental mice model of leukemia, we have shown that the dietary intake of inulin-type fructans lessens tumor cell proliferation in the liver, a phenomenon linked to propionate production (Bindels et al 2012). In the same model, the administration of pecto-oligosaccharides is able to improve cachexia, by decreasing the oxidation of fatty acids in the adipose tissue (Bindels et al 2015b). This shows that, depending on the context (obesity or, inversely, cachexia), the effect of prebiotic fermentation may generate differential effects, and improve host health, by changing the gut microbiota .

Conclusion

Even if the changes in gut microbiota by prebiotic appear as much more complex than previously thought, some bacteria or bacterial functions are particularly interesting to consider, in order to evaluate the relevance of this nutritional approach in the treatment or prevention of metabolic diseases.

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THE ROLE OF OLIGOSACCHARIDES IN HOST-MICROBIAL INTERACTIONS FOR HUMAN HEALTH

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Oligosaccharides, including those found in milk have the capacity to interact with both host and bacterial cell surfaces which in turn offers the host protection from pathogenic infection. There is evidence to suggest that the protection afforded to the host is not as a consequence of a single oligosaccharide activity but rather can be attributed to the many health promoting activities associated with milk oligosaccharides. For example, human milk oligosaccharides have been demonstrated to bind directly to invading pathogens, preventing host cell attachment and subsequent colonization of gastrointestinal epithelial cells (Manthey, Autran et al. 2014). Interestingly, oligosaccharides found in milk have been shown to have the potential to reduce the threat of infection in other organs by directly interacting with the bacteria. For instance, the ability of the oligosaccharide 6'-siallylactose to reduce the adhesion (Thomas and Brooks 2004) and invasion (Marotta, Ryan et al. 2014) of *Psudomonas aeruginosa* to lung cells has been demonstrated in vitro. Milk oligosaccharides have also been shown promote the adhesion of commensal bacteria to host receptors (Kavanaugh, O'Callaghan et al. 2013), which can also aid in the prevention of pathogenic establishment in the host. More recently, the immune-modulatory properties of bioavailable milk oligosaccharides and their capacity to change host cell surface glycosylation have been identified (Angeloni, Ridet et al. 2005). The ability of oligosaccharides to modulate cell surface glycosylation lead to a reduction in the adhesion of enteropathogenic *Escherichia coli* to host cells *in vitro*, further highlighting the protective capabilities of milk glycans. Human intestinal epithelial cells are coated in a protective mucin layer which acts as a barrier to pathogens (Dharmani, Srivastava et al. 2009). Oligosaccharides were also shown to have potential in altering mucin expression which may improve the protection these secretory proteins offer. For example, commercial oligosaccharides such as galacto-oligosaccharides have been shown to enhance the expression of mucin-associated proteins including MUC2, potentially promoting mucosal barrier function and conferring further resistance to pathogen infection (Bhatia, Prabhu et al. 2015).

Milk oligosaccharides were shown to modulate the transcriptomic response of gastrointestinal commensal bacteria such as the prototypical infant bifidobacterial strain, *Bifidobacterium longum* subsp. *infantis* (Kavanaugh, O'Callaghan et al. 2013). In this particular study, the authors highlighted the ability of the bifidobacterial strain to sense the presence of milk oligosaccharides and mount a transcriptomic response leading to an increased colonization potential of the bacterial species to a human colonic epithelial HT-29 cell line. Interestingly, many genes potentially involved in the adhesion process were seen to be upregulated after exposure to milk oligosaccharides. In addition to impacting the transcriptomic response of commensal bacteria, milk oligosaccharides from bovine and human milk have been shown to affect the transcriptional response of a human colonic epithelial HT-29 cell line (Lane, O'Callaghan et al. 2013). In this study, genes involved in the immune system, including cell surface receptors, interleukins and chemokines, were shown to be differentially regulated in response to oligosaccharides from both bovine and human colostrum, suggesting a possible role in protecting the susceptible neonatal gut from pathogenic infection. Interestingly, this study highlights the potential for bovine milk oligosaccharides to elicit a transcriptional immunological response similar to that observed for human milk oligosaccharides. This study highlights the potential of bovine milk as a potential source of oligosaccharides for use in infant formula where an important objective is to bridge the "oligosaccharide gap" which is evident between human breast milk and infant formula. Indeed, it is known that breast fed infants are less susceptible to pathogenic associated diarrhea (Morrow, Ruiz-Palacios et al. 2004, Newburg, Ruiz-Palacios et al. 2004) and achieve a higher cognitive and developmental score (Anderson, Johnstone et al. 1999, Wang, McVeagh et al. 2003) when compared to formula fed infants. Thus, it is clear that bovine milk may be an attractive source of milk oligosaccharides for formula supplementation in an effort to emulate the gold standard that is human milk.

Overall it can be seen that oligosaccharides, including those found in human and domestic animal milks as well as enzymatically produced oligosaccharides such as galacto-oligosaccharide, possess the capabilities to protect from pathogenic infection and in some cases increase commensal colonization through many different health promoting activities. The studies mentioned above have demonstrated the important roles oligosaccharides may play in maintaining the balance between reducing pathogenic infections and increasing commensal colonization potential. Such health promoting activities are modulated through direct bacterial interactions, immunomodulatory activities, promotion of gut barrier function and induction of protective transcriptional responses.

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SHORT-TERM IMPACT OF A CLASSICAL KETOGENIC DIET ON GUT MICROBIOTA: A PILOT STUDY

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Goal: The goal of this pilot study was to investigate the short-term effect of a classical ketogenic diet on gut microbiota
Background: The classical ketogenic diet (KD) is an isocaloric, high-fat (85 – 90 % energy), low-carbohydrate (2- 3 % energy) diet and is an effective treatment for GLUT1 DS and medically refractory epilepsy. The consumption of a high fat diet is associated with large alterations of microbiota in animal studies.
Study: Fecal samples were collected before and after 3 months on a classical KD in six patients diagnosed with GLUT1 DS and drug-resistant epilepsy.
Results: After 90 days on the diet there was a statistically significant increase in *Desulfovibrio* spp, a sulphate-reducing bacteria associated with inflammatory bowel diseases in humans
Conclusions: Although the small sample size limits our ability to draw definite conclusions, this report may prompt larger studies on the changes in microbiota induced by the KD to prevent potentially harmful long-term consequences
Key Words: classical ketogenic diet; gut microbiota
Conflict of Interest Disclosure: No conflicts to declare.
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Ketogenic diet (KD) is an isocaloric, high-fat, low-carbohydrate diet that induces ketone bodies production (i.e. b-hydroxybutyrate and acetoacetate), mimicking the biochemical changes of starvation (Tagliabue et al 2012). For classic KD, nutrient composition is expressed as the weight ratio of fat to protein and carbohydrate. The most common ratios are 4:1 and 3:1, equal to 3 or 4 g fat to 1 g protein +carbohydrates, providing at least 0.8 g/kg of protein for body weight. This means that 85-90% of the dietary calories are derived from fat and less than 2-3% are derived from carbohydrates. KD has been used since the 1920s as an adjuvant therapy in drug resistant epileptic patients suffering different types of seizure and epilepsy syndromes and it is the treatment of choice in Glucose Transporter 1 Deficiency Syndrome (GLUT 1-DS; OMIM #606777) (Klepper 2008, Veggiotti 2011). In epilepsy KD should be tried for at least three months to check for efficacy. For children who remain on the diet and achieve >50% seizure response, KD can be continued for years. In GLUT1 DS the KD may be a lifelong treatment.
Diet is a major factor driving the composition and metabolism of the colonic microbiota. The amount, type and balance of the main dietary macronutrients (carbohydrates, proteins and fat) have a great impact on the large intestinal microbiota. David et al (2014) demonstrated that the short-term consumption of diets composed entirely of animal or plant products alters microbial community structure and overwhelms inter-individual differences in microbial gene expression. The animal-based diet increased the abundance of bile-tolerant microorganisms (*Alistipes, Bilophila*, and *Bacteroides*) and decreased the levels of Firmicutes that metabolize dietary plant polysaccharides (*Roseburia, Eubacterium rectale*, and *Ruminococcus bromii*).
The consumption of a high fat diet was associated with large alterations in microbiota including a decrease in Bacteroidetes and an increase in both Firmicutes and Proteobacteria in animal models (Hildebrandt et al 2009). The increase in Proteobacteria (*B.wadsworthia*) was confirmed in mice by Devkota et al (2012) on a diet rich in animal-derived saturated fats. Several authors (Duncan et al. 2007, 2008, Russel et al 2011. Walker et al 2011) demonstrated a significant reduction in clostridial cluster XIVa, including *Roseburia intestinalis, Eubacterium rectale* (two species belonging to the Firmicutes phylum) and Actinobacteria, in obese individuals on a low-calorie, low-carbohydrate diet. In other terms, there is strong evidence that a high-fat diet may induce changes in gut microbiota in animal models and that this dismicrobism may produce deleterious effects on gut inflammation and intestinal barrier function, with the consequence of a metabolic endotoxemia leading either directly or indirectly to changes in the functioning of neurons, including vagal afferent neurons (De Lartigue et al. 2012). Since the composition of a classical ketogenic diet is characterized by a marked increase in fat mainly from animal sources and a reduction in carbohydrates and fiber, it is of great relevance to investigate whether a similar dismicrobism takes place in humans. We performed this pilot study to investigate the short-term effect of ketogenic diet on gut microbiota in patients diagnosed with GLUT1 DS and drug-resistant epilepsy.

Materials and Methods

Study design and patients
GLUT-1 DS is a rare disease. We prospectively enrolled 6 patients (3 females and 3 males, age range 8 – 34 years) diagnosed with GLUT 1-DS or medically refractory epilepsy at the Department of Child Neurology in Pavia. They underwent a dietary treatment with a classical ketogenic diet and were asked to collect fecal samples before and after three months on the diet.The study protocol received Institution Review Board approval and complied with all tenets of the Helsinki declaration. The patients or caregivers provided written informed consent before the beginning of the study. The main outcome measures were the changes from the baseline of large intestine microbiota composition through the analysis of faecal samples.

Ketogenic diet treatment
A non- fasting dietary protocol with at home gradual increase of ketogenic ratio was implemented at the Human Nutrition Research Center outpatient clinic. The usual caloric intake and food intolerances and preferences were evaluated for each patient by use of 7-day food diaries. After this evaluation the dietician worked out, for each patient, several KD plans with increasing ketogenic ratios. The macronutrient composition included a minimum of 0.8 -1 g per kilogram of body weight of protein from animal sources (e.g. eggs, milk, meat, poultry and fish). All the participants received a prescription of sugar-free multivitamin and mineral supplements according to their age and sex. No probiotic or prebiotic supplementation was provided in the first three months of treatment. All patients were instructed to start a 1:1 ketogenic diet at home and gradually proceed to 2: 1, 3: 1 or 4:1 ketogenic ratios in order to obtain blood values of beta hydroxybutyrate ≥ 2.0 mml/l Families were instructed to check blood daily during the induction phase and twice per week thereafter, and to report the values by e-mail.

Collection of faecal samples
Faecal samples were freshly collected in appropriate sterile tube containing 9 ml of liquid sterile transport medium (Amies medium), stored at refrigerated temperature and delivered to the laboratory within 24-48 hours from the recovery. Samples were taken at the beginning of the treatment (P samples) and after 90 days of ketogenic diet (D samples).

Total bacterial DNA extraction from faecal samples
0.2 gram of each faecal sample were submitted to DNA extraction using FastDNA SPIN Kit (MP Biomedical, USA) and FastPrep Instrument (MP Biomedical, USA), following the manufacturer's indications as regards the treatment of biological samples.
The extracted DNA was quantified using Qubit 2.0(Invitrogen™, USA), and Qubit® dsDNA HS Assay Kit (Invitrogen™, USA) to evaluate the concentration of DNA in ng/µl and diluted up to 5 ng/µl to standardize the amount of nucleic acid in each sample and eliminating a source of possible misinterpretation of the results.

RT – PCR analysis
The proper volume of RT - qPCR reagents was freshly prepared for each analytical session, according to the number of samples in duplicate, for a final reaction volume of 25 µl, including also a positive control (template belonging to the target genus). Serial dilutions of the reference DNA were prepared under sterility conditions, and in a dedicated area, to guarantee the lowest contamination probability. Both, target DNA and reference DNA were prepared in sterile microtubes and dilution made using sterile DNAse RNAse free water. The amount of the nine bacterial groups was calculated using standard curves derived from known concentrations of genomic reference DNA certified by DSMZ. Amplification mixture was prepared using RealMasterMix Sybr Rox 2.5X (5PRIME, Germany), according to supplier's instruction and primers were added to the mixture. Different protocols and primers were applied to quantify *Bifidobacterium* spp., *Lactobacillus* spp., *Clostridium perfringens, Enterobacteriaceae, Firmicutes, Bacteroidetes, Clostridium cluster XIV, Desulfovibrio spp and Faecalibacterium prausnitzii* (Haarman and Knol, 2005; Malinen et al. 2005; Wise and Siragusa ,2005; Huijsdens et al. 2002; Guo, 2008; Vigsnæs LK et al., 2011). Finally the RT - qPCR plates were prepared assembling the amplification mixture, and diluted DNAs, besides positive (reference strain or genus) control and a no template control (reaction mixture without any DNA addition). After plate preparation the thermal cycle was performed as reported by the quoted scientific papers without modifying the conditions documented by the authors. RT - qPCR results were checked on real – time during the cycle and stored in the Instrument Database until the elaboration of the data, on the basis of the specific dilution factor determined for each sample.

Data analysis
Variables are expressed as means and standard deviations. Because of the paired data, comparisons of continuous variables before and after 3 months of KD were performed by the paired t-test. All calculations were performed using SPSS version 17.0 for Windows (SPSS, Inc., Chicago, IL, USA). A value of $p < 0.05$ (two-sided) was considered statistically significant.

Results
All participants completed the 3-month protocol. Table 1 shows the daily dietary intake before and after the beginning of the KD. The total energy intake did not differ significantly before and after the completion of the study. On the classical ketogenic diet dietary fat and saturated fat increased from 37.6±4.7 % to 87.2±0.2% kcal ($p<0.001$) and 8.4 ± 3.1 % to 23.5 ± 8.5% ($p< 0.05$) respectively; dietary carbohydrates were reduced from 45.3±6.2% to 3.5 ± 1.6% kcal ($p<0.001$). All of the patients tolerated the diet well, and there were no adverse events.
Fecal microbiota composition was assessed by targeting specific bacterial groups and species by quantitative PCR. A general evaluation involving Bacteroidetes and Firmicutes phyla was firstly performed in order to check what previously assessed in mouse model by other authors, associating the consumption of a high fat diet with a decrease of Bacteroidetes and an increase in Firmicutes (Hildebrandt et al 2009). The six subjects submitted to ketogenic diet were sampled twice, at the beginning of the regimen (P samples) and after 90 days treatment (D samples). Due to the exiguity of the target population as well as to the variability in microbiome composition associated to genetic and environmental factors, the analytical data were elaborated by comparing the quantification of bacterial groups and species per single subject before and after treatment instead of a comparison between ketogenic-treated patients and healthy controls. No specific trends were observed neither for Bacteroidetes nor for Frmicutes among the study population. A slight increase in some subject was counterbalanced by a decrease in the rest of the community, therefore average pre-treatment values of Bacteroidetes and Firmicutes were of 7,68 and 9,13 log10 CFUs respectively, against 7,96 and 9,13 post-treatment.
These average data seem to indicate, when compared with similar values in healthy controls (data not shown), a general microbial depletion in ketogenic-treated subjects.
Fecal DNAs were also analyzed to quantify single bacterial genera of functional interest, both for their potentially positive impact on human health (lactobacilli, bifidobacteria, clostridia cluster XIVa) as well as for the putative detrimental effect attributed to their proliferation (enterobacteriaceae, desulfovibrio). Moreover, some bacterial species, recently emerged as target of gut healthiness, such as *Faecalibacterium prausnitzii,* or as dangerous opportunistic pathogen, such as *Clostridium perfringens*, were also quantified from faecal DNA.
The changes in microbiota observed in the six patients are illustrated in Figure 1. The average values of quantification of the analyzed bacterial groups were compared among pre- and post-treatment. A statistically significant increase in *Desulfovibrio* spp was observed after 90 days ketogenic diet ($p = 0,025$) while no other significant change was observed in the examined microbial groups after the end of the treatment period. When considered the dynamic of the microbiota composition of single subjects, it was not possible to draw conclusive remarks due to the high variability inside the small analyzed population. No significant trends were detectable except for the increase in *Desulfovibrio* clearly associated to the consumption of the high-fat regimen.

Discussion
The main results of this pilot study was a significant increase in *Desulfovibrio* spp a sulphate-reducing bacteria in the large intestine of patiens on a classical ketogenic diet for three months. This result is in accordance with observation by Devkota et al. (2012) in mice. They show that consumption

of a diet high in saturated (milk derived) fat, but not polyunsaturated (safflower oil) fat, changes the conditions for microbial assemblage and promotes the expansion of a low-abundance, sulphite-reducing pathobiont, (*Bilophila wadsworthia*). This was associated with a pro-inflammatoryT helper type 1 (TH1) immune response and increased incidence of colitis in genetically susceptible Il102/2, but not wild-type mice. These effects are mediated by milk-derived-fat-promoted taurine conjugation of hepatic bile acids, which increases the availability of organic sulphur used by sulphite-reducing microorganisms like *B. wadsworthia*. Together these data show that dietary fats, by promoting changes in host bile acid composition, can markedly alter conditions for gut microbial assemblage, resulting in dysbiosis that can perturb immune homeostasis. The data provide a plausible mechanistic basis by which Western-type diets high in certain saturated fats might increase the prevalence of complex immune mediated diseases like inflammatory bowel disease in genetically susceptible hosts.
Consistent with this hypothesis, previous studies in humans (Loubinoux et al 2002, Rowan et al 2009) have demonstrated an association between inflammatory bowel diseases and sulphate-reducing bacteria – which, like *B. wadsworthia*, can metabo¬lize certain sulphur-containing compounds. The by-products of this bacterial metabolism, namely hydrogen sulphide (H2S), might disrupt the epithelial tissue that lines the inside of the gut and that acts as a barrier against pathogens and toxins. Such disruption could magnify the effects of certain immune responses to antigens, of pro-inflammatory compounds produced by the gut microbiota, and of viral infections.
The ketogenic diet (KD) is an effective treatment for GLUT1 DS and medically refractory epilepsy and like other treatments it is not without side effects. The side effects encountered are related to the diet composition and the radical metabolic changes that results from a high fat, low carbohydrate and protein diet. Mild gastrointestinal disturbances are common side-effects of KD, mainly consisting in nausea, vomiting and diarrhea in the short–term In the long-term constipation is prevalent and is treated by increasing the fluid intake, use of polyethylene—glycol or non absorbable fiber or. No data are available for specific recommendations with reference to probiotic or prebiotic supplementation.
In summary, the major finding of this pilot study is that administering a KD for 3 months increases suphate-reducing bacteria in the large intestine. Although the small sample size limits our ability to draw definite conclusions, this report may prompt larger studies on the changes in microbiota induced by the KD to prevent potentially harmful long-term consequences. Sulphite-reducing bacteria could be considered easy-to-quantify and valuable biomarkers to evaluate the impact of saturated fats on human gut microbiome.

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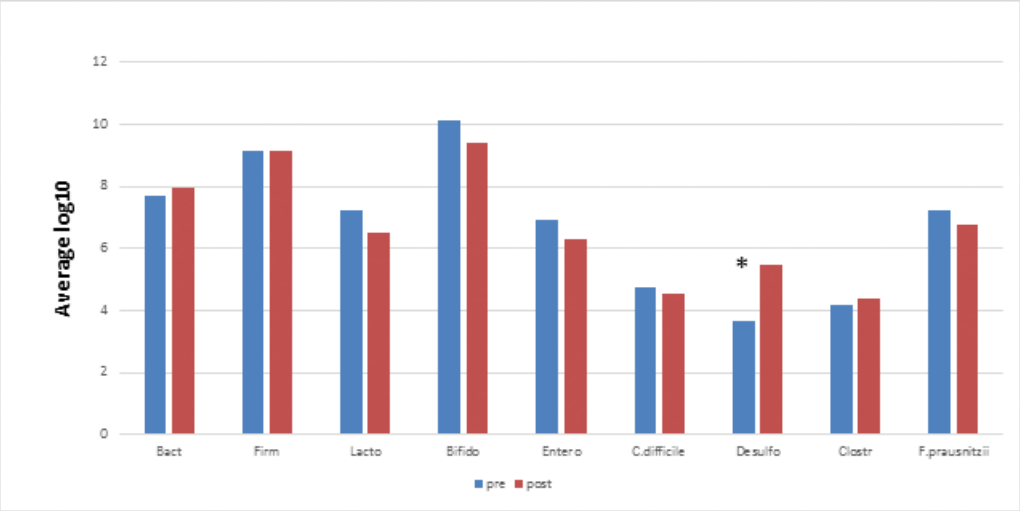
Table 1.Daily dietary intake before and after the beginning of the ketogenic diet					
	Pre intervention		**Post intervention**		*p value*
	Mean	SD	Mean	SD	
Energy intake (kcal/24 h)	1861,3	290,0	1891,3	317,1	0,184
Energy intake (kcal/kg)	40,6	18,1	41,2	17,9	0,147
Fat (g/kg)	1,6	0,6	4,0	1,7	0,011
Fat (% energy)	37,6	4,7	87,2	0,2	0,000
Satured Fat (% energy)	8,4	3,1	23,5	8,5	0,023
Monounsatured Fat (% energy)	10,3	3,4	15,7	1,2	0,008
Poliunsatured Fat (% energy)	2,8	1,2	4,3	2,7	0,371
Protein (g/kg)	1,7	0,7	0,9	0,2	0,027
Protein (% energy)	16,6	1,7	8,9	1,5	0,000
Carbohydrates (g/kg)	4,9	2,6	0,4	0,4	0,011
Carbohydrates (% energy)	45,3	6,2	3,5	1,6	0,000



THE ROLE OF VITAMIN D IN ALLERGIC DISEASE IN CHILDREN

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Keyword: vitamin D, asthma, atopic dermatitis.

Introduction

Vitamin D refers to a group of fat-soluble steroids among which vitamin D3 (cholecalciferol) is the most important compound for humans. The most common function of vitamin D3 is the regulation of calcium and phosphate homeostasis. Recently, vitamin D3 has been associated with cardiovascular disease, malignancies, microbial infections, autoimmune disorders and allergic diseases.[1]

Cholecalciferol can be ingested from the diet or synthetised in the skin by exposure to sunlight UVB radiation. Subsequently it's converted to 25-hydroxyvitamin D (25(OH)D) in the liver and after by the kidneys to1,25-dihydroxyvitamin D (1,25(OH)D) the biologically active form of vitamin D. This compound subsequently activates the vitamin D receptor (VDR), regulating the expression of genes involved in calcium metabolism, proliferation, differentiation, apoptosis, and immunity.[2]

Vitamin D receptor (VDR) was discovered to be present in a variety of tissues, suggesting the importance of the vitamin D system in various cellular and tissue functions.[3] One of the most important function is to modulate the immune system response both innate and adaptive.

Atopic diseases are Th2-dominant condition, characterized by the production of cytokines such as IL-4, IL-5, and IL-13, and the production of IgE by B cells.[4] Vitamin D has shown the ability to inhibit T_h2-type response by suppressing the production of both IL-4 and IL-13.[5]

Regulatory T cells (Tregs), play an important role in maintaining immune homoeostasis in response to allergen exposure by suppressing T_h2-mediated inflammation such as airway eosinophilia, mucous hypersecretion, and airway hyperresponsiveness.[6-7] Vitamin D can induce antigen-specific IL-10-producing Tregs that express low levels of the $CD4^+CD25^+$ Treg-associated transcription factor FoxP3.[8]

Vitamin D and its receptor (VDR) are both essential for development of natural killer (NK) cells and the expression of IL-4 and IFN-γ production.[9] NK cells are able to producing several pro-inflammatory cytokines such as IFN-γ, TNF-α and GM-CSF.[10] Vitamin D can suppress the pro-inflammatory cytokine IL-17 which has a key role in non-atopic asthma.[11] All these mechanisms could explain the growing evidence connecting vitamin D to allergic disease like asthma, atopic dermatitis and food allergy.

Vitamin D and Asthma

Several studies have found that low cord blood vitamin D levels are associated with increased risk of wheezing or recurrent lung symptoms in young children but not asthma.[12]

In a recent sistematic review13 several prospective studies measuring 25(OH)D in cord blood at birth or during pregnancy, did not find any association between 25(OH)D levels and asthma in children from 4 to 8 years. Hovewer, higher serum levels of 25(OH)D were associated with a reduced risk of asthma exacerbations[13]. In the Childhood Asthma Management Program (CAMP) study, the higher risks for severe asthma exacerbations leading to Emergency Department visits or hospitalizations was associated with vitamin D insufficiency (25OHD < 30 ng/mL).[14]

Recent experimental data suggest that vit D can potentially increase the therapeutic response to glucocorticoid and potentially be used as an add-on treatment in steroid-resistant asthmatic patients[15]. Xystrakis et al. [15] showed that typical impaired production of IL-10 by CD4+ regulatory T cell observed in steroid-resistant asthmatic patients could be reversed by the addition of dexamethasone and vit.D . In the same study the use of vit. D overcame the down-regulation of glucocorticoid receptor expression on CD4+ T cells induced by dexamethasone. About inhaled steroids, a study examining children with persistent asthma treated with inhaled steroids found a greater improvement of lung function over one year in children with vitamin D sufficiency versus deficiency.[16] Additionally, children with severe therapy-resistant asthma have been shown to have significantly lower vitamin D levels than children with moderate asthma.[17]

Vitamin D supplementation may potentially decrease the severity of asthma through a variety of mechanisms including effects on immune cells, prevention of predisposing infections[18], decreased inflammatory responses, improved lung function[19], and reduced airway remodeling.[20]

In a our recent study we investigated the relationship between Exhaled Nitric Oxide and vitamin D levels in 66 mild to moderate asthmatic children. Our data show a significant decrease in bronchial inflammation assessed by Exhaled Nitric Oxide ($p= 0.0018$) in children with vitamin D levels > 30 ng/ml.[21]

Brehm JM et al. associated high vit. D levels with reduced risks of hospitalization (OR, 0.05) and use of anti-inflammatory medications (OR, 0.18) in asthmatic children.14

Vitamin D and atopic dermatitis

Atopic dermatitis (AD) is a common chronic inflammatory condition characterized clinically by pruritus, eczematous lesions, and a defective epidermal barrier.[22]

Vitamin D is implicated in the stratum corneum barrier formation, by means of protein synthesis (such as filaggrin) and regulation of keratinocytes proliferation and differentiation.

Vitamin D stimulates the production and the regulation of skin antimicrobial peptides, such as cathelicidins.[23] Antimicrobial peptides show both a direct antimicrobial activity and an induced host cellular response by inducing cytokine release. Therefore vitamin D deficiency might predispose patients with AD to skin superinfection by Staphylococcus aureus or its superantigens.[24] Vitamin D deficiency is also associated with more severe skin lesions on localized body areas not exposed to sunlight.[25] UV light, administered in the controlled setting, is a widely recognized treatment for severe AD and the benefit of UV light exposure was due to improved vitamin D status.[26]

Several studies show that vitamin D deficiency is related to the severity of AD. Wang et al.[27] measured 25(OH)D levels in 498 children with AD and 328 non-allergic controls. AD severity, assessed by SCORAD score, showed inverse associations with serum 25(OH)D levels. Peroni et al.[28] showed that serum levels of 25(OH)D were higher in patients affected by mild AD compared to children with moderate or severe AD. On the other hand Chiu et al[29] found no statistically significant association between vitamin D levels and AD severity.

Vitamin D deficiency at birth is associated with higher risk of developing AD. In a birth cohort study of 239 newborns, low cord blood 25(OH)D levels were associated with higher risk of developing AD at ages of 1, 2, 3, and 5 years.[30]
In conclusion, systematic supplementation of vitamin D in childhood affected by AD currently cannot be recommended. Additional studies, with adequate sample size, longer duration of treatment, standardization of AD severity assessment, and adequate correction for confounding factors such as sun/UVB exposure, are currently needed.

Vitamin D and food allergy
Recently it was proposed that vitamin D is a risk factor for food allergy.
Vassallo et all.[31] hypothesize that Vitamin D deficiency predispose to more severe and frequent infections caused by common gastrointestinal pathogens due to altered production of antimicrobial peptides. Gut infections promote dysbiosis and impair intestinal barrier function. The increasing exposure to food antigens in genetically susceptible subjects may promote the development of food allergy. In vitro experimental data show that 1,25(OH)2D3 induces junction protein expression (ZO-1, claudin 1, claudin 2, and E-cadherin) and strengthens the tight junction complex improving the maintenance of mucosal barrier function.[32] About that, Chiu et al have proved that low cord blood vitamin D levels increase milk sensitization.[33]
There was a higher prevalence of food allergies in children living in areas with low exposure to sunlight (such as Australia) and in children born in autumn or winter.[34] Two recent large population-based studies have shown that low serum Vitamin D levels are associated with increased risk of peanut sensitization and challenge-proven peanut or egg-allergy.[35]

In conclusion, several data suggests that serum 25(OH)D levels are often insufficient in children with asthma atopic dermatitis and food allergy. A certain number of studies shows that vitamin D supplementation may have a role in allergic illness but there are studies that not confirm this hypothesis. Further clinical trial results are needed to provide conclusive evidence and to identify the optimal dosage, length of treatment and target serum 25(OH)D levels in allergic diseases.

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METAGENOME SEQUENCING OF THE HADZA HUNTER-GATHERER GUT MICROBIOTA

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Objective

Through human microbiome sequencing, we can better understand how host evolutionary and ontogenetic history is reflected in the microbial function. However, there has been no information on the gut metagenome configuration in hunter-gatherer populations, posing a gap in our knowledge of gut microbiota (GM)-host mutualism arising from a lifestyle that describes over 90% of human evolutionary history. In this scenario, we present the first metagenomic analysis of GM from Hadza hunter-gatherers of Tanzania - one of the last remaining hunter gathering populations - and western urban Italians from Bologna metropolitan area.

Methods

To investigate functional differences in the GM across the two groups, we performed shotgun sequencing on total stool DNA from 27 Hadza (age 8–70 years) and 11 Italians (age20–40 years) from Schnorr *et al.* Nat. Commun 2014. We generated 448.4 million of 2 x 100 paired-end reads, with an average of 11.8 million (±1.7 SEM) reads per subject.

Results

Hadza shows a unique enrichment in metabolic pathways that aligns with the dietary and environmental factors characteristic of their foraging lifestyle. We found that the Hadza GM is adapted for broad-spectrum carbohydrate metabolism, reflecting the complex polysaccharides in their diet. Furthermore, the Hadza GM is equipped for branched-chain amino acid degradation and aromatic amino acid biosynthesis. Resistome functionality demonstrates the existence of antibiotic resistance genes in a population with little antibiotic exposure, indicating the ubiquitous presence of environmentally derived resistances.

Conclusions

Our results demonstrate how the functional specificity of the GM correlates with certain environment and lifestyle factors and how complexity from the exogenous environment can be balanced by endogenous homeostasis. The Hadza gut metagenome structure allows us to appreciate the co-adaptive functional role of the GM in complementing the human physiology, providing a better understanding of the versatility of human life and subsistence.

WHAT WE CAN, AND CAN'T, LEARN ABOUT THE HUMAN ANCESTRAL STATE FROM STUDIES OF THE HADZA HUNTER-GATHERER GUT MICROBIOME

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Objective
The Hadza of Tanzania are modern-day hunter-gatherers who, because of where they live and their lifestyle, are often used as a proxy for Paleolithic human ancestors. Our study of their gut microbiome (GM) aims to explore ancestral qualities linked to a foraging lifestyle.

Methods
Fecal samples from 27 Hadza and 16 Italians were collected and pyrosequenced in the V4 gene region of bacterial 16S rDNA. Reads were clustered into operational taxonomic units (OTUs) at 97% identity. We compared species diversity using several different methods, and compared taxonomic profiles among our data and other published resources.

Results
The Hadza have high levels of microbial richness and diversity, and their GM shows differences in microbial structure between the sexes that reflects their sexual division of labor. They entirely lack Bifidobacteria, taxa known to correlate with health in Western populations. Furthermore, enrichment in *Prevotella*, unclassified *Bacteroidetes* and *Treponema*, and a peculiar arrangement of *Clostridiales*, may enhance Hadza ability to digest fibrous plant foods.

Conclusions
The Hadza present a unique arrangement of taxa that seems strongly linked to their foraging lifestyle. However, the unique aspects of the Hadza GM are influenced by the host genetic profile, the local environment, the social structure, and the diet of the Hadza. We cannot, therefore, describe the Hadza GM profile as our 'ancestral' profile. Instead, we need more studies of a variety of non-Western groups to look for similarities that may reflect components of a "core" ancestral microbiome, which has been altered or lost through Westernization.

THE MICROBIOTA-GUT-BRAIN AXIS: FROM ANIMAL MODELS TO PATIENTS WITH FUNCTIONAL GI DISORDERS

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KEYWORDS: Bacteria; IBS; brain; behavior; infection; antibiotics; probiotics.

Functional gastrointestinal disorders, of which the irritable bowel syndrome (IBS) is the prototype, are likely to be as heterogeneous in their etiology and pathogenesis as they are in their clinical expression. Historically, much emphasis has been placed on a primary role for stress and behavioral alterations as a driver for gut dysfunction in these conditions. However, there is increasing interest in the role of other environmental triggers such as enteric infection and antibiotic exposure and the entity of post-infective IBS is now recognized (1). Longitudinal studies following the outbreak of food or water contamination reveal that post infectious IBS may become chronic, thus raising questions regarding mechanisms that sustain the condition, including both intestinal and behavioral manifestations (1). Given the impact of infection or antibiotic exposure on resident intestinal bacteria, attention has focused on the role of the intestinal microbiota in the expression of these disorders (2).

A growing number of clinical studies have demonstrated compositional changes in the intestinal microbiota of IBS patients. To date, no microbial "signature" for IBS has been identified and it is evident that not all IBS patients exhibit demonstrable compositional changes in the microbiota. While there is been much variation in IBS-associated microbiota profiles, some trends have been identified and these include a relative decrease in Firmicutes, including Bifidobacteria and Lactobacilli, as well as a reduction in bacterial diversity. In addition, compositional instability over time has been documented in several studies. A smaller number of studies have shown changes in bacterial metabolism with a particular focus on the production of short chain fatty acids. Together, the changes in composition and function of the microbiota are referred to as "dysbiosis" and dysbiosis is now considered a putative mechanism for symptom generation in at least a subset of IBS (2,3)

Factors known to trigger the onset or relapse of IBS are also known, largely but not exclusively, on the basis of animal studies, to alter the microbial composition of the gut at least on a temporary basis. These include stress, antibiotic exposure, enteric infection and dietary change or indiscretion. Thus, the case may be made for a causal role for bacterial dysbiosis in symptom generation in at least a subset of IBS patients in whom the above described factors have been implicated. It is also known that changes in intestinal physiology, including motility, mucus production and entero-endocrine function alter the bacterial habitat and microbial composition. These bi-directional host-microbiota relationships provide a model in which dysbiosis, induced by infection or antibiotics, can be sustained (2).

Animal studies have provided proof of concept that perturbation of the microbial composition of the gut alters function not only in the intestine, but also in the brain. Transient shifts in microbiota profiles induced by antibiotic exposure have been shown to alter visceral pain responses to colorectal distention in mice (4). In that study, antibiotic induced dysbiosis was accompanied by low-grade inflammation - a finding in a subgroup of IBS patients. In addition, the enhanced pain responses following antibiotic exposure could be normalized by the administration of a probiotic, Lactobacillus paracaseii or by dexamethasone, suggesting that the enhanced pain response was a result of antibiotic-induced dysbiosis and involved a low-grade inflammatory response (4). Antibiotic-induced dysbiosis has also been associated with alterations in colonic transit in mice mediated by TLR4 (5). Taken together, these observations from animal studies indicate that perturbation of a previously stable intestinal microbiota can lead to changes in visceral pain responses as well as colonic motility - a profile of got dysfunction reminiscent of that found in humans with IBS.

