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CONFERENCE PROCEEDINGS

14th – 16th June 2011

Košice, Slovakia

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15:05 Coffee Break

15:25 SESSION 12

Chair: N. Boyko Co-Chair: A. Bomba

KEYNOTE Lecture

N. Boyko: Beneficial Bacteria in Traditional Fermented Products from Black Sea Area Countries and their Interaction with Gut Microbiota - Findings of the BaSeFood Project

KEYNOTE Lecture

H-Ch. Lin: Perinatal-Neonatal Microbiota Colonization Attenuate Inflammatory Disease Programming

A. Haslberger: Probiotic Strains Specifically Stimulate NFKb, Different miRNAs and Maturation in CACO II Endothelial Cells and in Dendritic Cells

H. L. Ventola: Effects of the Viability of Lactobacillus Rhamnosus GG on Rotavirus Induced Diarrhea in Neonatal Rats

B. Kim: Effects of Lactobacillus Plantarum CJLP243 on Growth Performance, Microbial Community and Acute-phase Response of Weaning Pigs Challenged with Enterotoxigenic Escherichia Coli

17:00 Young Scientist Award Presenting

Closing Speech

Farewell drink

Shifts in Microbial Population Fermenting Contrasting Indigestible Carbohydrate Sources Measured in the Intestines of Pigs and Using an In Vitro Model of the Pig Gastro-intestinal Tract

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An experiment was performed to compare the influence of indigestible carbohydrate (CHO) fermentation on intestinal microbiota composition *in vivo* and using an *in vitro* model of the pig gastro-intestinal tract. Three inulin and cellulose based semi purified-diets (5 % of inulin, 5 % of cellulose and 2.5 % of inuline + 2.5 % of cellulose) were fed to 3 groups of 4 piglets (about 25 kg). TiO₂ (0.5 %) was added as indigestible marker. After 3 weeks of adaptation to the diets, the pigs were slaughtered and digesta was sampled from the jejunum, ileum, caecum and 3 parts of the colon to measure pH and further bacterial DNA extraction. One week before slaughter, an *in vitro* gas fermentation test was performed in duplicate runs on inulin, cellulose and the mixture of inulin and cellulose using fresh faeces of the experimental pigs as bacterial inoculum. The gas production kinetics were measured until 72 h and modelled and fermentation broth samples were taken after 5, 8, 12, 24 and 72 h of fermentation for further microbiota characterisation. Total bacterial DNA was extracted from the *in vivo* and *in vitro* samples and q-PCR was performed to quantify total bacteria and genus used to measure the prebiotic index of CHO: *Lactobacilli*, *Bifidobacteria*, *Bacteroides*, *Clostridium* Cluster I and *Bacteroides*. DNA quantification in the extracts showed that the evolution was similar in both systems. *In vivo*, DNA concentration increased along the digestive tract until the second part of the colon and then decreased, while in the *in vitro* system, DNA increased until 12 to 24h of fermentation and then decreased ($P < 0.05$) This evolution was correlated to the fermentation kinetic of each CHO. In both models, inulin increased *Bifidobacteria* population. This increase was mainly located in the ileum in the digestive tract while *in vitro*, it was observed over all the duration of the fermentation. *Clostridium* cluster I populations increased *in vitro* and *in vivo* but the diet didn't influence this increase ($P > 0.05$). The diet did not influence the *Bacteroides* population ($P > 0.05$), except at the end of the colon where a higher level of *Bacteroides* was observed in the inulin diet. Consistently with *in vivo* results, inulin increased the *Bacteroides* population during the 72 h fermentation as compared to cellulose. Finally, the influence of the diets on *Lactobacilli* populations in both systems were not consistent. This can be ascribed to differences in pH between both systems. *In vivo*, pH varied between 6.81 and 5.75 depending on the diet and the portion of the GIT, while *in vitro*, the carbonate buffer doesn't allow the pH to drop below 6.7, reducing the competitive advantage of fast lactate production by *Lactobacilli* during the first hours of fermentation. Further developments of the *in vitro* method will need to tackle this buffering issue to allow proper highlight of prebiotic function of indigestible CHO.

