ABSTRACTS BOOK

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POSTER COMMUNICATIONS

AUTHORS

ORAL COMMUNICATIONS

GASTRO-INTESTINAL STRESS TOLERANCE OF LACTIC ACID BACTERIA FROM COMMERCIAL MILK-BASED PROBIOTIC DRINKS

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Keywords: Probiotic drinks, Gastro-intestinal stress, Lactic acid bacteria, PCR-DGGE.

INTRODUCTION
Many probiotic microorganisms are available in the market as functional foods, essentially milk-based fermented beverages. Their effectiveness is connected to the ability of probiotic strains to maintain their functional health characteristics for which they were originally selected, including the ability to survive transit through the stomach and small intestine.
OBJECTIVES
The aim of this study was the identification of microorganisms composing different commercial probiotic drinks and the ascertainment of their ability to survive gastro-intestinal (GI) stress, the main requisite to produce beneficial effects.
METHOD
Viable bacteria were enumerated by plate counts in different media. Denaturing Gradient Gel Electrophoresis-Polymerase Chain Reaction (PCR-DGGE) and sequencing were applied on pure isolates to verify their identity in species. Also, probiotic drinks were subjected to stresses characteristic of the GI tract to assess cell survival.
RESULTS
Results evidenced that detected concentrations were similar to those reported on the labels for almost all the probiotic drinks. However some discrepancies were observed between reported species and those ascertained through the identification. The GI stress test revealed that bacteria are strongly injured, and this fact was evidenced by a marked reduction in viable counts after the stress.
CONCLUSIONS
The present study documented the need to establish routinely and well-defined checks able to assess the real composition and efficacy of probiotic drinks.

THE VAGINAL MICROFLORA TUNES THE ANAEROBIC EPITHELIAL IMMUNE ENVIRONMENT

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INTRODUCTION
Epidemiologic studies implicate the disturbed vaginal microflora, characteristic for bacterial vaginosis (BV), in increased susceptibility to sexually transmitted and reproductive infections, and in adverse reproductive outcomes. In a large multicenter study of extremely low gestation age newborns we have recently shown that placental colonization with BV-associated bacteria increases while lactobacillus colonization decreases the risk of systemic inflammatory conditions after birth.
OBJECTIVES
In this study we tested the hypothesis that lactobacillus isolates of the normal microflora (L. acidophilus, L. crispatus and L. jensenii) and BV (P. bivia and A. vaginae) differentially modify the epithelial immune phenotypes.
METHOD / DESIGN
Human vaginal epithelial monolayers were grown in-vitro under conditions mimicking the anaerobic mucosal environment and colonized by vaginal bacteria for six days followed by assessment of epithelia-cell associated colony forming units (CFU), electron microscopy, apoptosis and innate immunity, applying ANOVA.
RESULTS
At consistently similar CFU counts and lack of apoptosis, all bacteria induced NF-B activation in the first 24h with weakest activation induced by L. acidophilus. Cytokines, chemokines, adhesion molecules and hypoxia mediators were differently expressed under continuous anaerobic conditions. Within six days of colonization A. vaginae persistently upregulated a plethora of proinflammatory proteins but not hypoxia mediated VEGF and less so the anti-inflammatory regulator IL-1RA. P. bivia caused selected cytokine increase at lower magnitude as compared to A. vaginae. Elafin levels decreased over the course of six days in the presence of all bacteria except Atopobium, which caused an elafin increase on day six. None of the lactobacillus isolates caused a significant increase of proinflammatory gene expression under the conditions of consistent colonization and equal CFU counts.
CONCLUSIONS
These findings suggest the beneficial role of lactobacillus species in maintaining a noninflammatory environment and incriminate A. vaginae in the development of persistent inflammatory responses in the anaerobic vaginal mucosal environment.

INFLUENCE OF THE TYPE OF DIET ON THE PHYTASE ACTIVITY OF INTESTINAL MICROBIOTA IN VITRO

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INTRODUCTION
Human intestinal epithelium is lacking of phytase activity – an enzyme that dephosphorylate myo-inositol hexakisphosphate, phytic acid or phytate (InsP6) to its derivatives (InsP5, InsP4, InsP3, InsP2, InsP) and inorganic phosphate. Therefore, in the human gut, intestinal bacteria are the main source of phytases and are responsible for the degradation of phytates.
OBJECTIVES
The aim of the study was to investigate whether diets different in the amount of phytate (conventional, vegetarian, breast-feeding) influence the ability of intestinal bacteria to degrade phytic acid. Design Faecal samples were obtained from adult omnivorous, vegetarians as well as from breast-fed infants. Diluted faecal bacteria were used for inoculation (107 CFU/ml) of modified microbiological media supplemented with 1 mM phytic acid. After incubation, the cell counts, the presence of lower myo-inositol phosphates and PCR-DGGE profile of bacteria were determined. Moreover, from each faecal bacterial culture up to 8 isolates were recovered, purified and genetically characterised.
RESULTS
Among bacterial groups, Bifidobacterium and Lactobacillus populations were characterized by the lowest ability to hydrolyze InsP6 (63-65% and 67-76% of NDPA, respectively) independently on the volunteer group investigated. E. coli and Bacteroides populations showed higher phytase activity (21-33% and 20-30% of NDPA). However, the highest level of hydrolyzed InsP6 (67%) and the lowest of NDPA (7%) was determined for non-selective medium inoculated with microbiota of vegetarian. Characteristics of isolates confirmed the individual-specific pattern of microbiota and allow to isolate and identify the most active bacterial isolates.
CONCLUSIONS
It has been shown, that the highest degradation of phytate occurred in the most complex cultures of faecal bacteria (in the non-selective medium) and was the highest in the vegetarian, lower in omnivorous adults and the lowest in breast-fed infants. This observation supports the hypothesis that the diet shape the metabolic activity of intestinal bacteria. Grant no. N N312 434337

INVESTIGATION OF ANTI-INFLAMMATORY PROPERTIES OF PROBIOTICS USING A HUMAN COLONIC MICROBIOTA MODEL AND MACROPHAGE CELL LINES

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INTRODUCTION
Current therapies to modulate the human gut microbiota to reduce gut derived endotoxin concentrations have been developed with the aim to improve inflammatory status. Probiotic bacteria are excellent biotherapeutics used to equilibrate the gut microbiota composition towards health promoting bacterial populations.
OBJECTIVES
The objective of this study was to investigate the anti-inflammatory properties of Bifidobacterium longum subsp. infantis ATCC 15697. The abilities to decrease colonic endotoxins, induce anti-inflammatory cytokines production and decrease pro-inflammatory cytokines secretion were all investigated.
METHODS / DESIGN
A semi-continuous human colonic microbiota model was set up. B. longum subsp. infantis ATCC 15697 was daily administered to the colonic microbiota model during 14 days. Colonic endotoxins, gram positive and gram negative bacteria were quantified in the human colonic microbiota model. RAW 264.7 macrophage cell lines were stimulated with bacterial supernatant from the colonic model. Concentrations of TNF-ALPHA, IL-1β and IL-4 inflammatory cytokines were monitored.
RESULTS
Results demonstrated that B.longum subsp. infantis ATCC 15697 significantly decreased colonic endotoxins, reduced TNF-ALPHA and IL-1β pro-inflammatory cytokines secreted, and increased IL-4 production.
CONCLUSIONS
B.longum subsp. infantis ATCC 15697 might exert anti-inflammatory properties, characterized with increased levels of anti-inflammatory cytokines and decreased levels of pro-inflammatory cytokines secreted, through a mechanism that involve decreased exposure to gram-negative bacteria-derived lipopolysaccharides. We expect to confirm in vivo the substantial beneficial effect of B.longum subsp. infantis ATCC 15697 on endotoxemia and inflammation-induced metabolic diseases.
Keywords : Gut microbiota, Probiotics, Inflammation, Endotoxins, Macrophages

ANALYSIS OF CLASS IIA BACTERIOCINS INTERACTION WITH HUMAN EPITHELIAL CELL PROTEINS

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KEYWORDS: bacteriocins, yeast two hybrid system, epithelial cell

INTRODUCTION

Bacteriocins are proteinaceous metabolites of bacterial origin. They posses antibacterial activity which is usually limited to a narrow group of organisms, most closely related to the producer. Class IIa bacteriocins family is highly homologous subgroup of peptides which are active against foodborne pathogen Listeria monocytogenes. They are considered safe and nontoxic agents devoid of allergenic properties and promising candidates for applications as a natural food preservatives.

OBJECTIVES

Recent studies have shown that the class IIa bacteriocins also have unconventional properties, with respect to eukaryotic cells. Ongoing research aimed at analyzing the impact of class IIa bacteriocins on human epithelial cells.

METHOD / DESIGN

Proquest Yeast two hybrid system was used to identify protein-protein interactions (Invitrogen). As a “bait” we used divercin AS7 (naturally produced by Carnobacterium divergens). As a “prey” we used proteins encoded by human epithelial cell cDNA library. We screened 106 yeast transformants.

RESULTS

We identified human epithelial proteins which interact with class IIa bacteriocin–divercin AS7. Among them ALDOA, MAPRE1, RPL13A, SSR2 show the strongest interactions.

CONCLUSIONS

We postulate these interactions have influence on importans molecular processes – modulation of translation. Further investigations are necessary to explain significance of identified interactions.

SNP ANALYSIS OF MPT OPERON REGULATORY REGION OF LISTERIA MONOCYTOGENES ISOLATES

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KEYWORDS: foodborne pathogen, bacteriocins, SNP analysis

INTRODUCTION

Class IIa bacteriocins family is group of antimicrobial peptides that are active against foodborne pathogen Listeria monocytogenes. Divercin AS7, naturally produced by Carnobacterium divergens AS7 is the promising candidate for applications as a natural food preservatives. However, resistant L.monocytogenes strain existance remains a major concern. In isolates of L.monocytogenes with differences in resistance to divercin AS7 the alternations in the mannose phosphotransferase system function were observed. Mannose phosphotransferase system is involved in transport and phosphorylation of carbohydrates across membranes. This protein complex includes domains IIA and IIB, soluble in the cytoplasm and two integral membrane proteins and IIC IID. The genes encoding individual subunits are located in the mpt operon under a control of regulatory region.

OBJECTIVES

Lack of expression of mpt operon results in resistance of L. monocytogenes strains to class IIa bacteriocins. Aim of this study was to identify changes in nucleotide sequences among mpt operon regulatory region.

METHOD / DESIGN

Divercin AS7 activity against L.monocytogenes was detected using an agar diffusion test. In order to detect changes in the promoter sequences among mpt operon we extract genomic DNA from L.monocytogenes isolates from food. Using species-specific primers we amplified fragments of mpt regulatory region. Amplicons were analyzed by PCR-SSCP and sequencing.

RESULTS

We have identified several SNP among mpt promoter sequence which could be correlated with the degree of resistance to divercin AS7.

CONCLUSIONS In our further studies we are going to analyse regulatory proteins binding to promoter sequences.

FUNCTIONAL CHARACTERIZATION OF THE P40 AND P75 PROBIOTIC FACTORS IN LACTOBACILLUS CASEI

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INTRODUCTION
The genomes of strains from the Lactobacillus casei/paracasei/rhamnosus group encode homologue proteins to p40 and p75 from Lactobacillus rhamnosus GG (LGG). These two proteins were identified as secreted factors in LGG that possess anti-apoptotic effect in intestinal epithelial cells and are able to prevent and treat DSS-induced intestinal injury and colitis in mice. p40 and p75 proteins contain aminohydrolase/peptidase (CHAP) and NLPC/P60 domains, respectively, that are usually present in cell-wall hydrolases.
OBJECTIVES
To characterize the functionality of p40 and p75 from the Lactobacillus casei BL23 strain.
METHODS
p40 and p75 were assayed for their cellular location with specific antibodies. Mutants in the corresponding genes were obtained by gene disruption and the enzymatic and epithelial cells binding activities of the recombinant proteins were assayed.
RESULTS
In Lactobacillus casei BL23 p40 and p75 are secreted to the growth medium and they display hydrolytic activities on cell-wall muropeptides. L. casei BL23 mutants in the p40 and p75 corresponding genes have an altered cell-wall phenotype. p40 and p75 from L. casei BL23 are able to bind mucus and human intestinal epithelial cells and, similar to the LGG proteins, stimulate the phosphorylation of the epidermal growth factor receptor (EGFR). Finally, immunofluorescence studies with epithelial cell lines show that p40 and p75 bind to the cellular surface at specific locations and that they can be internalized.
CONCLUSIONS
These results show that proteins belonging to the cell-wall metabolism machinery in L. casei/paracasei/rhamnosus are soluble probiotic factors involved in the health benefits of these bacteria.