Psychiatric comorbidity is common in IBS patients and there is an emerging literature demonstrating the ability of the intestinal microbiota to alter brain chemistry and function, as reflected by alterations in emotive behavior and cognitive functions. Initial studies focused on marked differences in brain chemistry and behavior between germ-free and colonized mice. In most cases, changes observed in germ-free mice could be reversed following bacterial colonization. These studies provided proof of concept that intestinal bacteria can influence the brain (6). This concept was further supported by the ability to adoptively transfer components of behavioral phenotype among different mouse strains via fecal microbiota. In studies that are more applicable to dysbiosis and IBS, brain chemistry and behavior was studied in conventionally-housed mice following antibiotic or diet-induced compositional changes in a previously stable microbiota. Antibiotic-induced dysbiosis resulted in a reduction in brain-derived neurotrophic factor (BDNF) in the hippocampus and anxiety-like behavior. These changes were maintained until the microbial composition of the gut reverted to normal following cessation of the antibiotics (7). Another study showed that a beef-enriched diet altered the microbial composition of the gut and was associated with changes in cognitive function in mice. Thus, animal studies provide proof of concept that intestinal dysbiosis can alter behavior (8), thus raising the possibility that the microbiota may contribute to the behavioral manifestations seen in at least a subset of IBS patients.

Work in our laboratory has begun to translate the above-described studies into man by providing evidence that the fecal microbiota of IBS patients can alter intestinal physiological and innate immune functions in mice. In these studies, germ-free mice were colonized with the microbiota from healthy controls or diarrhea-predominant IBS patients with or without evidence of psychiatric comorbidity. We found that mice colonized with the fecal microbiota from IBS patients, but not healthy controls, altered intestinal motility, ion secretion and epithelial barrier function. In addition, IBS microbiota-associated mice also showed evidence of innate immune activation as reflected by increased production of beta defensin. Mice colonized with fecal microbiota from healthy subjects or from IBS patients without anxiety or depression did not show behavioral changes. In contrast, mice associated with fecal microbiota from IBS patients with anxiety, as reflected by scores in the Hospital Anxiety and Depression (HAD) inventory showed evidence of anxiety-like behavior. Taken together, these results support the notion that the content of feces of selected IBS patients has the capacity to alter guts and brain function in mice reminiscent of the donor phenotype. We have attributed the changes to the fecal microbiota but we cannot exclude contributions from other microbes, such as fungi, or chemicals produced either by the microbiota or by the host in response to the microbiota. These studies now being extended to other subsets of IBS patients as well as to patients with untreated primary mood disorders.

If indeed the microbiota contribute to the intestinal and behavioral expressions of at least a subset of IBS patients, one might expect symptomatic improvement from microbiota- directed therapies. There is emerging evidence that this is indeed the case. Meta-analyses suggest a week but significant improvement in IBS symptoms following probiotic administration (9,10). These studies were not targeted to IBS patients in whom there was evidence of dysbiosis. There is also a study showing that the prebiotic trans-galacto-oligosaccharide promoted growth of Bifidobacteria and

significantly improved intestinal symptoms in IBS patients. There was also and improvement in anxiety scores in these patients (11) In addition, the antibiotic Rifaximin has been shown to improve symptoms in subsets of IBS patients, although the precise mechanism of action has yet to be elucidated (12).

In conclusion, there is good evidence from animal studies that experimentally induced intestinal dysbiosis can produce changes in gut function and behavior reminiscent of changes found in IBS patients. Moreover, the fecal microbiota from IBS patients appears to differ from that of healthy controls in as much as it has the ability to induce changes in the gut function and behavior in mice. Taken together these findings support a role for the intestinal microbiota in the intestinal and behavioral manifestations of at least a subset of IBS patients, and justify further examination of microbiota-directed therapies that include pre--and probiotics.

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SKIN MICROBIOME AND ACNE

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Introduction

The skin is a very particular and complex ecosystem, since, unlike the other epithelial surfaces of our body, is composed of diverse habitats with an abundance of folds, invagination and specialized niches that support a wide range of microrganisms. The primary role of the skin is to act as a physical barrier to protect the body from the potential assault of foreign organisms or toxic substances. Moreover, the skin represents the interface with the outside environment and for this reason it is colonized by an abundant and diverse collection of bacteria, fungi, viruses and mites that constitute the skin microbiome. The chemical and physical features of the skin select for unique sets of microrganisms that are adapted to the niche they inhabit and that are important for the correct physiological functioning of the skin. Multiple factors influence the skin microbiome composition: colonization is driven by the ecology of the skin surface and then depends on the density of the sebaceous glands, humidity, temperature, pH, endogenous host factors and exogenous environmental factors. For this reason microbiome composition presents an high intra- and inter-personal variability and it makes not easy to define precisely which is the microbiome composition of an healthy subject with respect to a pathological one. Under normal physiological conditions the skin maintains homeostasis between the microbiome and host. Disruption and perturbations in this balance and in the host-microrganisms relationship can result in infectious or inflammatory skin disorders such as atopic dermatitis, psoriasis, acne and rosacea.

SKIN MICROBIOME AND SKIN DISEASES

Skin microbiome is composed at least by 19 bacterial phyla. Major examples are *Actinobacteria* (51,8%), *Firmicutes* (24,4%), *Proteobacteria* (16,5%) and Bacteroides (6,3%). These four dominant phyla characterize also the microbiome of the gastrointestinal tract and of the oral cavity, even if they are present in different proportions. The majority of the identified species are *Corinebacterium, Propionibacterium* and *Staphylococcus* which abundance is strongly dependent on the specific skin site considered. In particular, skin can be divided in three distinct microenvironments: dry, moist and sebaceous each one characterized by its own resident microbial populations. Dry areas of the skin, such as arms and legs, are those with the less amount of microrganisms that include *Proteobacteria* and *Flavobacteriales*. Moist regions like axilla and the groin favor especially the growth of *Corinebacteria* and *Staphylococcus*. Finally, the face, the chest and the back that are skin sites with high density of sebaceous glands, represent suitable habitats for the colonization by lipophilic microrganisms such as *Propionibacterium* and *Malassezia*.
Many skin disorders are postulated to have an underlying microbial contribution considering that clinical improvement are observed after antimicrobial treatments. However, a real causative association between specific microrganisms and skin diseases has been identified in rare cases. Among cutaneous disorders with a correlation to microbiome, atopic dermatitis, psoriasis, rosacea and acne has to be mentioned. Alteration of the skin microbiome composition of a specific skin area has been described for each one of these pathologies. Atopic dermatitis has been associated with an increase in S aureus in the moist regions of the skin; psoriasis develops prevalently in the skin dry sites and the microbiome seems to present an increase in *Corynebacterium, Propionibacterium, Staphylococcus* and *Streptococcus*. Acne and rosacea evolve in the sebaceous areas of the skin and have been associated with an increase in P acnes and *Demodex follicolorum*, respectively. However, it is not clear yet if these skin microbiome alterations are responsible for the pathological condition or if they are the result of genetic and/or skin modifications due to the disease itself.

SKIN MICROBIOME AND ACNE

Acne is one of the most common skin diseases that affects about 85% of the adolescent population. It is a chronic inflammatory disease of the pilosebaceous unit with a non completely understood pathogenesis. Different factors contribute to acne onset and development. In particular, the basic disease mechanism is thought to involve increased sebum production, keratinocytes hyperproliferation, inflammation and altered bacterial colonization considering *P acnes* as the primary disease-associated bacterium. The exact sequence of these events is still under debate, but probably the androgen-induced increases in sebum production can be considered as the major physiopathological factor. Sebum is a complex and peculiar mixture of lipids produced by human sebaceous gland. Triglycerides and free fatty acids account for the predominant proportion of sebum weight (40-60%), followed by wax esters (19-26%), and squalene (12%). The least abundant lipid in sebum is cholesterol and its esters which represent about the 5% of total lipids. Skin microbiome composition is influenced by sebum production and then present age-associated changes due to a different bacterial colonization of the sebaceous areas. At birth, *Firmicutes* are higher in proportion respect to *Actinobacteria.* The puberty-associated maturation of the sebaceous glands leads to an increase in sebum production associated with an enrichment in *Actinobacteria* including *Corynebacterium* and *Propionibacterium* that is one of the most common commensal bacterium of the skin. *P acnes* hydrolizes sebum triglycerides releasing free fatty acids on the skin. This fatty acids production contribute to the acidic skin surface pH (pH ~ 5) that is able to inhibit colonization by many common pathogens including *S aureus* and *Streptococcus pyogenes.* Moreover, *P acnes* also produces propionic acid and secretes bacteriocines which can suppress the growth of other pathogenic microrganisms.
The shift from a commensal behaviour to a pathogen one can be attributed to the relative amount of *P acnes* between healthy and acne subjects. The P. acnes is, in fact, more frequently in pilo-sebaceous follicles of acne patients compared to normal subjects. Noteworthy, *P acnes* abundance is not always higher in acne patients compared with healthy individuals, but, a recent study, demonstrate that certain *P acnes* strains are highly associated with the pathology. These strains present unique genetic elements that may contribute to their virulence and pathogenicity. *P acnes* may lead to the disruption of the follicular wall inducing inflammatory reaction by several mechanisms. Secretion of hyaluronidases, lipases and proteases causes local injury and inflammation. Moreover, *P acnes* activates the classical and the alternative complement pathways and induces the production of pro-inflammatory cytokines and neutrophil chemotactic factors.

PROBIOTICS AND ACNE

Normalization of intestinal microflora by means of probiotics may offer beneficial effects for different skin conditions. 54% of acne patients presents marked alteration of intestinal microflora. Moreover, in acne subjects was found higher incidence of constipation generally associated with lower concentration of *lactobacilli* and *bifidobacterium*. One of the principal benefit that probiotics may exert in acne treatment is the reduction of inflammation

probably due to the downregulation of the release of inflammatory cytokines and the recruitment of T cells. Moreover, probiotics may counteract inflammation reducing sebum amount and therefore lowering P acnes follicular colonization. Oral supplementation of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* led to an improvement of clinical signs of acne. In particular, reduction of inflammatory lesions, total lesion count and clinical grade of acne were observed. Also topical probiotics treatment can lead to acne improvement. Acne subjects show impaired water barrier function. Sphingolipids, which have antimicrobial properties, are lower in acne stratum corneum respect to healthy subjects and this probably facilitates P acnes colonization. Application of probiotics such as *Streptococcus thermophilus* or *lactobacillus* can increase ceramide production accelerating the recovery of skin barrier function. The effectiveness of acne treatment with systemic or topical probiotics needs to be further investigated to confirm the results obtained until now. Anyway, supplementation of systemic probiotics could be recommended in acne patients receiving antibiotics in order to prevent or ameliorate some adverse effects associated with antibiotic alteration of intestinal microflora.

Conclusion

Technological advances, especially as regards the molecular techniques, have greatly increased the ability to identify and characterize microbial communities that inhabit the skin. This allows a greater understanding and definition of skin microbiome considering the intra and inter-personal variability due to the diverse factors affecting its composition. The association between a peculiar microbial profile and a specific skin disease is steadily increasing even if is not defined yet. Concerning acne, the attention has been focused on higher *P acnes* colonization and on *P acnes* diversity at strain level between acne patients and healthy subjects. The influence of gut microbiome on the health of the skin is another important aspect to be considered. An enhanced understanding of the skin microbiome and of its role in acne, as well as in other skin disorders, might lead to a better comprehension of their pathogenetical mechanisms and to the identification of novel therapeutic approaches and treatments.

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PROBIOTICS IN THE TREATMENT OF (VULVOVAGINAL CANDIDIASIS) VVC AND (BACTERIAL VAGINOSIS) BV

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The human vaginal microbiota plays an important role in the maintenance of a woman's health, as well as of her partner and newborns. When this predominantly *Lactobacillus* community is disrupted or decreased in abundance, Vaginitis may occur. Of the millions of cases of vaginitis each each year, most are caused by bacterial vaginosis (BV), followed by Vulvovaginal candidiasis (VVC). The dominance of lactobacilli in healthy vaginal microbiota and its depletion in BV and VVC has given rise to the concept of oral or vaginal use of probiotic *Lactobacillus* strains for treatment and prevention of vaginitis. Probiotics, defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host, are considered a valid and novel alternative for the prevention and treatment of female urogenital tract infections. Probiotics are well known for their ability to lower intravaginal pH, thus establishing a barrier effect against many pathogens. Some strains are also able to create additional and more focused antagonistic activities mediated by specific molecules such as hydrogen peroxide and bacteriocins. In any case, despite some undeniable positive evidence, other intervention studies have at least partially failed to highlight a statistically significant alleviation of BV and VVC symptoms. This is most likely attributable to the lack of a specific inhibitory activity of the strains used towards the bacteria commonly causing BV, such as *G. vaginalis* and *E.coli,* and VVC such as *C. albicans.*
Herein we present in vitro and clinical data to assess the effectiveness of specific probiotic strains in slow release vaginal tablet, for the topical treatment of BV and VVC, and the prevention of recurrences.

PROBIOTICS FOR INFANTILE COLIC

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Background

Infantile colic is usually a self-limited condition, typically resolving by 4-5 months of age. However, it may be very distressing to parents, hence, any safe and effective preventive and/or therapeutic measures would be desirable. Evidence suggests that gut microbiota plays a role in the development of infantile colic and that the gut microbiota in subjects with this disorder differs from the gut microbiota in an unaffected population. If so, it is logical to assume that manipulation of the gut microbiota with probiotics could be a preventive measure in the evolution of these disorders and also may play a therapeutic role.

Aim

To systematically evaluate evidence on the effectiveness of probiotics for treating and preventing infantile colic.

Methods

MEDLINE and the Cochrane Library were searched in April 2015, with no language restrictions, for relevant randomized controlled trials (RCTs) and meta-analyses.

Retults

Three independent RCTs showed that *L reuteri* DSM 17938 reduced crying times in infants with infantile colic in breast-fed infants.[1 2 3] However, one RCT that also involved formula-fed infants did not confirm this effect.[4] Three of these RCTs reported data on crying time on day.[1 2 4] Compared with placebo, the administration of *L reuteri* DSM 17938 reduced crying time on day 21 by approximately 43 minutes (mean difference, MD, -43 min/day, 95% CI -68 to -19). This was mainly seen in breast-fed infants (MD -57 min/day, 95% CI -67 to -46).[5] One recent RCT investigated the efficacy of *L reuteri* for preventing common functional gastrointestinal disorders in infants, particularly infantile colic, in both breast-fed and formula-fed infants. In the *L reuteri* group compared with the placebo group, a reduction of crying time by approximately 51 min/day at 1 month and 33 min/day at 3 months.[6]

Conclusion

The administration of *L reuteri* DSM 17938 is likely to reduce crying time in infants with infantile colic in breast-fed infants, but its role in formula-fed infants is less clear. More studies are needed. Recent data suggest that *L reuteri* DSM 17938 may be effective in the prevention of colic. This innovative approach needs further evaluation by an independent research team.

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IRRITABLE BOWEL SYNDROME

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Irritable bowel syndrome (IBS), as described by using the Rome criteria, includes symptoms of abdominal pain (AP) or discomfort associated with changes in bowel patterns (1). Studies have estimated the prevalence of IBS to range between 6% and 14% in children and between 22% and 35.5% in adolescents (2). A confident diagnosis, confirmation, explanation of pain experience and reassurance can by themselves be therapeutic. Specific goals of therapy include modifying severity and developing strategies for dealing with symptoms (1).
In adults, the Rome III committee recommends a sub-classification into different subtypes based on
the predominant bowel habit (constipation-IBS [C-IBS] or diarrhea-IBS [D-IBS]) (3). Several other authors consider that patients with symptoms of both constipation and diarrhea should constitute an alternating-IBS or a mixed-IBS subtype. In children, as shown by a recent study conducted at our Center, C-IBS seems to be the prevalent subtype, with a significantly higher frequency in girls, whereas D-IBS is more frequent in boys (4).
Despite a complete pathophysiologic understanding of this condition has not been achieved, a general consensus in the field is that, as most functional gastrointestinal disorders (FGIDs), IBS represents a disorder of the brain-gut axis. This bidirectional connection between the central and the enteric nervous system links emotional and cognitive centers of the brain with peripheral intestinal systems, such as intestinal motility or entero-endocrine and immune functions, which likely determine the clinical expression of most FGIDs (5).
The overall management of children with IBS should be tailored to the patient's specific symptoms and identifiable triggers. The biopsychosocial model for FGIDs, which highlights the importance of the child's physical and social setting as well as psychological comorbidities, represents the cornerstone of a multidisciplinary approach. The four major therapeutic approaches include: pharmacologic, dietary, psychosocial, and complementary/alternative medicine interventions.
Although there is limited evidence for efficacy of pharmacological therapies such as antispasmodics and antidiarrheals, these may have a role in severe cases. A Cochrane review concluded that only weak evidence exists regarding beneficial effects of pharmacological agents in providing relief from symptoms in functional AP in children (6). Antidepressants are among the most studied pharmacologic agents for FGIDs. In a randomized double-blind placebo controlled trial of 33 participants aged 12 to 18 years, patients who received amitriptyline were more likely to experience improvement from baseline in overall quality of life and reported a significant reduction in diarrhea, periumbilical pain and right lower quadrant pain. In a multicenter study by Saps et al., 83 children diagnosed with IBS, functional AP or functional dyspepsia (FD) were randomized to 4 weeks of placebo or amitriptyline; a substantial proportion of patients in both groups reported feeling better, but there was no significant difference between patients receiving amitriptyline and those receiving placebo (7).
Role of antibiotics in treatment of children with IBS remains controversial. The only rationale be- hind antibiotic therapy is to eradicate small intestinal bacterial overgrowth. Beneficial results were noted in a study of 50 children with IBS whose score to evaluate symptoms (AP, constipation, diarrhea, bloating, flatulence) improved after 1 month treatment with Rifaximin (8).
Various non-pharmacologic treatments are available for pediatric IBS. In a recent systematic review including 24 studies some evidence was found indicating beneficial effects of partially hydrolyzed guar gum (PHGG), cognitive behavioral therapy, hypnotherapy, and probiotics (LGG and VSL#3). Despite their common prescription in the daily clinical management of IBS (9), no beneficial effect has been detected for lactose-restricted dietary regimens or fiber supplementation other than PHGG.
In addition, as depression, anxiety and stress likely have a relevant role in the pathogenesis of these conditions, other therapeutic options such as behavioral therapy or hypnotherapy have been shown to be partially beneficial (10).
Probiotics play an emerging role as new therapeutic tools in FGIDs, due to the growing recognition of the importance of gut microbiota in influencing brain-gut interactions, and of the role played by intestinal infections in the genesis of AP-FGIDs (11). Recent preclinical data suggest that changes in the gut microbiota can affect brain signaling systems related to pain and associated emotional behavior. In rodents, the probiotics-induced modulation of gut microbiota has been shown to interfere with affective behavior, pain response and gene expression in the brain. Furthermore, the identification of neuroactive signaling molecules produced by bacterial components of the microbiota represents additional evidence of the remote effects in the central nervous system determined by signals generated in the gut. Therefore, probiotics are likely to have a relevant role in the management of FGIDs, by affecting the gut microbiota or by altering brain function and pain perception centrally (12).
Several trials, mostly on limited study populations, have assessed efficacy of probiotics in adults with FGIDs, bearing in general promising results. In children, however, fewer randomized clinical trials (RCTs) are available.
Guandalini conducted a double-blinded, placebo-controlled, cross-over RCT (13) examining the effect of the probiotic mixture VSL#3, a proprietary preparation consisting of a high concentration of eight different probiotic strains *(Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus bulgaricus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, and Streptococcus thermophilus),* in children and adolescents with IBS. The results showed that while taking VSL#3, patients experienced a significant improvement in the global subjective relief of symptoms score as well as in the score for abdominal pain/discomfort ($p < 0.05$). Similarly, significant benefits were found for bloating/gassiness and for caregivers' satisfaction with their child improvement, while no significant improvement was seen for diarrhea or constipation.
In 2014, a systematic review and meta-analysis (14) on the effect of different probiotics as a treatment for FGIDs in children and adolescents included nine trials, five of whom, including our study (28), focused on AP-related FGIDs, and four on bowel changes-related FGIDs (31–34). The meta-analysis concluded that the use of *Lactobacillus GG, Lactobacillus reuteri* DSM 17938 and VSL#3 significantly increased treatment success in affected children, particularly those with associated bowel changes. Of interest, *L. reuteri* DSM 17938's significant decrease of abdominal pain intensity persisted after the removal of the probiotic, indicating a lasting effect of the supplementation.
We recently assessed the efficacy of a probiotic based on a mixture of Bifidobacterium infantis M-63®, breve M-16V® and longum BB536® in children with IBS and FD. Our results showed that lower AP disappeared in a higher proportion of children who received probiotics; in patients who still referred lower AP after treatment with probiotics severity was reduced, unlike patients treated with placebo. In both groups, quality of life improved after treatment; nevertheless, this parameter achieved the best available score in a higher proportion of patients treated with probiotics (15).
Approximately 36–41% of children with gastrointestinal complaints use complementary and alternative medicine (CAM) each year (16). By definition,

complementary medicine is used alongside conventional medicine, while alternative medicine is used in place of conventional medicine. CAM includes techniques such as acupuncture, chiropractics, homeopathy, herbal medicine and spiritual healing. Evidence to support the use of CAM modalities in children is lacking, and there is a serious need for further research in this area.

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ORAL COMMUNICATIONS

EFFECT OF HYDROPINIC TREATMENT WITH CALCIUM BICARBONATE WATER PLUS L. REUTERI ON OROCAECAL TRANSIT IN PATIENTS SUFFERING FROM CHRONIC CONSTIPATION

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Introduction and Aim

Constipation is a common ailment in clinical practice, can sometimes be a clinical symptom of different organic diseases, but it often presents as a stand-alone problem that is not associated with any other pathology. Our study aims to evaluate intestinal transit time in patients suffering from chronic constipation after the administration of calcium bicarbonate water (Uliveto) based fluid associated to L. reuteri.

Methods

15 patients suffering from chronic constipation (average age: 41±5, 5 female and 5 male) and 15 healthy controls (average age: 40±7, 5 females and 5 males) were enrolled and were subjected to a lactulose breath test to determine orocaecal transit time. The study participants therefore began to assume a supplementation with 1,5 litres daily of calcium bicarbonate (fixed residue at 180 °C = 860 mg/l, bicarbonate HCO3- = 650mg/l, calcium Ca++ = 169 mg/l) water (Uliveto water) and L. reuteri (in form of tablets, at a dose of 108 CFU, twice daily) for 15 days. At the end of the hydropinic therapy, the patients were re-assessed by repeating the lactulose breath test and once again completing the questionnaire on gastrointestinal symptoms.

Results

Intestinal transit time was statistically slower in patients suffering from chronic constipation as compared with controls. All patients showed an alteration to orocaecal transit time. After 15 days therapy with the water supplementation plus L. reuteri, a statistically significant overall increase was seen in the orocaecal transit time in all patients.

Conclusion

After this we may affirm that supplementation with Uliveto water and L. reuterii resulted in improved intestinal transit time in patients suffering from chronic constipation. Further studies are necessary to established if this effect is linked to the supplementation with Uliveto water or L. reuterii or to the combination of both.

EFFECT OF HYDROPINIC TREATMENT WITH CALCIUM CARBONATE WATER PLUS L. REUTERI ON GASTRIC EMPTYING IN DYSPEPSIA

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Introduction and Aim

Dyspeptic syndrome has always been a health problem of great interest due to the fact that it is so widespread. This interest has then become even greater in recent years, due to the increase in pharmaceutical expenditure. In a significant percentage of dyspeptics, slowed gastric emptying underlies the symptoms. Treatments based on acid secretion inhibitors and prokinetics are often inefficient in actually eliminating or reducing symptoms. Hydropinic treatments based on thermal waters are often recommended and prescribed for this type of pathology. This study therefore aimed to assess the effect, after the administration of *calcium bicarbonate water (Uliveto)* based fluid associated to *L. reuteri*, on the gastric emptying of solids and on symptoms reported by a group of patients suffering from functional (non-organic) dyspepsia.

Methods

20 patients suffering from primary dyspepsia and 10 healthy (non-dyspeptic) controls were studied. All subjects were subjected to assume a supplementation with 1,5 litres daily of calcium bicarbonate (fixed residue at 180 °C = 860 mg/l, bicarbonate HCO3- = 650mg/l, calcium Ca++ = 169 mg/l) water (Uliveto water) and L. reuteri (in form of tablets, at a dose of 108 CFU, twice daily) for 10 days. Before and after the supplementation period, each subject involved in the study was subjected to a gastric emptying assessment by means of a '13c-octanoic acid breath test'. A clinical score was also used to assess changes seen in symptoms.

Results

In terms of mean +/- standard deviation results, dyspeptic subjects showed a clear improvement in emptying parameters (T1/2 and Tlag) after treatment, in addition to a reduction of average symptom scores.

Conclusion

Thermal treatment based on oligomineral water plus probiotic L. reuteri would appear to improve emptying of solids in dyspeptic patients. Medium – long term longitudinal studies were required to verify the persistence of this effect.

BIFIDOBACTERIUM ANIMALIS SUBSP LACTIS CNCM-I2494 RESTORES TIGHT JUNCTION PROTEINS LEVELS IN A CHRONIC LOW-GRADE COLONIC INFLAMMATION MOUSE MODEL

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Objective

Evidence has grown to support the effectiveness of probiotic strains in the management of gastrointestinal alterations which are mainly associated with deregulated barrier function. In particular, Bifidobacteria have been studied for their efficacy to prevent and to treat a broad spectrum of gut disorders. The aim of this work is to evaluate the effect of *Bifidobacterium animalis* subsp *lactis* CNCM-I2494 on intestinal barrier function

Methods

For this reason, we first achieve gut dysfunction using a DNBS-induced chronic low-grade inflammation model in mice. Then, markers of inflammation, barrier permeability and immune function were monitored.

Results

All the parameters pointed out the absence of an active inflammation process validating the model as a low-grade inflammation one. Nevertheless, barrier permeability, lymphocytes populations and colonic cytokines were found to be altered in challenged mice. CNCM-I2494 was able to restore the function of the intestinal barrier reducing intestinal permeability and to restore colonic goblet cell populations and cytokine levels. Furthermore, tight junction (TJ) protein levels were measured by qRT-PCR showing the ability of the studied strain to specifically normalize their level, especially evident for claudin-4 protein. Finally, CNCM-I2494 counterbalanced CD4+ lymphocyte alterations in both spleen and mesenteric lymphoid nodes (MLN) being able to restore the Th1/Th2 ratio altered by the DNBS challenge (which locally augments CD4+ Th1 cells) by increasing the Th2 response.

Conclusions

Taken together, these data suggest that *B. animalis* subsp lactis CNCM-I2494 can play an important role in restoring homeostatic level in disorders associated with low inflammation and increased colon permeability.

DESIGN OF EXPERIMENT APPROACH FOR DEVELOPMENT OF OAT BASED FOOD PRODUCT FORTIFIED WITH PREBIOTIC (HONEY) AS POTENTIAL PROBIOTIC VEHICLE

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Objective

Study aimed at isolation of proficient probiotics, their functional characterization, and application for development of oat-based fermented food product as potential probiotic-vehicle, using design of experiment (DOE) approach.

Methods

Tolerance of lactic acid bacteria (LAB) isolates under simulated gastrointestinal conditions was examined. Selected LAB were characterized for functional attributes like hydrophobicity, auto/co-aggregation, extracellular enzyme activity, antibacterial activity, antibiotic susceptibility etc.

Results

The LAB isolate M-13 (*Lactobacillus plantarum*) exhibited most of the functional properties of probiotics, and used for developing oat based fermented food product. Box-Behnken design-based optimized level of variables like concentration of oat, and honey, and incubation time, was 8.0%, w/v, 3.0 % w/v, and 48 h, respectively, that supported maximum growth of *L. plantarum* CFU/ml (15.98 CFU/ml). Among process variables incubation time was the most effective, and was followed by honey, and oat; interactive effect of honey and incubation time was maximum on growth of bacterium, and was followed by that of oat and honey, and oat and incubation time. In modified MRS (glucose replaced with probiotics like inulin, lactulose, fructooligosaccharides, or xylooligosaccharides) *L. plantarum* M-13 showed excellent growth. The probiotic-prebiotic (honey)-fortified food product developed was studied for shelf life at room temperature and under refrigeration.

Conclusions

L. plantarum M-13 may potentially be exploited as probiotic, and oat fortified with probiotic and probiotic (honey) could be a very healthy option.

INTESTINAL MICROBIOTA IS INVOLVED IN GENETIC INSTABILITY, INFLAMMATION, LONGEVITY AND LATENCY OF LYMPHOMA IN ATM DEFICIENT MICE

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Intestinal microbiota plays a role in nutrient metabolism, modulation of the immune system, arthritis, obesity and intestinal inflammation. When our lab moved from Harvard to UCLA we found a huge difference in genetic instability and longevity in Atm deficient mice. When we changed the intestinal microbiota back to conventional microbiota we could reproduce the phenotype at Harvard. We tested Atm deficient mice for genotoxicity, genetic instability, DNA damage, inflammation markers, cancer latency and longevity and high throughput sequencing of the intestinal microbiota. Isogenic mice from different housing facilities showed a four fold difference in life expectancy, a 4.5 fold difference in genetic instability and DNA damage. The onset of lymphomas was significantly 2.5 fold different. Metabolomics from the feces and urine showed bacterial metabolites with anticancer activity in the health beneficial microbiota. We sequenced the microbiota of both facilities and found Lactobacillus johnsonii 456 as dominant bacterial strain in the health beneficial microbiota.
Just this bacterium by itself reduced genotoxicity, reduced inflammation and reduced levels of cytotoxic T, NK and CD3 cells in the liver and blood. We also found similar differences in Trp53 deficient and even in wildtype mice. The underlying mechanisms is probably due to inflammation promotion or suppression mediated by the intestinal microbiota. The understanding of this effect may lead to a breakthrough in the understanding of the causes of carcinogenesis, which might lead to prevention of AT, a currently incurable progressive disease and possibly other cancer-prone DNA repair deficient diseases or even wildtype mice and people.

A DUAL-ENVIRONMENT CO-CULTURE SYSTEM TO BETTER EVALUATE EFFECTS OF FOOD INGREDIENTS ON INTESTINAL BARRIER INTEGRITY IN PHYSIOLOGICALLY RELEVANT CONDITIONS

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Objective

Appropriate intestinal barrier integrity is vital to prevent antigens and pathogens from entering the body and potentially causing disease. Conventionally, *in vitro* experiments to test the effects of food ingredients, including probiotic bacteria, on intestinal barrier integrity are carried out in an atmosphere of 5% CO2 in air because the human cell lines require oxygen. However, this does not accurately represent conditions in the human intestinal lumen, which contains limited oxygen in the small intestine and almost no oxygen in the large intestine. To overcome this limitation, we developed a novel dual-environment co-culture system where monolayers of epithelial cells receive oxygen from aerobic media in the basal compartment (below the cell layer, representing the underlying lamina propria), which is sealed off from the apical anaerobic environment of the workstation (above the cell layer, representing the intestinal lumen). We previously applied this system to study the interactions between a human obligate anaerobic bacterium, *Faecalibacterium prausnitzii*, and intestinal epithelial cells (*Cell Microbiol* 17:266-240).

Methods

The hypothesis of our current research was that anaerobic versus aerobic conditions alter physicochemical properties of food components (structure, charge, solubility) and this affects their interaction with intestinal cells. To test this we monitored the effect of three bovine milk proteins (purified casein, beta-lactoglobulin and lactoferrin) on the trans-epithelial electrical resistance (TEER) across epithelial cell layers (a measure of intestinal barrier integrity) in both conventional and apical anaerobic conditions over a 24 hour period.

Results

None of the three milk proteins altered TEER in conventional conditions, but all increased TEER (improved barrier integrity) compared to control medium in apical anaerobic conditions. This shows that the interactions between the milk proteins tested and host cells are different depending on the environment.

Conclusions

These data demonstrate that it is important to test the effects of food ingredients in more physiologically-relevant apical anaerobic conditions, in order to maximise the likelihood of successfully translating *in vitro* results into *in vivo* outcomes.

STUDIES ON THE IDENTIFICATION OF BIFIDOBACTERIA ISOLATED FROM HUMAN BREAST MILK OF INDIAN WOMEN

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Objective
This study was conducted to identify for the presence of Bifidobacteria in Human Breast Milk of Indian women.

Methods
A total of Thirty (30) breast milk samples of about 3-5 ml were collected asceptically from lactating women during the first week of delivery. They were subjected for screening of microflora by microbiological methods. We have observed mixed bacteria in these samples therefore we used a selective media supplemented with mupirocin (antibiotic). The colonies suspected for Bifido's were picked up for Gram's staining, Catalase and Fructose-6-phosphate phosphoketolase (F6 PPK) tests. The DNA was extracted from the identified Bifido's and subjected to PCR using genus and species specific primers. Subsequently the genome sequence analysis was carried out to know the variations and compared with already existing Bifidobacterium species available in databases viz. NCBI and GENBANK.

Results
The Bifido's identified as 'Y' and 'V' shaped in morphology were Gram positive and Catalase negative. The F6PPK test has shown the change in colour from yellow to dark brown indicating the genus Bifido which was further confirmed by PCR. The DNA sequencing results revealed that isolates from 6 samples out of 30 had 99% similarity and 4 were found to have 98 % with the existing *Bifidobacterium animalis* sub sps *lactis*.

Conclusion
We could conclude that human breast milk is a potential source for ***Bifidobacterium animalis*** **sub sps** ***lactis*** in Indian women. Since these are of human origin can easily colonize in human intestine and thus help in the treatment of various gastrointestinal diseases.

PHYSICIAN PERCEPTIONS ON PROBIOTICS: RESULTS OF A MULTINATIONAL SURVEY

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Objective
The objective of this study was to evaluate the knowledge, attitudes and current practices of physicians with regards to probiotics in 10 countries.

Methods
A closed-ended structured questionnaire was implemented in 10 different countries (Argentina, Peru, Spain, Italy, Hungary, Morocco, Turkey, Pakistan, India and China). Target and Sample Size: 90 to 190 physicians interviewed per country (General Practitioners-GP-, Pediatricians-P-, Gastroenterologists-G-). Total sample: 1670. Representativeness: adapted criteria according to each country's reality (quota method).

Results
85% doctors in 10 countries felt that they were somewhat or absolutely informed about probiotics, with the highest prevalence among G in China (100%) and GP in China (93%), India (91%). However 39% Moroccan physicians expressed a lack of information. Concerning probiotic definition 94% of Turkish doctors responded according to FAO/WHO criteria while in Pakistan only 39% of doctors did. Saccharomyces boulardii and Lactobacillus rhamnosus GG have been scientifically proven to work in acute infectious diarrhea and antibiotic associated diarrhea (46% and 30%) showing very different scores with no parallel with global guidelines. GPs are less aware of proofs on these strains in these indications whereas P remain the most aware target in the sample (36% boulardii/20% GG in GPs vs 51%/35% in P population). There is an international consensus on safety (84%) with no differences per target. Doctors do recommend probiotics to their family (82%) or themselves (68%). P recommend more frequently probiotics in acute diarrhea (in average 62,4 patients/100).

Conclusions
Most doctors feel well informed about probiotics.

CHOLESTEROL CONTENT OF LIGHVAN CHEESE: A NATURAL PROBIOTIC TRADITIONAL CHEESE

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Objective

Lighvan Cheese is a popular semi-hard cheese among Iranian consumers which is produced from raw sheep milk and ripened for three to five months. Presence of probiotics in this cheese is well documented. But concerns about cholesteol content of this cheese because of high fat content is considered by nutritionist and consumers. In this way, the study objective was to analyze the cholesterol content variation during the coagulation step and the ripening period.

Methods

The cholesterol content was analyzed by gas chromatography. In this way, unsaponifiable matter was was purified and accordingly injected without derivatization.

Results

Cholesterol in the initial milk was seperated in two phases during coagulation, one third in the whey and two third in coagulum. Average cholesterol content of cheese was 35 mg/100 gr chees. Regression analysis showed that the cholesterol changes during ripening was not significant ($p<0.05$), but the mean differences showed the least cholesterol content in the 3rd month.