Keywords: pig, gastro-intestinal model, indigestible carbohydrates, faecal microflora, real time PCR

Beneficial Bacteria in Traditional Fermented Products from Black Sea Area Countries and their Interaction with Gut Microbiota - Findings of the BaSeFood Project

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Introduction

It is known that traditional fermentation technologies offer considerable potential for stimulating development in the food industry because of their low cost, scalability, use of minimal energy and infrastructural requirements, and the wide consumer acceptance of fermented products in these countries. Efficient transfer and adaptation of these technologies is, however, often limited by inadequate basic scientific knowledge of the processes involved, and the lack of appropriate biological inoculants and process controls for these technologies. Another important issue of the BaSeFood project is to investigate the influence of selected foods and their microbial compositions on gut microbial communities on animal models and host immune function.

Methods

Sour rye bread and Sauerkraut (Ukraine), Tsiteli Doli Bread (Georgia), Kvass southern (Russian Federation), Elderberry soft drink (Socata) (Romania), Boza (Bulgaria) and Sautéed pickled green beans (Turkey) are fermented products that had been prioritised within BaSeFood project among of the dishes/beverages. The

extracts of all available for the study prioritised foods, drinks, selected plants and local berries were analyzed for their microbial contamination and ability to differently affect the representatives of gut microbiota *in vitro* and *in vivo*. The special efforts were made to isolate and examine the beneficial bacteria/microscopic fungi potential as a valuable source for the new functional foods products' developing.

Results

The *Lactobacilli* strains were poorly presented in all fresh and tested fermented samples; they were rarely accompanied with *Bifidobacteria*, enterococci and *Bacteroides*. *Bacteroides ovatus* had been isolated from garlic and tomato; *Clostridium butyricum* from parsley (root), carrot and Sauerkraut; *Bifidobacterium longum* was isolated from parsley (green), dill, celery; *Eubacterium lentum* was detected as only the isolate from onion. Two more genera were also investigated as potentially beneficial microorganisms: *Bacillus spp.* and *Corynebacterium non-pathogenic* species. They are widely distributed in nature and are mostly innocuous. These bacteria were mainly isolated from roots (except celery – for *Bacillus*) and parsley (for *Corynebacterium*). *Corynebacterium* had been detected as one of the bacterial compositions in plant native surfaces: tomato, bean, cucumber, pepper, pear and plum; apple (fresh) and melon (juice).

The plant originated Ags demonstrate high selectivity in their pro- (stimulating) and anti- (inhibiting) microbial properties against 33 chosen species of commensal gut bacteria *in vitro*; the changes of gut microbial associations were specific to each separately tested plant originated food Ag defined *in vivo* on mice model (BALB/c and SCID).

The molecular mechanisms involved of pro- and anti-inflammatory reactions triggered by the selected commensal microbiota, mutant strain(s) and diet antigens were further developed; selective "resistance" of human mucous membranes against various bacteria, bacterial- and food antigens had been generally clarified.

Discussion

Microbiological assays of traditional foods and drinks prioritised within BaSeFood project are potential sources of knowledge and experience of useful technological processes. The unique bacterial/microbial strains with their well-defined activities can be further exploited by food and pharmaceutical industries.

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Keywords: beneficial bacteria, pro- antimicrobial properties, fermented foods, gut microbiota, BaSeFood

Experimental Food Microbiological Issues Related to Human Life Support Inside Manned Space Modules: The MARS500 Programme

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In order to improve astronaut health, the application of microorganisms as Single Cell Proteins (SCP) integrators, in either nutraceuticals or probiotic foods has been investigated and further studies on the survival of beneficial microorganisms under space condition are needed.

As forthcoming space programmes are going to be mainly focused on long-term manned missions, an important issue will be the development of nutraceuticals and novel (or better) functional foods for both feeding and health of astronauts. Such an issue is fundamental for the success of future manned missions, so current research needs to investigate extensively to achieve feasible solutions for life support on space.

A previous experiment, carried out during the ESA ENEIDE mission implied the exposure of microorganisms inside the International Space Station (ISS) for a maximum time of 226 days; the aim of the experiment was to study the response of representative non pathogenic microorganisms to the environment inside the space vehicle and at different mission stages. Among the various bacteria tested some of interest for food applications were included: *Saccharomyces cerevisiae* (a commercial strain and a type strain), *Bacillus subtilis*, *Lactobacillus acidophilus*, *Enterococcus faecium* (a commercial strain and a type strain).