DIVERSITY OF SUPERANTIGEN GENES IN GROUP A STREPTOCOCCUS STRAINS ISOLATED FROM KINDERGARTEN INFANTILE POPULATION

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Superantigens are important virulence factors in the pathogenesis of invasive diseases caused by group A streptococcus (GAS). The aim of the present study was to characterize the streptococcal strains harboring SAg genes isolated from kindergarten infantile population, with or without clinical symptoms, including scarlet fever.
METHODS
The streptococcal isolates were identified using conventional tests (sheep blood haemolysis, bacitracin susceptibility, latex agglutination and API Strep). Production of cell associated virulence factors (adhesins) and soluble enzymatic factors (haemolysins and other pore-forming toxins, proteases, DNA-se and esculin hydrolysis) was assessed by inoculation of HeLa cells and respectively, specific enriched culture media containing different biochemical substrata, with standardized bacterial inocula prepared from fresh cultures. The GAS strains were investigated by PCR for the streptococcal superantigenic toxin (speA, speB, speC, speF, speG, speH, speJ, ssa, smeZ and speI) gene profiles.
RESULTS
All tested strains exhibited the ability of adherence to HeLa cells with a predominant diffuse-aggregative pattern. The soluble virulence factors implicated in the pathogenicity of the streptococcal strains were represented by beta-haemolysins, proteases and DNA-ses. Investigation of superantigen genes prevalence in GAS isolates showed that the most frequent alleles detected in these strains were the chromosomally located genes spef, speg and ssa, followed by speb, spec and speh, spei, smez. Conventional PCR techniques are important to differentiate the streptococcal strains harboring SAg genes irrespectively of their gene expression status, from the SAg gene-negative ones and thus, to clarify the role of SAags in Streptococcus pyogenes infection, which is still a matter of debate.

IMPACT OF COFFEE CONSUMPTION ON THE GUT MICROBIOTA: A HUMAN VOLUNTEER STUDY ANALYZED BY 16S PYROSEQUENCING

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KEYWORDS: Coffee, human gut, microbiota, Bifidobacterium

OBJECTIVES
The impact of a moderate consumption of an instant coffee on the general composition of the human intestinal bacterial population was assessed.
METHOD / DESIGN
Sixteen healthy adult volunteers consumed a daily dose of 3 cups of coffee during a 3 weeks period. Fecal samples were collected before and after coffee consumption and their intestinal bacterial composition was assessed by multiplex 16S pyrosequencing technology.
RESULTS
Although the global fecal profile of the microbiota was not affected by coffee consumption, the population of Bifidobacterium spp. significantly increased by a factor of two after the treatment period.
CONCLUSIONS
When analyzed by DGGE and FISH, this longitudinal study showed similar results (Jaquet 2009, Int. J. Food Microb). Our results confirm that the consumption of the coffee preparation obtained by water co-extraction of green and roasted coffee beans produces an increase in the numbers of the Bifidobacterium spp. population, a bacterial group of reputed beneficial health effects. In addition, our results showed that this effect is truly selective for some Bifidobacterium spp., and impacts no other members of the microbiota.

ACINETOBACTER BAUMANNII STRAINS ASSOCIATED WITH BIOFILM-BASED URINARY CATHETER-RELATED INFECTIONS: A MOLECULAR AND ULTRASTRUCTURAL STUDY

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INTRODUCTION
Acinetobacter baumannii (AB) has an increasing role as causative agent of hospital-acquired infections. Its long-lasting survival in clinical settings together with the antibiotic resistance are features that could be related with the ability for surface colonization and biofilm formation on medical devices such as urinary catheters.
OBJECTIVES
In this study we investigated the ability to adhere, to grow in sessile mode and to form biofilms of AB clinical isolates from urinary catheters. The possible correlation between AB lineages or the presence of specific carbapenem-hydrolyzing Class D b-lactamases (CHDL)-carrying plasmids and biofilm production was also investigated.
METHODS
The study included MDR-AB strains recovered from urinary catheters in patients of two different countries (2001-2011). Genomic based typing methods (PFGE; 2 MLST schemes) and characterization of CHDLs were performed by PCR and sequencing. Plasmid characterization by AB- PCR-based replicon typing (AB-PBRT), PCR mapping and sequencing was performed. The ability to adhere and to form biofilm in vitro was evaluated by the quantitative biofilm production assay. Field Emission Scanning Electron Microscopy (FESEM) and Confocal Laser Scanning Microscopy (CLSM) were used to characterize isolates under a sessile mode. RESULTS
The tested strains included different lineages and CHDLs (ST2/ST92-carrying blaOXA-23, ST2/ST98-carrying blaOXA-40 and ST15/ST103-carrying blaOXA-58) that demonstrated ability to strongly adhere and to grow in a sessile mode. Differences in adherence ability of isolates with different CHDLs plasmid encoded were not detected. The FESEM and CLSM analyses revealed the ultrastructural and tridimensional features of mature biofilms (24 and 48h), allowing the evaluation of biofilm formation, matrix appearance and bacterial surface.
CONCLUSIONS
Our data suggest the adhesiveness and the ability to form biofilm as typical virulence features of AB lineages associated with urinary catheter-related infections, which might explain their emergence and persistence, although a confirmation on a larger number of strains is mandatory.

SAFETY ASSESSMENT OF THREE PROBIOTIC STRAINS CNCM I-4034, CNCM I-4035 AND CNCM I-4036

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INTRODUCTION
Three probiotic strains were isolated from feces of breast fed infants and deposited in Institut Pasteur Collection. To use them such as ingredient in human nutrition, it is needed to test safety. Guide such as FAO/WHO 2002, EFSA QPS list, EFSA criteria in the evaluation of resistance were used.
OBJECTIVES
To evaluate the safety of the three strains (CNCM I-4034, CNCM I-4035 and CNCM I-4036) according to abovementioned guides. Bile salt hydrolase activity (BSH), resistance to antibiotics, D/L-lactate and biogenic amines production, oral toxicity and bacterial translocation in mice and genome sequencing were performed. Indeed were compared to commercial strains (B. bifidum and LGG).
METHODS
BSH activity was tested according to Kumar et al. (2006). Antibiotic resistance was evaluated following the criteria established by EFSA and minimum inhibitory concentration (MIC) was established. The concentration of D/L-lactic acid was tested using an enzymatic Kit. The production of biogenic amines was assessed by HPLC-UV using the methodology of Eerola et al. (1993). Oral toxicity and bacterial translocation were evaluated using acute ingestion assays in mice. Genome sequencing was performed on the 454 platform (Roche) to ensure absence of antibiotic resistance genes.
RESULTS
BSH activity was detected in the case of CNCM I-4035, being lower than other commercial strains. As a general rule, MIC values were lower than EFSA breakpoints. Strains have a minimum D-lactic production similar than commercial strains. Putrescine was not detected, cadaverine, histamine and tyramine were detected in low levels similar than commercial strains. There were no significant differences in food intake, body weight evolution, tissue weights and appearance of the internal organs between control and treated groups in murine model. Two strains contain plasmids without antibiotic resistance genes.
CONCLUSIONS
According to obtained results, the use of these strains in food or pharmaceutical products is SAFE.

OCCURRENCE OF BIOCIDE RESISTANCE GENES IN MULTIDRUG RESISTANT SALMONELLA, INCLUDING IN THE EMERGING CLONAL LINEAGES OF S. RISSEN AND S. TYPHIMURIUM MONOPHASIC VARIANT

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INTRODUCTION
Multidrug-resistant Salmonella is emerging worldwide with increasing involvement of particular clones. Also, resistance to biocide compounds frequently used as animal growth promoters and/or disinfectants might provide a better adaption of Salmonella to selective environments.
OBJECTIVES
To assess the occurrence of known biocide resistance genes among multidrug-resistant nontyphoidal Salmonella belonging to particular clonal lineages and obtained from different sources.
METHODS
Search of pcoD (copper), merA (mercury), silA (copper/silver), arsB (arsenic) and terF (tellurium) genes encoding resistance to compounds found in the animal setting (feed, disinfectants and environmental pollution) was performed by PCR/sequencing in 92 Salmonella isolates belonging to 16 serotypes recovered in Portugal (2000-2011). The isolates comprise the two emergent serotypes S.Typhimurium monophasic variant (n=32) and S.Rissen (n=30), and other 30 Salmonella isolates representative of multidrug-resistant clones from human/non-human sources. Antibiotic susceptibility was tested by disk diffusion/agar dilution method (CLSI). Clonality was established by PFGE/MLST. Antibiotic resistance genes, plasmid and integron backbones (PCR, RFLP and/or sequencing), transferability and genomic location (I-CeuI/S1 nuclease hybridization) was performed.
RESULTS
The pcoD and silA gene were detected in all S.Rissen (n=18; phenotype ASSuTTr; blaTEM-aadA-sul1/sul3-tetA-dfrA12; ST469). The majority of S.Typhimurium monophasic variant from the European clone (n=17; ST34; phenotype ASSuT; blaTEM-strA-strB- sul2-tetB) carried pcoD, merA and silA genes (chromossomally located) contrary to the Spanish multidrug-resistant clone (n=8; ST19) carrying the merA and silA on large non-conjugative IncA/C plasmids. The merA (n=18), silA (n=8) and pcoD (n=7) genes were also dispersed in isolates belonging to other multidrug-resistant clones/serotypes. The arsB and terF were only associated with the first extended-spectrum b-lactamase-producing (CTX-M-9) Salmonella strain isolated in Portugal.
CONCLUSION
Our data suggest that additionally to the antibiotic use, the biocides-metals, extensively present in animal production setting, might support the ongoing expansion of relevant clonal lineages, namely the S. Rissen and the S. Typhimurium monophasic variant.

A MICROBIOLOGICAL SURVEY OF THE HUMAN GASTRIC ECOSYSTEM IN THE SEARCH OF STRAINS WITH PROBIOTIC POTENTIAL

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INTRODUCTION
The human stomach seems to harbour a greater microbial diversity that that anticipated. Among the colonizing microorganisms, different lactic acid bacteria belonging mainly to the genera Lactobacillus and Streptococcus are present. These bacteria might be playing important roles in the maintenance of the healthy status of the host. Additionally, they could be used in the design of probiotic to counteract pathogens such as Helicobacter pylori.
OBJECTIVES
A microbiological study of biopsia samples from the gastric mucosa of healthy individuals was carried out by culturing and a metagenomic approach in order to search for lactic acid bacteria with desirable probiotic traits.
METHODS
Twelve biopsies of stomach were analyzed by culturing. Of these, DNA from four samples was amplified with universal bacterial primers and the amplicons pyrosequenced. Gastric lactobacilli isolates were characterized by a variety of in vitro assays which included, among others, production of antimicrobial compounds, antioxidative activity, acid resistance and ability to adhere to a gastric cell line. Results Occurrence of lactobacilli was evidenced by both culturing and pyrosequencing. Ten strains belonging to the species Lactobacillus gasseri (3), L. reuteri (2), L. vaginalis (2), L. fermentum (2), and L. casei (1) species were selected for further characterization. Strains showed good tolerance and survival to low pH and were all shown to be free of atypical antibiotic resistances. Species and strain specific differences in the production of H2O2, inhibition of Helicobacter pylori and adhesion to the gastric epithelium were encountered.
CONCLUSIONS
The presence of alive members of the genus Lactobacillus in the human stomach of healthy people was confirmed. A set of gastric lactobacilli was extensively characterized. Some strains showed beneficial properties, thus constituting good probiotic candidates.