Conclusions

The reduction in the third month of ripening could be explained by the ability of probiotics to absorb cholesterolwhich did not continue after bacterial lysis in the brine cotributing to cholesterol release. Cholesterol absorption was calculated as 42% of the initial content.The results indicates that Lighvan cheese should be ripened for three month to reach the least cholesterol content.

IMMUNOMODULATORY IN VITRO AND IN VIVO EFFECTS OF LACTOBACILLUS RHAMNOSUS AND ELDERBERRY EXTRACT ALONE AND IN COMBINATION

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Objective

Medicinal plants and probiotics have very high potential in immunomodulation. If numerous studies have investigated the effects of probiotics and plant extract alone in the immune response, the potential of their combination is less documented. As previously described, we selected an association between a probiotic strain and a plant extract, *Lactobacillus rhamnosus* GG and elderberry extract (EE), respectively, based on its ability to regulate cytokines expression involved in activation of immune cells. To complement our data, we examined the immunological properties of this association, both *in vitro* and *in vivo.*

Methods

Interleukin-8 (IL-8) production by HT-29 (human colorectal adenocarcinoma) cells was measured after incubation with *Lactobacillus rhamnosus* GG and/or EE.
BALB/c mice were vaccinated with Immugrip® and pre-treated with *Lactobacillus rhamnosus* GG in combination or not with EE. Vaccine-specific IgG response and secretory IgA were quantified by ELISA, in sera and in faeces, respectively.

Results

Lactobacillus rhamnosus GG and EE acts in a dose-dependent manner to decrease IL-8 production in HT-29 cells.
Pre-treatment of mice with *Lactobacillus rhamnosus* GG and EE enhanced the vaccine-specific IgG response and total IgA level in faeces when compared to non-treated mice.

Conclusions

These data confirm that the association between a probiotic and a plant extract presents immunomodulatory activities mainly through humoral immune response. Additionally, it modulates inflammatory response by decreasing IL-8 secretion. It is also noteworthy that *in vivo* effect on IgA secretion of the combination *Lactobacillus rhamnosus* GG and EE is stronger than the effects of *Lactobacillus rhamnosus* GG alone.

ELECTRON MICROSCOPIC INVESTIGATION OF PROBIOTIC BACTERIA INFLUENCE ON RAT INTESTINE MUCOSA IN DYSBIOSIS EXPERIMENTAL MODEL

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Objective

The aim of this study was to reveal the different influence of probiotic bacteria on ultrastructure of intestinal mucosa of rats with antibiotic associated dysbiosis.

Methods

Intestinal dysbiosis of male Wistar rats was induced by antibiotics. Probiotic bacteria *Lactobacillus rhamnosus* K32 (L), *Bifidobacterium longum* GT15 (B), *Enterococcus faecium* L3 (E) were introduced intragastrically for 5 days. Rats from the control group C1 did not receive probiotics. Animals from control group C2 did not receive antibiotics and probiotics. Intestinal mucosa ultrastructure was studied on ultrathin sections.

Results

The signs of slight inflammation were reveled in all the samples of mucosa except the C2 group. The recovery of the intestinal mucosa was determined only after consumption of probiotics. In group C1 a lot of microvilli on the surface of epithelial cells and the tight junctions were destroyed. Intercellular space was increased. Epithelial cells in groups C2, E and B were in a physiologically active state. The highest number of bacteria were on the mucosal surface in group C1. Bacteria were in intestinal lumen of group E, but their number was smaller than in C1. Bacteria in group E were separated from epithelial cells by layer of mucus. In group E there were many goblet cells. A large number of ribosomes were observed in epithelial cells in group C1.

Conclusions

The most significant violation of the microflora of the large intestine observed in children Ultrastructural changes in the intestinal mucosa after the correction of dysbiosis with probiotics depended on the kind of probiotic used.

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FEATURES OF THE PROBIOTIC ENTEROCOCCI INFLUENCE ON THE IMMUNE SYSTEM IN EXPERIMENTAL MODELS OF MULTIPLE SCLEROSIS AND INTESTINAL DYSBIOSIS

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Objective

Comparison of probiotic enterococci effect on microbiota and immunity of rats with experimental antibiotic-associated intestinal dysbiosis (EAID) and autoimmune encephalomyelitis (EAE).

Methods

EAID was induced by ampicillin® and metronidazole® (AM). EAE was obtained after injection of spinal cord homogenate (SCH). Enterococcus faecium L3 were introduced introgastrically 5 (EAID) or 14 (EAE) days. Gut microbiota were studied bacteriologically, by RT-PCR and metagenome analysis. Control groups of animal received PBS (C1), only antibiotics (C2) or SCH (C3). Populations of lymphocyte and cytokines in blood were analyzed using Flow Cytometry and ELISA.

Results

Intestinal dysbiosis signs were similar after introduction AM and SCH. The number of opportunisctic bacteria was increased, the content of lactobacilli, enterococci, bifidobacteria and faecalibacteria was reduced. Probiotic introduction led to the microbiocenosis recovery, disappearing of dysbiosis symptoms and EAE severity attenuation. It was accompanied by elevation of CD3+CD4+CD25+ cells during the latent period of EAE (7^{th} day) and after correction of EAID. The number of CD3+CD8+ T cells on the peak of clinical manifestations in EAE (14^{th}. day) was increased. The content of TGF-β (7^{th} day) and IL-10 (14^{th} day) were increased respectively due to EAE therapy. Elevation of concentration both cytokines was revealed after correction of EAID.

Conclusions

Despite the differences of the causes of dysbiosis, the changes of the microbiota composition can be similar after consumption of probiotic E. faecium L3. Immune response after exposure probiotic depends on the organism condition. It is necessary to consider the possibility of multi-directional action of probiotics on immunity.

ANTIVIRAL ACTIVITY OF DIFERENT PROBIOTIC STRAINS IN VERO CELL LINE

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Objective
The aim of the study was to evaluate the effects of probiotic strains metabolites on the reproduction of herpes simplex virus type-1 (HSV1).

Methods
Vero cells were infected with HSV1 and then incubated with supernatants of probiotic strains *Lactobacillus plantarum 8A-P3* and *Enterococcus faecium-L3* (EntA+EntB+ EntXa+EntXb+) applied in serial dilutions, with chemically synthesized peptides EntB and EntXb. Peptides were synthesized in situ by the solid-phase method with an "Applied biosystems 430A" synthesizer. They were added to the HSV1 60 minutes before incubation in concentrations 5 - 50 mcg/ml and then incubated in tissue culture. Acyclovir (Lek, Slovenia) 25 mcg/ml was used as antiviral drug control. Cytopathic effect of the virus was determined by light or immunoflourescence microscopy with serum containing antibodies to HSV1 after 48h incubation.

Results
HSV-1 alone caused the most profound cytopathic effect (100% cells). Addition of acyclovir reduced cytopathic effect for 50%. Supernatants obtained from *L.plantarum*, and *E.faecium* generated dose dependent effect from 60 to 37% of viral inhibition. *E.faecium* strain L-3 extract was 25% more active than *L.plantarum*. Extract from the strain L-3 contained demonstrated 80% antiviral activity against HSV1 in Vero cells as well as EntB and EntXb. These peptides inhibited the virus reproduction in dose dependent manner. Introduction of enterocins even in a minimum dilution reduced HSV1 cytopathic effect by 20%

Conclusions
Extracts of several probiotic bacterial strains express a specific activity against reproduction of HSV-1 *in vitro. Enterococcus faecium-L3* enterocins provide antiviral effects on HSV1 comparable to the effect of antiviral chemotherapy.

DOES PARTIALLY HYDROLYSED GUAR GUM HAVE A ROLE TO PLAY IN THE TREATMENT OF IRRITABLE BOWEL SYNDROME: A SYSTEMATIC REVIEW

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Objective
Irritable bowel syndrome (IBS) is a functional bowel disorder characterised by abdominal discomfort or pain that is associated with a change in bowel habit. It is one of the most common gastrointestinal disorders worldwide. Management has thus far proven challenging. Partially hydrolysed guar gum (PHGG) is a soluble fibre that demonstrates beneficial microbiota-modifying properties. Preliminary research has shown promising effects of PHGG in the treatment of a number of gastrointestinal conditions, including IBS. The aim of this review was to systematically evaluate the efficacy of PHGG in the treatment of IBS.

Methods
A computer-based search of MEDLINE, EMBASE, and the Cochrane Library was conducted in June 2015. A hand-search of the bibliographies of relevant papers, previous reviews, and authors' personal libraries was also undertaken. Trials were included in the review if they were human clinical trials (of any design) investigating the effects of PHGG on IBS-related symptoms or quality of life. There were no language restrictions. Eligibility assessment and data extraction were performed by two independent researchers.

Results
Nine trials were identified that met all eligibility criteria. Seven were open label trials and two were randomised, placebo-controlled trials. Heterogeniety in trial design and outcome precluded meta-analysis. All nine trials had results that were suggestive of the efficacy of PHGG.

Conclusions
PHGG shows promise in the treatment of IBS. A number of different mechanisms of action have been suggested, including modification of the gastrointestinal microbiota, normalisation of motility, and inhibition of substance P expression. Large-scale, randomised, controlled trials appear warranted.

THE ACTION OF DIFFERENT PROBIOTICS IN CORRECTING ACTIVITY OF INTESTINAL ENZYMES IN RATS AFTER ADMINISTRATION OF ANTIBACTERIAL AGENTS

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Objective
Comparison of different probiotics in restoring digestion in the intestine of rats after administration of antibacterial agents.

Methods
After administration of antimicrobials (ampicillin+methronidazole), rats were getting probiotics: "Laminolact" containing *Enterococcus faecium* L3, "Genobakt" (the mixture of bacteria *E. faecium* L3, *Lactobacillus rhamnosus* K32, *Bifidobacterium longum* GT15), Linex®, Bifiform®, or phosphate buffer (PBS), or after administration of water - PBS (control).

Results
In the absence of a probiotics after administration of antimicrobials, changes in the mucosa mass in the ileum and colon and the chyme mass in the colon, as well as the activity of the intestinal digestive enzymes were observed. Introduction of probiotics provided a correction of changes in the mass of intestinal mucosa. Concerning the chyme mass in the colon, the effect was only observed in the case of "Laminolact". After the introduction of probiotics, the activity of alkaline phosphatase (AP) in mucosa of the jejunum and in the intestinal chime was increased (to a lesser extent after Bifiform®). The change of maltase (M) activity in the small intestinal mucosa was less significant after Linex®, aminopeptidase M (AP-M) - after "Genobakt", glycyl-L-leucine dipeptidase (GL) - after Bifiform®. In the chyme the change of M-activity was the best reduced by "Genobakt", AP-M - by "Laminolact" and GL - by Linex® and Bifiform®.

Conclusions
Introduction of various probiotics to correct intestinal dysbiosis in rats differently affects the activity of intestinal enzymes involved in the metabolism of carbohydrates, proteins and lipids.

PROBIOTICS IMPROVE THE IRON ABSORPTION FROM A MEAL

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Objective
Iron deficiency is common and adding probiotics to meals could be one way to increase the iron absorption. The aim of this study was to test the hypothesis that non-heme iron absorption from a meal is improved by adding *Lactobacillus plantarum* 299v (Lp299v).

Methods
Iron absorption was studied in healthy women of reproductive age using a single-blind crossover design in two trials applying the double isotope (^{55}Fe and ^{59}Fe) technique. Study meals containing breakfast buns with margarine and orange jam were served for breakfast on four consecutive days. The first two days was control capsules containing iron given with the meal and the next two days was freeze dried Lp299v included in the capsules together with iron and given with the meal. The iron in the control meal was marked with ^{55}Fe and in the Lp299v meal with ^{59}Fe. The absorption of the iron isotopes was measured in blood and the obtained absorption values was normalised to a 40% iron absorption of a reference dose.

Results
In the first trial 14 subjects completed the study. The mean iron absorption from the meal with Lp299v was 22.4%, while the mean absorption from the control meal was 17.4% ($p=0.04$). In the second trial 28 subjects completed the study. The mean iron absorption in the meals with and without Lp299v was 24.5 and 20.9%, respectively ($p=0.003$).

Conclusions
Two clinical trials have shown that the non-heme iron absorption can be increased significantly with 23% (mean value) if Lp299v is included in a meal.

ANTI-OBESITY POTENTIAL OF LACTOBACILLUS SALIVARIUS LPLM-01 IN A MURINE MODEL OF DIET-INDUCED OBESITY

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Objective

To evaluate the metabolic effect of oral consume of the native probiotic *Lactobacillus salivarius* LPLM-01 on a murine model of diet-induced obesity.

Methods

48 C57BL/6 female mice were randomized into two groups and freely fed with either a high-fat diet (HFD, 24% fat) or a low-fat diet (LFD, 4.3% fat). After three months, the HFD group showed a significant weight gain when compared to the LFD group (36.3±7.5g vs 25.1±1.6g;$p<0.01$.) Next, each diet group was again randomized into two subgroups (n=10-12); one received a placebo, or placebo supplemented with $1x10^9$ CFU/g of LPLM-01. After three months of treatment, an insulin tolerance test and an oral glucose tolerance test were carried out. Plasma samples were taken for glycaemia, insulin and leptin analyses. The Body Mass Index (BMI) was calculated and subcutaneous and periovarian adipose tissues were taken. Statistical analysis was made.

Results

Supplementing the diet with the strain LPLM-01 does not significantly modify glucose tolerance or insulin response, but it did reduce body weight gain in both diet group when compared to the placebo ($p<0.05$). Weight loss was related to a reduction of BMI, fasting glucose, insulinemia, and subcutaneous and periovarian adipose tissues, without being statistically significant. However, weight loss was also related to a significant reduction of leptinemia in the HFD+LPLM-01 group, in comparison with the placebo group ($p<0.05$).

Conclusions

Supplementing the diet with the strain LPLM-01 has a beneficial effect on obesity, reducing weight gain in this model.

EFFICACY OF PROBIOTICS IN PATIENTS WITH LACTOSE INTOLERANCE - A PRELIMINARY STUDY

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Background

Lactobacillus bulgaricus and Streptococcus thermophilus produce lactase enzyme. Probiotics may alleviate lactose intolerance by modifying the intestinal flora into one which contains lactase-producing bacteria.

Aims

Assessing the efficacy of probiotics in improving lactose intolerance.

Methods

Patients were treated with a unique probiotic formulation (Bio-25, SupHerb, Israel) for 6 months. All patients completed a demographic questionnaire as well as a visual analog scale (VAS) for the assessment of the intensity and frequency of bloating, flatulence, abdominal pain and change in bowel habits, at entry, every 8 weeks and at the end of treatment period. Measurement of hydrogen levels (parts per million - ppm) at each of these time points was also performed. Study end points were: Improvement in symptom intensity or frequency, and the decrease below cut off point of 20ppm of the breath test. The Wilcoxon signed-rank test was used to compare symptom intensity and severity before and after treatment.

Results

Included eight symptomatic female patients with a positive lactose intolerance breath test. Mean age and mean body mass index (BMI) (kg/m2) were: 36.4±18.6 years and 25.2, respectively. Treatment with probiotics was associated with a significant improvement in the reported intensity of bloating ($z=2.55$, $p=0.11$) and flatulence ($z=2.21$, $p=0.027$); frequency of bloating ($z=2.06$, $p=0.039$) and flatulence ($z=2.04$, $p=0.041$); abdominal pain ($z=2.06$, $p=0.039$), and constipation ($z=2.07$, $p=0.039$). Lactose breath test was successfully normalized in two (25%) patients.

Conclusions

Treatment with probiotics may lead to symptomatic improvement in patients with lactose intolerance. A larger study is warranted to confirm our findings.

RESTORE AND MAINTAINING OF HUMAN GUT MICROBIOTA DURING THE ANTIBACTERIAL THERAPY

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Objective

The basic reason of the common adverse events of the antibiotic therapy are the antibiotic-associated gut microbiota changes. To solve this problem the combination of antibiotics with prebiotic was developed (ecoantibiotics). Combination of antibiotic with prebiotic could help to save the composition of the normal human intestinal microflora.

Methods

Several clinical trials were conducted to evaluate the antibiotic and prebiotic combination efficacy, safety, and influence on microbiota in comparison with antibiotic monotherapy. Antibiotics from group fluoroquinolones, macrolide, beta-lactam were used with the prebiotic in broad range of daily dose from 300 to 1200 mg. The microbiota condition was evaluated using microbiological methods, SCFA analysis, next-generation sequencing technologies.

Results

All evaluated combinations were comparable in efficacy with their analogues without prebiotic. The number of adverse events in investigated groups was less than in groups of comparative antibiotics ($p<0.05$).
Using microbiological methods in the amoxicillin with clavulanic acid study the number of patients with «normal» content of bifidobacteria and lactobacilli in antibiotic with prebiotic group was twiceas high than in the control group. Next-generation sequencing technologies based on 16S rRNA genes sequencing shown that the number of genera observed in the samples of reference drug group were low in comparison with the investigated group.

Conclusions

These results provide evidence of restores and maintains normal physiological and bacterial flora of the intestinal tract during antibacterial treatment. Ecoantibiotic decrease risk of side effects from antibiotic treatment and consequently can prevent multifactorial chronic disorders associated with disruption of intestine microbiocenosis after antibiotic therapy.

THE USE OF COMPLEMENTARY AND ALTERNATIVE MEDICINE IS FREQUENT IN PATIENTS WITH PANCREATIC DISORDERS

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Objective

Prevalence of Herbal remedies and other not conventional medicines (CAM) use in patients with pancreatic diseases and screen pancreatotoxicity.

Methods

Cross-sectional survey of consecutive patients seen at a pancreatic disorders outpatient clinic. Data were collected with questionnaire regarding demographics, CAM usage, reasons for CAM use, and respondent experiences of effects from CAM.
Descriptive statistics were used to analyse the prevalence of CAM use. Fisher or t-test were used to determine any association between CAM use, demographics and lifestyle factors.

Results

91 consecutive patients were enrolled (49.5% male; mean age 65+-11.7). The 42% of patients used CAM (44.7% male; mean age 65+-10.6) and the 21% for more then 1 year. 46% of patients with previous acute pancreatitis, 49% with chronic pancreatitis and 37% with IPMN used CAM. In most cases the use of CAM was for helping the standard therapies (31.5%) and for an overall feeling better (18%). 58% of patients reported advantages with treatment. CAM users were more often female (63% vs 47%), with higher school degree (42% vs 32%), performed physical activity more than once a week (42% vs 33%). However, none of these differences were statistically significant.
Two patients reported use of *serenoa repens* that has been associated with pancreatotoxicity.

Conclusions

The 42% rate of CAM use in patients with pancreatic disease is similar or higher to those reported in other GI diseases. 60% of patients report benefit with CAM. The use seems more frequent in female with higher education level and "healthier lifestyle". Patients might not be aware of potential pancreatotoxicity of CAM, which should be carefully considered by physicians.

THE EVALUATION OF EMULSION TECHNIQUE FOR MICROENCAPSULATION OF LACTOBACILLUS PLANTARUM WITH ALGINATE-RESISTANT STARCH CAPSULES

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Objective

Emulsion technique is one of the most successful methods of microencapsulation which have been used to improve the viability of probiotic bacteria in foods and in the gastrointestinal adverse conditions. Resistant starch as prebiotic can improve the viability of probiotic bacteria by providing additional protection. The present study aimed to evaluate the emulsion technique for microencapsulation of *L. plantarum* using alginate-resistant starch mixed gel.

Methods

A mixture of sodium alginate (2% w/v), and resistant starch (2% w/v), containing probiotic culture (1% v/v), was used in microencapsulation. The morphology of microcapsules was studied using scanning electron microscopy (SEM). Light microscopy was used for direct observation of living entrapped bacteria inside the capsules and their releasing process, after lugol staining. The metabolic activity of encapsulated bacteria was investigated by measurement of pH and optical density of inoculated MRS-broth medium. The stability of microcapsules was studied in bile salts solution (BSS), simulated gastric juice (SGJ), pancreatin enzymes solution (PES), and phosphate buffer solution (PBS), with or without 400 rpm mechanical shaking.

Results

The prepared microcapsules were spherical with the average diameter of 19.87 ± 1.49 µm, containing 1.7×10^9 cfu g-1 metabolically active bacterial cells. Encapsulation yield was obtained 30.35%. The stability of microcapsules were respectively, 60 and 90 min, in BSS and PES without mechanical shaking, and 30 min in the other tested conditions.

Conclusions

This study indicated that the emulsion technique of microencapsulation could be successfully applied to enhance the viability of *L. plantarum* in gastrointestinal adverse conditions.

CHEMICAL CHARACTERISTICS AND SURVIVAL OF PROBIOTICS IN CHEDDAR CHEESE FORTIFIED WITH PHENOLIC COMPOUNDS OF MANGO (MANGIFERA INDICA L.) KERNEL OIL

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Objective

A study was performed to assess the chemical characteristics and survival of probiotics in cheddar cheese fortified with mango kernel oil

Methods

Milk fat was partially replaced with mango kernel oil from 2.5% to 10%. Cheese milk was added with 2% bulk starter culture of *lactococcus lactis* ssp. *lactis, lactococcus lactis* ssp. *cremoris*, supplemented with *lactobacillus acidophilus* and *biofedobacterium bifedum* at 10^8/ml. Cheddar cheese prepared from 100% milk fat served as control, with no difference in starter and probiotics. Control and experimental samples were ripened at 8±2oC for 90 days, evaluated for chemical, microbiological and sensorial parameters at 0, 45 and 90 days.

Results

Total phenolic content of cheese formulated from 10% mango kernel oil were 62mg/g GAE, as compared to control, 0.14mg/gGAE. HPLC characterization of cheddar cheese showed the existence of chlorogenic acid, caffeic acid, quercetin in considerably higher concentrations over the control. Cheddar cheese fortified with 5% mango kernel did not have any inhibitory effect on the growth of *lactobacillus acidophilus and bifedobacterium bifedum*. Beyond this concentration, phenolics of mango kernel oil inhibited the starter and probiotics. Concentration of free fatty acids and organic acids in cheddar cheese fortified with mango kernel oil were not different at all the test intervals. Sensory characteristics of cheddar cheese fortified with phenolic compounds of mango kernel oil (5% oil), *lactobacillus acidophilus* and *biofedobacterium bifedum* at 10^8/ml was superior to control.

Conclusions

5% mango kernel can be added in the formulation of cheddar cheese with no effect on probiotics and sensory attributes.

AGING-RELATED CHANGES OF GUT MICROBIOTA COMPOSITION FROM NEW-BORN TO CENTENARIAN, CROSS-SECTIONAL STUDY

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Objective

It has been reported that the composition of human gut microbiota changes with age; however, the details have not been clarified.

Methods

Fecal samples of 367 healthy Japanese between the ages of 0 and 104 years were analyzed by high-throughput sequencing of amplicons derived from the V3-V4 region of the 16S rRNA gene.

Results

The relative abundance of Actinobacteria decreased dramatically after weaning and the decreasing trend continued through the life-span. Those of Firmicutes turned to be the most predominant phylum after weaning accompanied with an increase in the compositions of Bacteroidetes and Proteobacteria over 70 years old. Hierarchical Ward-linkage clustering based on the abundance of genera indicated five clusters, each with median (interquartile range) of age of 3 (0-35), 33 (24-45), 42 (32-62), 77 (36-84) and 94 (86-98) years old, respectively. Analysis based on bacterial co-abundance groups defined by Kendall correlations between genera revealed four patterns of microbiota each enriched in infant, adult, elderly and both of infant and elderly, respectively. In addition, functional properties prediction based on PICRUSt showed that the relative abundance of transporters decreased along with aging.

Conclusions

Our results indicate the existance of some patterns and turining points in the composition change of gut microbiota with aging. In addition, results of functional property prediction suggest that nutrients existing in the gut might play an important role in shaping the composition of gut microbiota.

DISCOVERY OF A CONJUGATIVE MEGAPLASMID IN BIFIDOBACTERIUM BREVE

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Objective

The objective of this study was to characterise a novel conjugative megaplasmid identified in *Bifidobacterium breve* JCM7017.

Results

Bifidobacterium breve is a common and sometimes very abundant inhabitant of the human gut. Genome sequencing of *B. breve* JCM 7017 revealed the presence of an extrachromosomal element, designated pMP7017, of more than 190 kb, thus representing the first reported bifidobacterial megaplasmid. *In silico* characterization of this element revealed several genomic features supporting a stable establishment of the megaplasmid in its host, illustrated by predicted CRISPR-Cas functions that are known to protect the host against intrusion of foreign DNA. Interestingly, pMP7017 is also predicted to encode a conjugative DNA transfer apparatus and consistent with this notion we demonstrate conjugal transfer of pMP7017 to representative strains of *B. breve* and *B. longum* subsp. *longum*. We furthermore demonstrate the presence of a megaplasmid with homology to pMP7017 in two *B. longum* subsp. *longum* strains.

Conclusions

These results demonstrate for the first time the presence of a conjugative megaplasmid in a Bifidobacterium strain and also demonstrate that this megaplasmid can be conjugally transferred to representative strains of *B. breve* and *B. longum* subsp. *longum.*

EXPOSURE OF LACTOBACILLUS ACIDOPHILUS AND LACTOBACILLUS CASEI TO 2.4 GHZ ELECTROMAGNETIC RADIOFREQUENCY RADIATION ENHANCES THE GROWTH OF THESE PROBIOTIC BACTERIA

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Objective

Lactobacillus acidophilus, a gram positive bacteria in the genus Lactobacillus, is believed to benefit health through producing vitamin K and lactase. On the other hand, *Lactobacillus casei* is also a beneficial bacteria which produces lactic acid to decrease the pH level in the digestive system and prevents the growth of detrimental bacteria. Probiotic products must contain more than 10 millions 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 to EMRR for 30, 45 and 60 minutes showed statistically significant increases (P=0.001, P=0.002, P=0.002, respectively) compared to those of sham-exposed bacteria. In this experiment, there was no difference between the growth in samples exposed to to EMRR for 15 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 min, the growth of *Lactobacillus casei* in samples exposed to to EMRR for 30, 45 and 60 minutes showed statistically significant increases (P=0.041, P=0.008, P=0.002, 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.

PROTECTIVE ACTIVITY OF LACTOBACILLUS RHAMNOSUS GG-DERIVED FACTORS ON PATHOGEN LIPOPOLYSACCHARIDE (LPS)-INDUCED DAMAGE OF HUMAN COLONIC SMOOTH MUSCLE CELLS

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Objective

Some beneficial effects of probiotics result to be determined by secreted probiotic-derived factors, identified as "postbiotic" mediators. Aim of this study was to evaluate if supernatants harvested from LGG cultures protect human smooth muscle cells (SMC) from LPS-induced myogenic damage.

Methods

L. rhamnosus GG (ATCC 53103 strain) was grown in MRS medium at 37°C and samples were collected in exponential phase, in early, middle and late stationary phases. Supernatants were recovered by centrifugation, filtered and stored at -20°C. The SMC culture was exposed for 24h to purified LPS (1µg/ml) of a pathogen strain of *Escherichia coli* (O111:B4) with and without supernatants. Postbiotics effects were evaluated on morphofunctional alterations and IL-6 production. Data are expressed as mean±SE ($p<0.05$ significant).

Results

LPS induced persistent significant 20.5%±0.7 cell shortening and 34.5%±2.2 decrease in acetylcholine-induced contraction of human SMC. These morphofunctional alterations were paralleled to a 365.65%±203.13 increase in IL-6 production. These effects were reduced in the presence of LGG-supernatants. Supernatants of the middle exponential phase already partially restored LPS-induced cell shortening by 57.34%±12.7 and IL6 increase by 145.8%±4.3 but had no effect on LPS-induced inhibition of contraction. Maximal protective effects were optained with supernatants of the late stationary phase with LPS-induced cell shortening restored by 84.1%±4.7, inhibition of contraction by 85.5%±6.4 and IL6 basal production by 92.7%±1.2.

Conclusions

The LGG-derived products are able to protect human SMC from LPS-induced myogenic damage. Novel insights are provided for the possibility that LGG-derived products could reduce the risk of progression to a postinfective motor disorder.

THE PERSPECTIVE USE OF NOVEL STRAINS LACTOCOCCUS LACTIS SSP. LACTIS FOR FOOD

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Introduction

The traditional use of the dairy lactococci in various food fermentations, the ability to synthesis of different kind of bioactive molecules, such as organic acids, bacteriocins and other antimicrobial agents. can be safety used in different food system (biopreservatives, probiotics and prebiotics). Among the probiotic correctors of normal microbiota special interest present the lactococci, isolated from national products with therapeutic–preventive effect. In light of the increased antibiotic resistance among pathogens, natural antimicrobial substances have attracted attention as an alternative means to prevent infection by pathogens. Screening of novel strains of *Lactococcus lactis* as the perspective for food system usefull for human health was performed.

Methods

We have isolated of effective strains from raw milk, milk products and also products of functional nourishment of mixed lactic acid and alcoholic fermentation from various climatic regions, which were widely used by people to prevent diseases of the gastrointestinal tract and cardiovascular system, to cure of tuberculosis etc. The phylogenetic analysis using the sequences of the 16S rRNA genes was performed. Biological and analytical HLPC, TLC, FAB-MS, FD-MMS-methods were carried out to determine of antimicrobial substances. The probiotic properties were determined as levels of resistance to bile and hydrochloric acids, also the presence of superoxide dismutase (SOD) activity using the xanthine oxidase-cytochrome c method. Proteolitic activity was determined at the various levels of pH (3,0; 4,2; 5,3; 7,0). It has been shown that lactococci influence on physiological properties of the organisms of laboratory animals. Biomodel of CBRB mouse females.

Results

According to microbiological properties and gene sequence of 16S rRNA isolated novel strains confirmed their taxonomic state as *Lactococcus lactis* ssp. *lactis* (GenBank database № DQ 255954, EF100777 - EF114305). Many strains inhibited growth of gram-positive bacteria only. But some of the selected strains expressed a broad spectrum of activity against pathogens: *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella* and fungi of *Aspergillus, Fusarium, Penicillum* genera, as well as *Rhodotorula aurantiaca, Candida albicans*. These strains have high antibiotic activity up to 3600 IU/ml compared to food preservative Nisapline (Applin & Barrett, UK).) and 1500-2700 IU/ml compared to antifungal antibiotic Nistatine. The individual antibiotic substances differed from each other by molecular mass, Rf values and biological propertiest. The main compound of antibiotic complex had polypeptide nature as nisin. Other component was a positively charged substance identified as peptide.built of the 20 amino acids, differed from nisin by molecular mass (M= 2589 Da) and activity against gram-positive and gram-negative bacteria. Components with a low molecular mass (Mr=506–829Da) were hydrophobic and contained aromatic groups, keto-, aldehydic and alkyl residues which were responsible for antifungal activity. Antimicrobial substances were identified as novel substances, that were absent in Berdy database BNPD. The perspective strains were resistant to the action of the bile acids at concentration of bile from 0.8 to 1.0% and hydrochloric acid. The strains possesses relatively high SOD activity (20 U/mg of protein) and has high proteolytic activity at different pH ($3{,}8\cdot10^{-3}$ -$23{,}8\cdot10^{-3}$ PU/ml).

Discussion

The novel effective strains of *L.lactis* ssp.*lactis* with broad spectrum action including antifungal activity were obtained. That is rare biological property for the strains of these species, which have status “GRAS” (absolutely harmless for human health and animals). Thus, the unique properties of this strain, somehow: stability at the condition of gastrointestinal tract, the spectrum of bactericidal and antifungal action to the pathogens, and relatively high superoxide dismutase and proteolytic activities, the absence of toxicity, make it possible to recommend it for the potential biopreservatives, probiotic and prebiotic cultures.

PHYSICOCHEMICAL PROPERTIES OF FUNCTIONAL SCAMORZA CHEESE FROM OVINE MILK

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Objective

The present study was undertaken to produce functional Scamorza cheese from Gentile di Puglia ewe’s milk by incorporating probiotic strains into the cheese matrix and to evaluate the physicochemical characteristics of Scamorza ewe’s milk cheese.

Methods

Gentile di Puglia ewe’s bulk milk was used for Scamorza cheese production. Cheeses were denoted S-CO for control Scamorza cheese, S-BB for Scamorza cheese made using a mix of *Bifidobacterium longum* and *Bifidobacterium lactis*, and S-LA for Scamorza cheese made using *Lactobacillus acidophilus* as probiotic strain. Probiotic cell recovery in cheese was 7.55±0.07 log10 cfu/g and 9.09±0.04 log10 cfu/g in S-LA and S-BB cheese, respectively. The derivatised free amino acids (FAAs) were separated and quantified by RP-HPLC. Total lipids from cheeses were extracted; derivatization was performed and FFA And CLA were separated using gas-chromatographic equipment.

Results

Probiotic cheeses displayed the highest levels of lactic microflora. The matured Scamorza cheese containing the mix of *B. longum* and *B. lactis* was characterized by significantly higher level of Gln, Ser, Arg, Ile, Leu whereas cheese containing *L. acidophilus* was characterized by higher levels of Tyr and Met. Total FFA content was the highest in S-LA, intermediate in S-BB and the lowest in S-CO cheese; in particular, Scamorza cheese containing *L. acidophilus* showed the highest level of vaccenic acid, oleic acid and total CLA.

Conclusions

Probiotic bacteria survived through the technological phases of pasta filata cheese production, maintained their specific metabolic pathways, and conferred functional properties to Scamorza ewe’s milk cheese.

POSTERS

RANGING STRAIN BLOCK ANALYSIS OF CANDIDA ALBICANS INFECTION RISK IN MIXED BIOTOPE BACTERIA-FUNGI CULTURES

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Objective

Earlier we proposed a general approach for screening and identification of leader strains within interacting microbial pools using ranging biofilm forming (BF). We demonstrated that the system "*Candida* (I) + *Lactobacillus* (II)" can serve the convenient sensitive coupled model to study molecular-cellular interactions between Gram positive bacteria and eukaryotic yeast like fungi. *C. albicans* (Ia) and *C. tropicalis* (Ib) are members of the main group of yeast like fungal infections. The aim was to evaluate multispecies II pool influence in respect of Ia and Ib BF on the example of urogenital biotope (UB) microcenoses.

Methods

Clinical strains of *L. acidophilus* (106, 124 [leader], 183a), *L. brevis* (104, 109, 143), *L. casei* (124b [leader], 183), *C. albicans* (3, 23, 26, 45, 116, 147, 161, 320 [antimycotic-resistant]), and *C. tropicalis* (*97, 112, 144, 162, 417, 433, 438, 897*) were of UB origin. Mono- and mixed (in optimal ratio) cultures in MRS were grown in polysterene micropanels (48 h, 37°C) in anaerobic conditions. BF was evaluated by crystal violet staining, stain extraction and measurement at 600 nm. The influence of *Lactobacillus* strain pool on each *Candida* strain in BF reaction was calculated and Candida strains were ranged. Species blocks in rows obtained were compared. Colony morphology and BF architectures were also studied.

Results

A. For variations of direction II (pool:7-8 strains)-I (pool:16 strains) blocks Ia1, Ia2, Ib, were identified. A1. II all strains: 23 > 161 > 320 > 147 > *144 > 97* > 45 > *438 > 897 > 112 > 417 > 162 > 433* > 116 > 3 > 26. **A2.** II without 124: 23 > 161 > 320, *144* > 97 > 147 >*112* ≥ *438* ≥ *897 > 417 > 433 > 162*, 116 > 3, 26 > 45. **A3.** II without 124b: 23 > 45 > 320 > 147 > 161, *438 > 144 > 97, 897 > 162, 417 > 433 > 112*, 116 > 26 > 3. *Lactobacillus* leaders influenced expression of block Ia2 (A2) or the whole compact block Ib (A3) in the row part of decreased BF. **B.** Early germ tube appearance and their fast growth were registered in case of strains 23,147 and 320.

Conclusions

Results indicate that *Lactobacillus* leader strains influence ranging populations of Ia depending on strain rate growth and resistant germ tube forming. *C. albicans* revealed more quick and more adaptive replies compared to *C. tropicalis*.