INCREASED GERMINATION ACTIVITY OF CLOSTRIDIUM DIFFICILE STRAINS ISOLATED FROM THE PATIENTS WITH RECURRENT INFECTION WITH C. DIFFCILE

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INTRODUCTION
Clostridium difficile is the principal pathogen causing pseudomembranous colitis (PMC) and antibiotic-associated diarrhea (AAD). Recently, recurrent cases of C.difficile-associated disease (CDAD) have become a difficult clinical problem, in terms of prolonged duration of hospitalization and increasing treatment costs. Various studies have reported that molecular and microbiological characteristics of isolates from CDAD and/or recurrent CDAD cases. However, reports for spore formation and germination abilities of C. difficile are very limited, although sporulation and germination are presumed as one of the most important factors in the pathogenicity of C. difficile.
OBJECTIVES
We performed DNA typing of clinical isolates and examined microbiological characteristics of isolates including germination and sporulation rates to investigate the correlation between molecular/microbiological characteristics of isolates and the recurrence of CDAD.
METHOD / DESIGN
Twenty isolates of C. difficile from 20 single infection cases and 53 isolates from 20 recurrent cases were used. DNA typing of isolates was performed by PCR ribotyping and PFGE analysis. The isolates from recurrent cases were divided into relapse and reinfection cases based on the results of PFGE analysis. Cytotocixity, antibiotic susceptibility and sporulation/germination rates of isolates were examined.
RESULTS
Sixteen (80%) out of 20 recurrent cases were identified to be relapse cases caused by the initial strain. All 73 isolates were susceptible to both vancomycin and metronidazole, but more than 80% of isolates were resistant to clindamycin, ceftriaxone, erythromycin, and ciprofloxacin. There was no correlation between DNA typing group, cytotoxicity and sporulation rates, and infection status. However, the germination rates of isolates from relapse cases were significantly higher than that of isolates from single and/or reinfection cases when incubated in the medium lacking the germination stimulant sodium taurocholate.
CONCLUSIONS
The germination ability of C. difficile may be a potential risk factor for the recurrence of CDAD.

CEFTRIAXONE THERAPY, INTESTINAL BETA-LACTAMASES AND INTERACTION WITH PROBIOTICS IN CHILDREN

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INTRODUCTION
The actual interest in probiotics is also determined by the intestinal side effects of antibiotics and increased resistance of intestinal bacteria.
AIM
We studied the effects of different commercial preparations of probiotics on the intestinal ecosystem during ceftriaxone administration in children to correct microflora imbalance.
METHODS
Fifty-one children (age 4-10 years) with respiratory tract infections were subdivided in eight groups and treated with ceftriaxone (1g/d) along with six different probiotic preparations (Lactobacillus, Bifidobacterium, Enterococcus, Saccharomyces) or lactulose for a week. The activity of betalactamases and other enzymes, flora composition, and ceftriaxone concentrations were determined in faeces before and after treatment.
RESULTS
Ceftriaxone caused a E.coli count decrease and enterococci increase. The administration of all probiotics along with ceftriaxone maintained the inhibition of E.coli, while the increase in enterococci was partially normalised. The effects on the other bacterial components showed more or less appreciable differences according to probiotic administered. The mean count of microaerophilic lactobacilli increased slightly, while different species of lactobacilli and bifidobacteria were isolated more frequently. Reduction in number of species of clostridia (from 6 to 3 different species) after therapy was observed. No C.difficile was detected in all children. The increase in lactobacilli and anaerobic gram-positive fermentative species contributed to maintain a low faecal pH (slight decrease of pH from 6.9 to 6.6). Bowel movements/day were reduced in probiotic treated children. Faecal beta-lactamase activity increased during antimicrobial therapy. In the probiotic treated groups the incidence was lower (25% - 30%). This is an unespected effect of bacterial strains selected for probiotic use.
CONCLUSIONS
Probiotics administered along with ceftriaxone at the start of antimicrobial therapy contribute to ameliorate the intestinal dysbiosis. Probiotics administration could afford protection against beta-lactamases production or activity. Their potential for resistance reduction in these conditions should be confirmed by further studies.

THE ORAL MICROBIOME BY A METAGENOMICS AND METATRANSCRIPTOMICS APPROACH

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INTRODUCTION

The oral cavity of humans is inhabited by hundreds of bacterial species and some of them play a key role in the development of oral diseases, mainly dental caries and periodontitis. More than half of these bacteria cannot by cultured by conventional methods and PCR-based molecular approaches are subject to important biases. The current work presents a metagenomic approach obviating PCR and cloning in order to study the oral microbiome.

OBJECTIVES

To describe the taxonomic composition and gene content of the total and active microbial community inhabiting the human supragingival dental plaque.

METHOD

Whole DNA and cDNA from dental plaque of healthy individuals was pyrosequenced, obtaining over 100 Mbp of sequence per sample with reads over 400 bp in length. Human DNA reads were filtered and the sequences assigned taxonomically and functionally to different databases. This taxonomic and functional analysis was compared between individuals, between oral and gut samples, and in oral samples before and after a meal.

RESULTS AND CONCLUSSIONS

In 24h oral biofilms, the composition of the metatranscriptome is different to that of the metagenome, indicating that the active fraction of the microbiota is only a part of the total composition and suggesting which species are actively contributing to biofilm formation. Several gene funcions appear over-represented in oral bacteria when compared to gut microorganisms, including genes for iron scavenging or osmotic and oxidative stress. The metatranscriptome of oral bacteria before and after a meal changed dramatically, indicating which species are probably related to sugar fermentation and the subsequent reduction in pH.

THE ACTIVE FRACTION OF HUMAN GUT MICROBIOTA

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INTRODUCTION
The human gut microbiota is considered one of the most fascinating reservoirs of microbial diversity hosting between 400 to 1000 bacterial species distributed among nine phyla with Firmicutes, Bacteroidetes and Actinobacteria representing around 75% of the diversity. One of the most intriguing issues relates to understanding which microbial groups are active players in the maintenance of the microbiota homeostasis.
OBJECTIVES
Here, we describe the diversity of active microbial fractions compared with the whole community from raw human fecal samples.
METHOD/DESIGN
We studied the microbiota distribution of faecal samples from four healthy volunteers. Active cells have been labelled with pyronin-Y, an RNA specific fluorescent dye. DNA from each fraction was then purified, and 16S rDNA gene was amplified. Amplicons were sequenced by 454 technology.
RESULTS
Bacterial families were observed to appear or disappear on applying a cell sorting method in which flow cytometry was used to evaluate the active cells by pyronin-Y staining of RNA. This method was able to detect active bacteria, indicating that the active players differed from that observed in raw fecal material. Generally, observations showed that in the active fractions, the number of sequences related to Bacteroidetes decreased whereas several families from Clostridiales (Firmicutes) were more highly represented. Moreover, a huge number of families appeared as part of the active fraction when cell sorting was applied, indicating reads that are simply statistically hidden by the total reads.
CONCLUSIONS
Obtained data clearly show that functional microbiota is different from total microbiota and that diversity distributions should not be deduced uniquely from DNA-based experiments.

YFIBNR OPERON IN KLEBSIELLA PNEUMONIAE

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INTRODUCTION
Klebsiella pneumoniae is an opportunistic pathogen that can cause pneumonia, septicemia, and nosocomal infections. Infections One factor that can contribute to the pathogenicity of K. pneumoniae is its ability to adhere to surfaces and form biofilms. The genus Klebsiella has been an alarming increase in the frequency and resistance of K. pneumonia strains in many countries, including Colombia.
OBJECTIVE
To analyze biofilm formation through the study of mutants with alterations in the genes coding for diguanylate cyclase (DGC) proteins involved in intracellular signaling.
Methodology: Using a gene replacement strategy, we obtained mutants in the genes of interest. Complementation assays were done by cloning K. pneumoniae gene copies in plasmid pBAD18. Phenotypes were verified by assays for biofilm formation and growth in indicator media. Gene expression was analyzed using real-time PCR and microscopic studies were carried out to observe the dynamics of biofilm formation.
RESULTS
Genetic and molecular analysis of the genes yfiR, yfiB, and yfiN showed that the ΔyfiR mutant has an increase in biofilm formation, while mutants ΔyfiB and ΔyfiN showed a decrease phenotype relative to the wild type. Our results are consistent with previous findings that indicate that this operon is involved in the synthesis of the second messenger c-di-GMP, results in enhanced biofilm formation due to overexpression of extracellular matrix components, in this case cellulose. Microscopic analysis the mutants strains also showed alteration in biofilm development consistent with the different predicted protein functions.
CONCLUSIONS
In this work we were able to establish that the yfiR gene of the yfiBNR operon, which is highly conserved in many γ–proteobacteria, also acts as a negative regulator of YfiN, which in turn affects c-di-GMP pools and production of cellulose and fimbria. Preliminary results suggest that yfiB, a probable transmembrane protein, likely responds to changes in oxygen and perhaps temperature.

BIFIDOBACTERIUM CECT 7765 IMPROVES METABOLIC AND IMMUNOLOGICAL DYSFUNCTION ASSOCIATED WITH OBESITY IN HIGH-FAT DIET FED MICE

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INTRODUCTION
Obesity has been related to phylum and group-specific changes in the microbiota, suggesting a role for specific bacteria in this disorder.
OBJECTIVE
To evaluate the effects of oral administration of Bifidobacterium CECT 7765 on metabolic and immune dysfunction in mice with high-fat diet (HFD) induced obesity.
METHODS/DESIGN
Adult (age 6¡V8 week) male wild-type C57BL-6 mice were fed a standard diet (SD) or HFD, supplemented or not with Bifidobacterium CECT 7765 for seven weeks (n„d 6/group). The following parameters were assessed: animal weight, serum levels of cholesterol, triglyceride, glucose and leptin, liver steatosis, white adipose tissue weight and adipocyte size, lipid micelles per enterocyte, functions of immunocompetent cells (macrophages and dendritic cells [DCs]) and composition and pro-inflammatory properties of the faecal microbiota.
RESULTS
Bifidobacterium CECT 7765 administration reduced serum cholesterol, triglyceride and glucose levels by 36, 25 and 35 %, respectively, in obese mice. This bacterial strain also induced a decrease in serum leptin levels in HFD-fed mice. The administration of Bifidobacterium CECT 7765 significantly reduced liver steatosis and the number of larger adipocytes (2000 to 4000 £gm2) in HFD-fed mice. These effects were associated with reductions in the number of fat micelles in enterocytes, suggesting reductions in dietary fat absorption. The strain also increased the macrophage oxidative burst, the ability of macrophages and DCs to induce cytokines (TNF-ƒÑ) in response to pathogenic bacterial components (LPS), and the ability of DCs to present antigens and to induce T lymphocyte proliferation. The bacterial strain also restored partially the composition of the gut microbiota of HFD-fed mice, increasing bifidobacteria and reducing enterobacteria numbers, which altogether led to reducing inflammatory signals coming from the gut.
CONCLUSION
Bifidobacterium CECT 7765 was shown to ameliorate both metabolic and immunological alterations related to obesity in HFD-fed mice.

BIFIDOBACTERIUM LONGUM CECT 7347 MODULATES INFLAMMATORY RESPONSES IN A GLUTEN-INDUCED ENTEROPATHY ANIMAL MODEL

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INTRODUCTION
Coeliac disease (CD) is an autoimmune disorder triggered by gluten proteins (gliadin) that involves innate and adaptive immunity where specific components of the microbiota could play an adverse or protective role in this disorder.
OBJETIVE
To study the possible protective effects of Bifidobacterium longum CECT 7347 in an animal model of gliadin-induced enteropathy.
METHOD/DESIGN
Newborn Wistar rats were randomly distributed into seven different groups (n=6 per group): 1) artificially reared (AR); 2) AR and fed B. longum CECT 7347; 3) AR and fed gliadin (GP); 4) AR and fed GP and B. longum CECT 7347; 5) AR sensitised with 1,000 U IFN-ƒ× administered intraperitoneally immediately after birth; 6) sensitised animals (AR) fed GP; 7) sensitised animals (AR) fed GP and B. longum CECT 7347. Weanling rats were fed 50 ƒÝg gliadin/day in a single dose during the 10 days, and a provocative dose of gliadin 100 ƒÝg ƒî2 hours before sacrifice. Tumour necrosis factor-ƒÑ and interleukin (IL)-10 were determined in homogenised jejunal tissue sections by ELISA. T-cell sub-populations (CD4+, CD8+ and CD4+/Foxp3+) were monitored in peripheral blood samples by flow cytometry.
RESULTS
Feeding gliadin alone reduced peripheral CD4+ cells, increased CD4+/Foxp3+ T and CD8+ cells, while simultaneous administration of B. longum CECT 7347 exerted opposite effects. Animals sensitised with IFN-ƒ× and fed gliadin exhibited increased NFƒÛB mRNA expression and TNF-£\ production in tissue sections. B. longum CECT 7347 administration increased NFƒÛB expression and IL-10, but reduced TNF-£\, production in the enteropathy model. In sensitised gliadin-fed animals, CD4+, CD4+/Foxp3+ and CD8+ T cells increased, whereas the administration of B. longum CECT 7347 reduced CD4+ and CD4+/Foxp3+ cell populations and increased CD8+ T cell populations.
CONCLUSIONS
B. longum CECT 7347 attenuates the production of inflammatory cytokines in the intestine and the peripheral T-cell phenotype in an animal model of gliadin-induced enteropathy.

PROTECTIVE EFFECT OF A LACTOBACILLUS PLANTARUM STRAIN IN AN ANIMAL MODEL OF GASTROINTESTINAL INFECTION ASSOCIATED TO ENTERIC SALMONELLA TYPHIMURIUM.

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INTRODUCTION
Salmonella is an important etiologic agent in men and other animals, and it can cause zoonotic diseases. Objective: To determine the protective role of a Lactobacillus plantarum strain in a mouse model of Salmonella typhimurium ATCC 14028 infection.
DESIGN
BALB/c female mice were distributed in groups, forming a probiotic group (treated with a daily dose of 109 CFU/mouse of probiotic strain for seven days previous to the infection, and then infected with 108 CFU/mouse of Salmonella), an infection group (only infected with Salmonella), a probiotic control group (only received the L. plantarum treatment) and a negative control group. Haematological samples and stool tests were continuously analysed, and there were scheduled sacrifices to prove the grade of infection and/or protection. Results: S. typhimurium colonised animals from the inoculated groups, being found 6-7 days after the infection in intestines, spleen, and liver. Concentrations of the bacterium up to 107 CFU/g were found in the stool tests; the counts of the Lactobacilli group were always significantly lower than those observed in the infection group. The probiotic strain was quantified in faeces and confirmed 2 days after the infection by PCR, in concentrations of 105 CFU/g. In the haematological aspect, there was an increase of neutrophils and monocytes, and a decrease of lymphocytes. In the histological aspect, there was damage in the liver and spleen of infected animals. Statistically, there was a higher survival rate and better growth rates in the animals that had received the probiotic strain.
CONCLUSION
A model of infection with S. typhimurium in BALB/c mice was effectively established. The application of the Lactobacillus strain had success in significantly reducing the mortality of the treated animals, which may be caused by an in vivo antagonist effect and the stimulation of key immunological components.

CHARACTERIZATION OF KLEBSIELLA PNEUMONIAE FIMBRIAE INVOLVED IN CATHETER ASSOCIATED URINARY TRACT INFECTIONS.

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INTRODUCTION

K. pneumoniae is capable of colonization and biofilm formation on urinary tract catheters, which are standard medical devices in hospital and nursing homes, e.g. 21 to 50 % of patients being catheterized. Catheter associated urinary tract infections (CAUTI) are often caused by K. pneumoniae. The role of fimbriae is here studied in vitro and in vivo.

OBJECTIVES

To characterize the importance of K. pneumoniae fimbriae in biofilm formation in vitro, and development of CAUTI in vivo.

METHOD / DESIGN

Isogenic type 1 and type 3 fimbriae mutants of the clinical isolate C3091 were tested in a biofilm microtiter plate assay to determine fimbriae's role in biofilm formation.
A murine model of CAUTI to determine fimbriae's role in vivo.

RESULTS

The in vitro assay indicated that K. pneumoniae needs both types of fimbriae for biofilm formation.;The assay was only performed once and will be repeated to confirm the results obtained. The in vivo experiments strongly indicated that the two types of fimbriae could compensate for each other. Loss of type 3 fimbriae lowered the bacterial counts, while loss of both types severely diminished the infection ability in catheterized mice.

CONCLUSIONS

The obtained results from the murine CAUTI model strongly confirm previous in vitro results by Stahlhut et al. (2012), establishing the roles of type 1 fimbriae and type 3 fimbriae. In conclusion, type 3 fimbriae are more important compared to type 1 fimbriae for the ability of K. pneumoniae to cause CAUTI. Furthermore, K. pneumoniae can use one fimbrial type to compensate for the other type being lost, but if both are lost, the virulence is severely compromised.

POSTER COMMUNICATIONS

HYDROGEN PEROXIDE PRODUCTION BY VAGINAL LACTOBACILLI DEPENDS ON SPECIES AND STUDY GROUP

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INTRODUCTION
Vaginal health depends significantly upon the composition of microbiota. Lactic acid bacteria maintain the ecological equilibrium by protecting against pathogens and microbiota imbalance. Hydrogen peroxide (HP) is one of the most important antimicrobial compounds produced by vaginal lactobacilli. While screening them as potential probiotics the detection of HP production is essential.
OBJECTIVE
To assess the HP production in vaginal lactobacilli of healthy women and women of infertile couples, the latter being the partners of men with or without inflammatory prostatitis (IP).
METHODS
Altogether 135 strains were investigated, of them 70 originated from women of infertile couples (48 from partners of healthy men, 22 from partners of IP-patients) and 65 from healthy women. Lactobacilli were identified by sequencing of 16S rDNA fragment. For HP detection, the strains were plated on TMB agar containing tetramethyl-benzidine and horseradish peroxidase, and incubated for 48 h at 37°C under anaerobic conditions. After 30 min exposure to air, HP producing colonies turned blue.
RESULTS
Most of Lactobacillus crispatus (89%) and Lactobacillus jensenii (80%) strains while only 46% of Lactobacillus gasseri strains produced HP ($p=0.005$ in comparison with L. crispatus, $p=0.056$ in comparison with L. jensenii). Lactobacilli originating from healthy women ($p=0.037$) and partners of healthy men ($p=0.029$) expressed stronger production of HP than partners of IP-patients. In case of L. jensenii, higher number of strong HP producers originated from partners of healthy men than partners of IP-patients ($p=0.047$).
CONCLUSIONS
Our study suggests that HP production is species-specific while the species that are associated with more stable vaginal environment (L. crispatus, L. jensenii) are stronger producers. In addition, HP production depends on donors' contingent since it is lower in strains that originate from partners of IP-patients. Therefore healthy women and partners of healthy men are preferred as potential donors of putative probiotic bacteria.

EFFECT OF SOURDOUGH WITH BIFIDOBACTERIA ON THE QUALITY CHARACTERISTICS OF WHOLE RYE-WHEAT MIXED BREAD

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INTRODUCTION
Whole grains can be modified by sourdough fermentation to improve nutritional value or promote healthiness of cereal foods. The sourdough has great potential to modify the digestibility of starch by raising the lactic and acetic acid levels and increasing the mineral bioavailability.
OBJECTIVE
Develop new cereal-based products, with increased nutritional quality, by using intestinal bifidobacteria as new starter for sourdough, and to optimize whole rye-wheat mixed bread quality.
METHOD/DESIGN
A factorial design was constructed to study the effects of whole rye flour (0, 25, 50, 75, and 100%) and the sourdough levels (0, 10, and 20%) in the formulation of bread dough preparation. Response surface plots were obtained to show the single effects and the interactions among the both factors. Bread quality was evaluated by the specific bread volume, slice shape, crumb texture profile, digital image analysis, crumb and crust colour, dietary fibre, myo-inositol phosphates levels and sensory evaluation.
RESULTS
The whole rye flour inclusion into the bread formulation significantly affected the final product quality in terms of specific volume and crumb firmness, while both soluble and insoluble fibres increased significantly. The products with sourdough inoculated with Bifidobacterium pseudocatenulatum ATCC 27919 showed similar technological quality as their homologous samples without sourdough, with the exception of the crumb firmness. Sourdough significantly increased the levels of organic acids in fermented dough. The inoculation of the bifidobacterial strain contributed to phytate hydrolysis resulting in bread with significantly lower phytate levels. The sensory analysis showed lower ratings for breads with high concentration of rye flour than for control breads nearly irrespective of the degree of flour replacement by sourdough.
CONCLUSIONS
Breads with 25% of whole rye flour were highly accepted as evidenced by overall acceptability scores in comparing with whole wheat breads, with higher nutritional value by inclusion of sourdough fermented with bifidobacteria.

APPLICATION OF PHYTASES FROM BIFIDOBACTERIA IN THE DEVELOPMENT OF CEREAL-BASED PRODUCTS WITH AMARANTH

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INTRODUCTION
The inclusion of whole grain products in the daily diet provides a nutritional benefit. However, whole grains contain significant amounts of phytic acid (myo-inositol hexakisphosphate, InsP6) or its salts (phytates), a well known inhibitor of mineral, proteins and trace elements bioavailability.
OBJECTIVE
The objective of this investigation was to develop new bakery cereal-based products with improved nutritional quality, by including whole amaranth flour and phytases from intestinal bifidobacteria.
METHOD/DESIGN
Two different whole amaranth flour levels from Amaranthus cruentus (25 and 50%) were used in bread dough preparation and were comparatively evaluated with control ones (100% wheat flour and 100% whole wheat flour). The effects of the inclusion of purified recombinant phytases from Bifidobacterium infantis ATCC15697 and B. pseudocatenulatum ATCC27919 on phytate levels of each bread formulations were also analyzed comparing to its counterpart without phytase (as negative control) and with commercial fungal phytase (as positive control).
RESULTS
The quality of the final products was analysed by the loaf specific volume, width/height ratio of the central slice, crust and crumb colour, crumb hardness and phytate levels. Breads samples made with 50% of amaranth flour showed a significant change of all the studied parameters, in comparison with control ones. However, the 25% formulated bread samples showed similar technological quality as the control white bread. The phytases inclusion did not affect the final product quality, with the exception of a slight increment on loaf volume and a softening of the crumb. The addition of the phytases contributed to the hydrolysis of phytate and decreased significantly its level in breads with 25% of amaranth.
CONCLUSIONS
As a general trend, 25% of whole amaranth flour could be used as a replacement for wheat flour in bread formulations, improving the nutritional value, with a slight depreciation in the quality.

CHARACTERISATION OF LACTOBACILLI AND BIFIDOBACTERIA FROM HUMAN COLON SUITABLE AS POTENTIAL PROBIOTIC AGENTS

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INTRODUCTION
Gastrointestinal (GI) functions in health can be modulated by gut microbiota and isolation of favourable bacteria may lead to identification of novel probiotic agents. We sought to select a small number of candidate Lactobacillus and Bifidobacterium isolates from human colon suitable for further characterisation and delivery as potential probiotic agents influencing movements of the intestine.
OBJECTIVE
To develop a rational approach for selection of candidate organisms, prior to investigating their potential to modulate intestinal motility.
METHODS
Bifidobacteria and lactobacilli were isolated on selective media from mucosal biopsies taken with informed consent at routine colonoscopy of the right sided colon. Patients with a diagnosis of GI infection, colorectal cancer or inflammatory bowel disease were excluded. Isolates were identified by biochemical tests or mass spectrometry. Growth characteristics, tolerance of bile and acid, and autoaggregation were assayed by standard methods. A fluorescence-based assay for adhesion of bacteria to porcine mucin was applied.
RESULTS
Recent human isolates differed widely in a range of characteristics that have been proposed to identify useful probiotic organisms, and few organisms performed well in all tests. Adhesion to mucin correlated closely with adhesion to Caco-2 cells in control assays with probiotic standards, and was reproducible and simple to perform. There was limited correlation between mucin adhesion and autoaggregation, although autoaggregation has previously been suggested as a surrogate test for adhesion.
CONCLUSIONS
A combination of mucin adhesion, measured by fluorescence, with bile and acid tolerance provides a simple and rational screening scheme with which to select candidate organisms likely to survive oral administration and remain within the GI tract for an acceptable time, prior to conducting physiological tests on how they might influence intestinal motility.