A PEA (PISUM SATIVUM L.) SEED EXTRACT MODULATES COLONIC MICROBIOTA COMPOSITION IN THE DSS MODEL OF MOUSE COLITIS

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Objective

Nutritional management of IBD patients is key for the amelioration of the disease together with drug therapy. Pea (*Pisum sativum* L.) seed extracts have revealed to possess anti-inflammatory and immunomodulatory activities. The present study evaluates the effects of a pea seed extract (PSE) on the contents and mucosa-associated microbiota composition of the colon. Soy BBI, a serine protease inhibitor similar to that present in the seed, was used as control.

Methods

Male C57BL/6J mice were assigned to four groups: one non-colitic and three colitic. Colitis was induced by incorporating DSS (3.5%) in drinking water for four days, after which DSS was removed. Treated groups received orally PSE (15 g/kg•day), or pure soy Bowman-Birk inhibitor (BBI) (50 mg/kg•day), starting 14 d before colitis induction, and maintained for 9 d thereafter. Bacterial counts of specific groups in the colonic contents and in the colonic tissue were determined by rt-PCR. In addition to total bacteria, the bacterial groups studied were lactocbacilli, bifidobacteria, the *B. coccoides* / *E. rectale* group, *C. leptum*, enterobacteria, Escherichia / Shigella and bacteroides.

Results

The administration of PSE or soy BBI restored bacterial counts, partially or totally, to values in healthy mice in both the colonic contents and the colonic tissue. The bacterial groups mainly affected were total bacteria, lactobacilli, bifidobacteria and the *B. coccoifdes*/*E. rectale* group.

Conclusions

PSE and BBI were able to prevent the DSS-induced effects on the colonic microbiota composition, being their effects due, at least partially, to the presence of active BBI.

DEVELOPMENT AND USE OF A PROBIOTIC STRAINS TO REDUCE THE INFECTION OF *HELICOBACTER PYLORI* IN BALB/C MICE

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Objective

Colonization of *H. pylori* on the mucous layer of gastric epithelium is thought to cause gastritis and peptic ulcer disease. Some lactic acid bacteria (LAB) strains have been proved to be useful as adjuncts to the treatment and prevention of gastritis. The purpose of this study is to develop a probiotic with high protective effect against gastritis.

Methods

LAB strains from different origins were evaluated for their antagonistic effect against *Helicobacter pylori* (HP). Three strains selected by inhibition activity on HP were evaluated for their basic probiotic properties, including tolerance to gastric acid, bile salt and adherence of intestine cells. In the *in vivo* study, four-week-old BALB/c mice were infected with HP three times in three days followed by three times in three weeks. Then, mice were fed with LAB cells three times in the following week. Reversed order for HP and LAB feeding were also performed. Afterward, these mice were sacrificed, their stomachs were removed, and the bacteria cells including HP were counted. In addition, the urease activity was assayed.

Results

Results showed that a strain of Enterococcus faecium demonstrated higher antagonistic effect against the infection of H. pylori in vitro and in vivo when compared with two commercial available strains, ie, L. gasseri and L. acidophilus. Moreover, even in heat killed form, this strain preserve its protective function against the infection of H. pylori.

Conclusions

In conclusion, this strain may be used as potential a probiotic to prevent human from gastritis and peptic ulcer disease.

PROBIOTICS AND GUT MICROBIOTA: IMMUNO & NEUROMODULATORY EVIDENCES

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Objective

Probiotics have been proposed as preventive and therapeutic measures to restore the healthy composition and function of the gut microbiome. Expanding evidences are pointing to the role of these bacteria in modulation of various integral aspects of human health and well-being. The objective of the prospective study is to feature the probiotic attributes, antioxidant potential, anti-inflammatory and neuromodulatory ability of novel indigenous lactic culture, *Enterococcus faecium* (EF) CFR 3003 and *Lactobacillus rhamnosus* GG (LGG) MTCC 1408.

Method

EF was examined for resistance to *in vitro* gastric acidity, bile toxicity, pepsin and trypsin tolerance. EF and lyophilised cell-free culture supernatant (LCS) was assayed for *in vitro* antioxidant ability. The ability of EF, LGG and their LCS to induce the secretion of anti-inflammatory cytokine interleukin-10 (IL-10) and to suppress the induction of pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) was evaluated using the murine macrophage-like cell line, J774.1. Furthermore, the propensity of these probiotic supplements to modulate endogenous oxidative markers and redox status in mice brain was investigated.

Results

EF showed significant resistance to gastro-intestinal stress conditions. The results indicated that EF could withstand acid stress at pH 1.5, 2 and 3. The bacterium also survived at a bile salt concentration of 0.45% and tolerance was also observed towards pepsin and trypsin. EF produced lactic acid as a major metabolic product followed by butyric acid. LCS of EF exhibited potential DPPH antioxidant activity (82.21±0.021 %), reducing power ability (A700nm=0.64±0.11), ascorbate autooxidation inhibition activity (16.50±0.16 %) and oxygen radical absorbance capacity (ORAC value=128.34±3.34 umol Trolox equivalent/g of LCS). EF, LGG and their LCS possessed anti-inflammatory effect by negatively modulating TNF-α production and up-regulating IL-10 levels in LPS stimulated J774A.1 macrophage cell lines. Mice provided with oral supplements of EF and LGG for 28 days significantly lowered cytosolic oxidative markers, enhanced antioxidant enzyme activities with concomitant increase in gamma-aminobutyric acid (GABA) and dopamine (DA) levels in various brain regions.

Conclusion

The current study highlights the protective role of these bacteria against damaging oxidative radicals and inflammatory cytokines. It is hypothesized that their intake could be a potential approach for immunomodulatory and neuroprotective advantage.

Key words: Probiotics, Antioxidant potential, Anti-inflammatory activity, Neuromodulation, Mice, Gamma-aminobutyric acid, Dopamine

HOW PROBIOTIC BACTERIAL LECTINS AND GLYCOCONJUGATES PARTICIPATE IN MUCOSAL INNATE IMMUNITY

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Objective

Lectin systems (LS) recognize glycoconjugates (GC). Interaction between LS and GC systems (GCS) results in active multifunctional network of LS-GCS combinations participating in regulation of any biorecognition system. The aim was to propose concept (based on own works) of "Probiotic bacterial LS (PBLS)-Mucosal GCS (MGCS)" system increasing mucosal innate immunity.

Results and Proposals

We described multifunctional human PBLS which recognize multivalent polymeric MGCS. *Concept*. PBLS supporting human health (separately or in combinations with other antimicrobials and antipathogen reagents and factors) are important permanent contributors to biotope distant protection against pathogens. Being metabolomebiotics, PBLS form reversible specific complexes to natural and artificial MGC that allow building on duty dynamic network of antipathogen-directed biological cascades of reactions and communication signals of mucosal innate immunity. Secreted molecular and cell surface network PBLS -MGCS (potentially synbiotic supersystem) serves as important directed synergistic system contributing to biotope microbiocenosis antipathogen resistance and Cross-Talking to human protective cells/ tissues/ organs to support prolonged healthy status of mucosa. This network influences mucosa pores, affine texture and microecological redistribution in biotope. Mucosal innate immunity *strategies* of PBLS, MGCS and PBLS -MGCS against microbial and viral pathogens, early tumor like mucosal cells are proposed. Recognition activities of artificial glycoconjugates cofunctioning to LS are compared. Probiotic molecular-cellular synbiotic network system cofunctions to antibodies, cytokines, enzymes, antibiotics and antipathogen phytolectins.

Conclusion

Proposed concept and strategies against pathogens represent new insights to mucosal innate immunity involving protective network PBLS-MGCS in organism.

CONTRIBUTION OF COLONIC MACROBIOTA TO RESTING ENERGY EXPENDITURE AND SUBTRATE UTILIZATION

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Objective

The microbiota was found to play a role in obesity-related metabolic syndrome. Our aim was to investigate the contribution of the gut microbiota to resting energy expenditure and overnight fast substrate utilization.

Methods

This study was prospective controlled non-randomized open-label trial. It was carried out in a tertiary care university-affiliated medical center. The study population included 16 healthy subjects (eight females) referred for routine colonoscopy to department of Gastrointestinal and Hepatic Diseases. Resting energy expenditure was evaluated in the morning of the colonoscopy after bowel preparation and was repeated several days before or >1 month after the procedure.

Results

Resting energy expenditure increased significantly (from 1535±210 before to 1591±194 kcal/day after, $P < 0.038$) and the respiratory quotient decreased significantly (from 0.82±0.04 before to 0.77±0.03 after, $P < 0.0002$) after bowel preparation. Based on protein excretion in the urine, fat utilization was shown to have increased significantly at the expense of carbohydrates, but no significant change was observed in protein utilization. As a percentage of total caloric expenditure, carbohydrates contributed 46.1 ± 14.1% to the caloric expense before colonoscopy and 29.7 ± 10.0% after it ($P < 0.0044$), while fat contributed 29.9±11.0% before bowel preparation and 44.5 ± 11.3% after it ($P < 0.0077$) No significant change was found in protein contribution.

Conclusions

These results indicate that microbiota may participate in human metabolism and substrate utilization and therefore may affect energy expenditure measurements. This can also indicate the contribution of the microbiota to weight and body composition.

LACTIC ACID BACTERIA FROM ARMENIAN «MATSUN» AS A BASIS FOR THE NEW FUNCTIONAL FOOD PRODUCTS

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Objective

Traditional Armenian «*Matsoon* » (*matsoni, matsun*) is produced using the home made so called "spontaneous starters", it can be considered as a potential pool of the strains with probiotic properties. More than 300 strains of lactic acid bacteria (LAB) were isolated from different samples of traditional acid-lactic product «Matsun» from rural households from different regions of Armenia.

Methods

For isolation and cultivation of LAB the standard methods were applied. Determination of probiotic properties was carried out according to WHO requirements.

Results

The probiotic properties of several isolated LAB strains (proteolytic activities, resistance to enzymes of GIT, antibiotics, growth at pH=3,0-8,0 range) were investigated. It was showed, that they have high antioxidant activity, ability to adhesion and inhibited the growth of multidrug resistant human pathogens. Strains with best probiotic, organoleptic and technological parameters were selected for the creation of new products of functional nutrition. It was shown, that during co- cultivation of LAB strains with different combinations and in different conditions, both the synergic and antagonistic effects were observed. After co-cultivation of certain strains of LAB, the increase in antimicrobial activity was about 50%. The increase of antimicrobial activity can be attributed to the synergistic effect of metabolic products of probiotic bacteria. Investigation of obtained best combinations during 12-15 days showed improved organoleptic properties (sweet taste, low acidity), and antimicrobial activity.

Conclusions

So, strains of LAB, isolated from Armenian matsoun, can be recommended as a basis for creation of new symbiotic preparation for functional assignment with antimicrobial properties.

MULTIPROBIOTICS AS AN ALTERNATIVE MEANS CORRECTION OF PATHOLOGICAL CHANGES IN THE SALIVARY GLANDS AT LONG-TERM HYPOACIDITY

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Objective

It is known, that long decrease in gastric secretion leads to the development of hypergastrinemia and to pathological changes in digestion organs. Very important there is a search of ways to correction of these undesirable consequences. Application of probiotics is one of such ways. In complex treatment of acid diseases of digestion organs are used probiotics. Uses of probiotics not only correct infringements of microecology of a digestive path, but also positively influence on activity of immune and endocrine systems. Multiprobiotic of the last generation «Symbiter Acidofilic» shows by itself the mutualistic symbiosis of 14 cultures of probiotic bacteria (bifidobacterium, lactobacillus, lactococcus and propionibacteria) with the high concentration of viable cells (10^{11-12} CFU/dos.), has a wide spectrum of physiological valuable properties with synergism of the most essential probiotics properties. Concentrated biomass of living cells of microorganisms symbiosis, CFU/sm^3, not less than: lactobacillus and lactococcus – $6{,}0\times10^{10}$, propioniacidic bacteria – $3{,}0\times10^{10}$, bifidobacteria – $1{,}0\times10^{10}$, acetoacidic bacteria – $1{,}0\times10^{6}$ is a content of 1 dose of «Symbiter Acidofilic» (10 ml). It does not need additional activating, but begins to show the action from the oral cavity, because it is a living biomass of cells, but not liofilisate, in which microorganisms are in anabiosis. By modern presentations, the mechanism of positive action of probiotic is based on variability properties of indigenic microflora.

Methods

The substantiation of experimental efficiency of multiprobiotic «Symbiter Acidofilic» for the correction of pathological changes in tissues of salivary glands under conditions of hypergastrinemia was a research objective. Experiments are executed on 71 rats-males of line Vistar, weight 180-250g. Animals within 28 days entered omeprasole (14 mg/kg of weight) and multiprobiotic «Symbiter Acidofilic» together and separately. Development of the hypergastrinemia verified by the maintenance gastrine in blood plasma of rats (59,0±35,5 pg/ml, in comparison with investigated animals – 170,7±90,7 pg/ml).

Results

In the homogenate of salivary glands defined activity of ornithinedecarboxylase, α-amylase, general proteinases, NO-ergic system and the maintenance of nitrites, molecules of average weight, oxidative modificated proteins and ingibitors of the general proteinases. Under conditions of long omeprazole introduction pathological changes in salivary glands tissues appear: intensification of free-radical oxidation, disbalance of proteolysis by decompensated type, increased activity of α-amilase, disbalance of polyamines and NO-ergic systems. We determined that the activity of NO-ergic system under conditions of correction was 1.18 ($p<0.05$) times greater, than without correction, and the maintenance of nitrites – 1.02 times ($p<0.05$). Also correction of the hypergastrinemia by multiprobiotic «Symbiter Acidofilic» led to the increase of the ornithinedecarboxylase activity 1.2 times more ($p<0.05$), α-amylase 1.08 times more ($p<0.05$) and to the decrease of the maintenance of molecules of average weight 1.11 times ($p<0.05$),

oxidative modified proteins 1.08 times ($p<0.05$) and to the increase of the inhibitors of the general proteinases maintenance 1.06 times more ($p<0.05$).

Conclusions

Under conditions of long hypergastrinemia pathological changes in salivary glands tissues appear: intensification of free-radical oxidation, disbalance of proteolysis by decompensated type, increased activity of α-amilase, disbalance of polyamines and NO-ergic systems. Correction of omeprazole-inducted hypergastrinemia using multiprobiotic «Symbiter Acidofilic» normalises synthesis of regulatory polyamines, NO, α-amylase, proteolysis and reduces of the free-radical processes.

EVALUATION OF ANTIMICROBIAL ACTIVITY, SOME FUNCTIONAL AND PROBIOTIC PROPERTIES OF ENTEROCOCCUS SPP. ISOLATED FROM MILK

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Bacteria of the genus *Enterococcus* are ubiquitous Gram-positive, catalase-negative cocci that often occur in large numbers in vegetables, plant materials, and foods, especially those of animal origin such as dairy products. Enterococci have been used in many different applications as starters or adjunct cultures, and in foods they seems to have a major role in improving flavour development and quality of cheese. The role and the application of enterococci in food and health and more specifically in meat and dairy products has been reviewed recently.

The aims of this study were to determine antibacterial activity, some functional and probiotic properties, and to evaluate the safety of Enterococcus spp. that isolated from milk.

Isolation of Enterococci; the diluted homogenates were plated on M-17 medium and azide agar medium. The isolates were tested from their ability to grow in M17 broth at 10 and 45°C with 6.5% NaCl or at pH value adjusted to 9.6. Ribotyping was performed with a RiboPrinter Microbial Characterization System (Qualicon Inc., Wilmington, DE) and the standard *Eco*RI DNA preparation kit as described in the manufacturer's operations. Antagonistic activity screening was investigated well diffusion assay. The amount of produced lactic acid, hydrogen peroxide, proteolytic activity of the lactic acid bacteria was determined.

The studied strain inhibited the growth of selected tested LAB, Gram positive and Gram negative bacteria. *E. faecium* IN4 strain was negative for the tested virulence factors and did not present multi-resistance to antibiotics. The strain was resistant to physiological concentrations of bile salts and lysozyme. Almost all tested strains showed resistance to a pH range from 3.0 to 6.5, and to phenol and they showed tolerance to bile acid within 24 h.

The usage of Enterococci with probiotic properties in fermented milk products may enhance safety and quality of these products. Further studies are needed.

RELEASE OF ACE INHIBITORY AND ANTIOXIDANT HYDROLYSATES DURING IN VITRO GASTRO-INTESTINAL DIGESTION OF PUMPKIN SEED PROTEIN

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Objective

The use of plant proteins in the formulation of novel functional food products has been focus of scientific research. These proteins during gastrointestinal digestion can release bioactive peptides which can possess multiple bioactive properties (antihypertensive, antioxidant, anti-inflammatory and hypocholesterolemic) and they may play significant role as health promoting agents in functional food or nutraceuticals. The objective of the study was to investigate whether the major storage protein (cucurbitin) obtained from pumpkin (*Cucurbita pepo*) oil cake, previously reported as potential functional food additive, can be hydrolysed by the digestive enzymes.

Methods

A two stage *in vitro* digestion model system (first by pepsin and then with α-chymotrypsin and trypsin, simultaneously) was used to simulate the process of human gastrointestinal digestion. The hydrolysis characteristics, angiotensin I-converting enzyme (ACE) inhibitory and antioxidant activity of cucurbitin, during *in vitro* digestion, were determined.

Results

Final digests of cucurbitin showed significant ACE inhibitory activity (IC_{50}= 0.301±0.04 mg/ml. Also, 2, 2-diphenyl-1-picryl hydrazyl (DPPH) and 2, 2-azinobis 3- ethyl benzo-thiazoline-6-sulphonic acid (ABTS) radical cation activities and reducing power of cucurbitin were enhanced by *in vitro* digestion, therfore resultant digest acts as a radical quencher and reducing agent.

Conclusions

The above results showed that obtained hydrolysates of pumpkin oil cake protein are promising natural ACE inhibitors and antioxidant peptides with potential use as ingredients for functional food. Therefore, the benefits of cucurbitin are extended beyond its role as a potential additive used to improve functional properties of food, because when ingested, it may promote human health.

METABIOTICS OF LACTOBACILLUS RHAMNOSUS 2012 AGAINST THE HUMAN GUT MICROBIOTA

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Objective

During past decades increased the emergence of multidrug-resistant (MDR) bacteria that are resistant to many commercially available drugs. So one of the most promising concepts can be the use of metabiotics, obtained from lactic acid bacteria with probiotic properties. The metabiotics from *L.rhamnosus* 2012 from the culture collection of SPC "Armbiotechnology" NAS RA were used.

Methods

Metabiotics were purified from culture liquid by chromatographic methods. The resistance of bacteria to antibiotics determined by standard antibiotic disks method.

Results

More than 60 strains of human gut pathogens were isolated from patients in the Infection Hospitals of Yerevan. It was shown, that human pathogens showed low resistance to fluoroquinolones (17.4-18.2 %), were highly resistant to a beta-lactams (50-100%) and aminoglycosides (about 70%). The influence of partially purified metabiotics of *L.rhamnosus* BTK 2012 on the multidrug-resistant human pathogens shown, that it inhibited the growth of pathogenic bacteria with different efficiency. *Staphylococcus aureus* strains were 44% sensitive to metabiotics in minimal concentration, sensitivity of *Proteus mirabilis* was 60% and different *Salmonella* species was 80%. At the same time metabioitics do not inhibited the growth of *Esherichia coli* strains. It was shown, that *Lactobacillus rhamnusus* 2012 metabiotics in the same concentration do not affect on the LAB strains growth, belonging to different genera and species. While LAB strains shown high sensitivity to investigated antibiotics (about 70%).

Conclusions

Overage statistical results proved that applied metabiotics of *L.rhamnosus* 2012, do not concede to antibiotics by their antibacterial activity and do not harm the commensal microbiota.

PROBIOTIC LACTOBACILLUS STRAINS ACTIVITIES ON AUTOPHAGY AND INFLAMMATORY RESPONSE

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Objective

In this preliminary work, we aim to characterize in which extent probiotic lactobacillus strains, or their derived-culture supernatants, are able to modulate inflammatory response and autophagy, a cellular process that stands at the crossroads of metabolism and immune cellular defense in host cells. Given broad clinical implications of autophagy disorders in socioeconomically relevant diseases, including inflammatory bowel diseases, characterization of probiotic strains able to stimulate this process is of prime interest.

Methods

Human epithelial cell lines (Hela and Caco-2) or macrophages (THP-1) were treated with a panel of live Lactobacillus strains or their culture supernatants (grown in either biofilm or planktonic form). In parallel, an inflammatory response was triggered by treating cells with lipopolysaccharide or by infecting them with *Salmonella* Typhimurium, and monitored by RT-qPCR (cytokines mRNA levels). Autophagy activity in host cells was monitored by following mRNA levels of a panel of autophagy-related genes (RT-qPCR) and by assessing LC3-II protein conversion (Western blot).

Results

Our results demonstrate that the biofilm-derived supernatants of Lactobacillus strains display enhanced immunomodulatory effects compared to corresponding planktonic supernatants. Preliminary results show that Lactobacillus strains are able to stimulate transcription of autophagy-related genes and favor activation of the process.

Conclusions

In addition to their anti-inflammatory properties, boosted by biofilm mode of life, *Lactobacillus* strains might have beneficial effects by stimulating autophagy.

PHENOTYPIC CHARACTERIZATION OF A LACTOBACILLAR FIMBRIAL OPERON THROUGH RECOMBINANT EXPRESSION IN LACTOCOCCUS LACTIS

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Objective

To characterize the adhesive properties and induced host immune-cell responses of *Lactobacillus ruminis* ATCC 25644 sortase-dependent piliation.

Methods

Immuno-electron microscopic analysis was conducted using antiserum specific for the predicted *L. ruminis* backbone-pilin subunit (called LrpA). The pilus operon (so-called *lrpCBA*) was cloned into *Lactococcus lactis* for the recombinant production of surface-localized wild-type (GRS1224) and LrpC tip pilin-deleted (GRS1225) LrpCBA pili. Adhesion experiments with *L. ruminis* and recombinant-piliated lactococcal cells were performed using human gut-derived HT-29 and Caco-2 cell lines, and extracellular matrix proteins (fibronectin, collagen I and IV). Modified HEK293 cells expressing human Toll-like receptor 2 (TLR2) were used for characterizing the immunomodulatory effects of native and recombinant LrpCBA piliation.

Results

Immuno-EM verified that *L. ruminis* ATCC 25644 is surface piliated, this then consistent with genomic predictions that this strain contains a clustering of genes for sortase-dependent piliation. Binding experiments with recombinant lactococcal clones revealed the LrpCBA pilus has an affinity for collagen and fibronectin, and intestinal Caco-2 and HT-29 cells. Owing to the lowered adhesiveness of GRS1225, the LrpC pilin subunit can be seen to have a focal role in the pilus-mediated binding. LrpCBA-piliated lactococci showed a general reduction in TLR2-regulated NF-kappaB responses, despite *L. ruminis* cells rousing a heightened immune response. Levels of NF-kappaB signaling dropped sharply when bacterial cells were heat treated.

Conclusions

L. ruminis ATCC 25644 surface piliation not only mediates cellular adherence to ECM proteins and hum gut epithelial cells, but it also has an immune-dampening effect in the form of lessened TLR2-dependent responses.

TESTING OF ANTIMICROBIAL ACTIVITY OF GASTROINTESTINAL ISOLATES AND THEIR USE FOR THE EDAM TYPE CHEESE

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Objective

Probiotic bacteria additive into food products is a very perspective area of applied research. These microorganisms should have primarily a beneficial effect on consumer´s health. Their presence in foodstuff can positively influence also the sensory properties and production of antimicrobial substances can improve their safety and their ability to stay preserved.

Methods

The goal of the work was to test the antimicrobial activity of selected members of the genus *Lactobacillus* and *Bifidobacterium* of the gastrointestinal origin towards dairy cultures and selected undesirable microorganisms. There was used agar diffusion assay supplemented by PCR method detecting a gene for the production of Class IIa bacteriocin (particularly effective against *Listeria* sp.).
Additionally, the influence of tested probiotic bacteria on sensory and chemical properties of the Edam type cheese and the total content of these bacteria during cheese maturation were observed. Mini-cheeses were produced under sterile laboratory conditions and probiotic strains were added into the milk together with starter culture.

Results

The influence of the basic dairy cultures was strain-specific. At all used lactobacilli there was found a gene for production of bacteriocin and using the plate method there was proved its inhibitive effect on the growth of *Listeria innocua*.
The determined content of lactobacilli throughout the process of maturation (90 days) was above 10^6 CFU/g. Sensory properties of these cheeses were satisfactory.

Conclusions

The gained results show possibility to use the tested probiotic lactobacili for the production of the maturing cheeses. Nowadays we are testing this information during a pilot plant production.

THE ROLE OF MULTIPROBIOTIC "SYMBITER® ACIDOPHILIC" CONCENTRATED IN THE IMPROVEMENT OF TREATMENT OF NSAIDS-GASTROPATHY

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Objective

The aim of our work was to evaluate the effectiveness of combined treatment by proton pump inhibitors pantoprazole (P) and multiprobiotic "Symbiter® acidophilic" comcentrated (M)) in patients with NSAIDs-gastropathy in comparison with standard therapy (P).

Methods

We observed 60 patients who used NSAIDs for more than 1 month with endoscopically diagnosed erosive NSAIDs-gastropathy. The mean age of these patients was 63,2±6.0. The fecal microflora has been analyzed by bacteriological culture methods. Patients were randomized and placed into two equal groups. The control group was treated with P (20 mg 2 times daily) for 28 days. The main group received combined therapy: P (20 mg 2 times daily) for 4 weeks and M (10 ml per day) for 20 days. Over 1 month after the beginning of treatment we repeated all examinations which were done before.

Results

Over 1 month in the control group erosive lesions of GM were observed in 16 patients (over the length of the study, the number of erosions significantly decreased, but nevertheless they didn't disappear). In the main group in all patients erosions were absent. In the main group the height of epithelium in antrum and the sectional area of the parietal cells after the treatment was higher to compare with control group on 29,6% ($p<0,001$) and 56,8% ($p<0,001$) consequently.

Conclusions

The inclusion of multiprobiotic in the standard scheme of treatment of NSAIDs-gastropathy leads to the total healing of gastric mucosa with the improving of histo-morphological peculiarities and eliminates the detected dysbiosis in all patients.

ELUCIDATING THE MECHANISM OF WEISSELLA-DEPENDENT LIFESPAN EXTENSION IN CAENORHABDITIS ELEGANS

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Objective

Weissella species are found in Korean traditional fermented vegetables, kimchi and the intestinal tracts of humans and other animals. The mechanism whereby lactic acid bacteria extend the lifespan of *Caenorhabditis elegans* has previously been elucidated, however, the role of *Weissella* in the lifespan extension of *C. elegans* has not been studied.

Methods

Weissella koreensis, Weissella cibaria or *Escherichia coli* OP50 was administered to *C. elegans* and lifespan was measured. To evaluate the effect of retarding aging, lipofuscin accumulation, locomotory activity and body size were measured. Expression of aging related genes was analyzed by qRT-PCR and lifespan in loss-of-function mutants was measured to elucidate the target of *Weissella* species.

Results

W. koreensis and *W. cibaria* significantly ($p < 0.05$) extended the lifespan of *C. elegans* compared with *E. coli* OP50 and induced expression of several genes related to lifespan extension (*daf-16, aak-2, jnk-1, sod-3* and *hif-1).* Oral administration of *Weissella* lowered the accumulation of lipofuscin and increased locomotory activity. Moreover, *Weissella*-fed *C. elegans* had decreased body size, brood size, and pharyngeal pumping rate compared with *E. coli* OP50-fed worms. Further, lifespan was extended in loss-of-function mutants of *sod-3*, *hif-1* or *skn-1* but not in loss-of-function mutants of *daf-16*, *aak-2* and *jnk-1*, which highlights the potential role of these genes in *Weissella*-induced longevity in *C. elegans*.

Conclusions

Weissella species extend *C. elegans* lifespan by activating DAF-16 via the c-Jun N-terminal kinase pathway which is related to the stress response and the AMP-activated protein kinase-pathway that is activated by dietary restriction.

PROTECTIVE EFFECT OF A PROBIOTIC MIXTURE ON ACUTE DIARRHEA IN A RAT MODEL

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Objective

To determine the antidiarrheal activity of a probiotic mixture (Lactibiane Imedia) using the castor oil model in rats.

Methods

Three groups of rats ($n = 8$) were orally treated either with a probiotic mixture (Lactibiane Imedia, 30.10^9 CFU/kg), a standard drug (loperamide, 10 mg/kg), or a vehicle (spring water) one hour before castor oil (10 ml/kg) administration. Immediately after ingesting the castor oil, each animal was kept in an individual cage and observed for 4 hours. The following parameters were monitored: onset of diarrhea, total number of faeces, faecal weight and consistency, body weight loss and pain.

Results

Pretreatment of rats with the probiotic mixture significantly delayed the onset of diarrhea compared to the vehicle-pretreated rats. It reduced the number of diarrheal episodes by 81% and decreased the total weight and wetness of the faeces. The loss of body weight and the pain score were also decreased. The standard drug (loperamide) was effective in inhibiting diarrhea and loss of body weight but failed to reduce pain.

Conclusions

The tested probiotic mixture has a beneficial effect in diarrhea as well as in associated pain and could be an interesting alternative to anti-motility drugs.

EXPERIMENTAL COMPARATIVE STUDY OF MONO- AND MULTI PROBIOTIC EFFICACY OF STRESS-INDUCED ULCER PROPHYLAXIS IN RATS

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It was shown the effectiveness of probiotics application in *Helicobacter pylori* eradication by a number of studies. Some works also revealed gastroprotective properties of probiotic strains. The mechanisms of such action aren't completely understood. Furthermore it's interesting to establish which strains show the most positive effect on gastric mucosa (GM) in condition of ulcerative impact. So, the aim of the current work is to assess the prophylactic action of probiotic strains of genera *Lactobacillus* and *Bifidobacterium* on erosive and ulcerative lesions in GM caused by stress and investigate probiotics influence on the state of mucus barrier in the rats stomach.

The study was carried out on 42 white rats, which were divided into 6 groups. The 1st group were injected with water during 14 days (stress-control group), other groups were treated with probiotics *Lactobacillus casei (L. casei), Bifidobacterium animalis (B. animalis)* VKL, *B. animalis* VKB, the mixture of *B. animalis* VKL and VKB, and the mixture of all three strains accordingly. Probiotics were injected at a dose of 3.2×10^{10} CFU/kg and were dissolved in water (2.5 ml/kg). After 14 days of treatment rats of all groups were subjected to 3-hour water immersion restraint stress. The size of erosions and ulcers in GM was measured immediately after stress. In order to estimate the state of mucus barrier the concentration of free oxyproline, fucose and hexuronic acid were measured by the standard biochemical techniques.

It was established that the area of ulcers in GM in rats which were treated with monostrain probiotics *B. animalis* VKL and VKB didn't differ from stress-control group. Contrariwise introduction of *L. casei*, probiotic mixture of *B. animalis* VKL and VKB and the mixture of all three strains accelerated cicatrization of stress-induced gastric lesions. The total area of lesions in GM decreased by 37% ($p<0.05$) in rats, which were injected with *L. casei*, by 27% ($p<0.05$) in those injected with the probiotic mixture *B. animalis* VKL and VKB and by 42% ($p<0.05$) – with the probiotic mixture *L.casei, B. animalis* VKL and VKB in comparison with rats which got water. One of the mechanisms of the gastroprotection of these probiotic strains is prevention from mucus barrier degradation, which was evident in decrease of free fucose and hexuronic acid content. Thus, the obtained data suggest that the probiotic mixture *L.casei, B. animalis* VKL and VKB was the most effective in prophylaxis of stress-induced gastric ulcer in rats. These results confirm the expediency of probiotics use for the prevention of stress-induced lesions in the GM and the relevance of the further investigations of probiotics action mechanisms.

ALIVE MULTISTRAIN PROBIOTIC IN OBESITY PREVENTION

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Objective

According to new WHO analysis prevalence of children obesity has significantly increased in past few years. In European countries it varies from 11% up to 33%. Metabolic impairment in youth may cause more pronounced changes in adult. Therefore, the prevention of obesity in childhood is an urgent challenge of modern science. So, the aim of the study was to investigate the effect of periodic administration in youth of alive multistrain probiotic Symbiter on obesity in rats induced by neonatal injection monosodium glutamate (MSG).

Methods

The study was carried out on 60 white rats that were divided into 3 groups: intact, MSG- and MSG+Symbiter groups (10 male and 10 female rats in each group). Newborn rats of MSG- and MSG+ Symbiter groups were administered with MSG (4 mg/g, 8 µl/g, subcutaneously) at 2nd -10th days of life. Since the age of 1 month, rats of MSG-group were treated with water (0.25 ml/100 g), rats of MSG+ Symbiter groups – with Symbiter (1.4×10^{10} CFU/kg) dissolved in water (0.25 ml/100 g). Introduction had been performed intermittently (two-week courses alternated with two-week breaks) for 3 months. In 4-month rats anthropometrical parameters and visceral adipose tissue (VAT) mass were estimated, and adiponectin in serum and leptin in VAT were measured by ELISA.

Results

In 4-month rats we diagnosed the changes of the anthropometrical parameters and significant increase of VAT mass that confirmed development of visceral obesity. In male rats, there were more pronounced changes. Symbiter reduced the obesity both in males and females. The use of probiotic therapy led to recovery of adiponectin level and leptin level that were changed under obesity. Adiponectin concentration in serum grew by 90% ($p\leq0.05$) in males and by 40% ($p\leq0.05$) in females which were treated with multiprobiotic compared with rats injected with water. Symbiter also diminished leptin concentration in males and females to control values.

Conclusions

Thus, the introduction of melanin prevents MSG-induced obesity in rats and recovers the endocrine function of adipose tissue.

A PROPIONIBACTERIUM EXTENDS THE LIFESPAN EXTENSION OF CAENORHABDITIS ELEGANS

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Objective

Propionibacterium freudenreichii subsp. *freudenreichii* is used in the fermentation of Emmental cheese and has been known to improve human gut immunity. The aim of this study is to evaluate the effect of *P. freudenreichii* on the lifespan extension of *C. elegans.*

Methods

P. freudenreichii and control *E. coli OP50* were administered to *C. elegans* and lifespan, lipofuscin accumulation, locomotory activity, and body size were measured. Lifespan of loss-of-function mutants, daf16, daf-2, skn-1, jnk-1, mek-1, pmk-1, sek-1, daf-7, dbl-1, sir-2.1, and daf-12, were measured to elucidate the mechanism of lifespan extension of *C. elegans*. In addition, pathogen killing assay using *Salmonella typhimurium* was performed.

Results

P. freudenreichii significantly ($p < 0.05$) extended the mean lifespan of *C. elegans* and increased pathogen resistance of *C. elegans* compared with *E. coli OP50.* Oral administration of *P. freudenreichii* lowered the accumulation of lipofuscin, increased locomotory activity, and decreased body size. Among the tested loss-of-function mutants, loss-of-function mutants of pmk-1 and dbl-1 did not extend the lifespan of *C. elegans*, which suggests that the stimulation of innate immune system of *C. elegans* by *P. freudenreichii* might be involved in the extension of lifespan and pathogen resistance of *C. elegans*.

Conclusions

P. freudenreichii might extend lifespan of *C. elegans* and increase pathogen resistance of *C. elegans* by overall enhancing innate immunity through P38 MAPK (mitogen-activated protein kinases) and TGF-beta pathways.

INTESTINAL MICROBIOCENOSIS IN PATIENTS WITH DIFFERENT PHENOTYPES A-AND HYPOGAMMAGLOBULINEMIA

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Objective

Primary immunodeficiencies with a- and hypogammaglobulinemia are infectious and autoimmune clinical phenotypes. The purpose - study of intestine microbiota condition at the patients with different phenotypes of a -and hypogammaglobulinemia.

Methods

The intestine microbiota condition was estimated by the excrement bacteriological inoculation, the respiratory hydrogen test with lactulose, by the maintenance definition of the short chain fat acids AI in excrements using GLC, and by a method of cytofluorometry PBMC was phenotyped. 11 patients were examined, 4 - with autoimmune (AP), 7 - with an infectious phenotype (IP) of the CVID and XLA.