TIMING AND DURATION OF PROBIOTICS ADMINISTRATION- A CRUCIAL POINT TO MAXIMIZE POSITIVE EFFECTS IN DIFFERENT GASTROINTESTINAL DISORDERS

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INTRODUCTION

The use of selected probiotic strains are the basis for a satisfactory clinical outcome in gastrointestinal disorders. Nevertheless, timing and duration of probiotics administration remain to define.

AIM

Effects of different schedule of probiotics administration in various pathologic conditions presenting gastrointestinal disturbances.

RESULTS

Probiotics administration for treatment of acute infectious diarrhea in children may vary from one to three weeks; the early administration at onset of symptoms seems induce more satisfactory results as reduction of diarrhea duration and number of movements/day. The early administration in preterm low-body-weight infants, prolonged until discharge, induced positive effects in Necrotizing Enterocolitis The incidence and severity of Antibiotic-Associated-Diarrhea and Recurrent-C.difficile-Infection is reduced by selected strains; their administration along with antibiotic therapy seems more satisfactory. Prolonging administration after the end of antibiotic therapy should accelerate microflora normalization. In chronic pancreatitis the short course of intestinal antibiotic therapy followed by 1 month of probiotic administration improved intestinal symptoms and clinical parameters. In Premenstrual Syndrome, one month of probiotics administration improved gastrointestinal symptoms with further amelioration after three months. Similar results were obtained in autistic children, after 1 month of high-dose mixture probiotics administration. In chronic inflammatory bowel diseases the prolonged administration (range from 2 to 12 months) seems improve the clinical outcome as remission rate of Ulcerative Colitis in both children and adults. Multistrain probiotic treatment was effective for maintenance of remission of chronic-relapsing-pouchitis for nine months or until relapse, while a single strain in acute pouchitis was ineffective. Four weeks was thought to be the minimum duration of intervention in Irritable Bowel Syndrome patients; prolonging treatment maintained the initial partial positive effect without further clinical improvement.

CONCLUSIONS

The timing and the duration of probiotics administration, along with strain characteristics and appropriate dose, are crucial points to obtain positive effects in intestinal disorders of different etiology.

PRODUCTION OF POLYAMINES BY DIFFERENT STRAINS OF L. PLANTARUM AND ITS EXPRESSION IN FOOD AND HUMANS.

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Polyamines are aliphatic molecules with amine groups distributed along their structure. In humans the source of polyamines may be endogenous or exogenous supplied by the diet. Polyamine synthesis is necessary for cell growth, tissue reparative processes, immunity and the synthesis of proteins and nucleic acids. The polyamines of microbial origin are formed by the decarboxylation of amino acids.

AIM

To assess in vitro the ability of human Lactobacillus strains to produce poly- and biogenic amines from different amino acids, the corresponding genes and their expression in vivo.

MATERIAL AND METHODS

The production of poly- and biogenic amines (putrescine, cadaverine and histamine) from precursor amino acids (arginine, glutamine, lysine, ornitine and histidine) by 5 lactobacillus (incl. L. plantarum Inducia, Tensia) and 4 gram-negative strains (Escherichia coli, Pseudomonas aeruginosa), was tested using gas chromatography (GC). The corresponding genes were detected by PCR with specific primers. The amount of polyamines in probiotic and control cheeses (n=3) and in urine samples (n=24) of consumers was also assessed.

RESULTS

Lactobacilli, compared to enterobacteria, were able to produce putrescine (median 0, range 0-2.1 vs. median 14.4, range 1.8-2228.5; $p<0.0001$) in very small amounts. The production of putrescine in vitro by L. plantarum Inducia was 6 times higher compared to that of L. plantarum strain Tensia (3.1 vs. 0.5 mg/L).and in cheese it was proportional to that in vitro (24.67 vs. 1.32 mg/kg). A significant increase of acPut was found ($p=0.02$) in urine of volunteers consuming cheese comprising L. plantarum Inducia for 3 weeks, not detected in consumption of control and cheese comprising L. plantarum Tensia

CONCLUSION

The amount of putrescine produced in vitro by the L. plantarum strains (Inducia and Tensia) both from the decarboxylation media and cheese, as assessed by GC, was in a good concordance with its concentration in urine of cheese consumers.

SUPERNATANT OF Bifidobacterium breve CNCM I-4035 DECREASES ADHESION OF Salmonella typhi TO CACO-2 CELLS

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INTRODUCTION
Intestinal epithelial cells, are important participants in the mucosal immune response and therefore must respond to a variety of stimuli, including commensal and pathogenic bacteria. Adhesion of pathogenic bacteria on enterocytes is a prerequisite of colonization and is involved in the mucosal immune response. Probiotic bacteria and their products may provide protection against intestinal damage induced by pathogens colonization, but the underlying mechanisms are still largely unknown.
OBJECTIVES
The aim of this study was to determine whether Bifidobacterium breve CNCM I-4035 or their metabolism products, inhibit the Salmonella typhi CECT 725 adhesion on intestinal Caco-2 cells.
METHODS
Caco-2 cells were exposed to 1-2x108 CFU/ml of Salmonella typhi CECT 725, Bifidobacterium breve CNCM I-4035 and/or 4% of neutralized and not neutralized culture supernatant of Bifidobacterium breve CNCM I-4035 (17 and 24 h, 37°C, anaerobically) lyophilized and concentrated 10x during 4h. After of washing three times, total DNA was extracted and the adhesion inhibition was evaluated by quantitative PCR using specific primers for Salmonella and Bifidobacterium.
RESULTS
Only 17h culture supernatant of Bifidobacterium breve CNCM I-4035 (neutralized and not neutralized) but not the bacteria inhibits Salmonella typhi CECT 725 adhesion to Caco-2 cells($P<0.001$).
CONCLUSIONS
Neutralized and not neutralized culture supernatant of Bifidobacterium breve CNCM I-4035 inhibits Salmonella typhi CECT 725 adhesion to epithelial cells.This effect seems be due to some substances produced by the probiotic bacteria and indeed present in culture supernatants. This work has been funded by HERO Spain, Contract no.2143 Fundacion General Universidad de Granada Empresa

ADDITION OF PHYTASE-PRODUCING BIFIDOBACTERIA AND ITS INFLUENCE ON IRON BIOAVAILABILITY OF WHOLE WHEAT BREAD

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INTRODUCTION
Whole wheat breads are valuable sources of dietary fiber, vitamins, minerals, and trace elements. However, the presence of myo-inositol hexakisphosphate or phytate (InsP6) inhibits mineral bioavailability (BA) due to its chelating properties and technological actions are needed to improve essential micronutrients BA.
OBJECTIVES
In this study, the potential use of phytase-producing Bifidobacterium strains as starter on breadmaking process (direct or indirect) and implications in iron solubilisation and uptake were assessed.
METHOD/DESIGN
Whole wheat breads fermented with B. longum spp infantis ATCC 15697 and/or B. pseudocatenulatum ATCC 27919 were the materials of the current investigation, for which total iron content (measured by atomic absorption spectrophotometry and InsP6 and myo-inositol pentakisphosphate (InsP5) concentrations (measured by high pressure liquid chromatographic) were analysed. These samples were subjected to an in vitro digestion/Caco-2 cell model to evaluate iron BA. Ferrozine was used to determine the total amount of soluble iron present in the dialysates and intracellular ferritin quantification (Spinreact) as a measure of bioavailable iron.
RESULTS
The addition of bifidobacteria significantly reduced the InsP6 + InsP5 concentrations compared to control samples. Breads made either through direct or indirect process with bifidobacteria had similar iron concentrations ranged between 31.7 - 35.8 µg/g (dry matter). The dialyzable iron contents in samples with bifidobacteria were increased 2.3-5.6-fold and its dialyzability was improved by 2.6-8.6% compared to controls. However, this was not reflected in an increase of iron uptake by Caco-2 cells as was predicted by the phytate/Fe molar ratios higher than 1.
CONCLUSIONS
The results support the usefulness of phytase-producing bifidobacteria to reduce phytate during breadmaking process and to increase iron bioaccessibility, although, the extent of reduction appears to be insufficient to improve iron bioavailability to Caco-2 cells. Further refinement of the use of phytase-producing bifidobacterial strains and/or bread-making technological processes is encouraged for improving mineral uptake.

USE OF A BATCH FERMENTATION SYSTEM TO STUDY THE EFFECT OF THE PEA PROTEIN HYDRDOLYSATES ON HUMAN INTESTINAL BACTERIA

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INTRODUCTION
Hydrolysis of food proteins is responsible for formation of myriad of peptides and amino acids that display properties different to ones of native proteins and are highly biologically active. A broad spectrum of scientific studies must be conducted in order to determine the biological impact of such food hydrolysates. The role of the intestinal microbial ecosystem is crucial in maintenance of the homeostasis of the human organism and therefore any development of new hydrolysate-based products need to consider the impact of such products on the gut microbiota. Although pea seeds are of significant nutritional value due to their high contents of proteins, carbohydrates and fibre, they are also responsible for health inconveniences resulting from their susceptibility to digestion and occurrence of antinutritional compounds. The enzymatic degradation may pass over these nutritional obstacles by liberating hydrolysates empowered to exert their impact on bacterial intestinal ecosystem thus influencing the physiology of a consumer.
OBJECTIVES
Therefore, the aim of this study was to evaluate the influence of pea protein hydrolysates on the proliferation and metabolic activity of colonic bacteria.
METHOD/DESIGN
The analyses were conducted with the use of experimental batch-type simulator models imitating human intestinal conditions. Changes in bacterial biodiversity will be determined with a culture approach. The fermentative activity of the intestinal microbiota will be assessed by the analysis of the synthesis of SCFA by HPLC.
RESULTS
The pea protein hydrolysates beneficially modulated bacterial physiological activity and thus are predisposed to be used as a potential modulators of the composition and metabolic activity of gut microbiota.
CONCLUSIONS
The results obtained here may help modulate the balance of the gut microbiota of a healthy individuals. In addition, this studies may also impact on the food industry for production of supplements with defined biochemical composition and biological activity for health-promoting effects.

ENZYMATIC HYDROLYSATES OF RICE PROTEINS FROM RICE MILK SUBSTITUTE AS MODULATORS OF PHYSIOLOGICAL ACTIVITY OF GUT MICROBIOTA

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INTRODUCTION

Food proteins have been perceived hitherto purely as a source of nutrients indispensable for maintaining life. However, latest findings indicate that they may release biologically active peptides in a consequence of enzymatic degradation. Such hydrolysates may affect the balance of intestinal bacteria and their adhesive potential, thus influencing the health status of the host. Many people use in their daily diet milk substitutes such as rice milks. Consumed milk substitutes undergo modifications due to enzymatic hydrolysis by proteinases present in the GI tract. Peptides and glycopeptides released by the hydrolysis of proteins may significantly modulate the condition and activity of intestinal ecosystem, particularly bacteria.

OBJECTIVES

This study aimed at the estimation of the impact of hydrolysates of proteins from rice milk on proliferation and adhesion of human intestinal bacteria.

METHOD/DESIGN

The proteins from the rice milk were subjected the enzymatic (pepsin-pancreatin) hydrolysis imitating the processes occurring in vivo. Such prepared substrates were used to study their impact on the proliferation rate of bacteria and their adhesion to the epithelial cells. Caco-2 cell line was used as a common model to study bacterial adhesion. The impact of bacterial proliferation rate was examined with the fluorescent marker DAPI, whereas their biodiversity was examined with FISH technique.

RESULTS

Hydrolysates from rice milk substitute influenced the microbial proliferation rate depending on the bacterial metabolic potential. Additionally, examined hydrolysates modulated the adhesion of gut bacteria to the epithelial surface, thus influencing the maintenance of the intestinal barrier.

CONCLUSIONS

Rice milk proteins’ hydrolysates may be considered as potential modulators of physiological activity of gut bacteria. Hence, they may impact the balance of gut microbiota as well as the maintenance and strengthening of the intestinal barrier. In consequence, they may modulate a human health status. Studies conducted within the project no : N N312 305940

YOGURT AND KEFIR HYDROLYSATES AS MODULATORS OF BACTERIAL ADHESION

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KEYWORDS: yoghurt cultures, kefir cultures, fermented food, bacterial adhesion, Caco-2 cell line

INTRODUCTION

Food products, such as yogurt and kefir, are not solely the source of nutrients shaping the proper physiological condition of a human but are also the source of substances displaying a high biological activity that, when reaching the intestinal ecosystem, influence homeostasis. Food proteins reaching the intestine undergo degradation in the process of hydrolysis and in consequence may act as modulators of bacteria adhering to the epithelium thus influencing the intestinal barrier. Adhesion is one of the bacterial strategies indispensable to for colonization of the intestinal environment. In a broader context, nutrients are able to bring about the alteration of the health status of the host.

OBJECTIVES

Therefore, the aim of this study aimed at investigating the influence of yoghurt and kefir hydrolysates on the adhesive properties of the human intestinal bacteria.

METHOD/DESIGN

The yogurt and kefir products underwent the enzymatic (pepsin-pancreatin) hydrolysis imitating the processes occurring in vivo. Such prepared substrates were used to study their impact on the adhesion of gut bacteria to the epithelial cells. Caco-2 cell line was used as a commonly used model to study bacterial adhesion.

RESULTS

Yogurt and kefir hydrolysates proved to modulate the adhesion of gut bacteria. The observed effect resulted from a simultaneous interactions of bacteria specific to a particular host as well as the tested substrate.

CONCLUSIONS

Yogurt and kefir hydrolysates may significantly modulate the adhesive potential of human gut bacteria. In consequence, they may impact the maintenance as well as strengthening of the intestinal barrier, which consists of the intestinal bacteria, thus contributing to selective permeability and protecting the human organism from invasion of harmful microorganisms as well as food allergens.

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IMMUNOMODULATING AND TOLEROGENIC PROPERTIES OF FERMENTED WHEY BEVERAGES

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INTRODUCTION
Occurrence of allergic reaction to cow milk proteins (CMP) at an earlier phase of life increases the risk of occurrence of constant form of CMP allergy and can result in development of reactions to other foods, as well as reactions upon inhalation. Therefore, it is very important to "teach" the organism to tolerate CMP by stimulation of immune system for proper regulation of immunological response.
OBJECTIVES
The aim of the study was the modulation of immunological mechanisms by fermented whey products with lowered immunoreactive potential in organisms with induced CMP allergy.
METHOD
Mice were sensitized with CMP. Animals after sensitization were fed with fermented whey products. After 4-week feeding period mice were sacrificed, selected organs were collected to perform analysis: - Spleen – the evaluation of the number of lymphocytes secreting IgA and IgG - ELISPOT assay. Moreover, cultures were run from isolated spleen cells for determination of IL-4, TGF-beta, INF-gamma and IL-10 secretion - ELISA. - Caecum content– analysis of bacterial species diversity – PCR-DGGE. - Small intestine and its content– determination of IgA - ELISA. - Blood – determination of IgA, IgG and IgE - ELISA.
RESULTS
In the study it was found that stimulation of animal cells with the fermented whey beverages increased the total IgA+ and IgG+ cells in the spleen and decrease total IgE in blood serum. This study reports the effect of the oral administration of fermented beverages on the cytokine production changes. It was observed IL-10, IFN-gama and TGF-beta increase in mice fed with whey beverages. Qualitative determination of caecum microflora by PCR-DGGE revealed changes in microbial community pattern.
CONCLUSIONS
This work has characterized immunomodulatory effects of fermented whey beverages. All strains used in this study heave the ability to down-regulate pro-inflammatory cytokines. These results indicate the tolerogenic aspect of used beverages.

DOES PEA ALBUMINS MODULATE T CELL RESPONSE AND COLONIC MICROFLORA IN MICE?

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INTRODUCTION
Food allergens entered human gastrointestinal track every day. They stimulate different cells of mucosal immune system what give opportunity to balance between immunity and tolerance.
OBJECTIVES
Possibility of modulation T cell response and colonic microflora in mice by immunization with pea albumins.
DESIGN
Pea albumins were delivered to mice in three ways: orally by 10-consequence days in presence of CT or Alum as adjuvant, and intraperitoneally, trice once a week, with Freud Adjuvant. 14 days later serum and fecal samples were collected and specific IgG and IgA were assigned by ELISA
METHOD
On the 35th day from beginning mice were terminated and cells from spleen, mesenteric lymph nodes and head and neck lymph nodes were isolated for culturing and phenotyping CD4 and CD8 T lymphocytes. Microbial populations attached to the colonic epithelium were assessed. The higher level of specific IgG and IgA was found in mice immunized intraperitoneally. Alum as adjuvant was the less effective in inducing any antibodies response. Looking for T cells profile immunization with CT as adjuvant, expressed the highest percentage of CD4+ and of CD8+, 51.8% and 25% respectively, in head and neck lymph nodes. Mesenteric lymph nodes T lymphocytes profiles are clearly dependent from chosen way and adjuvant for immunization. Using CT in oral immunization induced 48.3% CD4+ and 14.9% CD8+ in lymphocytes population. Alum gave about half les number of CD4+. Intraperitoneally immunization gave effect in significant decreasing CD4+ and CD8+ levels. That of course has an impact for expression regulatory cells, which regulate immune response in autoimmune diseases and food allergy. Two clear populations are observed in CD8 population from group intraperitoneally immunized. Non significant differences in bacteria numbers were found in all tested groups.
CONCLUSION
Controlled antigen delivery could be used for immune response modulation for food allergy treating or preventing.

IMPACT OF NON-DIGESTIBLE CARBOHYDRATES ON GUT MICROBIOTA AND METABOLITES IN THE HUMAN COLON

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Molecular methodologies used to survey the human colonic microbiota has revealed that most of the abundant bacterial species belong to the Bacteroidetes and Firmicutes with the latter mainly represented by Lachnospiraceae and Ruminococcaceae families. Despite geographic and inter-individual variation in microbial composition two butyrate-producing species, Faecalibacterium prausnitzii and Eubacterium rectale, which belong to these two families are amongst the most abundant species in the healthy colon. The abundance of these and other key species is influenced by the gut environment, including pH, and the amount and type of dietary carbohydrates consumed. In humans non-digestible dietary carbohydrates including resistant starch, pectin and oligosaccharides that escape digestion by host enzymes provide the major energy source for bacteria in the colon. In controlled human dietary intervention studies decreasing the carbohydrate content of the diet from approximately 400 g/d to 24 g/d resulted in a decrease in faecal butyrate concentrations correlating with a decline in E. rectale/Roseburia abundance. Moreover, decreasing carbohydrate intake also resulted in an increase in levels of hazardous metabolites such as N-nitroso compounds with a concomitant decrease in antioxidants derived from plant phenolics. Altering dietary carbohydrate type also influences microbiota composition. When 14 male volunteers were provided with a diet high in either resistant starch or non starch polysaccharides (NSP), analysis of 16S rRNA sequences from their faecal microbiota revealed blooms in the abundance of certain bacterial species, including Ruminococcus bromii, particularly when volunteers were consuming a resistant starch-enriched diet. In vitro analyses of four dominant amylolytic species, Eubacterium rectale, Bacteroides thetaiotaomicron, Bifidobacterium adolescentis and Ruminococcus bromii, revealed that the latter possesses an exceptional ability to degrade starch particles. It is therefore evident that both the amount and type of carbohydrate in our diets has a major impact on microbial composition and metabolism that have the potential to influence health.

BIFIDOBACTERIUM ANIMALIS BB12 AND LACTOBACILLUS RHAMNOSUS GG HAVE COMMON ADHESION PROTEINS ON THEIR ENVELOPE SURFACE

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KEYWORDS: probiotic, adhesion, EF-Tu, GAPDH, GroEL

INTRODUCTION

Bacteria in order to colonize the gastrointestinal tract have to be able to adhere to the intestinal surface. Several proteins have been indicated as adhesion molecules for attachment to epithelial cells. These are S-layer proteins, EF-Tu, GAPDH, GroEL, TPI, Mub, and MapA.

OBJECTIVES

Envelope surface proteome analysis for presence of common multifunctional proteins presence.

METHODS

B. animalis Bb12 and L. rhamnosus GG were cultured at 37ºC for 20 hours in anaerobic conditions in MRS or BHI. Surface proteins were stripped from cells' envelope and analyzed by Western-Blot for presence of EF-Tu, GroEL, and GAPDH.

RESULTS

Both B. animalis Bb12 and L. rhamnosus GG contain multifunctional proteins: EF-Tu, GroEL, and GAPDH on their surface. B. animalis Bb12 grown in BHI broth contain higher amounts of the proteins on their envelope surface as compared to MRS grown cells.

CONCLUSIONS

The adhesion property (as well as others) of certain probiotic strains differ in particular conditions that may indicate their specific niche in gastrointestinal tract.

ACKNOWLEGMENTS. This study was supported by the research grant no. N312 272640 of the Polish Committee for Scientific Research in years 2011-2014.

BILE ACIDS CHANGE ADHERENCE OF LACTOBACILLUS RHAMNOSUS GG AND BIFIDOBACTERIUM ANIMALIS BB12 TO ENTEROCYTES

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KEYWORDS: probiotic, adhesion, bile, enterocyte

INTRODUCTION

In order to demonstrate beneficial effects probiotic bacteria need to colonize the gastrointestinal tract at least temporarily. The best results of probiotic administration are achieved when the strain is able to adhere to the intestinal surface. However the property of bacteria can vary depending on changing environmental conditions in distinct parts of gastrointestinal tract.

OBJECTIVE

We investigated the adhesion to enterocytes of Lactobacillus rhamnosus GG and Bifidobacterium animalis Bb12 grown in presence of bile acids.

METHODS

The bacterial strains were cultured in broth supplemented with 0.05% bile salts and 3H-thymidine radiolabeled, then added to apical site of confluent monolayer of 21-days-differentiated Caco-2 cells cultured on porous filter cell culture inserts. After 1.5h incubation the inserts were washed to remove unattached bacteria. The amount of adherent bacteria by means of radioactivity was measured by liquid scintillation.

RESULTS

Bile salts in growth medium reduced L. rhamnosus GG and increased B. animalis Bb12 adherence to Caco-2. L. rhamnosus GG possesses low or none bile salts hydrolases activity since growth rate of the strain in presence of 0.05% bile salts was heavily reduced. The growth of B. animalis Bb12 is also decreased by bile salts but in lesser extent.

CONCLUSIONS

In our experiment 0.05% bile salts were used which constitute an approximately 1 mM concentration. In human colon the concentration of bile salts ranges from 10 mM in duodenum to 2 mM in ileum and further decreases to an average of 0.43 mM in cecum. The adhesion property (as well as others) of certain probiotic strains differ in particular conditions that may indicate their specific niche in gastrointestinal tract.

ACKNOWLEGMENTS. This study was supported by the research grant no. N312 272640 of the Polish Committee for Scientific Research in years 2011-2014.

LEPTOTRICHIA BUCCALIS PREVALENCE IN THE HUMAN RESIDENT ORAL MICROBIOTA Strelnikova N.V., Antonova A.A., Koltsov I.P., Polovova E.B., Chirikova E.L. The Far Eastern State Medical University, Khabarovsk city, Russia Keywords: Leptotrichia, oral microbiot

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Keywords: Leptotrichia, oral microbiota

INTRODUCTION

The Leptotrichia buccalis are normal inhabitants of the human oral cavity. The Leptotrichia genus bacteria are colonizing saliva, dental plaque,interdentally spaces, tongue mucosa. Bacteria can causing bacteriemia, sepsis, abscess and inflammatory processes. In Khabarovsk region increased incidence of inflammatory lesions of the oral mucous membranes.

OBJECTIVES

Identify the occurrence of Leptotrihia genus bacteria in the healthy adult and child population of the Khabarovsk to address the issue that bacteria are the possible etiology of inflammatory processes of the oral cavity.