Results

It was revealed more significant increase of opportunistic pathogenic water producing microflorae at the patients with AP: 143.8+53.4 ppt, at the patients with IP - 131.8 + 46.5 ppt, more significant decrease in quantity of bifido - and lacto bacterium was established at the patients with IP on the average group - 2.1 x 10 ±1Ig. During GLC in the AP AI was lowered in the area of distinctly negative values (IP it was lowered to the direction of weakly negative ones). At the AP case the increase of cytotoxic cells number was not accompanied by the reduction CD4+lymphocytes: IRI-1.2±0.1, unlike the patients with IP-0.6±0.1. TLR4 expression strengthening at the patients with AP (63±8 %) in comparison with the norm (20±4 %) and the patients with IP (21±3 %).

Conclusions

The noted distinctive features can become the basis for the forecast of a course and the differentiated introduction of additional therapeutic means of treatment of primary agammaglobulinemia.

SURVIVAL OF LACTOBACILLUS ACIDOPHILUS LA-5 IN SYNBIOTIC APPLE ICE CREAMS UNDER IN VITRO GASTROINTESTINAL CONDITIONS BY PROPIDIUM MONOAZIDE qPCR

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Objective

The quantitative Real Time PCR (qPCR) is a technique that complements the plate count method, which considers only the cultivable population fraction. Therefore, the aim of this study was to evaluate the survival of *Lactobacillus acidophilus* La-5 in apple ice creams produced with milk, soymilk, and/or whey protein isolate + inulin, under *in vitro* simulated gastrointestinal conditions throughout 84 days of storage at -18 °C, using propidium monoazide qPCR (PMA-qPCR).

Methods

Employing a simplex-centroid mixture design, seven ice-cream-making trials and an axial point randomly chosen were produced containing *L. acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* Bb-12. The survival of La-5 was quantified at days 7, 42, and 84 in different periods (0h, 2h, 4h, and 6h) of the simulated gastrointestinal conditions by PMA-qPCR.

Results

In general, the viability of La-5 in all synbiotic apple ice creams produced was about 8 log cfu^{-1} at 0 h, and showed a significant decrease to around 5 log cfu^{-1} after 6 h assay ($p<0.05$), similarly to the ice cream produced with only milk. In addition, La-5 survival rate remained around 70% after 6 h assay. These findings indicated that the La-5 count under *in vitro* simulated gastrointestinal conditions was not affected by the replacement of milk by soymilk and whey protein isolate + inulin.

Conclusions

In conclusion, the use of soymilk and whey protein isolate + inulin instead of milk showed to be feasible in order to obtain a food matrix that may provide protection to the probiotic under stress conditions.

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DEVELOPMENT OF PROBIOTIC VEGETABLE JUICES FERMENTED BY DOCUMENTED PROBIOTIC LACTOBACILLUS STRAINS

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Objective

The aim of the study was to investigate the suitability of three documented probiotic bacteria (*Lactobacillus rhamnosus* GG, *Lactobacillus casei* Shirota, *Lactobacillus plantarum* 299v) as starter culture to ferment vegetable juices and determine the activity and the survival of these strains to develop a functional, non-dairy probiotic product.

Methods

The growth, the acidification, the viability and the activity of the probiotic strains were investigated in carrot, tomato and red beet juice. The number of living cells was determined by traditional microbiology method. The viability of bacteria was studied in a 4-weeks storage experiment at 30 ºC, 23 ºC and 5 ºC. The activity of probiotic cells was investigated by their dehydrogenase activity with MTT colorimetric assay.

Results

All of the investigated probiotic strains grew well on the vegetable substrates (reached the 10^9/ml cfu cell concentration after 24 hours) and decreased the pH below to an appropriate low value, however in the enzyme activity and in the viability were significant difference between the strains, which was influenced also by the vegetable juice. In the carrot juice, stored at 5 ºC, all strains showed good viability during the 4 weeks, however in some cases we obtained enough high living cell concentration during the storage period also at 23 ºC.

Conclusions

According to our results the documented probiotic *Lactobacillus* strains can be used as starter culture for vegetable fermentation. Nevertheless the type of the vegetable, the parameters of the fermentation and the storage considerably influence the behaviour of the probiotic starter strain.

PHENOLICS RELEASE DURING STORAGE AND IN VITRO DIGESTION OF BISCUITS ENRICHED WITH CHERRY POMACE

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Objective

The aim of this research was to evaluate influence of storage and *in vitro* digestion on phenolics release in biscuits enriched with encapsulated cherry pomace extract.

Methods

Cherry pomace phenolics were extracted with 50% ethanol, suitable for food applications. After that, extract was encapsulated with whey proteins by freeze-drying method. Encapsulated powder was incorporated in biscuits (15%) to enchance phenolics content and control their release properties. Phenolics content in buscuits during 10 weeks of storage and *in vitro* digestion in simulated gastric (SG) and intestinal (SI) fluids was determined.

Results

Phenolics content, after 10 weeks of storage, in enriched biscuits increased from 23.23 to 116.16 mgGAE/100g buscuit, while in control biscuits decreased from 24.39 to 14.52 mgGAE/100g buscuit. *In vitro* digestion of enriched biscuits indicated higher release of phenolics from biscuits in SI (85.31 mgGAE/100g biscuit), than in SG fluid (24.58 mgGAE/100g biscuit).

Conclusions

Results of this study showed good stability, recovery and release of phenolics incorporated in food in encapsulated form during storage and digestion. In this way, their role in prevention of oxidative damage of lipids and DNA by free radicals in GI tract is enabled and enhanced. Based on these results, encapsulated product may be further studied in developing other food products that promote health.

BRAN FERMENTATION WITH PROBIOTIC AND NON-PROBIOTIC LACTOBACILLUS STRAINS TO DEVELOP A FUNCTIONAL INGREDIENT FOR SOURDOUGH PRODUCTION

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Objective

The aim of the study was to select probiotic and non-robiotic *Lactobacillus* strains according to their antifungal activity and fermentation properties for oat bran fermentation. The application of fermented bran as functional starter component for sourdough preparation was also examined as well as the applicability of fermented bran-enriched sourdough for bread making. We have compared also the effects of different components (bran, bran with lactobacilli and fermented bran) on the protein profiles of breads.

Methods

Lactobacilli were counted by pour plate method, the yeasts and the mesophilic aerobic microbes were counted by surface-spread plate method. The antifungal activity of the investigated lactobacilli was studied against six moulds and against four baker's yeasts by double-layer agar spot method. The protein profiles of breads were investigated by 2 DE (two-dimensional electrophoresis) analysis.

Results

Three *Lactobacillus* strains (*Lb. delbrueckii* subsp. *bulgaricus* 397 and *Lb. curvatus* 2768 and *Lb. casei* Shirota) with good antimicrobial and fermentation properties were selected to ferment oat bran to develop a fermented bran enriched sourdough. The fermented bran had not any significant influence on the commercial baker's yeasts and the prepared sourdough had positive effect on the properties of bread, among others on the protein profiles and the shelf-life of the sourdough bread.

Conclusions

The results suggest that the lacto-fermentation is a potential bioprocessing technology to develop from bran a functional ingredient for sourdough production, which could be used after all for sourdough bakery products.

EFFECTS OF NEUROACTIVE AMINES ON THE GROWTH CHARACTERISTICS OF LACTOBACILLUS ACIDOPHILUS NK-1

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Objective

Stress frequently causes *dysbiosis*, disrupting the balance among the bacterial inhabitants of the gastro-intestinal tract. Catecholamines released into the bloodstream under stress stimulate the growth of opportunistic intestinal pathogens [1]. The *goal* of this work was to elucidate the effects of catecholamines and related neuroactive compounds on the growth characteristics of beneficial lactobacilli exemplified by the strain *Lactobacillus acidophilus* NK-1.

Methods

An *L. acidophilus* NK-1 culture was grown for 48 hs anaerobically on the modified MRS medium with cysteine. The tested growth variables included colony-forming unit formation, optical density at 520 nm, and medium acidification. Since these variables behaved in unison, only the CFU data are presented below.

Results

Norepinephrine, the main stress-associated neurochemical, drastically (~4-fold) increases the CFU number at concentrations as low as 1 nM; however, CFU formation is inhibited at very high norepinephrine concentrations (> 10 μM). Dopamine and epinephrine stimulate CFU formation less efficiently and at relatively high concentrations. Serotonin contained in the chromaffine granules of the intestinal mucosa only insignificantly (by ~25%) stimulates CFU formation. The proinfammatory agent histamine increases the CFU number 2.5-3-fold at a concentration of 1 μM.

Conclusions

The results obtained provide evidence that neurochemicals produced under stress stimulate the proliferation of a beneficial (probiotic) bacterial strain. These findings point to an ambivalent influence of stress that should increase the growth of both beneficial and detrimental [1] bacteria in the GI tract and, therefore, presumably intensify their antagonism.

DIFFERENT COMPONENTS OF LACTOBACILLUS AMYLOVORUS DSM16698T CELL WALL ARE ABLE TO COUNTERACT THE ENTEROTOXIGENIC E. COLI K88 INDUCED MEMBRANE DAMAGE IN INTESTINAL CELLS

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Objective

Lactobacillus surface molecules may play a role in pathogen exclusion/competition and mucosal barrier maintenance in intestine. We previously showed that *L. amylovorus* DSM16698^T reduced the enterotoxigenic *E. coli* (ETEC) K88 adhesion and prevented the pathogen-induced disruption of tight junction (TJ) and cytoskeleton proteins through IL-10-mediated signaling involving IL-8 down-regulation in intestinal cells. In the present study we investigated the role of *L. amylovorus* DSM16698^T surface molecules in the protection against ETEC-induced membrane damage.

Methods

Purified cell wall fragments (CWF) from L. amylovorus DSM16698T were either uncoated (U-CWF) or coated with recombinant S-layer proteins (S-CWF). Differentiated Caco-2 cells on Transwell filters were treated with ETEC, or with S-CWF or U-CWF either alone or simultaneously with ETEC for 2.5 h, or pre-treated with S-CWF or U-CWF for 1 h before ETEC addition. TJ and adherens junction (AJ) proteins were analyzed by immunofluorescence and Western blot. Membrane permeability was determined by phenol red passage. NFkB expression was measured by Western blot.

Results

We show that S-CWF protected the cells from the TJ and AJ injury, increase in membrane permeability and up-regulation of NFkB expression induced by ETEC either when the cells were pretreated or co-incubated with the pathogen. U-CWF pretreatment, but not simultaneous treatment with ETEC, inhibited membrane damage and prevented NFkB increase.

Conclusions

These results suggest that the various components of *L. amylovorus* cell wall may counteract the damage caused by ETEC and that different mechanisms are involved in the protection exerted by S-layer proteins and uncoated cell walls.

IMPACT OF SELECTED FOODBORNE LACTIC ACID BACTERIA ON ENERGY METABOLISM IN THE MODEL ORGANISM CAENORHABDITIS ELEGANS

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Objective

The nematode *C.elegans* is widely used as a model system for research on aging, development and host-pathogen interaction and, recently, to evaluate the beneficial effects of probiotics. In a previous work we demonstrated the impact of supplementation with a complex, foodborne microbiota, derived from Mozzarella di Bufala Campana cheese, on *C.elegans* energy metabolism. Herein we report the effects exerted by the single species of the consortium, namely *Lactobacillus delbrueckii*, *L. fermentum* and *Leuconostoc lactis*, on worm's physiology.

Methods

Bacteria were grown in MRS medium and used to feed worms. Animals were analyzed in terms of life span, larval development, brood size, fat storage and bacterial colonization capacity. NMR spectroscopy-metabolomic profiles were obtained for selected bacteria.

Results

Among the species tested, *Lactobacillus delbrueckii* induced phenotypes more similar to those observed with the whole consortium. Identification of *L. delbrueckii* as subsp. *lactis* prompted us to analyze also the effects induced by other subspecies, i. e. *bulgaricus* and *delbreuckii*, as well as a commercial *lactis* subspecies. We demonstrated that *L. delbrueckii subsp. lactis* impacted more severely on lifespan, larval development, fat accumulation as well as gut colonization capacity, probably depending on different metabolome profiles of the three subspecies. On the contrary, *bulgaricus* subspecies displayed probiotic features.

Conclusions

Overall our data indicated that *L. delbrueckii* is the principal responsible for the impact of foodborne consortium on *C.elegans* metabolism. Moreover, we have demonstrated that feeding worms with different *L. delbrueckii* subspecies results in opposite effects on host metabolism.

AMENSALISTIC ACTIVITY OF HUMAN LACTOBACILLUS STRAINS ISOLATES

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Objective

Recently, the attention has been focused on the properties of probiotic bacteria, in particular *Lactobacillus* spp. Among various characteristics that a good probiotic must possess there is the production of compounds with antimicrobial activity. These substances, named bacteriocins, are peptide molecules with a broad inhibitory spectrum, different mechanisms of action and biochemical characteristics.
Our aim was focused on the production of these substances by human *Lactobacillus* strains isolates, which, in our previous work, have demonstrated good resistance to the conditions of the gastrointestinal tract *in vitro*.

Methods

For this purpose, we used ten clinical isolated strains. The induction was assessed by stressed conditions (33°C, 18 hours), different media (MRS, LSM, M17) and in the presence of inducers (pathogens killed by tyndallization, glycerol, fructose). Inhibition of growth was determined by using a bioassay with *Escherichia coli* ATCC 25922, *Enterococcus. faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and *Sarcina lutea ATCC 9341.*

Results

Our results demonstrate that these strains produce substances that inhibit pathogens growth on agar well diffusion test with significant differences depending on the conditions considered. Briefly, due to the stressing growth condition the production of antibacterial substances was reported showing inhibition-zone diameters between 18mm - 32mm.

Conclusions

Further investigations are ongoing to isolate and to characterize these substances by chemical analytical methods, with good forecasts to discover new molecules.

OBSERVATIONAL PROSPECTIVE CLINICAL STUDY ON LACTOBACILLUS PLANTARUM IN WOMEN WITH BACTERIAL VAGINOSIS/AEROBIC VAGINITIS

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Objective

To evaluate lactobacillus plantarum(LP) p17630 in the treatment of women with bacterial vaginosis/aerobic vaginitis.

Methods

This is an observational, prospective, multicenter study. Eligible were women: >=18 years with moderate/severe leuchorrea and/or itching, vaginal burning and dryness and diagnosis of bacterial vaginosis(BV) and/or aerobic vaginitis(AV). Severity (four levels scale) of symptoms was collected at study entry and follow up visit (15 days after end of treatment).

Women were treated with: -BV: oral metronidazole 250 mg, 2 x 2/die/7 days or vaginal clindamicine cream 2%, 5 g/die/7 days- AV: vaginal Chloramphenicol 500mg/3 days. Women were also proposed a treatment with LP p17630 (1 vaginal capsule for 6 days, then a capsule per week for 2 weeks). Resolution of clinical infection was defined as: absence of clue cells and negative results for at least 2 Amsel criteria (for BV) and/or absence of clinical symptoms and vaginal pH normal or score Donders <3 (for AV) and /or culture negative.

The study was approved by Ethics Committees.

Results

A total of 94 patients were enrolled: of those 48 (51,1%) were treated with LP. At the follow up visit, 40 women treated with LP reported clinical resolution (83,3%). The corresponding value in no treated with LP women was 22 women (47,8%) ($P<0.05$). Resolution rate was similar in women with BV or AV. No adverse event was reported in both groups.

Conclusions

This observational study suggests that LP given in association with specific treatment may improve clinical resolution in women with BV and AV.

LACTOBACILLUS ACIDOPHILUS AND L. PARACASEI STABILITY AND THEIR RESISTANCE TO SIMULATED GASTROINTESTINAL CONDITIONS IN SYNBIOTIC AND PROBIOTIC JUSSARA (EUTERPE EDULIS) SORBETS

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Objective

To evaluate the stability during storage and the resistance to simulated gastrointestinal conditions of *Lactobacillus acidophilus* LAC03 and *L. paracasei* BGP1 added in probiotic and synbiotic sorbets, by using jussara (*Euterpe edulis*) as the main raw material.

Methods

Four formulations of sorbet were developed: with *L. acidophilus* (LA) or *L. paracasei* (LP) and each one with polydextrose (LAP and LPP). To determine stability, cell counting was performed after 0, 7, 15, 30 and 60 days. For in vitro analysis, which was performed after 21 days, each sample was added to simulated gastric condition (sodium chloride, pepsin, pH 1.8±0.1) and aliquots were collected for viable cell counting after 0, 60 and 120 minutes; the remaining fluid was added to enteric condition (sodium chloride, pancreatin, trypsin, bile salts, pH 6.5±0.1) and analyzed after 0, 90 and 180 minutes.

Results

There was no reduction in the number of microorganisms ($p>0.05$) during storage (LA: 8.53 log cfu/g, LAP: 8.64 log cfu/g, LP: 8.71 log cfu/g and LPP: 8.57 log cfu/g). However, all formulations presented reduction in the number of cells after 60 minutes of exposure to the simulated gastric condition ($p<0.05$); that reduction was kept constant until the analysis conclusion, with a total decrease of 3.9 log cycles for LA, 4.1 for LAP, 4.4 for LP and 5.1 for LPP.

Conclusions

LAC03 and BGP1 maintained their viability throughout the storage period for all sorbets and were able to resist to the adverse conditions of simulated gastrointestinal fluids, ensuring the functionality of the products.

COMPARISON OF CHOLINE -TRIMETHYLAMINE LYASE (cut-C) GENE EXPRESSION LEVEL IN INTESTINAL MICRO BIOTA: BY RT-QPCR

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Objective

Intestinal microbiota anaerobically converts dietary ingredients such as choline, phosphatidylcholine and carnitine into trimethylamine (TMA) by choline trimethylamine-lyase (*cut-C*) gene. TMA further converted to trimethylamine-N-oxide (TMAO) in the host liver. Which in the serum correlated with several diseases, including cardiovascular disease (CVD), trimethylaminuria (fish-malodor syndrome) and nonalcoholic fatty liver (NAFLD) etc,.Thus, it is important to evaluate the gene expression level of choline trimethylamine-lyase (*cut-C*) gene of different gut microorganisms.

Methods

Eleven bacterial strains were used. All strains were grown on nutrient broth medium at 37^{0}C for 24 hr and bacteria number was adjusted to approximately 1–3×10^{8}CFU/ml. Primers targeting specific *cut-C* genes were designed. The *cut-C* gene expression level was evaluated by Reverse transcription quantitative polymerase chain reaction (RT-qPCR).

Results

Eleven gut bacteria strains were screened for their cut-C gene expression. Three PCR primer sets were designed for all these 11 strains. The amplification sizes were targeting the cut-C gene of *Escherichia coli*, *E. fergusonii*, *Proteus mirabilis*, *Klebsiella pneumonia*, 421 bp; *Collinsella aerofaciens*, *Providencia rettgeri*, *Providencia alcalifaciens, Providencia rustigianii* 172 bp and *Clostridium spp* 211bp respectively. RT-qPCR data revealed that the *Providencia rustigianii* showed the highest while *Collinsella aerofaciens* showed the lowest expression levels of *cut-C* gene.

Conclusions

We have compared the *cut-C* gene expression level of some gut microrganisms. This preliminary information might be useful in better understanding the TMA producing bacteria, which may play the major roles in many correlated diseases. Further analyses by LC-MS/MS for the TMA production level of these strains will be undertaken.

AN INFORMATIVE STUDY ABOUT PROBIOTIC USE BETWEEN SLOVAK CANCER PATIENTS

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Objective

More than 80% of cancer patients supposed to use complementary and alternative medicine, and also dietary supplements including probiotics. We have decided to determine what aspects most likely influence the probiotic use between Slovak cancer patients.

Methods

Between March and December 2014, 499 patients hospitalized at National Cancer Institute were asked to evaluate their overall experience with probiotic use during the course of their disease. The cohort consist of 323 women (64.7%) and 176 men (35.3%), 91.6% were undergoing chemotherapy (2.8% together with radiotherapy) and 8.4% had no anticancer therapy. Three most frequent diagnoses were gastrointestinal tumours (26.0%), breast cancer (19.0%), lymphoma (14.3%), and 50.7% of patients belong to early age category (60-74 years). Statistical analysis was done by Fisher´s exact test.

Results

Probiotic usage was recorded in 27.8% of whole patients´ cohort (72% of women and 28% of men, respectively) with duration ≤1 month – 44.6%, ≤ 6 months – 32.4%, ≥1 year – 23.0%. Moreover, 70.5% of patients favoured taking probiotics other days then receiving chemotherapy and side effects experienced only 9.4%. Regarding to complementary medicine and dietary supplements, 40.1 % of patients took supplements with probiotics while 59.9% prefered dietary supplements alone (vitamine C, B, Mg^{2+}, Aloe Vera, green barley, Oyster mushroom).

Conclusions

Our pilot results of a questionnaire study have shown the differences in probiotic use due to the gender, cancer type and taking of dietary supplements. Although experimental studies showed decreasing toxicity related to anticancer chemotherapy or radiotherapy, safety of probiotic use in immunocompromised cancer patients must be taken to account.

AN IN VITRO DIGESTION MODEL TO EVALUATE THE EFFECTS ON GUT MICROBIOTA OF CACIOCAVALLO CHEESE NATURALLY ENRICHED WITH OMEGA 3 FATTY ACIDS

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Objective

The aim of the study was to evaluate the effects of changes in fatty acid profile after digestion on Italian cheese Caciocavallo. Milk for cheesemaking was from cows supplemented in animal diet with flaxseed or from control cows.

Methods

Friesian cows were supplemented with 500 g/day of flaxseed for one month versus a control group without supplementation; the effects of fatty acids composition from Caciocavallo on gut microbiota composition were investigated by an in vitro digestion model. In vitro alternatives determine endpoints such as the bioaccessibility of nutrients and non-nutrients or the digestibility of macronutrients (e.g. lipids, proteins and carbohydrates) and are used for screening and building new hypotheses; the digested cheese was analysed by gas chromatography to evaluate the fatty acid present once artificially digest.

Results

The results of fatty acid profile of digests reflected the fatty acid profile of cheese. The increase of two-fold in C18:3omega3 found in Caciocavallo cheese from flaxseed group was found also in Caciocavallo cheese digest from flaxseed group (0.70 vs 0.36 ± 0.10 in Caciocavallo cheese and 0.77 vs 0.32 ± 0.02 in Caciocavallo digest). The differences in Caciocavallo digests were even more evident after 90 days of ripening.

Conclusions

The increase of C18:3omega3 in digests from Caciocavallo of flaxseed group can have beneficial effects on the gastrointestinal tract and consequently on human health.

THE STUDY OF GUT BACTERIA FROM HIV/AIDS PATIENTS

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Objective

According to the growing evidence about the role of microbial community colonizing the human gastrointestinal tract in AIDS etiology, we have decided to test the internalization capacity of HIV patients´ bacteria in HL-60 cell line and normal human lymphocytes.

Methods

For this pilot study, bacterial clones from rectal swabs of 8 HIV-infected antiretroviral therapy (ART) naive Slovak and American patients were isolated. Selected clones were co-cultivated with HL-60 cells and human lymphocytes for 1 hour, respectively. Afterwards, intracellularization ability was tested by gentamicin protection assay. For comparison, bacteria from colon cancer patients were used. Moreover, 19 HIV/AIDS patients were using of probiotics for three months and the viral load was detected.

Results

Internalization capacity of HIV patients´ bacteria was 5 to 10 times higher comparing to colon cancer bacteria. About 50-60% of tested bacteria were competent to lyse HL-60 partially (patient bacteria M1, M22) or completely (patient bacteria K1, M12). We have detected partial (patient bacteria P3, M22) and complete lysis (patient bacteria P1, K1, M1, M12) also after co-cultivation with normal human lymphocytes. Tested bacteria were mostly characterized as *Escherichia coli*. After three months of probiotics application the remission of the viral load was found in 61% of tested patients.

Conclusion

Higher intracellularization and lysis ability of HIV patients´ bacteria suggest gut bacteria of HIV patients may contribute to HIV/AIDS progression. Our pilot results confirm the positive effect of three-month probiotics administration. Application of probiotics after anti-retroviral therapy may help to restore normal colonisation of gastrointestinal microflora of AIDS patients.

EFFECT OF BETA-GLUCAN ADDITION ON SURVIVAL OF LACTOBACILLUS ACIDOPHILUS IN LOW FAT STIRRED PROBIOTIC YOGHURT

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Objective

Yogurt is the most important and common probiotic carrier in dairy industries. One of the most important problems to produce probiotic yogurt is the low viability of such bacteria and its sensitivity to low pH. Prebiotics can improve probiotic's growth and survival. In this study, barley beta-glucan as a fat substitute was added to the low fat yoghurt and survival of *Lactobacillus acidophilus* was investigated as a probiotic.

Methods

Beta-glucan was added to the yoghurt at four levels: 0.25, 0.5, 0.75 and 1 %w/w. Survival of *Lactobacillus acidophilus* was evaluated at 1st, 7^{th} and 14^{th} days of storage. Also, its physicochemical properties (WHC, synersis, viscosity, pH and acidity) were determined at 7^{th} day of storage.

Results

Lactobacillus acidophilus count increased significantly with beta-glucan addition up to 0.75 % w/w ($p<0.05$) because of its prebiotic effect and buffering capacity. Survival of bacteria in yoghurt, including 1 %w/w beta-glucan, was less than other samples. Also, the results showed that the treatment had significant ($p<0.05$) positive effects on the physicochemical properties of yogurt up 0.5% beta-glucan.

Conclusions

Results of this research indicated that beta-glucan could be used successfully as a functional fat replacer in low fat synbiotic yogurts at 0.5 %w/w as a prebiotic accompanying with creating good texture for low fat yogurt.

THE EFFECTS OF BENEFICIAL BACTERIA ON ORAL PATHOGENS AND CARIES DEVELOPMENT

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Objective

The aim of this study is to evaluate the effects of lactic acid bacteria on salivary mutans streptococci (MS) counts and caries formation under a high cariogenic challenge.

Methods

Thirty-two Wistar rats were equally divided into four different groups: group 1, normal diet; group 2, sugar-rich diet; group 3, sugar-rich diet and *L. plantorum* 167.P6.5 isolated from fermented meat products; group 4, sugar-rich diet and *L. rhamnosus* M17-10.2 isolated from the human oral flora. The offspring were also infected with *Streptococcus sobrinus,* excluding those in the group 1. MS counts were determined during the study. After 8 weeks of weaning, the animals were killed, and the number of carious lesions was determined.

Results

The MS count ratios at the end of 8 weeks were significantly lower than those at the end of 4 weeks in the group administered *L. rhamnosus* ($p < 0.05$). A strong correlation was found between the total MS count and sulcal caries scores ($r = 0.507$). It was also observed that the caries ratios of the probiotic-applied groups were lower.

Conclusions

These results suggest that lactic acid bacteria, especially those originating from an oral source, may serve as therapeutic agents against oral pathogens and dental caries.

TRANSFERABILITY OF THE ANTIMICROBIAL RESISTANT GENE IN COMMERCIAL PROBIOTIC LACTOBACILLUS

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Objective

Lactobacillus strains have long history of use in food and extensively been used as probiotics. Introducing probiotic products containing lactobacilli to the market involves stepwise process that requires to be carefully controlled in order to obtain a safe product. Amongst other criteria, it is recommended to screen the potential probiotics for their antibiotic resistance spectrum. Before launching a probiotic product into the market, it is important to consider that any probiotic strain does not contain transferable antibiotic resistance genes. Even though fermented milks, including probiotic products, are generally regarded as safe and stable, this study looked in to the antibiogram of selected lactobacilli from different fermented dairy products.

Methods

Lactobacillus isolates; 6 from from fermented milks of UK/Europe markets, 2 commercial cultures and 3 type strain cultures were compared for their susceptibility to antimicrobials. The study consisted of determination of Minimal Inhibitory Concentration (MIC) for 25 antimicrobials and detection of antimicrobial resistance genes using polymerase chain reaction (PCR).

Results

All isolates presented intrinsic resistance to ciprofloxacin, daptomycin, amikacin, sulfisoxazole and nalidixic acid. Also, they were found susceptible to penicillin, ampicillin, linezolid, amoxicillin/clavulanic acid, ceftiofur, nitrofurantoin, quinupristin/dalfopristin, tigecycline and trimethoprim/sulfamethoxazole. However, variable resistance was noticed among tested isolates to ceftriaxone, chloramphenicol, erythromycin, gentamycin, kanamycin, lincomycin, streptomycin, tetracycline, vancomycin, cefoxitin, and tylosintartarate. None of the isolates contained any of the 28 studied antimicrobial resistance genes.

Conclusions

The present *in vitro* study implies that these isolates are safe for use as probiotics in this respect.

EFFECT OF SIBO ON GASTRIC MYOELECTRIC ACTIVITY DISTURBANCES IN PATIENTS WITH LIVER CIRRHOSIS

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Background

Liver cirrhosis (LC) promotes excessive bacterial growth, which may impair gastric myoelectric activity and cause dyspepsia.

Aim of the study

Evaluation of SIBO influence on gastric myoelectric activity disturbances in patients with LC.

Material and Methods

Study included 9 patients with LC (6 men, 3 women, aged 47.7 ± 9.3 years), and 9 healthy people - control group (5 men and 4 women, aged 45.8 ± 10.2 years). Gastric myoelectrical activity (EGG) were examined using a 4-channels system electrogastrography in the fasting state, after a meal (hormonal stimulation), and after drinking water (neurogenic stimulation). Dyspeptic symptoms were identified using the questionnaire. The presence of SIBO was determined by a breath test using Gastrolyzer.

Results

EGG. Patients with LC demonstrated impaired fasting EGG. The standard meal and water test did not cause the improvement of the EGG indicators, in contrast to the control group ($p < 0.05$).
In the patients group was noted a correlation between the presence of SIBO and dyspeptic symptoms: epigastric discomfort ($r = 0.42$, $p = 0.04$), whereas in the control group were correlation between the presence of SIBO and epigastric discomfort ($r = 0.61$; $p = 0.04$) and bloating ($r = 0.7$; $p = 0.001$). Patients with SIBO had significantly lower value of normogastria (48.2±15% vs. 66±6.2%; $p = 0.04$) than patients in the control group with SIBO.

Conclusions

Preliminary results show disturbances of gastric myoelectric activity in patients with liver cirrhosis. SIBO observed in patients with LC correlated with dyspeptic symptoms and disorders of EGG.

EFFECTS OF FERMENTED COW'S MILK PRODUCT WITH HEAT-KILLED LACTOBACILLUS PARACASEI CBA L74 ON HUMAN ENTEROCYTES

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Objective

Fermented cow's milk with *Lactobacillus paracasei* CBA L74 (FM-CBAL74) exerts a preventive effect against childhood infectious diseases. We evaluated if this effect is at least in part related to a direct interaction with human enterocytes

Methods

Human enterocytes (Caco-2) were stimulated for 48 hours with FM-CBAL74 at different concentrations. Cell growth was assessed by colorimetric assay (MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); innate immunity peptides synthesis, beta-defensin-2 (HBD-2) and cathelicidin (LL-37), by ELISA. Cell differentiation, tight junction proteins (zonulin and occludin), HBD-2 pathway, and anti-inflammatory modulation were analyzed by Real Time PCR using enterocytes RNA.

Results

FM-CBAL74 stimulates in a dose-dependent manner cell growth (+600%,p<.05), HBD-2 (+1018%,p<.05), LL-37 (+3400%,p<.05) synthesis; and lactase (+65%,p<.05), zonulin (+167%, p<.05) and occludin (+177%,p<.008) expression with maximal effective doses between 11.5 and 115 mg/ml. Same effective FM-CBAL74 doses stimulate expression of toll like receptor-2 (+170%, p=.007) and transcription factor NF-kB, (+333%,p<.05); and down-regulate inflammatory mediators expression (COX-2, -48%; iNOS, -37%, p<.05). The effects of FM-CBAL74 are dependent on a thermo-stable component/s.

Conclusions

Through a direct interaction with the enterocytes FM-CBAL74 regulates cell growth and differentiation, innate immunity, and inflammatory mediators expression. These actions are responsible at least in part for the positive effect observed in children.

BREAST MILK BUTYRATE AS PROTECTIVE FACTOR AGAINST FOOD ALLERGY

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Objective

Breast milk is considered a protective factor against food allergy. The major short chain fatty acids, butyrate produced by gut microbiota exerts positive effect on immune system. We aimed to see whether butyrate concentration in human milk is able to prevent food allergy in animal model.

Methods

We determined by gas cromatography butyrate concentration in 40 samples of mature breast milk collected from lactating women (aged 21-42 years), receiving a mean content of 12.2 g (SD±3.45)/d of dietary fiber). 4-week-old female C3H/HeOuJ mice were sensitized by oral route with β-lactoglobulin (BLG) plus cholera toxin (CT) as an adjuvant in the presence or absence of butyrate. Acute allergic skin response, anaphylactic symptom score, body temperature, intestinal permeability, anti-BLG IgE, IL-4 and IL-10 production were assessed soon after oral food challenge.

Results

Mean butyrate concentration in breast milk was 0.47 mM (SD±0.15). This means that a breastfed infant receives a daily dose of butyrate of about 20 mg/Kg body weight. The same concentration was able to significantly prevents CMA in the animal model, as suggested by a dramatic inhibition of acute allergic skin response, anaphylactic symptom score, body temperature decrease, intestinal permeability increase, anti-BLG IgE, IL-4 and IL-10 production (p<.05).

Conclusions

Our data support the role of butyrate as effective human milk component able to prevent food allergy.

NAVY BEAN SUPPLEMENTATION ALTERS MICROBIAL ACTIVITY AND COMMUNITY STRUCTURE AND IMPROVES GUT BARRIER INTEGRITY IN LEAN AND OBESE MICE

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Objective
To determine the effect of 20% cooked navy bean (NB) flour supplementation on the aspects of gut barrier integrity, microbiota activity and community structure in lean and obese mice.

Methods
Male C57Bl/6 mice consumed i) NB flour diet (Bean) or an isocaloric control diet (Control) for 3 weeks (lean, n=10/diet), or ii) a high fat diet (HF, 60% kcal fat) or isocaloric high fat plus NB flour diet (HF+Bean) for 12 weeks (obese, n=12/diet).

Results
In lean mice, Beans i) increased colon crypt area and mucus production, ii) increased mRNA expression of MUC1, REG3gamma, occludin and JAM-A, and iii) reduced serum lipopolysaccharide levels compared to Control ($P \leq 0.05$), indicative of enhanced antimicrobial defense and gut barrier integrity. Furthermore, Beans i) increased microbial-derived cecal short chain fatty acids acetate, butyrate, and propionate and ii) altered the colonic microbial community structure by increasing abundance of Prevotellaceae (71-fold) and reducing Peptostreptococcaceae (145-fold), Clostridiaceae (13-fold), Streptococcaceae (8-fold), Peptococcaceae (3.5-fold), and Rikenellaceae (2.5-fold) versus Control (16S rRNA sequencing). In obese mice, bean supplementation similarly altered both microbiota activity and community structure. The compromised mucosal barrier integrity component of the obese phenotype was attenuated by beans, wherein permeability to FITC-dextran was reduced by 30% in the HF+Bean versus HF ($P \leq 0.05$). Additionally, relative epididymal visceral adipose tissue weight was 16% lower in the HF+Bean versus HF group ($P \leq 0.05$).

Conclusions
In lean and obese mice, bean supplementation enhances gut barrier integrity and alters microbiota activity and community structure which helps to mitigate the severity of the obese phenotype.

DEVELOPMENT OF AN ANDEAN POTATO PRODUCT WITH FOLATE-PRODUCING LACTIC ACID BACTERIA

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Objective
Folate is a B-group vitamin that cannot be synthesized by humans and must be obtained exogenously. It is known that certain lactic acid bacteria (LAB) strains can produce this vitamin. The aim of the study was to select folate-producing LAB in order to obtain a novel fermented potato product with elevated folate levels.

Methods
Several LAB strains were isolated from Tocosh (a traditional Andean fermented potato product) and cultured in folate-free culture medium (FACM). The LAB which produced the highest amounts of folates (determined by a microbiological method) were selected for the fermentation of two Andean potato varieties (*S. tuberosum* spp *andigena* churqueña and collareja). Sterile cooked purees were inoculated and samples were collected at 0, 8 and 24 hours. Cell growth, pH and total folate were determined.

Results
From a total of 63 tested strains, 40 could grow in FACM after 7 subcultures. *Lactobacillus* (*Lb.*) *sakei* 2T2MM10 and 2T3MS8, *Lb. fermentum* T3M3, *Lb. paracasei* 3T3M3 and 3T3MS2 produced the highest folate concentrations in FACM (between 29 and 138 ng/ml). These were used to ferment the two potato varieties. Although all 5 selected strains were able to grow and produce folates in the potatoes, *L. sakei* 2T3MS8 showed the highest increase after 8h incubation, with folate concentrations reaching between 300-400 ng/g which is more than 3 fold the amounts of the unfermented potatoes (100±30ng/g).

Conclusions
The adequate selection of LAB could be used to produce novel potato based products with elevated folate concentrations.

BACTERIOCIN-PRODUCING LACTOBACILLUS STRAINS ISOLATED FROM DIFFERENT LOCAL SOURCES.

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Bacteriocins of lactic acid bacteria have a great potential for application as a preservatives in food industry and in medicine as an alternatives to antibiotics.

Objective
The aim of this work was to screen for bacteriocin-producing lactic acid bacteria out of the strains isolated from a range of traditional fermented products and study of their spectrum of antimicrobial action.

Methods
Thirty nine strains of lactic acid bacteria were isolated from the kumis (fermented horse milk), shubat (fermented camel milk) and sauerkraut. The ability to produce bacteriocin was investigated by agar-spot test described by T.Klaenhammer et. al. (2012).

Results
Two LAB isolates that produce antimicrobial peptides were identified as *Lactobacillus plantarum* strains by biochemical methods and 16S rDNA gene sequencing. The proteinaceous nature of antimicrobial substances was established based on the loss of antimicrobial activity after treatment with proteinase K and pepsin. Of these strains, *L. plantarum 42* produce a bacteriocin which inhibits the growth of two *E. faecalis* strains and five *E. faecium* strains and *L. plantarum 44* produce a bacteriocin against *P. aeruginosa* strain.
L. plantarum 42 and *L. plantarum 44* strains were found to be resistant to bile salts (0.6%), NaCl (8%) and pH 3, susceptible to most antibiotics.

Conclusions
Taking into consideration the clinical importance of *E.faecalis, E.faecium* and *P. aeruginosa*, the isolates *L. plantarum 42* and *L. plantarum 44* shows potential for the production of probiotic and functional foods.

LACTOBACILLUS RHAMNOSUS K32 AS ADJUVANT IN MUCOSAL RECOMBINANT CHIMERIC POLYPEPTIDE VACCINE

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Objective
To evaluate an adjuvant activity of different forms of *L.rhamnosus* after vaccination with recombinant protein.

Methods
L.rhamnosus K32 in live, hot inactivated and disintegrated form were tested. Recombinant molecules PSPF and Su2 representing *S.pneumoniae* and *S.agalactiae* protein vaccines based on surface proteins were studied. Mice were treated nasally with different forms of *L.rhamnosus* combined with chimeric antigens. The same procedure was repeated 21 days later. In parallel chimeric antigens without probiotics were administrated. Specific IgG and IgA were evaluated in serum and nasal washing in dynamics. The protective efficiency were tested on the model of peritoneal S.*pneumoniae* or *S.agalactiae* infection and clearance rate was estimated by the number of pathogen counts in spleen.

Result
L.rhamnosus in different forms provided an adjuvant effect regarding PSPF polypeptide. Mice that received probiotic adjuvants had significantly higher level of specific IgG and IgA antibodies in comparison with PSPF group. On the contrary, the Su2 polypeptide were more immunogenic without adjuvants. Vaccine protective efficiency coincided with adaptive specific immune response level. Su2 polypeptide and polypeptide PSPF combined with live or non-viable *L.*rhamnosus provided the best protection against *S.agalactiae and S.pneumoniae* in mice model.

Conclusion
Taking together this finding support the hypothesis that in general probiotics could be used as mucosal vaccine adjuvants. At the same time this property are not universal. The problem of probiotic application have to be solved after individual testing the antigen-probiotic pare.

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ANTIVIRAL ACTIVITY OF THE ENTEROCINS ENTB AND ENTXB AGAINST INFLUENZA VIRUS IN MDCK CELL LINE

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Objective

Enterocins EntB and EntXb are the low molecule peptides produced by probiotic *Enterococcus faecium L3.* The aim of our study was to study the effect of chemically synthesized bacteriocins EntB and EntXb on influenza virus reproduction.

Methods

The peptides were chemically synthesized by the solid-phase method with an "Applied biosystems 430A" synthesizer using Na-Boc protected amino acid derivatives. The crude synthetic peptides were purified on semi-preparative HPLC column. The A/Perth/16/2009(H3N2) influenza virus was grown in chicken embryos and stored at -70°C. We estimated inhibition of viral reproduction in MDCK cell line NBL-2. Confluent monolayer was overlaid with 100 µl of EntB or EntXb serially diluted in DMEM containing 2 µg/ml TPCK trypsin. After 60 minutes of incubation the 100 µl of virus were added at 10-0.1 multiplicity of infection (MOI). The cytopathic effect (CPE) was evaluated after 72 hours by staining with 0.5% crystal violet as decrease of the optical density at 630 nm (OD_{630}).

Results

The EntB at a concentration of 0.005 mg/ml demonstrated 23% CPE reduction after influenza A(H3N2) virus infection at 10 MOI and 100% inhibition at 1 MOI. The 0.025 mg/ml of EntXb had shown 14% reduction of virus CPE at 10 MOI, 57%- at 1 MOI and 100% inhibition at 0.01 MOI.

Conclusions

Both EntB and EntXb demonstrated antiviral activity against A(H3N2) influenza virus in MDCK cells. These data confirmed the anti-viral activity of the *E. faecium L3* enterocines against respiratory viruses representing a challenge to the public health system.

IN VITRO EVALUATION OF LACTOBACILLUS ACIDOPHILUS AND BIFIDOBACTERIUM ANIMALIS SUBSP. LACTIS SURVIVAL IN SIMULATED GASTROINTESTINAL FLUIDS AFTER APPLICATION IN SEMISWEET CHOCOLATE

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Objective

It was compared the survival in simulated gastrointestinal conditions of commercial probiotics before and after application in semisweet chocolate.

Methods

Lactobacillus acidophilus (LA3) and *Bifidobacterium animalis* subps. *lactis* (BLC1) (SACCO, Italy) were cultivated twice in MRS broth (Acumedia, USA) at 37 °C for 18 h. Cells were collected by centrifugation and added to the chocolate at 10^9 CFU/g. Initially, the microorganisms were exposed to simulated gastric fluid (SGF, pH 1.8) for 2 h, followed by exposure to simulated intestinal fluid (SIF, pH 6.5) for 3h, and enumerated in each step. To determine the hydrophobicity profile of the cell wall of probiotics, the cells were resuspended in KNO_3 and added to xylene, chloroform and ethyl acetate, and the absorbance of aqueous phase was analyzed at 600 nm.

Results

With regard to hydrophobicity profile of probiotics, LA3 presented a more hydrophobic and acidic cell wall surface when compared to BLC1, which suggests the two microorganisms have the potential to adhere to intestinal epithelium. Populations of free LA3 and BLC1 reduced, respectively, 2 and 4 logarithmic cycles, after exposure to gastrointestinal fluids, but when these probiotics were added in chocolate, there was no reduction on bacterial populations.

Conclusions

The semisweet chocolate protected LA3 and BLC1 from adverse conditions of the digestive process. Furthermore, the studied probiotics have hydrophobicity profile which could allow the adhesion to the intestinal epithelium.

POSITIVE EFFECT OF A NEW SYMBIOTIC FORMULATION CONTAINING LACTOBACILLUS PARACASEI B21060 IN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Objective

Probiotics have been proposed for the treatment of dyslipidemia. We aimed to evaluate efficacy and tolerability of a new symbiotic formulation containing a combination of the probiotic *Lactobacillus paracasei* B21060 and prebiotics (arabinogalactan, xilooligosaccharides) in the treatment of children affected by familial hypercholesterolemia (FH).

Methods

Prospective, randomized, case-control study involving otherwise healthy FH subjects (6–12 yrs) consecutively observed at two Tertiary Centers for Pediatric Nutrition. Two groups of 6 months intervention: active group, received a low saturated fats diet plus the symbiotic (2.5×109 cfu, bid); control group, received low saturated fats diet alone. The plasmatic lipid profile was assessed by peripheral blood sampling at baseline (T0) and after 6 months of intervention (T1). Same subjects were re-evaluated after additional 6 months of observation (T2) from the end of the therapeutic course.

Results

40 FH children were enrolled (20 in active group receiving the symbiotic: 8 male, (±8.4 yrs), BMI 17.6; 20 in control group receiving a placebo: 8 male, median age 7.5 yrs, BMI 17.0). At T1 a reduction of C-LDL, total cholesterol, LDL/HDL ratio was observed in both groups, but the differences were significant only in active group. At T2 patients in active group showed stable total cholesterol and C-LDL serum level. Adherence to symbiotic doses was >90%.

Conclusions

The symbiotic containing *Lactobacillus paracasei* B21060 is able to significantly reduce lipid biomarkers in children with FH. The treatment was well accepted and tolerated by patients. Our results further support the efficacy of this therapeutic strategy against pediatric FH.

IMPACT ON GUT MICROBIOTA OF TWO CANDIDATE PROBIOTIC STRAINS WITH A PROTECTIVE EFFECT AGAINST FOOD ALLERGY

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Food allergy has significant effects on morbidity and quality of life and can be costly in terms of medical visits and treatments. Clinical and experimental studies have shown alterations in the sequential bacterial colonization of the gut in westernized countries that could be responsible for a T-helper balance deviation, a major factor in the rise of allergic diseases. A modulation of the gut microbiota may therefore contribute to prevention and management of allergic diseases and this notion supports the use of probiotics.

Two *Lactobacillus* strains (*L. rhamnosus* LA305 and *L. salivarius* LA307), able to induce regulatory responses *in vitro,* were tested in a murine model of food allergy to beta-lactoglobulin (BLG). Their impact on the T-helper balance was studied through quantification of cytokines secretion by BLG-stimulated splenocytes (systemic impact) or lymph node cells (local impact). Caecal microbiota was assessed by culture methods and quantitative real-time PCR.

Both strains decreased allergen specific IgE, IgG1/IgG2a ratio and MCP-1, then showing a protective impact on sensitization and allergy

LA305 showed a regulatory immune effect, inducing IL-10 secretion at both local and systemic levels while LA307 appeared to decrease production of all cytokines. Both strains induced a decrease of *Enterobacteriaceae* level while LA305 increased *Staphylococci* colonization and LA307 decreased *Enterococci* colonization.

These results show that the two probiotic strains are protective in a mouse model of food allergy through different immunoregulatory mechanisms, and modulate the gut ecosystem, mainly impacting subdominant microbiota.

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HUMAN IGE-REACTIVE PROTEINS IDENTIFIED IN LACTOBACILLUS CASEI LCY

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Objective

Although lactic acid bacteria (LAB) are recognized as safe and may exert beneficial effects on humans, the results of clinical studies with allergic patients show a varying, also negative, influence of LAB on therapeutic effects. Therefore, this study examines the ability of bacterial proteins to react with human IgE obtained from allergic patients.

Methods

A strain representing one of the most common LAB species (*Lactobacillus casei*) used in the food industry was selected. Cells of *L. casei* LcY were disrupted and separated into surface proteins (SP), cytoplasmic proteins (CP) and proteins of the cell wall/membrane (CMF) fractions. Immunoblotting of the bacterial protein fractions revealed that two proteins from CMF reacted with pooled human sera characterized by high IgE levels, although no reactions with sera of healthy people were found.

Results

The proteins were identified by mass spectrometry as cyclopropane-fatty-acyl-phospholipid synthase and carboxylate-amine ligase. *In silico* analyses of the potential allergenic properties of the proteins showed that they are not cross-reactive proteins with already known allergens, but have predicted B-cell linear epitopes that may be responsible for initiating sensitization.

Conclusions

To the best of the authors' knowledge, this study describes for the first time the human IgE-reactive specific proteins of lactic acid bacteria.

SIMULATED DIGESTION PROCESS REDUCED COW'S MILK PROTEINS IMMUNOREACTIVITY LOWERED DURING FERMENTATION OF BUTTERMILK

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Objective

The proteolytic activity of lactic acid bacteria (LAB) is vital for their growth in the food matrix and differs significantly among strains. Moreover, the proteolysis, which is an effect of bacterial enzymatic activity, provides essential components that determine the sensory qualities of products and may also change immunoreactivity of milk proteins.

Methods

Analysis of immunoreactivity of α-la, β-lg, α-casein, β-casein, κ-casein and bovine serum albumin and lactoferrin was investigated with competitive ELISA method after fermentation of sweet buttermilk with *L. casei* LcY and *L. delbrueckii* subsp. *bulgaricus* 151. A three-stage simulated digestion process (pooled saliva – pepsin – pancreatin with bile salts) was conducted on the fermented buttermilk products. Presence of immunoreactive milk proteins was analyzed applying immunoblotting of fermented buttermilk products with human allergic sera and detection of IgE reactive milk proteins.

Results

The immunoreactivity of buttermilk fermented with both tested strains was significantly ($P<0.001$) reduced for all tested proteins α-la, β-lg, α-casein, β-casein, κ-casein, bovine serum albumin and lactoferrin. The immunoblotting of fermented buttermilk with human allergic sera revealed α-casein as the only allergenic milk protein. Moreover, the simulation digestion process further lowed (up to 20 times) the immunoreactivity of milk proteins.

Conclusions

This study shows that a fermentation of buttermilk with *L. casei* LcY and *L. delbrueckii* subsp. *bulgaricus* 151 strains can be successfully applied for lowering of the immunoreactivity of sweet buttermilk proteins.

EFFECTS OF L.RHAMNOSUS GG ON FOOD ALLERGY-RELATED MITOCHONDRIAL DYSFUNCTION: EVIDENCES FROM A MICE MODEL OF PEANUT ALLERGY

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Objective

Immune function and mitochondrial activity are related. Mitochondrial dysfunction plays a role in the pathogenesis of asthma. We aimed to see whether if these features are present also in food allergy, and if they could be modulated by a nutritional intervention with an extensively hydrolyzed casein formula containing the probiotic *L.rhamnosus* GG (EHCF+LGG).

Methods

4-week-old female C3H/HeOuJ mice were sensitized by oral route with five weekly doses of peanut extracts plus cholera toxin as adjuvant in the presence or absence of a 14-day pre-treatment with EHCF+LGG. Liver mitochondrial respiration rates were evaluated polarographically in isolated mitochondria in the presence of succinate or palmitoyl-L-carnitine using the Clark electrode, soon after oral food challenge. The carnitine-palmitoyl-transferase (CPT) and aconitase activities were measured spectrophotometrically. H_2O_2 yield was assayed by following the linear increase in fluorescence due to the oxidation of homovanillic acid in the presence of horseradish peroxidase.

Results

We found in sensitized mice a lower state 3 respiration rate in presence of succinate and decreased fatty acid oxidation than controls (-36%, $p<.05$), although no difference in CPT activity was observed. Moreover, an increased oxidative stress in sensitized group was proven by inactivation of aconitase activity (-25%, $p<.05$) and higher H_2O_2 yield (+52%,$p<.05$). Pre-treatment with EHCF+LGG exhibited an improvement of mitochondrial function (+85%, $p<.05$) and redox state (-57%,$p<.05$) if compared to sensitized group. No changes on CPT activity was observed in mice receiving EHCF+LGG.

Conclusions

Food sensitization induces mitochondrial dysfunction and increases oxidative stress at liver level. EHCF+LGG efficiently prevents these effects.

CHANGES IN GUT MICROBIOTA IN CHILDREN AFTER VIRAL GASTROENTERITIS

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Objective

The aim of the study to identify changes in the microbiota in children after rotavirus, norovirus and mixed rota-norovirus gastroenteritis.

Methods

The study involved 40 children aged 1 – 7 years with severe rotavirus (20 children, group I), norovirus (10 children, group II) and mixed rota-norovirus (10 children, group III) infections. All patients received combined therapy including infusion, enzymes, probiotics including *Enterococcus faecium* and *Bifidobacterium longum* (Bifiform, Denmark). Violations of the intestinal microflora were investigated on 21st of disease day using bacterial seeding method and RT-PCR "colonoflor" in faeces. Hydrogen breath analyzer Gastro Gastrolyzer (Bedfont, United Kingdom) was used for bacterial overgrowth syndrome (BOS) detection.

Results

Changes in gut microbiota were detected in 31 (77,5%) samples: 17 (85%) from group I, 4 from group II, 9 from group III. Patients without gut microbiota changes had high levels of Faecalibacterium prausnitzii and low *Bacteroides fragilis/Faecalibacterium prausnitzii* ratio. Overgrowth of *Klebsiella pneumoniae* (42,5%), *Staphylococcus aureus* (27,5%) or *Pseudomonas aeroginosa* (22,5%) was detected in most of cases (57,5%). In 20% of patients overgrowth was accompanied by reduction of normal symbiotic microflora. Clostridium difficile was found in 1 sample by RTR-PCR. BOS was diagnosed in 4 children (10,0%).

Conclusions

The most significant violation of the microbiota were observed in children with a mixed rota-norovirus infection. Study of intestinal microbiota using various methods can lead to more accurate assess of the degree of impairment, as well as contribute to a more adequate designation corrective therapy.

GUT MICROBIOTA PROFILING IN PEDIATRIC INFLAMMATORY BOWEL DISEASE

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Objective

Colonic dysbiosis contributes to inflammation in inflammatory bowel disease (IBD), characterized by decreased prevalence of protective bacteria and concomitant increasing in harmful bacteria. Aim: to investigate gut microbiota composition.

Methods

Thirty five patients (age range 3-21 years) were enrolled at Pediatric Hospital Bambino Gesù, Rome; 21 IBD (5 CD, 16 UC): 13 mild-moderate disease activity, according to PCDAI-PUCAI and histology, 8 remission, 14 healthy age matched controls (CTRLs).

For each patient, fecal samples were collected by aspiration during colonoscopy and were pyrosequenced for microbiome analysis. Bowel preparation was with polyethileneglycole, the day before; 6/35 with disease activity were in metronidazole or ciprofluoxacine; 3/35 (2 disease activity) in probiotics and 7/35 (4 disease activity) had cow's milk protein free diet.

Results

A total of 115,242 sequencing reads were obtained from a total of 35 samples. Dominant phyla were Actinobacteria, Bacteroidetes, Firmicutes, Verrucomicrobia, Proteobacteria and Tenericutes, with a predominance of Firmicutes, Bacteroidetes and Proteobacteria in IBD patients. Kruskal-Wallis analysis showed statistically significant differences in OTUs relative abundance for *Eubacterium dolichum*, Lachnospiraceae, *Dialister*, *Erysipelotrichaceae*, *Ruminococcus gnavus*, Gemellaceae, Epulopiscium, Clostridiaceae. Gemellaceae, prevalently associated to disease activity with a remission pattern intermediate between activity phase and CTRLs ($p<0.05$). Sequence β-diversity, assessed by unweighted unifrac algorithm, showed clear clustering of the disease acivity-linked sequences.

Conclusions

These preliminary data depict composition, ecological diversity, and "enterogradient"-like patterns of gut microbiota of IBD pediatric patients *vs.* CTRLs. The analysis showed no statistically significant differences in relative abundance at phylum and family/genus level for patients' and CTRLs groups, while statistical significance was reported for stratified patients under disease activity and remission *vs.* CTRLs at family/species level.

We are waiting for biopsy *vs.* fecal microbiota evaluation and co-correlation between antibiotic and probiotic administration *vs.* OTUs panel variation in order to fulfill IBD-related microbiota profiling.

PROBIOTIC POTENTIAL OF A HIGH GABA PRODUCING STRAIN, LACTOBACILLUS BREVIS FEM 1874, ISOLATED FROM TRADITIONAL "WILD" ALPINE CHEESE

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In the last years it has been shown how probiotic may potentially impact on neuropsychiatric conditions, by modulating brain functions via the gut:brain axis. γ-aminobutyric acid (GABA) is a major inhibitory neurotransmitter which also works in the periphery through immuno-modulation and regulating adipocyte function. Importantly, GABA is produced in high amounts by certain lactobacilli. In *Lactobacillus brevis* ATCC 367, GABA production depends on the activity of two genes *gadA* and *gadB*, expressed as an operon, regulated by *gadR*, as well the presence of an antiporter *gadC. L. brevis* FEM 1874 was isolated from a traditional "wild" fermented Alpine cheese and characterized for putative probiotic traits. This novel strain is bile salt hydrolase (BSH) positive, carries *gad* genes and is able to produce high levels of GABA in pure culture compared to over 100 other local cheese isolates. We studied this strain in terms of growth on different prebiotic substrates, ability to survive upper gut acid and bile challenges and genetically characterized its GABA production related genes. *L. brevis* FEM 1874 appears to be a strain with promising probiotic properties and might be an important adjuvant in production of functional dairy products. However, further investigations are needed to assess whether this strain produces GABA within the gastrointestinal tract and how this may impact on host immune system and possibly brain function.

PROBIOTICS PERSISTENCE IN GUT MICROBIOTA OF ALLERGIC CHILDREN ADDRESSED BY SYSTEMS BIOLOGY BASED PROFILING

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Objective

Gastrointestinal (GI) tract is a complex ecosystem consisting of more than 10^{14} bacteria. Microbiota plays an important role in health and it is also linked to the risk of GI diseases as inflammatory bowel diseases, irritable bowel syndrome, celiac disease and allergies, like asthma and eczema. A new approach in the treatment of allergies is addressed to regular consumption of probiotics that induce restoring intestinal permeability, reinforcing gut immunologic barrier functions, and reducing pro-inflammatory cytokines. Therefore the correct identification and enumeration of probiotic bacteria is necessary to investigate the viability and activity of these microbes in the gut microbiota.

Methods

The aim of this work was evaluate the probiotic administration effects and the metabolic alterations on gut microbiota of allergic patients (es. atopic dermatitis, and food allergies) in early infancy, by systems biology driven approaches consisted in genomics (Real-Time-PCR), metagenomics (pyrosequencing) and metabolomics (GC-MS/SPME).

Results

The metagenomic analysis showed a clear separation between healthy children (CTRL) and allergic patients. Within the patients some differences were detected among subjects during probiotic administration period, in particular Proteobacteria and Bacteroidetes were prevalent in the patients, while Firmicutes were higher in CTRL. Moreover, RT-PCR experiments discriminated *Bifidobacterium* spp. and *Lactobacillus* spp. By GC-MS/SPME analysis, around 200 volatile organic compounds were detected and the level of esters, alcohols and aldehydes were higher in patients respect to CTRL.

Conclusions

Through the omic approach is possible correlate the gut modulation to allergic diseases, in order to improve the clinical condition of patients, through "systems medicine" models.

DEVELOPMENT AND APPLICATION OF MULTIPLEX PCR, DNA BIOCHIP AND REVERSE TRANSCRIPTION REAL-TIME PCR METHODS FOR THE DETECTION OF IMPORTANT LACTIC ACID BACTERIAL STRAINS IN COMMERCIAL FERMENTED MILK

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Lactic acid bacteria (LAB) play an important role in food fermentation processes. Fermented milk starters are often combined with multiple LAB strains with specific characteristics in terms of flavor or health properties. The presence of multiple and closely related species in these products make the differentiation and enumeration of those probiotic strains difficult, due to similarity in growth requirements and overlapping biochemical profiles of the species. Recent reports have shown that the identity of recovered microorganisms does not always correspond to the information stated on the product label. The aim of this study was to develop rapid, reliable and easy methods based on multiplex PCR, DNA biochip and Real-Time quantitative reverse transcription-PCR(RT-rtqPCR) methods to differentiate among important lactic acid bacterial strains. In this study, we used the groESL, heat shock protein and tuf gene to design multiplex PCR, DNA biochip and RT-rtqPCR methods for the detection of important lactic acid bacterial strains in commercial fermented milk. Primer sets for the detection of Lactobacillus acidophilus, L. brevis, L. casei, L. actobacillus delbrueckii subsp. bulgaricus, L. fermentum, L. plantarum, L. reuteri and L. sakei were tested with target bacterial strains. The primer sets used in this study generated amplicons with expected sizes. In this result, we have shown the identity of recovered Lactobacillus spp. correspond to the information stated on the product label. The multiplex PCR and DNA biochip for the detection of L. acidophilus(A), Bifidobacterium animalis subsp. lactis(B), Bifidobacterium longum subsp. longum(B), L. delbrueckii subsp. bulgaricus(L) and Streptococcus salivarius subsp. thermophilus (S) were developed. DNA biochip demonstrated that the hybridization patterns are in compliance with designed patterns either from single target or multiple target bacterial strains in fermented milks. Eleven fermented milks products available in markets were assayed for the presence of ABLS and the total cell counts of Lactobacillus spp., Bifidobacterium spp. and S. thermophilus. Total culturable counts of Lactobacillus and S. thermophilus determined with MRS agar plates were $6.09 \pm 2.33 \times 10^7$ CFU/mL and total bifidobacteria counts determined with MRS agar containing 0.05% L-cysteine plates were between $6.12 \pm 2.67 \times 10^7$ CFU/mL. The viable cell counts and bacteria species for LAB in fermented milks were corresponded to those labeled on the products and the Chinese National Standards (CNS) 3058 standard. Identification of species is a important issue to be verified for the compliance of fermented milk with the required product specifications in terms of accurate species labeling and, if appropriate, to support health claims that could be associated with added probiotics. In this study showed that DNA biochip and RT-rtqPCR has better detection and identification for verification of accurate bacterial counts and species labeling in fermented milk.

VAGINAL COLONIZATION OF LACTOBACILLUS ACIDOPHILUS LA-14 AND LACTOBACILLUS RHAMNOSUS HN001 AFTER ORAL ADMINISTRATION IN HEALTHY WOMEN

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Objective

A balanced microbiota is essential for the maintenance of healthy vaginal environment. Lactobacilli spp. have been tested for their effectiveness in preventing bacterial vaginosis (BV). Lactoferrin is an iron binding glycoprotein present in secretions of mammals including milk, which exerts both immunomodulating and antimicrobial activities.
The aim of the current double blind, randomized, placebo controlled study was to determine if oral consumption of a combination of two probiotics (*L. acidophilus* La-14 and *L. rhamnosus* HN001) with bovine lactoferrin would lead to the detection of the consumed probiotic strains in the vagina of healthy women.

Methods

Forty healthy women were administered one capsules of investigational product twice daily for 2 weeks. Vaginal swabs were collected at 0, 1, 2 and 3 weeks and analyzed by PCR.

Results

The current study showed that vaginal *L. rhamnosus* HN001 and *L. acidophilus* La-14 levels were significantly increased. In particular, *L. acidophilus* significantly increased on days 14 and 21 while *L. rhamnosus* significantly increased on days 7 and 21.

Conclusions

The consumption of *L. acidophilus* La-14, *L. rhamnosus* HN001 in combination with bovine lactoferrin leads to vaginal detection, even 1 week after consumption was stopped. The results from the current clinical trial show for the first time the capability of orally consumed selected lactobacilli strains to reach and colonize the vagina. Our study highlight the potential use of a mixture of *L. acidophilus* La-14, *L. rhamnosus* HN001 in combination with bovine lactoferrin for the management of urogenital tract infections and contribute to vaginal health.

IN SILICO APPROACHES FOR THE IDENTIFICATION OF PUTATIVE BACTERIOCIN GENE CLUSTERS FROM THE HUMAN MICROBIOTA

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Objective

The gut is a rich source of antimicrobial-producers with the potential to alter intestinal communities in a beneficial way for human health. With this in mind, several studies have used traditional culture-dependent approaches to successfully identify bacteriocin-producers from the mammalian gut. Here we present alternative *in silico* techniques with the aim to detect potential bacteriocin-encoding gene clusters using genomic and metagenomic data from the gastrointestinal subset of the human microbiome project and compare the density of these clusters to other body sites.

Methods

In silico strategies to identify novel gene clusters are now also being utilised to take advantage of the vast amount of data currently being generated by next generation sequencing technologies, usually in the form of a BLAST-based approach. This poster presents alternative approaches including a Profile Hidden Markov Model pipeline and other freely-available genomic tools, such as BAGEL3, that can be applied to both genomic and metagenomic data.

Results

These techniques have resulted in the identification of numerous putative bacteriocin gene clusters from the human microbiota, including important members of the gastrointestinal tract microbiota that have not previously been associated with bacteriocin production.

Conclusions

These *in silico* techniques, and others, are a powerful tool for the identification novel biosynthetic gene clusters in a culture-independent method and have the potential to vastly improve our arsenal of microbiota-modulating probiotics.

LACTOBACILLUS BREVIS FEM1874 FOR THE DEVELOPMENT OF GABA-ENRICHED CHEESE

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Probiotic microorganisms have recently been shown to impact on brain development and function through the gut:brain axis. *Lactobacillus brevis* FEM1874 isolated from Traditional Mountain cheese has been reported produce high concentrations of gamma-aminobutyric acid (GABA) and to possesses Bile Salt Hydrolysis activity *in vitro*. GABA is synthesized from glutamate which is the most common amino acid in cheese. The aim of this study was to test the ability of the strain to convert glutamate to GABA during cheese production. Twenty experimental micro-cheeses were produced using a commercial starter strain (10^7 CFU/mL) and FEM1874 as adjunct culture. Four different concentrations (10^2, 10^3, 10^4, 10^5 CFU/mL) of FEM1874 were tested in quadruplicate. In order to follow the microbial evolution, samples of milk, curd and cheese after 20 days of ripening were enumerated in selective media. The control and experimental samples showed a similar trend, suggesting that both milk-resident and starter strains grew during ripening. However, the load of mesophilic lactobacilli in all experimental curd samples was higher than the control. The concentration of GABA and glutamic acid in cheese samples after 20 days of ripening was quantified by UHPLC-HQOMS. The amino acidic profiles showed that while the concentration of *Lb. brevis* FEM1874 in milk increased, the amount of both glutamic acid (from 284±97 to 202±44) and GABA (from 154±48 to 83±28) significantly decreased during cheese production. These results suggested that the experimental strain converted the glutamic acid to GABA, but that GABA may have subsequently been converted to succinate by GABA transaminases.

GUT MICROBIOTA META-OMICS CHARTS SUPPORTING CF PATIENTS' LABORATORY AND CLINICAL MANAGEMENT

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Objective

Cystic fibrosis (CF), is a disorder affecting the exocrine glands of the respiratory, digestive and reproductive systems and there appear to be a link with the gut microbiota, including a possible association with its dysbiosis. High-throughput meta-omics-based approaches may actually assist in unveiling this complex network of symbiosis modifications. The aim of this work was to investigate the gut microbiota composition and modulation of CF patients by omic approach.

Methods

Thirty-one faecal samples from either CF patients and healthy children (HC) (age range 0-6 years) were collected at Bambino Gesù Children's Hospital. The metabolomic analyses were performed by GC-MS/SPME and ^{1}H-NMR, while metagenomic analysis was carried out by 454 pyrosequencing platform.

Results

About 200 volatile organic compounds, 150 shared between HC and CF children and 50 belonged only to CF patients were detected and quantified by GC-MS/SPME and about 20 molecules characterized with ^{1}H-NMR.The inter-individual variability of molecules levels resulted high. The level of esters, alcohols and aldehydes were higher in CF patients. On the contrary, SCFA were higher in HC than CF. ^{1}H-NMR analysis, showed lower levels of amino acids and uracil in CF patients. Metagenomic indicated *Firmicutes* as most abundant phyla in HC, while the abundance of *Bacteroidetes* and Proteobacteria varied according to the sample analyzed.

Consclusions

By this integrated approach it's possible to generate personalized "omics" charts that can be used for the monitoring of the nutritional state of the child and for the evaluation of gut absorption in CF patients, hence provide a translational medicine tool.

GUT MICROBIOTA PROFILING IN NASH/NAFLD PATIENTS BY 454 PYROSEQUENCING AND GC- MASS SPECTROMETRY

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Objectives

Non-alcoholic fatty liver disease (NAFLD) and its more severe form (non-alcoholic steatohepatitis, NASH) are multi-spectrum diseases involving hepatic fat accumulation occurring in the absence of excessive alcohol intake.

The aim of this work was to investigate gut microbiota composition in term of OTU and metabolite abundances in 4 groups of subjects (NAFL [nr. 27], NASH [n. 26], Obese patients [n. 8], recruited at Pediatric Hospital Bambino Gesù of Rome and healthy age matched controls (7-16 years) [n. 54]).

Methods

All samples submitted to DNA extraction, 16S rRNA amplification and pyrosequenced by Next generation sequrncing platform. Also, facal metabolite was extracted and analyzed by Gas chromatography mass spectrometry (GC-MS).

Results

A total of 918,876 sequencing reads were obtained from a total of 115 samples. The majority of the sequences were assigned to six dominant phyla Actinobacteria, Bacteroidetes, Firmicutes, Verrucomicrobia, Proteobacteria and Tenericutes, with a predominance of Firmicutes and Bacteroidetes, respect to the others in all type of subject categories. A Kruskal-Wallis analysis indicated that Firmicutes and Actinobacteria were statistically prevalent in the patients than in the CTRL children and that the opposite was true for Bacteroidetes.

Different classes of volatile organic compounds (VOCs) such as *i.e* ketones, aldehydes, indoles, alcohols, phenols, alkanes, alkenes, lactones, terpenes, esters, and sulfur compounds, in patients and compared to controls.

Conclusions

These data are able to depict composition, ecological diversity, and "enterotype"-like patterns of gut microbiota of NASH/NAFLD pediatric patients.

INULIN REDUCES PRODUCTION OF TMA DURING FERMENTATION OF RED MEAT BY THE HUMAN GUT MICROBIOTA IN VITRO

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Objective

Certain metabolites produced by the gut microbiota, like the biogenic amine Triethylamine (TMA) appear to increase the risk of cardiovascular disease upon colonic fermentation of choline or L-carnitine [1] [2]. Here, we investigated the ability of the prebiotic inulin to impact gut microbiota protein fermentation and production of potentially toxic metabolites using NMR based metabonomics and in vitro faecal batch cultures.

Methods

Fermentation of meat digested using an in vitro digestion process were conducted using 24 h pH-controlled anaerobic batch cultures and fresh faeces from 5 healthy human donors (n=5). For each donor, faecal fermentations of the negative control (no substrate), inulin (1% w/v), beef (0.5% w/v) and beef + inulin (1.5% w/v) were run over 24 h. Cell free supernatant samples taken at 0, 5, 10 and 24 h fermentation were analyzed with proton magnetic resonance spectroscopy (1H-MRS) and multivariate analysis (MVA) to determine the change in metabolite profiles. Fecal microbiota composition is being analyzed by fluorescent in situ hybridization and 16S rRNA community profiling.

Results

Metabonomic analysis suggested that inulin improved faecal microbiota protein metabolism, by increasing the consumption of amino acids, such as tryptophan and phenylalanine, but at the same time reducing the production of potentially toxic metabolites including TMA, 5-Aminopentanoate (a precursor of cadaverine) and phenol.

Conclusions

In the presence of inulin, fermentation of meat by the gut microbiota was associated with reduced production of toxic metabolites. This supports the notion that increased consumption of fermentable fiber or prebiotics like inulin may be one means of reducing the production of harmful metabolites by the gut microbiota [3].

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THE INFANTMET STUDY: CHARACTERISATION OF INFANT-DERIVED GUT MICROBES

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Objective

In terms of infant health, it is imperative to understand how early infant nutrition influences the development of a healthy gut microbiota. Thus, the INFANTMET project was set about to provide the scientific community with a greater understanding of what constitutes a healthy gut microbiome at birth and how marked changes which occur at birth can shape our microbial community in later life. The aim of the study was to examine the evolving composition and functionality of the intestinal microbiota in healthy full term infants exclusively fed breast milk. A unique bank of intestinally derived bifidobacteria and lactobacilli were isolated from healthy breast fed infants over the first year of life for their characterization and potential use as future probiotics in infant nutrition. Bacteriocins and exopolysaccharides are just two examples of valuable pharmabiotic substances which will be examined for their ability to confer a competitive advantage in a complex microbial environment and provide protection against the invasion of pathogenic bacteria.