METHOD

Randomized investigated a group of children from 1 year to 18 years old and adults to 70 years, both urban and rural populations. Scraping the back of tongue was performed. Two smears were stained by Gram. Due to the fact that bacteria have typical morphology - gram-negative rods, we counted the number of Leptotrihia, the length of the cell. Cultures were grown under anaerobic and then aerobic atmosphere.

RESULTS

Statistically valid data were obtained. In children Leptotrihia determined in 95% of the microscopic method and 30% confirmed by culture method. In adults determined by microscopic Leptotrihia genus bacteria in 98.9% of surveyed and 28% of the culture method. We found that the regional norm for healthy residents of Khabarovsk region can be considered constant presence of bacteria in the mouth in amounts of 1-2 to 10-15 in the field of view of the microscope. The length of the bacteria reached 10-15 microns, rarely up to 20-30 microns.

CONCLUSIONS

As a resident Leptotrichia buccalis can cause a local Leptotrichia-infection of the mouth and serve as the role of opportunistic emergent pathogen of the new century.

IMPROVEMENT OF ANTIOXIDATIVE STATUS OF GUT WITH PARTICULAR LACTOBACILLI IN SALMONELLA TYPHIMURIUM MURINE MODEL

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S.enterica Typhimurium causes inflammation and oxidative stress in gut. We aimed to evaluate the impact of administration of lactobacilli on the antioxidative status of ileum in mice infected with S. Typhimurium. Material and methods. A total of 173 NIH line (47, 72 and 54 in studies I-III, respectively) 6 weeks old male mice (Kuopio, Finland) were recruited to the studies. The mucosa of ileum was obtained during autopsy on the 10th day following challenge with S. Typhimurium. Lipid peroxidation LPO and ratio of oxidised and reduced glutathione (GSSG/GSH) were tested spectrophotometrically. The impact of the administration of L. acidophilus and L. fermentum DSM 14241 in milk for 5 consecutive days before and 10 days after challenge with S. Typhimurium was evaluated in study I. The mice of control group were fed with milk containing both lactobacilli for 15 consecutive days. In study II and III the L. fermentum in drinking water was added to ofloxacin (OFX) treatment of the S. Typhimurium infection for 8 days. Results. The values of LPO and GSSG/GSH of intestinal mucosa were significantly higher in S. Typhimurium challenged mice as compared to the control animals ($p<0.01$; $p < 0.001$ and $p < 0.003$, respectively) in all studies. The reduction of the values of LPO was detected in study I and II ($p<0.05$, $p = 0.002$, respectively). In study III the GSSG/GSH level decreased in both L. fermentum containing treatment schemes if compared to S.Typhimurium infected mice ($p=0.006$; $p=0.046$). Conclusions. Thus, the reduction of tested oxidative stress indices (LPO and GSSG/GSH) in treatment regimens supplemented with lactobacilli show the possibility to improve the antioxidative status of gut during infection.

LOCAL LEPTOTRICHIA BUCCALIS ASSOSIATED INFECTIONS Strelnikova N.V., Turkutyukov V. B., Polovova E.B., Vdovenko E.S. The Far Eastern State Medical University, Khabarovsk, Russia The Vladivostok State Medical University, Vladivostok, Russia Keywords: Le

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INTRODUCTION
The Leptotrichia buccalis are normal inhabitants of the human oral cavity, intestines and genital tract. The Leptotrichia genus bacteria as oral commensales are colonizing biotopes: saliva, dental plaque, interdentally spaces, gingival pockets, tongue mucosa. Bacteria with the weakening of immunity can leave their habitats, causing bacteriemia, sepsis, abscess. Increased incidence of inflammatory lesions of the oral mucous membranes, clinically similar to Candida-associated fungal inflammation, but fungi of the genus Candida are not cultivated.
OBJECTIVES
Identify the value of Leptotrihia genus bacteria in the patient's of the Far East to address the issue that anaerobic bacteria are the etiology of oral cavity infections.
METHOD
Randomized investigated a children group of patients from 1 year to 8 years old and adults 46 to 72 years. Scraping of back of tongue was performed. First, two smears were stained by Gram. We counted the number of Leptotrihia, the presence of oral microbiota koadhesion also considered desquamated epithelium and the level of histadhesion. Identification Leptotrichia buccalis was carried out using Anaerotest 23 and ATB analyzer system.
RESULTS
Leptotrihia genus bacteria determined in 100% of the microscopic and 38% confirmed by culture method in patients with stomatitis, gingivitis, glossitis, juvenile Vincent stomatitis. The bacteria in the mouth are amounts of 15-20 to 30-50 in the field of view of the microscope. The number of epithelial cells is 6-8. The length of the bacteria reached 15-30 microns, rarely up to 50-60 microns. The Leptotrihia genus bacteria are difficult to cultivated forms.
CONCLUSIONS
The resident Leptotrichia buccalis cause a local Leptotrichia-infection of the mouth and act as the role of emergent pathogen.

CHARACTERIZATION OF PROBIOTIC MICROORGANISMS.

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INTRODUCTION
One of important criteria for selection of probiotic microorganisms is their resistance to the conditions of human gastrointestinal tract. During their transition through the digestive tract, they are negatively affected by bile salts, digestive enzymes, low gastric pH, etc. Efficacy of probiotics depends on their ability of epithelial colonization, which could be particularly affected by the ability of adhesion, hydrophobicity and autoagregation of cells.
OBJECTIVES
The aim of this work was to test some of probiotic properties in eighteen strains of the genera Lactobacillus, Bifidobacterium and Enterococcus originating from the Collection of Dairy Microorganisms Laktoflora (Czech Republic). Selected strains were compared in terms of tolerance to bile salts, hydrophobicity and ability of autoagregation.
METHODS
For testing the tolerance to bile salts, bovine bile at concentrations of 0 %, 0.3 %, 0.5 % and 1.0% w/w was used. Cell surface hydrophobicity was determined by MATH methods as affinity of tested strains to hexan as solvent. Autoagregation was determined by measuring absorbance at 600 nm during the cultivation of tested strains in PBS buffer at intervals of 2, 4, 7 and 24 hours.
RESULTS
The highest inhibition of bacterial growth in the presence of bile salts occurred during the first eight hours of measurement. After eight hours of incubation, strains began repeatedly to grow slowly at 0.3 % and 0.5 % w/w concentrations of bile salts. The highest ability to autoagregation was detected in strain B. animalis subsp. lactis CCDM 366. The highest hydrophobicity was observed in strains Lbc. rhamnosus CCDM 233, B. animalis subsp. lactis CCDM 366 and Lbc. fermentum RL23.
CONCLUSIONS
Based on the results, strains Lbc. rhamnosus and B. animalis subsp. lactis showed a good colonization potential.
Acknowledgement This study was supported by Grant No. QI111B053 of National Agency of Agricultural Research, Ministry of Agriculture of Czech Republic.

THE BIOFILMS TOLERANCE TO ANTIMICROBIALS – BIO-NANOTECHNOLOGICAL APPROACHES TO FIGHT AGAINST BIOFILM FORMATION ON MEDICAL DEVICE AND OTHER SURFACES

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Sessile/adherent microbial growth in biofilms is predominant, biofilm' cells being metabolically more efficient, well protected and resistant to all stress conditions. Biofilms are developed on wet surfaces, including medical "ecosystems" (cellular / inert surfaces), but also pipes and food processing equipment surfaces. The biofilm formation on medical devices and implants is involved in nosocomial infections, while bacterial adherence and biofilm development on surfaces in food industry is responsible for serial food contamination and consecutive health problems. Recent publications stated that 60 to 85% of all microbial infections are biofilms associated infections, difficult to solve due to the behavioural resistance of biofilm' cells. The mechanisms of this resistance or tolerance are: i) failure of antibiotics to penetrate into the depth of a mature biofilm due to the self generated biofilm matrix; ii) matrix its self by accumulation of antibiotic degrading enzymes; iii) the persister cells, less susceptible to antimicrobials due to nutrient limitation in the depth of a biofilm; iv) changes in the gene expression pattern in irreversibly adherent bacterial cells and mutations (hipermutators cells, influenced by stress conditions, with an adaptive role). The molecular studies have shown that biofilm's formation and behavior are controlled by intercellular communication mechanisms mediated by signal molecules (both in bacteria and microfungi), involved in quorum sensing mechanism (QS), gene expression (e.g. virulence) regulation and resistance to stress conditions. Considering the differences in physiology and susceptibility to antibiotics of biofilm embedded cells, there are necessary new approaches based on different mechanisms of action, alternative/ complementary to antibiotherapy, for the prevention and treatment of biofilm associated infections. Also, we need now environmentally safe, eco-friendly materials and production methods, using natural compounds like vegetal antimicrobial compounds and QS inhibitors carried by (bio)nanoparticles, able to penetrate the biofilm reticular matrix and reach the target cells, without any selective pressure.

NANOBIOSYSTEMS FOR THE RELEASE CONTROL OF NATURAL PRODUCTS WITH MICROBICIDAL AND QUORUM SENSING INHIBITORY ACTIVITY

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INTRODUCTION

Microbial biofilms formed on cellular or inert substrata (as medical devices and other wet surfaces) are involved in 60 to 85% of all infections. Considering the major feature of biofilms, respectively the high resistance or tolerance of biofilm embedded cells to all kind of antimicrobials, there are necessary new approaches based on different mechanisms of action, alternative or complementary to antibiotherapy, for the prevention and treatment of biofilm associated infections.

OBJECTIVES

The present work aims to describe the synthesis and characterization of functionalized magnetite nanoparticles with natural products (essential oils and usnic acid) for the release control and an increased penetrability into biofilms of the natural active fractions with proved microbicidal effect or quorum sensing inhibitors (QSI) activity and to obtain a more efficient antipathogenic strategy for biofilm related infections.

METHOD / DESIGN

The microbial strains were isolated from different clinical specimens and were identified by Vitek II automatic system. Fe3O4/natural products - nanoparticles (core/shell) were characterized by X-Ray Diffraction (XRD), Fourier Transform-InfraRed Spectrometry (FT-IR) and Brunauer–Emmet–Teller (BET) porosimetry. The nanoparticles were used for covering treatment of the prosthetic devices represented by catheters. The obtained modified surfaces were subsequently used for the in vitro study of the microbial biofilm development. The microbicidal effect of some fractions was assayed by viable cell counts and the inhibitory effect of the usnic acid on biofilm formation and architecture was assessed by Confocal Laser Scanning Microscopy (CLSM).

RESULTS

The results proved that the nanobiosystem show a significant antibiofilm activity, proved by the decrease of the biofilm viable cells and by the CLSM images.

CONCLUSION

Our results are highlighting the opportunity of using functionalized magnetite nanoparticles with natural products (essential oils and usnic acid) for the developing of efficient antibiofilm strategies as coating prosthetic devices and other surfaces.

VIRULENCE HALLMARKS OF ENVIRONMENTAL BACTERIA ISOLATED FROM WASTEWATER AND RECEIVING RIVERS

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The aim of the study was to investigate the diversity of natural water microbial populations found in natural surface water and wastewater and to assess their virulence characteristics.
METHODS
Five samples of wastewater and four of surface water were collected and analyzed through a standardized membrane filter method. The bacterial isolates were further identified using biochemical tests (multitests systems and API systems). The virulence tested features were: adherence and invasion capacity on HeLa cells by Cravioto adapted method, adherence on inert substrata quantified by slime test, production of extracellular enzymes and exotoxins (haemolysins and other pore-forming toxins, amylase, mucinase, gelatinase, caseinase, aesculin hydrolysis).
RESULTS
A total of 64 bacterial strains were identified, most of them beloging to: Klebsiella, Citrobacter, Enterobacter, Aeromonas, Burkholderia, Cryseomonas, Pseudomonas, Enterococcus and Escherichia coli. The most aquatic strains isolated from wastewater and natural surface water exhibited high capacity of adherence to the cellular substrate (62,22% and 64,7 respectively) demonstrating the potential to colonize the eukaryotic host cells and to initiate an infectious process, augmented by several extracellular enzymes and microbial products associated with bacterial pathogenesis, including: in strains isolated from wastewater lipase (19%) and lecithinase (13%), while in strains isolated from natural surface water esculin hidrolysis (76, 47%) and gelatinase (47,05%). The genes encoding their expression was determined by PCR. While the possession of any one of these virulence factors does not mean that a microbe can cause disease, it is generally believed that their known association with pathogenesis is one requirement for the development of infection.
CONLUSION
Virulence factors are essential for the ability of a microbe to cause disease therefore determination of the presence or absence of these factors in naturally occurring bacterial strains will be of great help to epidemiologists and medical microbiologists.
Keywords: virulence hallmarks, environmental bacteria, virulence, wastewater.