Methods

Using traditional culture based techniques bifidobacteria and lactobacilli were isolated from infant faecal samples (taken at 1, 4, 8 and 24 weeks) and stored at - 80 ˚C. Screening for antimicrobial producing lactobacilli was carried out using *L. bulgaricus* LMG 6901 as an indicator strain and subsequent antimicrobial producers were analysed for bacteriocin activity in agar well-diffusion assays. Lactobacilli were also screening for exopolysaccharide-production using selective media supplemented with 5% sucrose. Subsequently, pulse field gel electrophoresis (PFGE) was used for the identification of each isolate at the strain level.

Results

Currently the most abundant *Lactobacillus* species identified in these infants throughout the first 24 weeks of life are *L. casei, L. rhamnosus* and *L. paracasei.* Out of a total of 600 Lactobacillus isolates characterised thus far, three have been identified as potential bacteriocin producers, as well as one EPS-producing *L. paracasei* strain. PFGE has also identified individual specific strains between infants, with resident strains identified throughout the first year of life.

Conclusions

Further investigation is necessary to determine survivability of these strains under different conditions and their ability to adhere to intestinal epithelium cells will be analysed. Results from this study will provide intestinally derived probiotic strains isolated from healthy breast fed infants with particularly advantageous properties suitable for infant nutrition and health.

GLYCOMACROPEPTIDE REDUCES INTESTINAL EPITHELIAL CELL BARRIER DYSFUNCTION AND INFECTION OF ENTEROHEMORRHAGIC AND ENTEROPATHOGENIC ESCHERICHIA COLI IN-VITRO

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In recent years, the potential of glycosylated food components to positively influence health has received considerable attention. Milk has proven to be a rich source of biologically active glycans. Bioactivities associated with milk derived glycans include antimicrobial, immunomodulatory, anti-adhesion, anti-inflammatory, and prebiotic. One such glycoprotein, Glycomacropeptide (GMP), can be isolated from κ-casein after hydrolysis with chymosin during cheese making.

Objective

In this study, GMP was investigated for its ability to inhibit the adhesion of a variety of pathogenic *E. coli* strains to HT-29 and Caco-2 intestinal cell lines.

Methods

The protective effects of GMP against *E.coli* infection were assessed using adhesion and transwell migration assays. **Results** GMP significantly reduced pathogen adhesion, albeit with a high degree of species specificity toward enteropathogenic *Escherichia coli* (EPEC) (O125:H32 and O111:H2) ($P<0.05$) and enterohemorrhagic *Escherichia coli* (EHEC) (12900 O157:H7) ($P<0.01$). The pre-incubation of intestinal Caco-2 cells grown as monolayers on Transwell inserts with GMP reduced pathogen translocation and barrier dysfunction as represented by a decrease in transepithelial electrical resistance (TEER).

Conclusions

As demonstrated in this study, GMP is an effective *in vitro* inhibitor of adhesion and epithelial injury caused by *E. coli* and has potential as a bio functional ingredient in foods marketed to improve gastrointestinal health.

KGLP-1-EXPRESSING PROBIOTIC IMPROVES DYSLIPIDAEMIA BUT NOT GLUCOSE METABOLISM IN A DIET INDUCED OBESE RAT MODEL

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Objective

The human gut hormone glucagon-like peptide 1 (GLP-1) is an insulinotropic peptide with potential as a therapy for type-2 diabetes mellitus and cardiovascular health. The aim of this study was to assess the efficacy of a recombinant probiotic synthesizing GLP-1 in attenuating weight gain and improving glucose and lipid metabolism in diet-induced obese rats.

Methods

Twenty Long-Evans rats were fed a high-fat diet for six weeks followed by a three week exposure to 109 CFU/rat/day of either *Lactobacillus paracasei* NFBC 338 transformed to express KGLP-1 - a long-acting analogue of GLP-1 - or the isogenic KGLP-1- control strain which solely expressed the pNZ44 plasmid. Animal weight gain, food and water intake were continuously assessed; body composition and glucose tolerance (OGTT) were assessed at the end of the treatment.

Results

While the strains survived gastric transit well, no alterations in satiety, weight gain, body composition or glucose tolerance was observed between the two groups. In addition, serum GLP-1 levels were not found to be elevated in the GLP-1+ group animals. However, serum LDL cholesterol and triglyceride levels of the KGLP-1+ group were ~20% lower than those of the control group. This led to improved TG:HDL and LDL:HDL levels in GLP-1+ animals.

Conclusions

These data suggests that the KGLP-1-expressing probiotic had a beneficial effect on lipid metabolism. KGLP-1 was not detected in the fasted serum, suggesting that the peptide acted on enteric cell GLP-1 receptors to modulate chylomicron formation and did not reach pancreatic β-cells in biologically relevant quantities.

EFFECTS OF DIETARY ADMINISTRATION OF GABA AND GABA-PRODUCING BACTERIA LACTOBACILLUS BREVIS DPC 6108 ON THE DEVELOPMENT OF DIABETES IN A STREPTOZOTOCIN RAT MODEL

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Objective

The aim of this study was to determine whether gamma-aminobutyric acid (GABA)-producing *Lb. brevis* DPC 6108 has the potential to attenuate or prevent type 1 diabetes (T1D) *in vivo.*

Methods

Sprague-Dawley rats (n=15/group) received an injection of streptozotocin (STZ) to induce T1D while the non-diabetic control group received an injection of citrate buffer vehicle only. Diabetic and non-diabetic control groups received placebo [4% (w/v) yeast extract in dH2O], while the other three diabetic groups received one of the following dietary supplements: 2.6 mg/Kg BW GABA (low GABA), 200 mg/Kg BW GABA (high GABA) or ~10^9 *Lb. brevis* DPC 6108 bacterial cells.

Results

STZ induced T1D decreased body weight ($p<0.05$), increased intestinal length ($p<0.05$), increased corticosterone levels ($p<0.05$), increased anxiety ($p<0.05$) and stimulated hyperphagia and polydipsia; features all unaffected by dietary interventions. Insulin and c-peptide levels were decreased ($p<0.05$), whereas glucose was increased ($p<0.001$) in all diabetic groups. Glucose was decreased ($p<0.01$) in diabetic rats receiving *Lb. brevis* DPC 6108, compared with diabetic-controls. Microbial composition and diversity were affected by T1D. Diversity in diabetic rats supplemented with low-dose GABA was not reduced ($p>0.05$), compared with non-diabetic controls, while all other diabetic groups showed reduced diversity ($p<0.05$).

Conclusions

Lb. brevis DPC 6108 attenuated high levels of glucose caused by diabetes. T1D affected microbial composition and diversity, however low dose GABA supplementation attenuated diabetes-associated reductions in microbial diversity. Intestinal microbiota and their metabolites appear to be involved in complex interactions in the gastrointestinal ecosystem which may alleviate diabetes, although the mechanisms are yet unclear.

USE OF NEW IN VIVO MODEL (GALLERIA MELLONELLA) TO EVALUATE THE EFFICACY OF TWO PROBIOTICS STRAINS AGAINST INTESTINAL PATHOGENS

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Objective

In vitro tests are not sufficient alone to assess the antimicrobial activity of probiotics, and in vivo models are required to provide supplemental data. In this study, we evaluated the wax moth larva *Galleria mellonella* as an in vivo invertebrate model to determine the inhibition of the enteropathogens *Salmonella tiphymurium and Listeria monocytogenes* by two probiotic strains of *Clostridium butyricum* and *Lattobacillus rhamnosus,* respectively.

Methods

In vivo tests. Different concentrations of each probiotic strain were injected into groups of 20 *G. mellonella* larvae 2 h before the inoculation of the lethal concentrations of each enteropathogen. The number of dead and survived larvae was recorded daily.
In vitro tests. The inhibitory activity of the probiotic strains of *C. butyricum* or *L. rhamnosus* was evaluated using the agar spot test.

Results

The probiotic strains of *C. butyricum* and *L. rhamnosus* increased the survival of *G. mellonella*, with approximately 30% and 50% of the larvae inoculated with either *S. tiphymurium* or *L. monocytogenes*, respectively, being still alive at the end of the experiments.
Both probiotic strains showed a good *in vitro* inhibitory activity *vs* each of the two enteropathogens.

Conclusions

In conclusion, we have demonstrated that the probiotic strains of *C. butyricum* and *L. rhamnosus* exerted a good protective effect against the enteropathogens *S. tiphymurium* and *L. monocytogenes* in the *G. mellonella* in vivo model. Thus, the new larva model can be a useful preliminary model in addition to the in vitro tests for assessing the antimicrobial efficacy of probiotics.

CHARACTERIZATION OF PROBIOTIC PROPERTIES IN HUMAN VAGINAL LACTOBACILLI STRAINS

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Objective

To characterize vaginal lactobacilli for their probiotic properties and to compare the probiotic potential.

Methods

The *Lactobacillus* strains were isolated from vaginal samples by conventional cultivation and sequencing of the 16S rDNA fragment. We detected the production of hydrogen peroxide H2O2 by the tetramethyl-benzidine-peroxidase assay and lactic acid by gas chromatography, antagonistic activity against *E. coli, Candida* and *Gardnerella vaginalis* strains with modified methods. The auto-aggregation and safety of *Lactobacillus* strains by detection of haemolytic activity, antibiotic susceptibility, and presence of certain resistance genes (*tet, erm*).

Results

A total of 135 vaginal lactobacilli belonged to *Lactobacillus crispatus* (56%), *Lactobacillus jensenii* (26%) and *Lactobacillus gasseri* (18%). Most of *L. crispatus* (89%) and *L. jensenii* (86%) strains produced H2O2. The best lactic acid producers were L. gasseri compared to *L. crispatus* and *L. jensenii* ($p<0.0001$; $p<0.0001$, respectively). *L. crispatus* strains showed significantly higher anti-*E. coli* activity compared to *L. jensenii*. *L. gasseri* strains expressed significantly lower anticandidal activity. There was no significant difference in antagonistic activity against *G. vaginalis strains*. None of the tested lactobacilli caused haemolysis. Twelve lactobacilli strains were able to autoaggregate. Although phenotypical resistance was not found to ampicillin, chloramphenicol, erythromycin, gentamycin, tetracycline and vancomycin, *erm*(B), *tet*(M) and *tet*(K) were detected. All strains were resistant to metronidazole, trimethoprim/sulfamethoxazole and kanamycin.

Conclusions

Our study revealed *L. crispatus* as the most frequent species in vaginal samples. Moreover, *L. crispatus* strains produced H2O2 and had antagonistic activity against *E. coli* and *Candida* spp. Therefore a potential probiotic candidate could be found among *L. crispatus.*

IN VITRO DETERMINATION OF FUNCTIONAL ACTIVITY OF HEMICELLULOSE B FRACTION FROM SANTALUM ALBUM L. AS A CANDIDATE PREBIOTIC MOLECULE

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Objective

To determine the prebiotic efficacy of the highly branched hemicellulose B fraction from *Santalum album* L. to confirm the role which plant cell wall may play, in development of novel prebiotics as a source of diverse polysaccharides with complex structures.

Methods

Cell wall material from Sandalwood suspension culture was fractionated by sequential extraction with imidazole, followed by Na_2CO_3, and finally with KOH. The hemicellulose-rich alkaline fraction was neutralized and alcohol precipated to obtain the fraction of interest. Prebiotic activity was assessed by measuring the growth (cfu mL^{-1}), and O.D after 24 h of growth of the probiotic strains on 1% of the lyophilized and characterized polysacchaide or 1% glucose, relative to the change in the aforementioned parameters of *E.coli* ATCC 25922 grown under same conditions. *Lactobacillus rhamnosus* ATCC 7469 and *Lactobacillus acidophilus* MTCC 10307 were the probiotics used.

Results

The fraction of interest, chemically xylan and mannan in nature, was used as the substrate. For *L. rhamnosus* the highest score obtained was on Inulin (1.005), followed by that on candidate prebiotic (0.72) and on FOS (0.695). For both *L. acidophilus* and *L. rhamnosus*, growth on the saccharide was akin to that on glucose, as there were no marked differences when compared statistically. The growth of pathogenic *E. coli* was significantly less on hemicellulosic fraction ($p<0.05$) in contrast to that on glucose, monitored over a time span of 48 h.

Conclusions

Cell wall carbohydrates, especially hemicelluloses, owing to its heterogeneity, can potentially bring novelty in the domain of prebiotics research.

THE INFLUENCE OF ESCHERICHIA COLI NISSLE 1917 ON NATURAL COMPOUNDS WITH ANTIOXIDANT PROPERTIES

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Objective

Intestinal microorganisms have an effect on the health of the host, positive or negative, depending on properties of the bacterial strain. The mechanism of action of a bacterial strain can be different (ranging from an effect on metabolism of xenobiotics to an influence on enzymes). The aim of our study was to find whether probiotic strain of *E. coli* Nissle 1917 (EcN) has an effect on natural compounds with antioxidant properties.

Methods

Our preliminary *in vitro* study was performed with a live bacterial suspension of EcN (10^8 CFU/ml) and with silymarin (solution of 24 µg/10 µl) or with 50 µM solution of quercetin. Probiotic strain of EcN has been incubated with added substrate for 24 h. Also, three control samples (only bacterial suspension of EcN, the medium, or the medium with substrate) were incubated for 24 h. Then, all samples were centrifuged, processed and measured by LC/MS.

Results

In case of silymarin as a substrate added to EcN, the increased levels of taxifolin have been measured in comparison with control samples. Taxifolin is a silymarin compound present there in very low concentrations. After incubation with EcN, its concentration has been increased by 63 %. In case of quercetin, two hydroxylated metabolites of quercetin have been determined in comparison with control samples.

Conclusions

Our preliminary results suggest that bacterial strain of EcN causes a cleavage of glycoside from taxifolin glycoside and therefore causes a formation of free taxifolin compound. Moreover, EcN have also an ability to form hydroxylated metabolites of quercetin.

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FAECAL TRANSPLANTATION FOR MICROBIOTA RECOLONISATION

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Objective

Driven by rapid advances in molecular technology, the description of new taxa has been occurring at an accelerated rate, resulting in a better understanding of the relationships between microbes and host physiology. However, many cultivation strategies remain insufficient with respect to growing obligate anaerobes from the gastrointestinal tract. As research into the advantages of faecal transplantation continues, developing a defined community of microorganisms that represents the indigenous microbiome of healthy individuals has become increasingly important. Therefore, the objective of this study was to employ strictly anaerobic, cultivation-dependent methods in order to study how previously uncultivated members of the gut influence their environment.

Methods

To isolate fastidious anaerobes, stool samples were used as inocula under anoxic conditions. Variables such as substrate availability, pH, salinity, temperature and gaseous head-space were altered, and strict anaerobic culture techniques were employed in order to recover these difficult to grow microorganisms.

Results Sequencing of PCR-amplified small subunit ribosomal RNA (16S rRNA) genes from pure culture isolates revealed identities relative to the nearest cultivated phylogenetic neighbour. These cultivars are undergoing further characterisation with respect to key physiological traits known to confer health benefits and/or mitigate deleterious processes.

Conclusions

The gut microbiome harbours many previously unclassified microorganisms. Targeted anaerobic cultivation has generated an evolving inventory of isolates through which physiological characteristics can be exploited to improve human conditions. Health-associated microbes can now be combined into complex and defined mixtures, making standardisation of donor stool communities possible for those who would benefit from faecal transplantation.

CONSUMPTION OF THE HUMAN MILK STRAIN BIFIDOBACTERIUM BREVE CECT7263 MIGHT IMPROVE SYMPTOMS OF INFANT COLIC

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Objective

To evaluate the effects of the consumption of an infant formula supplemented with the probiotic strain *B.breve CECT7263* in neonates from 1 to 12 months of life.

Methods

A randomized double blinded controlled study including 154 infants at the age of 1 month was conducted. Infants were assigned randomly to either infant formula supplemented with *B. breve*, or the same formula without the probiotic strain. The primary outcome of the study was the growth of infants. Secondary outcomes were fecal concentration of Short Chain Fatty Acids (SCFA), flatulence, regurgitation and bouts of crying. This study was carried out according to the Helsinki declaration, and the protocol was approved by the Regional Ethics Committee.

Results

The z-scores of weight, length and head circumference for age were calculated based on the WHO Child Growth Standards and no significant differences between groups were observed. The population of the study did not differ from the standard and no significant differences were detected between the groups of the study. No significant differences were observed in the odds to suffering flatulence or regurgitation however infants receiving probiotic formula had lower risk of having colic than those receiving standard formula (OR = 0.569; $p<0.001$). Similar concentrations of SCFA were observed in both groups.

Conclusions

The consumption from 1 to 12 months of life of an infant formula enriched with the human milk probiotic strain *Bifidobacterium breve* CECT7263 is safe. Furthermore, the consumption of this probiotic strain might improve symptoms of infant colic.

REDUCED ATOPIC SENSITIZATION IN BABIES BORN BY CAESAREAN SECTION SUPPLEMENTED WITH LACTOBACILLUS RHAMNOSUS GG

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Background
Children born by Caesarean section (CS) have less lactobacilli in faecal microflora and therefore have a higher risk of atopic sensitization and allergy compared with naturally delivered children. Perinatal supplementation of probiotics including lactobacilli to mothers and CS babies of atopic parents reduces the development of allergy. However, also babies of non-atopic parents can develop allergy and need prevention.

Objective
To reduce the risk of atopic sensitization/allergy with *Lactobacillus Rhamnosus GG* (LGG) supplementation in the first three months of life after CS.

Methods
157 babies were born by CS from January 2010 to October 2014: 75 received 5 billion daily LGG supplementation from 1 to 3 months (Group A) and 82 did not because enlisted after the age of 3 months (Group B). 274 babies naturally delivered was the control Group C. Health visits were done in Paediatric Primary Care at 1, 3, 6, 9, 12, 24, 36 months, Skin Prick Test (SPT) at 6, 12, 24, 36 months.

Results
Babies with at least one SPT+ were 8% in Group A vs. 19.5% in Group B (Fisher test p=0.0414). Most of them was born from non-atopic parents. Babies SPT+ in Group C were 13.1%. Allergy developed in 2.7%, 4.9% and 5.8% of the babies respectively.

Conclusions
LGG given in the first 3 months of life to CS babies can reduce the risk of atopic sensitization until 3 years of age. Follow up research will continue until 6 years to assess the potential of LGG to prevent allergic diseases.

CAN 2 APPLES A DAY DECREASE CVD RISK AND MODULATE THE GUT MICROBIOME IN MILDLY HYPERCHOLESTEROLAEMIC SUBJECTS?

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Objective
Apples are a rich source of polyphenols and fiber. An important proportion of these bioactive components escape digestion in the upper intestinal tract and reach the colon where they can be transformed by the gut microbiota (1). A randomized, controlled, crossover, dietary human intervention study was performed (AVAG study) to test the hypothesis that 2 apples a day can beneficially modulate the gut microbiome and cardiovascular health in mild hypercholesterolaemic subjects.

Methods
Forty volunteers, (23 women, 17 men) with a mean BMI 25.3±3.7 kg/m2 and age 51±11 years, consumed 2 apples (Renetta Canada variety) or 100 ml of a sugar matched control apple drink (containing no fibre and low polyphenols) daily for 8 weeks in a random order separated by a 4-week washout period. Blood, urine and faecal samples were collected before and after each treatment. A high resolution LC-MS metabolomics with minimal sample cleanup was performed using an LTQ Orbitrap XL mass spectrometer to identify putative biomarkers. Changes in faecal populations were identified using fluorescence in situ hybridization (FISH).

Results
Preliminary results show a significant diet interaction for total cholesterol (TC) (P=0.04) and a trend for vascular cell adhesion molecule-1 (VCAM-1) (P=0.076). Metabolomics analysis have identified a number of dose dependent biomarkers – including various microbial classes of apple polyphenols. FISH analysis indicates a small change in selected bacterial groups and a 16S rRNA community profiling is currently being conducted.

Conclusions
Consuming 2 apples a day may beneficially affect cardiovascular health and modulate microbial composition and metabolic output.

References
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THE INFLUENCE OF MULTIPROBIOTIC ON GASTRIC ACID SECRETION IN RATS IN CONDITIONS OF LONG-TERM HYPOACIDITY

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Objective

It is known that long-term hypoacidity of gastric juice is risk factor for carcinogenesis in stomach and leads to dysbiosis which accelerates the development of neoplastic changes in stomach. The aim of the study was to investigate effect of multiprobiotic "Symbiter® acidophilic" concentrated" (Symbiter) as drug for prophylaxis of dysbiosis, on changes in gastric acid secretion (GAS) evoked by GAS blocker omeprazole (OM).

Methods

The rats were divided into 3 groups. The rats of the 1st (control) group were injected with 0.2 ml water (intraperitoneally (i.p.)) and 0.3 ml water per os once a day during 28 days. The rats of the 2nd group were injected with OM (14 mg/kg, i.p.) during 28 days. The rats of the 3rd group were injected with the same dose of OM and Symbiter (0,14 mg/kg, per os) (the firm "O.D. Prolisok") during 28 days. In a day after last injection of drugs by method of perfusion of isolated stomach (Ghosh, Shild, 1958) we investigated GAS stimulated by pentagastrin (26 mkg/kg) and histamine (3 mg/kg).

Results

It was established that after 28 days of OM injection GAS stimulated by pentagastrin and histamine decreased in 2 ($p<0,01$) and 1.4 ($p<0,01$) times respectively in comparison with control. In a day after 28 days of simultaneous injection to the rats OM and Symbiter GAS stimulated by pentagastrin and histamine was at a level controls.

Conclusions Positive effect of Symbiter on GAS is the result of the dysbiosis elimination, reduce IFN-gamma in blood serum which cause microorganisms of Simbiter (Pilipenko, 2015).

THE INFLUENCE OF DIET ON THE OCCURRENCE OF BIFIDOBACTERIA AND LACTOBACILLI

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Among the GIT microbiota, bifidobacteria and lactobacilli represent important commensal groups. Diet is a major factor driving the composition and metabolism of the colonic microbiota. The diet of vegetarians contains amount of plant derived polysaccharides compared to the conventional protein-rich diet. Substrate preferences of bacteria are often species-specific. Therefore, we can expect a different species occurrence of bifidobacteria and lactobacilli in the faeces of people on a different diet.

Occurrence of bifidobacteria and lactobacilli in faecal samples of donors on conventional diet (10) and vegetarians (10) was determined using cultivation on selective media. Totally 400 isolates selected based morphology and microscopic characteristics were identified using the MALDI TOF MS method.

In the faecal samples of donors on conventional diet, bifidobacteria and lactobacilli were detected in counts of 9.36±0.57 and 5.00±1.17 log CFU/g of faeces, respectively. Similar results were detected in vegetarians in counts of 9.62±0.35 log CFU/g of bifidobacteria and 4.66±1.15 CFU/g of lactobacilli. The statistical difference between these groups was not significant.

Totally 6 species of the genus *Bifidobacterium* were detected. The obtained data showed that the species composition of bifidobacteria is relatively stable depending on the diet. In the digestive tract of tested groups *B. adolescentis* and *B. longum* commonly occurring in adults were mostly detected. While 13 different species of the genus *Lactobacillus* were identified. The occurrence of lactobacilli species was found more varied depending on the diet of individuals without differences specific to the tested groups.

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ANALYSIS OF THE DURABILITY OF THE BENEFICIAL EFFECTS OF LACTOBACILLI ON MICE FED HIGH FAT DIET

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Objective

Western life style, and high calorie diet in particular is causing major health problems such as insulin resistance, hepatic steatosis and heart disease in modern times. High fat diet induces similar changes in mice, such as increased body weight, hipercholesterolemia and accumulation of triglycerides in the liver. These changes can be ameliorated to some extent by the administration of *Lactobacillus* species. The focus of this study was to test whether the beneficial effects of lactobacilli on mice fed high fat diet, such as lowering of cholesterol levels, and reduction of triglyceride accumulation in the liver are long lasting.

Methods

Mice on high fat diet were given *Lactobacillus plantarum* WCFS1 or *Lactobacillus rhamnosus* LA68 for three months followed by a two month wash out period. Mouse sera was collected and various parameters analyzed. Liver tissue was analyzed for triglyceride levels.

Results

Upon the completion of wash out period mouse weight was still significantly lower in the supplemented groups compared to the high fat diet group, but total cholesterol, HDL and LDL, insulin and leptin levels were increased in all experimental groups on high fat diet, where as adiponectin level was lowered. Trigliceride level in the spleen was elevated in all groups on high fat diet.

Conclusions

Our results show that the beneficial effects of Lactobacilli on total body weight are more durable than the effects on blood cholesterol, and that the mice which have received probiotics although leaner, are not healthier than their counterparts on high fat diet only.

THE EFFECT OF LACTOBACILLUS HELVETICUS L10 SUPPLEMENTATION ON RESPIRATORY INFECTIONS AND MUCOSAL IMMUNITY IN ELITE ATHLETES

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Objective

The aim of this study was to evaluate if *Lactobacullus helveticus L10* supplementation during 14 weeks in winter can influence the duration, severity and incidence of URTI in the population of elite athletes with high training loads, as well as immune markers.

Methods

A randomized, double-blind, placebo-controlled parallel-groups study was conducted. Thirty-nine elite athletes were randomized either to placebo (n=19) or probiotic (n=20) group. Probiotic group received *Lactobacillus helveticus* L10, $2x10^{10}$ CFU. The athletes filled in training diaries and health questionnaires weekly. The resting blood and saliva samples were collected at baseline and after 14 weeks. Salivary IgA was measured by enzyme linked immunosorbent assay (ELISA).

Results

The mean duration of URTI in probiotic group (7.25±2.9 d) was significantly shorter than in placebo group (10.64±4.67). The number of symptoms in probiotic group (4.92±1.96) was significantly lower than in placebo group (6.91±1.22). Severity and incidence of URTI didn't differ between the treatments. Salivary IgA level in probiotic group didn't change significantly over the study (p=0.26), but decreased significantly in placebo group (p=0.0059).

Conclusions

Probiotic strain *Lactobacillus helveticus* L10 is beneficial nutritional supplement for reducing the length and number of URTI symptoms, which might be due to better mucosal immunity integrity maintenance.

A SYNBIOTIC MIXTURE OF SCGOS/LCFOS AND BIFIDOBACTERIUM BREVE M-16V IS ABLE TO RESTORE THE DELAYED COLONIZATION OBSERVED IN CAESAREAN SECTION DELIVERED INFANTS

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Objective

The objective was to determine the effect short-chain galacto- and long-chain fructo-oligosaccharides (scGOS/lcFOS) and *Bifidobacterium breve* M-16V in restoring the delayed colonisation in C-section delivered infants.

Methods

In a multi-country, double-blind, randomised controlled study, 153 infants born by C-section were randomly assigned to receive either an infant formula supplemented with scGOS/lcFOS/B.breve M-16V or with scGOS/lcFOS or a standard infant formula from birth until age 4 months. Stool samples were collected at day 3, day 5, week 4, week 8, week 12, week 16, and week 22 (6 weeks post-intervention). Bifidobacteria, *B.breve* M-16V, pH and SCFA were assessed. Safety and tolerance were recorded.

Results

In the synbiotic group, the proportion of bifidobacteria was higher at D3/D5 (p=0.006) and this effect remained significant until 1 month of age (p=0.029) compared to the control. The prebiotic group showed a significant bifidogenic effect at 1 month of age (p=0.048) compared to the control. In the synbiotic group, *B. breve* M-16V was detected in 37% of the infants at week 22. A significant lower faecal pH and a higher acetate level were observed in the synbiotic group from the first days of life and this remained significant until 1 month of age compared to the control. All formulas were well tolerated and all groups showed a comparable safety profile. A lower number of subjects with adverse events of eczema/atopic dermatitis was reported in the synbiotic group (n=3) compared to the control (n=10) and prebiotic group (n=9).

Conclusions

An infant formula with scGOS/lcFOS and *B. breve* M-16V is able to restore the delayed colonization and positively modulate the gut ecosystem in C-section delivered infants.

PROBIOTIC COMMERCIAL PRODUCTS IN IRAN

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Objective

evaluation of present state of commercial production of probiotics in IRAN

Methods

Internet search and personal findings

Results

The materials with probiotic characteristics can be divided in two larg groups : traditional foods and new materials with probiotic effects . Some probiotic traditional foods have prolong histories in IRAN and they have some diversities related to geographic and cultural aspects, but most of them are dairy fermented products with different animal origin especially yogurt.

There are some probiotic products in IRAN that are produced in industrial scale as the following:

1. Foods containing probiotic microorganisms:
1.1. Dairy products : yogurt, probiotic yogurt (with additional probiotic microorganisms),probiotic doogh (Iranian yoghurt drink),Kefir,Symbiotic cheese.
1.2. Probiotic cake
1.3. Probiotic biscuit
1.4. Non alcoholic probiotic malt beverage
1.5. Probiotic additive for food industry
2. Drugs:
2.1. Oral drop/ sachet: Probiotic- prebiotic formulation for infants and young children
2.2. Oral capsules: with different formulations for elderly people, women, all of the family member and for people needed special care
2.3. Vaginal tablet/gel
3. Animal probiotic feed (Heat resistant probiotic additives)

Moreover, some foods, food additives and different kinds of drugs imported to IRAN and are used by people for huaman beings or animals.

Many researchs have performed yet or in current about using probiotics as food additives, drugs, etc in IRAN.

Conclusions

It is concluded that there is a growing trend for production and consumption of probiotic products in IRAN.

COMPARISON OF THE EFFECT OF VINEGAR WITH CHOLORHEXIDIN MOUTH WASH AGAINST *MUTANS STRREPTOCOCCI*

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Objective

Dental caries is the most common infectious diseases that are involved all groups, ages and classes of society people. Among the oral bacteria, *mutans streptococci* especially *Streptococcus mutans* and *Streptococcus sobrinus* are known as the most important microbial agents in dental caries.*Streptococcus sanguis* and *Streptococcus salivarius* are considered as bacterial agents of dental plaque and dental caries too. Dental caries treatment imposes heavy costs in all countries. Although there are different chemical antimicrobial agents for the prevention of of dental caries, but their important side effects have reported. Therefore, Many attempts have done for finding alternative safe medications specially with using - natural ingredients. The aim of this research is the determination of antimicrobial effects of vinegar against cariogenic bacteria

Methode

The antimicrobial effects of different concentrations of vinegar against *Streptococcus mutans, Streptococcus sobrinus, Streptococcus sanguis and Streptococcus salivarius* are evaluated with disc diffusion ,well plate and microtitre plate methods. Also, the effect of this material on biofilm formation by mentioned bacteria were studied.

Result

The results showed vinegar significantly reduced growth of the mentioned oral stereptococci. MIC of vinegar for *Streptococcus mutans*, *S. sobrinus, S. sanguis and S. salivarius* was ./125, ./125, ./0625,./0625 and its MBC for them was ./5, ./5, ./125, ./125 respectively. Also, vinegar significantly reduced biofilm formation from 93.45 to 80.26 of investigated streptococci. Antimicrobial effect of vinegar on each tested microbial isolates showed no significant difference with chlorhexidine mouthwash.

Conclusions

This results indicated the potential capacity of vinegar for production of effective and safe anti dental caries mouthwash.

Key words: Dental caries, *Mutans streptococci*, Vinrgar, Cholorhexidine Mouth wash

THE ANTI-INFLAMMATORY PROPERTIES OF GINGER CONSUMPTION BETWEEN IBD EXPERIMENTAL MODELS

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Objective

Growing data suggest that ginger (*Zingiber officinale*) has important compounds (gingerol, shogaol) that give it antioxidant and anti-inflammatory properties. This study evaluated the ginger anti-inflammatory role aftected by gut microbiota composition and activities between colitics rats (5% DSS).

Methods

Rats were divided randomly into control and 1% fresh ginger supplementation (diets). Rats were sacrificed one weeks later. Colon to body weight ratio was evaluated then each colon homogenized and diluted (wt/vol 1/10) in normal saline. Also faecal samples were collected before and fter colitis induction (0, 24, 48, 72, 96 h, and day 7) then placed in sterile tubes, weighed and diluted five times. One-tenth ml of each dilution (colon and faecal) was placed in selective media for the isolation of *Bifidobacteria, Clostridium histolyticum,* SRB and *Lactobacillus*. However, SCFAs analysis still under progretion in addition to the colonic histobithology.

Results

Results showed valuable effects of ginger consumptions against DSS induced colitis. Also colon/body weight ratio (index of tissue edema) was markedly decreased in the colitis rats after administration of ginger. However, the colonic microbiota counted is relatively higher in the prebiotcs (*Bifidobacteria* and *Lactobacillus*) in the group that was receiving ginger (colon and faecal) in contrast to no ginger.
In contrast, the *Clostridium and* SRB numbers were lower in rats with ginger supplementations.

Conclusions

Current research shown that ginger consumption caused significant increase in prebiotcs presented in the colon and feacal samples; that was previous conifirmed by us as diets and gut microbiota interactions modulating the development of colitis.

COMPARATIVE STUDY BETWEEN RIFAXIMIN-ALPHA AND LACTOBACILLUS CASEI DG IN PATIENTS WIYH DIARRHEA PREDOMINANT IRRITABLE BOWEL SYNDROME

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Objective

Irritable Bowel Syndrome (IBS) is one of the most common functional gastrointestinal disorders, characteristic of modern society, with high health-related costs and significant socioeconomic impact. The aim of this study was to evaluate two different therapeutic strategies after 3 months using the IBS-QOL questionnaire (symptoms remission, QOL improvement) and 3 anorectal volume sensation tests (first perception, urge to defecation and pain onset) in order to objectify the treatments effectiveness.

Methods

Between march 2013 and february 2014, 62 patients were included into a prospective study at the National Institute of Infectious Diseases "Prof. Dr. Matei Bals" Bucharest. All of them were diagnosed with diarrhea predominant IBS based on Rome III criteria, normal colonoscopic exam, negative fecal calprotectin and oncological biomarkers. The patients were randomized in two groups: group A – 30 patients were treated with permanent Otilomium bromide 3 tablets daily and Rifaximin-alpha 4 tablets daily 10 days per month, every month; group B – 32 patients received permanent Otilonium bromide 3 tablets daily and Lactobacillus casei DG 1 tablet daily 10 days per month, every month. All these patients were invited to complete an IBS-QOL questionnaire at the time of diagnosis and 3 moths after. Each patient received a journal.

Results

Statistical analysis pointed out the prevalence of female patients (62,7%) with average age of 42,3 ±10,72 years. In the initial study cohort females were more likely to have a lower QOL then males (90,13 vs 77,40, p=0,06). Three months follow-up evaluation showed a similar symptomatic improvement in both groups, but the QOL and cost effective were better in the Lactobacillus casei DG group.

Conclusions

We observed an overall improvement of symptoms in both studied groups, with better results on QOL and cost effective treatment in the group receiving Lactobacillus casei DG. Setting this study as an initial point we decided to develop a larger study to improve the results by widening the period of monitoring.

GUT MICROBIOTA PROFILING IN AN INFANT COLONIZED BY MULTIRESISTENT GERM CANDIDATE TO LIVER TRANSPLANTATION

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Objectives

Aim of this study was to investigate gut microbiota composition in a 9 months' old patient affected by chronic liver disease, candidate to liver transplantation and colonized by multiresistent germs. Due to high infective risk linked to surgical procedure and immunosuppressive post transplant therapy, we have monitored the plasticity of the gut microbiota. Indeed, so far no clear indications on preventive antibiotic treatments for gut decontamination, nor management of these patients have been described.

Methods

In order to determine the gut microbiota ecology of the patient colonized by *Klebsiella pneumoniae* multi-drug resistant strains (KP-MDR), 7 fecal samples were compared to 8 samples from each of her parents, also to evaluate fecal transplantation. All fecal samples were pyrosequenced and Operational Taxonomic Units (OTUs) distributions described for child and parents' microbiotas.

Results

Beta diversity showed three distinct sample groups. The majority of the sequences was assigned to Bacteroidetes and Firmicutes in parents, while Proteobacteria and Firmicutes were predominant in the infant microbiota. A Kruskal-Wallis analysis indicated that in the baby microbiota a reduction of Bacteroidetes and an increase of Proteobateria were evident. At species level, the infant microbiota was limited to Enterobacteriaceae (including KP-MDR), *Streptococcus*, *Veilonella dispar*, *Lactobacillus*, while the parents' microbiotas were polymicrobic and prevalently constituted by *Fecalibacterium prausnitzii*, Lachnospiraceae, *Bacteroides*, Ruminococcaceae.

Conclusions

These data show the high dysbiosis level of the gut microbiota of a KP-MDR pediatric patient, prevalently monomicrobic, against the microbiota diversity within the family, suggesting the usefulness of further monitoring and possible interventions to restore eubiosis.

EFFECTS OF CONCOMITANT ADMINISTRATION OF VITAMIN K1 AND LACTOBACILLUS RHAMNOSUS GG ON THE PROLIFERATION AND APOPTOSIS OF COLON ADENOCARCINOMA CELLS

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Objective

In the last years, the anticancer effect of the vitamin K1 has been demonstrated due to its ability in inhibiting the growth, mainly of liver but also of colon cancer cells. Also probiotics, including Lactobacillus rhamnosus GG, have shown potentialities in contrasting colon cancer, being able to significantly affect cell proliferation and polyamine metabolism. A strengthening of the probiotics action, due to a synergistic effect with the concomitant administration of vitamin K1, has been postulated. Therefore, the aims of the study were to investigate in three differently graded human colon cancer cells (Caco-2, HT-29, and SW480) the effects of increasing concentrations of vitamin K1 (from 10μM to 200μM) alone or in combination with viable Lactobacillus rhamnosus GG (10^8 CFU/ml), administered up to 72h, on the cell proliferation, cell cycle, and apoptosis.

Methods

The proliferative response was measured by MTT test. The cell cycle was evaluated by the Muse Cell Cycle kit. The apoptosis was measured by the quantitative PCR method with SYBR1 green dye (for Bax and Bcl-2 mRNA expression) and the Muse Annexin V/Dead Cell kit.

Results

In all the tested cell lines, after 24h of treatment vitamin K1 caused a significant antiproliferative and proapoptotic effect, starting from 100μM. The effect was enhanced by the concomitant administration of Lactobacillus rhamnosus GG that proved to act by arresting the cell cycle.

Conclusions

Therapeutically, combinations of vitamin K1 with Lactobacillus rhamnosus GG may represent a suitable option for chemoprevention and/or treatment in future strategies for colorectal cancer management.

TESTING OF SYNBIOTIC PROPERTIES OF HUMAN MILK OLIGOSACCHARIDES AND PROBIOTIC BACTERIA

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Objective

The gut microbiota of newborn is quite unstable and the mode of delivery plays an important role in infant gastrointestinal colonization. Modern time allows influencing the composition of infant gut by probiotics, prebiotics and synbiotics. Human milk oligosaccharides (HMOs) are the first prebiotics for infant. The aim of this study was to determine the ability of probiotic strain (from Infloran) to compete *in vitro* with faecal microbiota with addition of HMOs.

Methods

The ability of rifampicin-resistant bifidobacterial mutant, which was added in order to compete with infant faecal samples (from infants born by caesarean section without bifidobacteria), was tested in three media - medium with HMOs, human milk (HM) and control medium (CM). Survival ability of rifampicin-resistant mutant was monitored after cultivation on modified W + SP agar supplemented with mupirocin, rifampicin and acetic acid. After that, rifampicin-resistant mutant was reidentified using species specific PCR.

Results

Combination of mutant (probiotics) and HMOs (prebiotics) reduced counts of clostridia and gram-negative bacteria and exhibited synbiotic effect. Significantly lower counts of G- bacteria and *Escherichia coli* were found after *in vitro* competition in HM, than on HMOs compared to the CM. In contrast, inoculated rifampicin-resistant mutant utilized all media and it grew in the similar counts.

Conclusions

In vitro competition confirmed that bifidobacterial strain is suitable probiotic for infants and in combination with HMOs or HM act as a suitable infant synbiotic and it is able to inhibit potentially pathogenic bacteria.

ACTIVITY OF AGRIMONIA EUPATORIA L EXTRACT IN CLINICAL ISOLATES OF HELICOBACTER PYLORI

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Objectives

Helicobacter pylori (Hp) infects about 30% of the western world population and about 80% of the population in developing countries. Hp is associated with severe pathologies, including peptic ulcer and gastric cancer. Treatment of Hp infection requires the simultaneous use of at least two antibiotics and a proton pump inhibitor. Many naturally occurring compounds found in dietary and medicinal plants have been shown to possess antimicrobial activities. A combination of antibiotics with plant extracts that possess antimicrobial activity are, therefore, being developed. The main objective of this study is to evaluate the potential of plant extract of *Agrimonia eupatoria* L (Ag) to inhibit the growth of Hp clinical isolates.

Methods

Four Hp clinical isolates and Hp ATCC 43504 were grown on Columbia agar supplemented with horse blood (CBA) under microaerophilic conditions. Minimum inhibitory concentrations (MIC) of Ag (25 to 5 mg/ml) extracts were determined by broth microdilution method (CLSI 2013 M45-A2) using Mueller Hinton broth (MHB) supplemented with fetal serum bovine. Tests are performed in triplicate.

Results

Ag extract presented a CMI of 25 mg/ml for all clinical isolates and 20 mg/ml for Hp ATCC.

Conclusions

Ag is a medicinal plant well characterized, and with anti-inflammatory anti-oxidants properties, used in various pathologies including gastrointestinal diseases. This study demonstrates that extract has activity anti-Hp. Therefore, this extract may be helpful in association with the traditional therapeutics.

IN VITRO EVALUATION OF ANTIFUNGAL EFFECT OF LACTOBACILLUS ISOLATED FROM URINE OF CAMEL AGAINST CANDIDA ALBICANS, GLABRATA AND KRUSEI

reihaneh amini [1], Mohammad Rabbani Khorasgani [2]
Graduate Student, Department of Microbiology, Faculty of Biology, University of Isfahan, isfahan, Iran [1], Associate Professor, Department of Microbiology, Faculty of Biology, University of Isfahan, isfahan, Iran [2]

Objective

Camel's urine has significant antimicrobial activities against some pathogenic microorganisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Antimicrobial effect of camel urine can be due to the presence of lactobacilli in it. In this study isolation of *Lactobacillus* from the urine of camel and its effect against some strains of *Candida* (as important agent of hospital-acquired fungal infections with increasing risk of anti fungal resistance development) is reported.

Method

Isolation of *Lactobacillus* from camel urine was performed with biochemical methods and confirmed by molecular method. The antifungal effect of isolated *Lactobacillus* against 3 *Candida albicans, glabrata* and *krusei* isolated from vagina evaluated using microtitre plate method and the MFC and MIC determined.

Results

The isolated *Lactobacillus* had not fungicidal effect against the *Candida* isolates but inhibited of their growth (MIC90 was 3.75×10^7).

Conclusion

Results of this study showed that lactobacilli may express antifungal effect against *Candida* and it has the potential of prevention and control of *Candida* infections.

EFFECTS OF PROBIOTICS IN PATIENTS WITH DIABETES MELLITUS TYPE 2: A STUDY PROTOCOL FOR A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

Shaun Sabico [1], Majed Alokail [2], Nasser Al-Daghri [2], Philip McTernan [1]
Warwick Medical School, Warwick University, Coventry, United Kingdom [1], King Saud University, King Saud University, Riyadh, Saudi Arabia [2]

Objective

Low grade chronic inflammation is observed in patients with DMT2. Endotoxin derived from gut bacteria, may act as a potent inflammatory stimulant. Probiotics, which are believed to contain health promoting live microorganisms, may influence circulating endotoxin levels. Ingestion of live probiotic cultures may alter gut microbiota in a beneficial manner to reduce inflammation; no information is available whether or not they do so in patients with T2DM. Therefore, the aim of this study is to characterize the beneficial effects of probiotics on circulating endotoxin levels and other biomarkers related to systemic low-grade inflammation in patients with T2DM.

Methods

One hundred and twenty consenting adult Saudi T2DM patients [naïve or newly diagnosed and without co-morbidities], will be enrolled in this clinical trial and randomized to receive daily placebo or probiotics (Ecologic®Barrier) for 26 weeks in a double-blind manner. Inflammatory and metabolic markers will be measured and faecal samples analysed. Measurements/samples will be obtained at baseline and after four, eight, 12/13 and 26 weeks of treatment.

Conclusions

The study is now in its first half and preliminary results will be presented. It is expected that the probiotic product will induce beneficial changes in gut microbiota, reduce the systemic inflammatory state through altering systemic endotoxin levels and, as such, reduce the systemic inflammatory response observed in T2DM subjects.

Trial Registration

ClinicalTrials.gov Identifier: NCT01765517

SUPPLEMENTATION WITH MUCILAGES OF IRVINGIA GABONENSIS SEEDS AND TRIUMFETTA CORDIFOLIA BARK: ANTIOXIDANT EFFECT AND LIPID PROFILE ON DIETARY-INDUCED HYPERLIPIDEMIC RATS

Woguia Alice Louise [1], Ngondi Judith Laure [2], Boudjeko Thaddée [1], Chakocham Raoul [2], Oben Enyong Julius [2]
University of Yaoundé 1, Biotechnology center Nkolbisson, Yaoundé, Cameroon [1], University of Yaoundé 1, Department of Biochemistry, Yaoundé, Cameroon [2]

Objective

We examine the antioxidant properties and the potential effect on lipid profile, food intake, weigh intake of *Irvingia gabonensis* seed and *Triumfetta cordifolia* bark mucilages in male albino rats.

Methods

The two plant mucilages (200 mg/kg body weight) and 300 mg/kg of cholesterol were administered by oral gavage for 30 days to male Wistar rats with dietary-induced hyperlipidemia. The food intake, the weight gain and the lipid profile was monitored. Antioxidant marker enzymes such as catalase (CAT), superoxide dismutase (SOD), and Non enzymatic marker of oxidative stress thiol (-SH) group and MDA were determined in the liver and heart.

Results

Results indicate that all rats receiving mucilage showed a significant reduction of dietary intake. Rats with *Triumfetta cordifolia* mucilage supplement have the highest decrease. Weight gain was significantly lowered in animals receiving the high fat diet plus cholesterol and mucilage administration compared with rats receiving the reference mucilage (galactomane from seeds of *Ceratonia siliqua*) and the high-fat diet only. Triglycerides were 2-fold lower in the group receiving *Triumfetta cordifolia* mucilage than in the high fat group. Furthermore, SOD activity was significantly increased in the liver of rats treated with *Irvingia gabonensis* and in the heart of rats that received *Triumfetta cordifolia* mucilages compared to group of untreated rats. The MDA level increased ($P<0.05$) in hypercholesterolemic rats and significantly depleted ($P<0.05$) in treated rats liver.

Conclusions

The two mucilages indicate antioxidant, cardioprotective and hepatoprotective properties. They can be used as complementary or alternative agents in the management of the deleterious effects of hyperlipidemia diet.

WHEAT BRAN SOURDOUGH-LIKE FERMENTED: A POTENTIAL SOURCE OF PREBIOTICS AND PROBIOTICS

Federica Manini [(1)], Milena Brasca [(1)], Daniela Erba [(2)], Maria Cristina Casiraghi [(2)]

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Objective

This study aims to increase the amount of wheat bran's bioactive compounds through a sourdough-like fermentation process, in order to use the fermented bran as a functional ingredient. The solubilization of arabinoxylans, the main non-starch polysaccharides of bran, deserve particular attention because of the positive influence on glucose metabolism and the prebiotic potential of the resulting soluble arabinoxylan-oligosaccharides.

Methods

Soluble fiber, water-extractable arabinoxylans were determined before and after bran fermentation. Bioprocessed bran was fermented *in vitro* with human faecal inoculum in order to test the modulation of the growth and the activity of some intestinal bacteria, by means of DAPI and FISH techniques and short chain fatty acid determination.
Leuconostoc mesenteroides, *Leuconostoc citreum*, *Lactobacillus brevis*, *Lactobacillus curvatus*, *Lactobacillus sakei*, *Lactobacillus plantarum* and *Pediococcus pentosaceus* strains isolated from the fermented bran were characterized by probiotic properties, such as acid and bile tolerance, anti-listeria activity and adhesion ability to Caco-2 cells, as well as exopolysaccharide production and safety.

Results

Bran fermentation contribute to fiber (+30%) and arabinoxylans solubilization (+ 80%). Fermented bran seems to induce the *in vitro* production of butyrate, a protective agent against colon cancer. Moreover, *Pediococcus pentosaceus* CE65 could be a candidate for use as probiotic.

Conclusions

The current study suggests that sourdough-like fermentation is an efficient means for the nutritional enhancement of bran and provides additional information for the future purpose to add fermented bran as a functional ingredient. Moreover, wheat bran sourdough proved a good source of interesting microbial strains with potential for future applications.

FERMENTATION PATTERN OF PECTIC POLYSACCHARIDES FROM PRUNES (*PRUNUS DOMESTICA*) VARY ACCORDING TO THEIR CHEMICAL STRUCTURE

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Prunes, the dried fruits of *Prunus domestica*, possess large amounts of pectic polysaccharides which can be structurally divided into homogalacturonans (HG) and type I arabinogalactans (AGI). Previously, a Fehling treatment was applied to prune pectic crude fraction (PWH) to separate these distinct chemical structures yielding fractions SF (composed of AGI) and PF (composed of HG). In the present study, an *in vitro* fecal fermentation model was used to access the putative prebiotic property of prunes pectins as well as to investigate how different structures contribute to their fermentation properties such as gas production, pH, and short chain fatty acids (SCFAs). After 24 hours fermentation, fractions PWH and SF resulted in the highest total SCFAs production, significantly greater than fructooligosaccharides (FOS). As expected, SCFAs production was accompanied by an increase in gas and drop in pH. Regarding propionate, fraction SF was highest among the materials, however, for butyrate production, the crude fraction PWH performed better. The findings suggest that the AGI portion in PWH is mostly responsible for propionate production, while HG portion of pectins in PWH may be important for butyrate production. Interestingly, the isolated homogalacturonan (fraction PF) was poorly fermented, with gas, pH and SCFA production similar to the negative control. This outcome could be associated with low water solubility presented by the isolated HG and/or the removal of methyl groups from the core HG after Fehling treatment, and will be further investigated. Overall, these results indicate that chemical features of prune pectins are closely related to their fermentation profile. Moreover, fractions PHW and SF were butryogenic and propiogenic, and may be potential prebiotics.

OLIGOSACCHARIDES CONSUMPTION AFFECTS NEONATAL GUT CONNECTOME

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Oligosaccharides (OS) are commonly added to infant formulae to better mimic maternal milk but their physiological impacts are only partly described. In adult animals, OS stimulate colonic fermentation and entero-endocrine cells (EEC) proliferation [1, 2], and intestinal microbiota affects enteric nervous system (ENS) [3, 4]. We thus assumed that neonatal OS consumption could modulate the gut connectome, of which ENS and EEC are major components [5].

Suckling rats were supplemented with FOS or GOS/inulin mix (2.9 g.kg-1), or control solution from days 5 to 14 and sacrificed at 14d. Densities of colonic EEC, and expression of different genes related to EEC and ENS were measured by immunohistochemistry (chromogranin A and GLP-1) and qPCR, respectively.

Both OS impacted EEC differentiation (increased neuroD1, proglucagon and PYY, decreased Pax4) but only FOS increased the proportion of GLP-1 positive cells. Although only GOS significantly decreased the colonic neurons number (decreased Elavl4), both OS decreased neuromediators expression (Chat, VIP and neuronal Nos1), which would result from reduced neuronal activity (decreased neuronal Nos1/Elavl4 ratio). OS did not affect the number of glial cells (unchanged GFAP and Sox10 expression). However, both OS induced decreases in S100beta expression, suggesting that glial cells activity was deregulated by OS consumption.

Finally, very early OS supplementation impacted the gut connectome. Considering its pleiotropic roles, these changes could have several immediate and programming consequences on physiological functions regulated by the gut-brain axis such as intestinal epithelial barrier homeostasis or food sensing regulation, what requires further studies including follow-up until adulthood.

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GLOBAL AND EU FUNDING TO FIGHT AGAINST OBESITY EPIDEMIC

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Key-words: EU, Obesity, Funding, Horizon 2020

Obesity epidemic is one of the main issue the EU is investing in. The number of adults dying each year because of obesity or overweight is around 3.4 million, thus proving the wide spreading of obesity-related health problems which is almost doubling if compared to the situation occurring 20 years ago. In this respect, several preventive measures have been taken worldwide. The WHO and the European Commission have always been committed to tackle this relevant social issue. As far as WHO is concerned, several initiatives have been taken since 2004 to fight against obesity related issues, as well as to promote healthy diets regular physical activity. As a matter of fact, the WHO has launched the "Global Action Plan for the prevention and control of noncommunicable diseases 2013-2020.This Action Plan, built on the WHO Framework Convention on Tobacco Control and the WHO Global Strategy on Diet, Physical Activity and Health, will contribute to progress on 9 global NCD targets to be attained in 2025, including a 25% relative reduction in premature mortality from NCDs by 2025 and a halting of the global obesity rates to those of 2010. More specifically, as for childhood obesity, the high-level Commission on Ending Childhood Obesity (ECHO), was established by WHO's Director-General. In addition, as for the EU, in May 2007, The European Commission established a Community Strategy to comprehensively address the issues of overweight and obesity; the strategy has been turned into a Green Paper, *the Paper A Strategy on Nutrition, Overweight, and Obesity-related health*, which focused on the actions that could be taken at local, regional, national and European level to reduce the risks associated with poor nutrition and limited physical exercise. The main instrument through which this strategy has been implemented is Horizon 2020, the new funding opportunity released by EU aiming at promoting research in Europe. In H2020 several actors ranging from international organizations to Member States actors in the private sector as well as for the public sector are encouraged through several initiatives to work together in the field of nutrition. The EC play a key role in creating relevant and effective partnerships working together to advance the state of the art in this research area, as well as establishing a common framework for research and cooperation targeted to this relevant social issue. The EU commitment in this respect has been actually testified by several funded ongoing projects which are specifically focused on a better understanding of the reasons why an ever increasing number of people are becoming obese. Childhood obesity is also one of the most relevant issue, since it can bring to a higher risk of disease in the adult life. The third pillar of H2020 focuses in particular on several societal challenges, where the topic Innovative, Sustainable and Inclusive Bio-economy specifically supports research projects in the obesity related area, following the previous funding initiatives under FP7. Among the various EU funded projects on obesity and overweight related issues there is, for example, the SATIN Project, - www.satin-satiety.eu – which is developing foods which accelerate satiation, by making people feel fuller faster and for longer, thus helping them control their weight. Moreover, the Full4Health Project - www.full4health.eu/project - is exploring the interconnections between food and satiety and the mechanisms behind feeling full. As for childhood obesity, two key projects funded by the EC are I.Family Project - www.ifamilystudy.eu/project-information/ - and the EarlyNutrition Project - www.project-

earlynutrition.eu/ . The first one is studying the eating habits of children and their families in 8 EU countries. second one is investigating the interconnections between obesity – in childhood and in adult life – and the eating habits and lifestyle of mothers before and during pregnancy, and the children during infancy, in order to detect the ways in which these elements affect obesity and overweight related diseases later in life. H2020 has provided a new insight on the obesity related issues by funding projects which are strongly multidisciplinary in methodologies and approach. A concrete example of this *embeddedness* is The NoHoW Project - nohow.eu/the-project/- where concrete evidences are provided on how mobile phone apps and other technologies can effectively help people to lose weight and maintain their weight loss.
Recently, interest has been drawn towards the role of the intestinal microbiota as a potential novel contributor to this epidemic. It is estimated that the human adult intestines contain more than 1014 bacteria from over 1000 species. The genetic material of the intestinal microbes, collectively named the microbiome, exceeds the magnitude of the human genome over 100 times.Aberrant compositional development of the gut
microbiota precedes overweight, offering new possibilities for preventive and therapeutic applications in weight management.
In conclusion, it should be highlighted that EC funding remain the main opportunity to promote research in EU countries, such as Italy, where the GDP devoted to R&D unfortunately does not reach 1%. Thus, the Italian effort is significantly lower than the effort made in this field by other countries i.e. the Scandinavian countries, Japan or U.S, where R&D remain one of the focus area of the national policies.

IN VITRO COMPARISON OF LACTOBACILLUS FERMENTUM AND MICONAZOLE/FLUCONAZOLE AGAINST DIFFERENT CANDIDA SPECIES

Francesca Deidda
Biolab Research Ltd., Novara, Italy

USEFULNESS OF SELECTED BENEFICIAL BACTERIA IN THE TREATMENT OF ACNE

Adele Sparavigna
Institute of Clinical Research and Bio-Engineering Derming

THE ROLE OF BIFIDOBACTERIA IN THE PREVENTION OF INFANT COLIC

Gianni Bona
Director of the Pediatric Clinic, Università degli Studi del Piemonte Orientale, Novara, Italy

BIFIDOBACTERIA IN THE PREVENTION OF THE COMPLICATIONS OF CHILDHOOD OBESITY

Prof. Gianni Bona
Director of the Pediatric Clinic, Università degli Studi del Piemonte Orientale, Novara, Italy

LACTOBACILLUS SALIVARIUS DLV1 IN THE TREATMENT OF PSORIASIS

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Laboratory of Clinical Chemistry and Microbiology, IRCCS Galeazzi Orthopaedic Institute, Milan, Italy

COMPARISON OF THE EFFICACY BETWEEN CLOTRIMAZOLE AND LACTOBACILLUS FERMENTUM LF16 (DSM 26856) IN ASSOCIATION WITH LACTOBACILLUS ACIDOPHILUS LA02 (DSM 21717) IN THE TREATMENT OF ACUTE CANDIDIASIS

Filippo Murina, Dr. Franco Vicariotto
Outpatient Department of Vulvar Disease, V. Buzzi Hospital, University of Milan-Italy

NEW STUDIES ON BIFIDOBACTERIUM LONGUM W11

Luca Mogna
Biolab Research Ltd., Novara, Italy

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GENERAL INFORMATION

DATES
September 13-15, 2015

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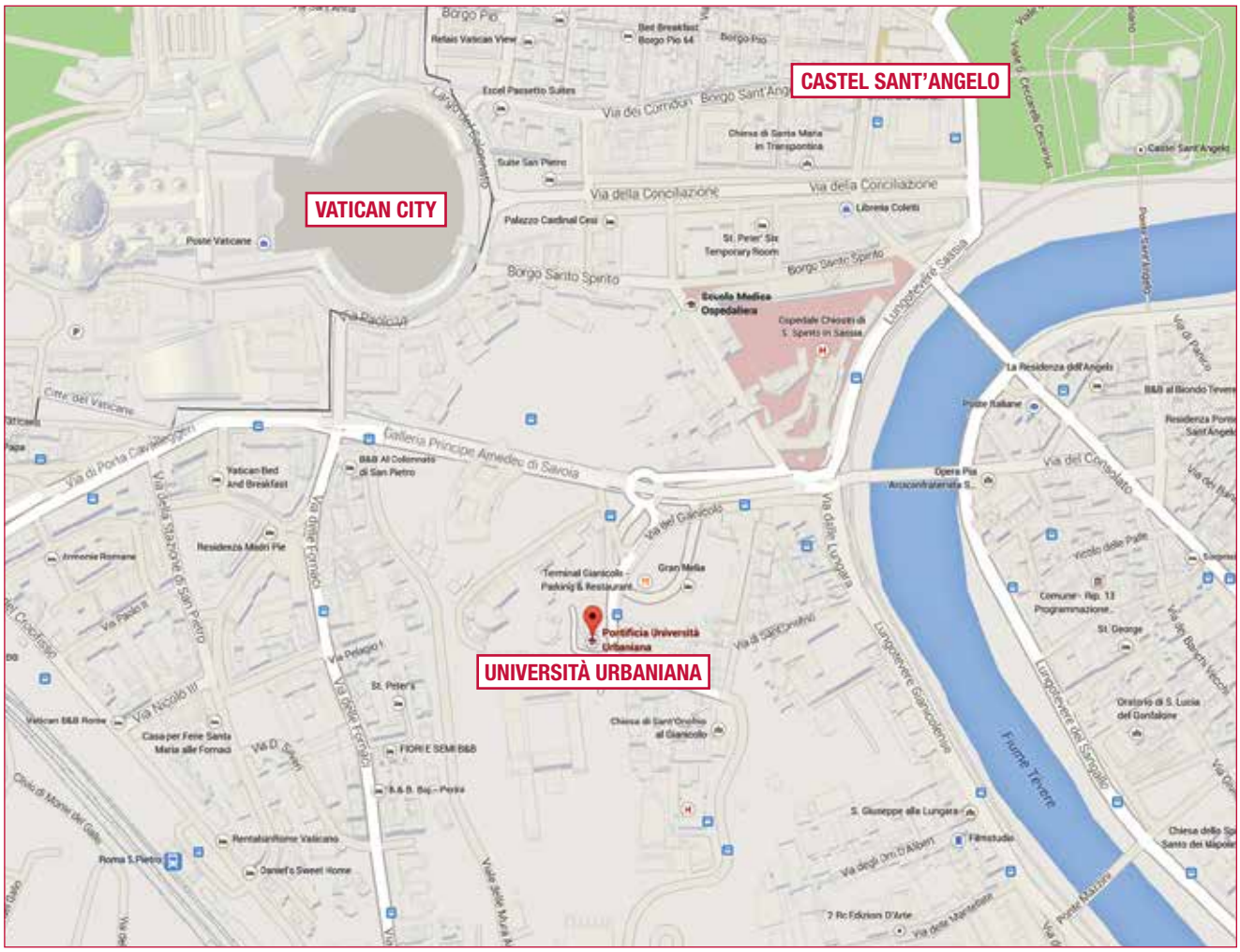
LANGUAGE
English will be the official language of the Meeting.

CLOTHING
Informal for all occasions.

CLIMATE
September in Rome is still hot but unpredictable.

BADGES
All participants and 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	€ 150,00
Dieticians/Nutritionists	€ 150,00
Nurses	€ 100,00
Members Mediterranean Task Force for Cancer Control	€ 150,00
Members AIIPA, Gruppo Miaf-Federchimica	€ 150,00
Under 35*	€ 150,00
Pediatric Day**	€ 200,00
Under 35 and ESPGHAN member for 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:

Participants

- Admission to scientific sessions, technical exhibition
- Final Programme
- Selected proceedings and abstract
- Coffee corner and lunch
- Opening ceremony and welcome cocktail
- Certificate of attendance
- Italian CME certificate (to whom entitled)

Accompanying Persons

- Opening ceremony and welcome cocktail

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, 2015, 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

Business lunch and coffee/tea 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 contact the 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 Ignazio Marino. 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.
Rental cars 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 € 8 c.a. one-way or € 11.00 c.a. return, taking 40 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 19:20. 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 € 3.90 one-way, taking 40 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. BusShuttle or ATRAL Line runs a service similar to Terravision. Their stop near Termini is about 20 metres up the road from Terravision's. Cost is € 4 for a single.
The fixed fare for a taxi ride to the city centre (inside the Aurelian Walls) is € 30, 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. Rental cars 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:
06 - 3570 Radio Taxi
06 - 5551 Samarcanda
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:

By taxi - We recommend you to only use licensed taxis available outside the station.
Telephone number main taxis companies:
06 - 3570 Radio Taxi
06 - 5551 Samarcanda
06 - 4994 La Capitale
By public transport - Follow the signs for "Station" of Leonardo express.
Take the train for Stazione Termini and get off at the Station - BUS 64 stop at Lgt. Sassia (S.Spirito Hospital) from this station there will be 350 metres walking

• From Ciampino Airport:

We advice to take a taxi available outside the airport.

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: probiotics2015@emec-roma.com
Website: www.probiotics-prebiotics-newfood.com

ORGANISING SECRETARY DESK AT THE MEETING VENUE WILL BE OPEN AS FOLLOWS:

DAY	DATE	FROM	TO
Sunday	September 13	8.30 a.m.	8.00 p.m.
Monday	September 14	8.00 a.m.	7.00 p.m.
Tuesday	September 15	8.00 a.m.	3.00 p.m.

ORAL COMMUNICATIONS

Oral communications sessions are scheduled as follows:

September 15, AULA C from 08.30 a.m. to 1.00 p.m.

POSTERS

Poster authors are kindly requested to hang the poster in the poster area from 10.30 a.m. on September 13 and remove it after 1.00 p.m. on September 15. 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:

• **"8th Probiotics, Prebiotics & New Foods - for microbiota and human 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
Infermiere
Infermiere pediatrico

Rif. n. 134203 - Crediti assegnati 10,5

Per aver diritto ai crediti ECM è necessario frequentare il 100% 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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YAKULT

&

ATHENA PHARMA ITALIA

CA.DI.GROUP

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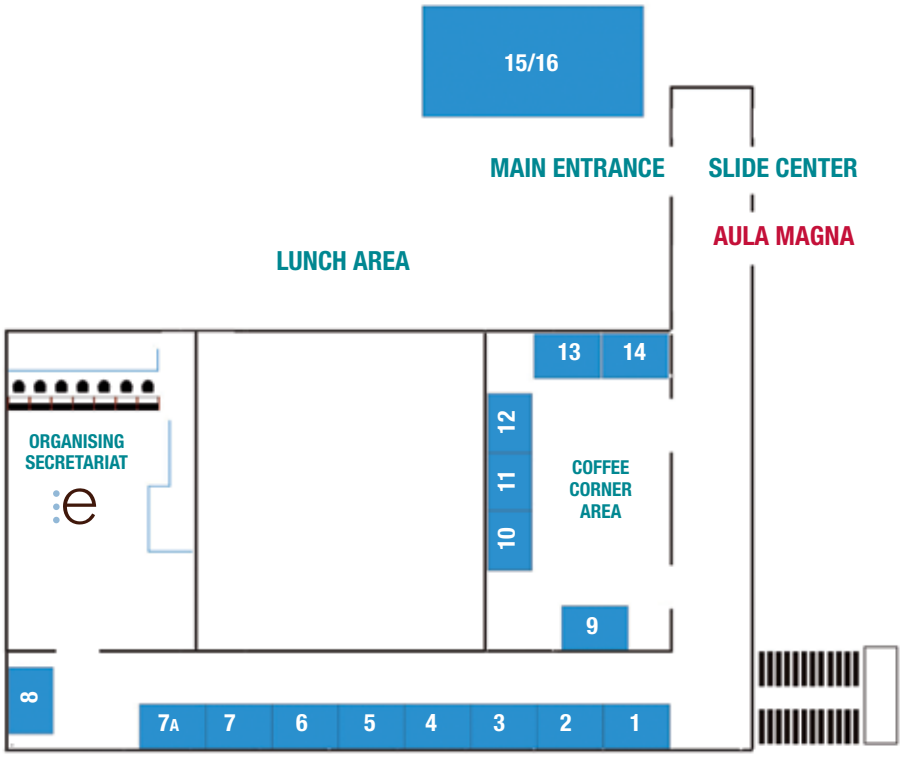
SOFAR

VALEAS

CEC EDITORE

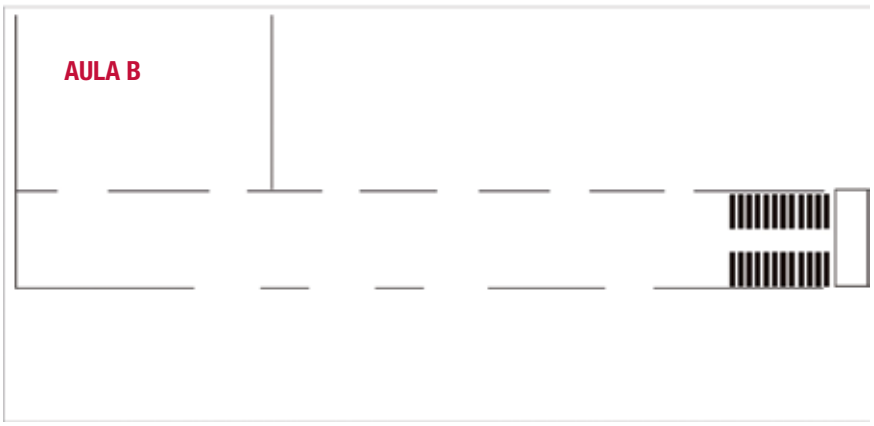
FLOOR PLAN - LEVEL I



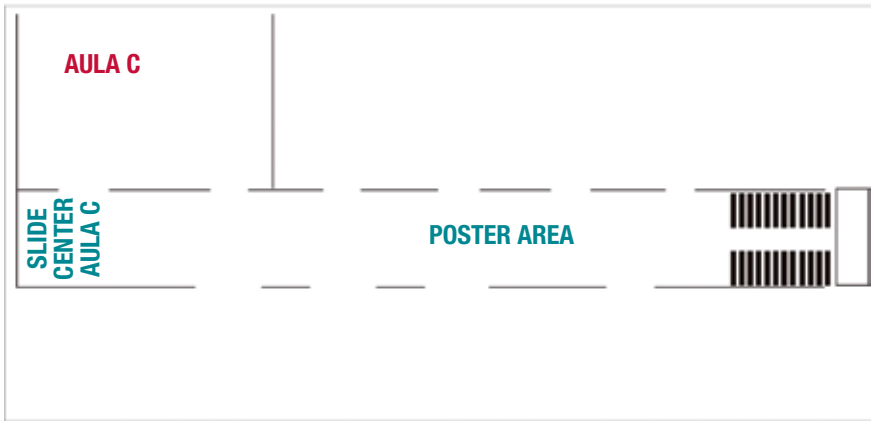
EXHIBITORS

1) VALEAS
2) OMEGA PHARMA
3) NÓOS
4) CA.DI.GROUP
5) ATHENA PHARMA ITALIA
6) GUNA
7) SOFAR
7A) S.I.I.T.
8) YAKULT
9) ITALCHIMICI
10) FARMACEUTICI PROCEMSA
11) ALFA WASSERMANN
12) FARMACEUTICI DAMOR
13) PROBIOTICAL
14) PROBIOTICAL
15) DICOFARM
16) AG PHARMA

FLOOR PLAN - LEVEL II



FLOOR PLAN - GROUND LEVEL



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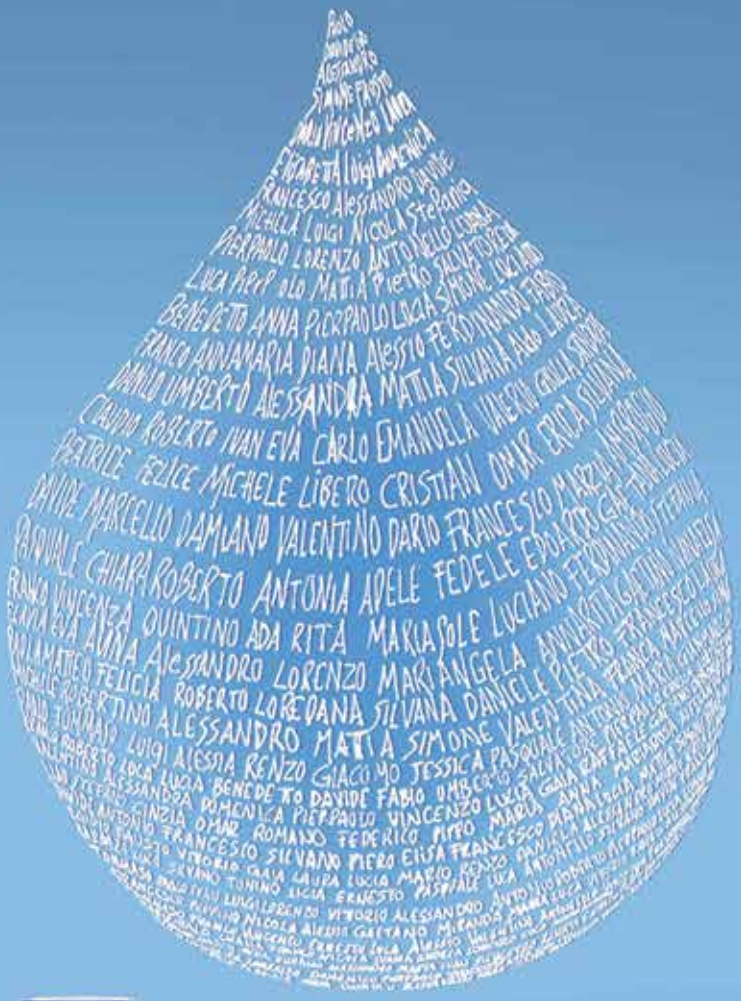

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