QUALITATIVE AND QUANTITATIVE ASSESSMENT OF DROSOPHILA MELANOGASTER NATIVE MICROBIOTA

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INTRODUCTION
Drosophila melanogaster represents a genetically tractable model for studying the mechanisms used by commensal microorganisms/infectious agents to colonize the healthy individuals.
PURPOSE
Our purpose was to characterize the cultivable members of the microbial consortium of both Oregon wild-type and selected mutants of D. melanogaster. Material and methods: This study focused on describing the casual microbiota in adult males of wild-type Oregon and two D. melanogaster mutant lines, namelyl(3)S057302 and ?Cop14a. The flies were reared on sterilized standard cornmeal-molasses medium at 25°C. The evaluation of every considered fly strain were performed in triplicate, each consisting in three adult males. Homogenization and the ten-fold subsequent dilutions were performed in sterile saline. From each 10-2 and 10-3 diluted homogenates were derived three gelose-agar plates which were incubated at 37°C overnight under aerobic conditions and the morphological characteristics of the bacterial colonies and the number of CFU/plate were recorded. Colonies representing each morphological type were streaked for isolation on gelose, blood and MacConkey agar. Following incubation, single colonies were examined for catalase, oxidase and Gram staining and they were submitted to specific identification schemes for enteric bacteria (API 20E), non-fermentative Gram-negative bacilli (Api 20NE), Gram-negative cocci (Biolog) and Gram-positive bacilli (conventional biochemical tests). The statistical significance of the results was analyzed using GraphPad Prism software.
RESULTS
Our study emphasizes that there are qualitative and quantitative differences in the cultivable microbiota of wild-type Oregon versus mutant lines, which unexpectedly yielded fewer colonies. The commensal microbiota proved to be of a relative low diversity, the commonly found taxa being primarily represented by Enterobacteriaceae (Providencia sp. and Serratia sp.), Bacillus sp. and, occasionally, enterococci.
CONCLUSION
The phenotypic and numeric analysis of cultivable native microbiota indicates that the laboratory breed analyzed D. melanogaster lines bear a low-diversity bacterial community, mainly represented by enterobacteria and Gram-positive, sporulated bacilli.

DENATURING GRADIENT GEL ELECTROPHORESIS CHARACTERIZATION OF GUT MICROBIOTA IN MICE FED WITH DIFFERENT TYPES OF FAT.

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INTRODUCTION
We have previously reported a culture dependent study on the effect of different high fat diets on the intestinal microbiota of mice. Some authors have studied the effect of high fat/high calorie diets and have detected changes on the microbiota. However, there is no study on the specific effect of different types of fat, even though physiological effect of each is quite distinctive.
OBJETIVES
We have started a research line on the specific effect of different types of fat on gut bacterial populations and their correlation with some physiological variables.
METHODS/DESIGN
For three months, we have fed mice with three high fat diets (virgin and refined olive oil and butter) and with a control diet. Evolution of symbiont population in feces has been studied using culture independent methods by amplifying and separating the V3 region of 16S DNA (Denaturing Gradient Gel Electrophoresis) and the most representative bands have been sequenced.
RESULTS
Comparison of the different DGGE profiles throughout the experiment will be shown as well as the result of a database search for each sequenced band.
CONCLUSIONS
A preliminary study of results indicate that different fats have a distinct effect on gut microbial composition, as already observed in culture dependent studies.
Acknowledgements: This work has been financed by the Andalusian Government (Consejería de Economía, Innovación y Ciencia. Proyecto de Excelencia Ref. AGR 6340) and by the University of Jaén (UJA2009/13/03)

OLIVE OIL RICH DIET AND INTESTINAL MICROBIOTA. EFFECTS ON PLASMA LIPID PROFILE

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INTRODUCTION
During last years, several studies have correlated changes in gut microbiota with obesity and disturbance in lipid metabolism. However, no data is available about the effect of different lipids on these parameters.
OBJECTIVES
The aim of this study was to determine the effects of high fat diets, with different fatty acid profile and polyphenol content, on intestinal microbiota, and its relationship with blood lipid levels.
METHOD / DESIG
Mice were divided in four groups (n=10). Each group was fed during three months with one of the following diets: C = control diet (standard laboratory mice diet, 3% fat). O = olive diet (high fat diet, 20% olive oil), VO = virgin olive oil diet (high fat diet, 20% extra-ecologic virgin olive oil) and B = butter diet (high fat diet, 20% butter). At the end of the experiment, feces were obtained to analyze the colonic microbiota. Blood samples were obtained in order to determine lipid plasmatic levels (total cholesterol, HDL-cholesterol and triglycerides).
RESULTS
High fat diets increased total levels of cholesterol and HDL-cholesterol with respect to standard diet, but the highest HDL/total-cholesterol ratio was obtained for VO diet. Statistically significant difference was only established for VO vs C in the analysis of triglycerides, with lower levels in virgin olive oil diet. With respect to the fecal microbiota and as previously reported, counts of firmicutes remained unchanged all through the experiment but there was a significant reduction on counts in Bilis Esculine Agar both for B and O diets but not for VO.
CONCLUSIONS
These results support a role of fatty acid and polyphenols in the effects of high fat on colonic microbiota. This changes could be associated with changes in plasmatic lipid profile and with the development of dyslipidemia and related diseases, such as obesity, hypertension and metabolic syndrome.

CHANGES IN THE INTESTINAL MICROBIOTA ASSOCIATED WITH MUCOID ENTEROPATHY IN RABBITS

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The rich microbial community present in the caecum of rabbits carries out the final and most important part of feed digestion. Mucoid enteropathy (ME) is a serious disease of young rabbits with a high mortality rate. The etiology of this disease is unknown although the pathology concentrates in the gastrointestinal tract and sick animals show dehydration, increased mucus production, diarrhea and finally cecal impaction. To determine the cecal microbial diversity and specific bacterial groups related with mucoid enteropathy disease compared to healthy rabbits. Three rabbit groups of 10 individuals (total 30) were studied: ME diseased, and two healthy controls with and without antibiotic treatment. The microbial composition of the intestinal content was analyzed by real-time PCR and 16S gene amplicon sequencing. More than 288.000 bacterial sequences were identified. Principal component analysis at the genus level showed that healthy control and antibiotic groups were very similar whereas the ME group was significantly different. Healthy rabbit intestinal microbiota was dominated by Alistipes (28.5%), Ruminococcus (21.4%), Akkermansia (12.7%), Subdoligranulum (11.1%) followed by Anaerotruncus (3.7%), Rikenella (3.5%), Papillibacter (3.4%) and others. Antibiotic treatment did not modify significantly the microbial diversity compared to healthy control samples. In samples from rabbits with ME, a significant increase of Bacteroides (33.2%), Akkermansia (22.7%), Rikenella (8.7%) and Clostridium (3.5%) was observed, and, a significant decrease of Alistipes (6.4%) and Ruminococcus (3.5%). Furthermore, Lysinibacillus (3%) and Citrobacter (2.7%) were only detected in ME samples. Interestingly, a number of species so far only described in human intestinal microbiota were detected in ME samples. Animals suffering ME displayed a remarkable dysbiosis, with colonization by some typically human fecal bacteria. Further investigations will determine if the susceptibility for the ready colonization by exogenous gut saprophytes is related to the primary cause of the disease, or it is just a secondary effect.

BIOFILMS IN CHRONIC BACTERIAL PROSTATITIS NIH-II

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To day, Prostatitis-P represent one of the emerging problems in males between 20-40 years, with important effects on fertility and quality of life. In Italy their prevalence is increasing (13.3%), accompanied by prostatic calcifications in 56% of the studied patients. Cat II-NIH chronic-bacterial-prostatitis-CBP-NIH-II are the most frequent: their aetiology theoretically involves the whole range of bacterial species (Mazzoli et al., 2007, 2011). All these microbes are able to form biofilms and infect prostate cells. Recently (2010) Mazzoli demonstated that bacteria isolated from genito-urinary samples, (total ejaculate, prostate/seminal vescicles secretions, intra-prostatic calcifications-PC) of CP-NIH-II-patients were able to produce in-vitro biofilms, detectable by culture and the electron microscopy. Bacterial population studied consisted in 150 clinical bacterial strains isolated from CP-NIH-II-patients presented to the Florence STDs Center from all Italy in 2008-2009. Quantitative assay of biofilm production was performed by the classic Christensen micro well assay. Globally 84,6% of the strains produced a consistent biofilm. The ability of the “preformed biofilm” and of “in formation biofilm” to resist to three different fluoroquinolones molecules was studied. Inhibition of biofilm formation was observed mainly among all the gram-negatives isolates, with a strain-dependent inhibition trend in biofilm in formation. Emerging Gram positives were mainly resistant. During biofilm formation in vitro cristallization phenomena were also observed. In 2010 Mazzoli has demonstrated by electron microscopy and culture that prostate calculi from patients affected by CBP-NIH-II were formed by calcified bacterial biofilms. These phenomena can well explain, in P, microbiological detection difficulties, symptoms persistence and antibiotic treatments high resistance in vivo and support prostatitis frequent chronicity. In addition these results provide intriguing evidence for a role of inflammation, bacteria mediated, in the biogenesis and the process of calcification formation in the prostate and may give important insight into prostate chronic inflammation as a potential contributing factor to prostate carcinogenesis.

ANAEROBIC CULTURED HUMAN INTESTINAL FLORA TRANSPLANT

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INTRODUCTION
Clostridium difficile associated disease (CDAD) with frequent watery stools, painful bowel movements is most probably depending on a disruption of the intestinal balance. We have inoculated a culture of anaerobe faecal microbes as an enema or by nasoduodenal tubing to a large number of patients.
OBJECTIVES
A special culture containing an unknown amount of faecal microbes regularly recultivated for more than 15 years under strict anaerobic conditions, has been transplanted to severalpatients.
METHOD / DESIGN
Faeces was obtained from a healthy Scandinavian donor on an ordinary Western diet. The faeces was investigated for absence of hepatitis A, B and C, Cytomegalovirus, Epstein–Barr virus, HIV, Rotavirus and Calicivirus. Furthermore, the faeces was screened for absence of Salmonella, Shigella, Campylobacter, Yersinia and Clostridium difficile. The faeces has been re-cultivated regularly anaerobically and the cultures have meanwhile been stored at -70oC. Patients with at least 3 diarrhoeal relapses after antibiotic treatment including metronidazole and/or vancomycin have received the faecal transplant after informed consent.
RESULTS
Most patients healed within the first week after the transplant and experienced a dramatic increase in life quality after a life with diarrhoea during several months. Some more patients healed after a second enema and several patients have recovered after receiving the transplant by nasoduodenal tubing. Some few patients improved, e.g., experienced less severe symptoms - in particular a reduction in the stool frequency.
CONCLUSIONS
In patients with relapsing CDAD, where antibiotic treatment with metronidazole or vancomycin does not solve the problem, faecal culture transplant can be an alternative. Freeze drying the culture and investigations using different techniques to identify functionally active microbes by e.g., 454-based barcoded pyrosequencing and other molecular techniques, are on-going. Obviously, this anaerobe culture has maintained its bio-therapeutical potential even after being sub-cultured at the laboratory for a long time.

AUTISM AND THE INTESTINAL MICROBIOME-OMPOSITION, FUNCTION AND BIOMARKERS

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Data from developed country reflect a dramatic increase in the number of autistic children. Out of the many factors that have been proposed being involved in this increase, alterations in the child's gut microbiota are increasingly focused upon, Presence/absence of groups of specific microbes, as clostridia, desulfovibrios and Sutterella have been reported. Temporary improvements in clinical functional status as well as relief in abdominal discomfort, often present in autistic children, have been found following dietary alterations as well as antibiotic and probiotic therapy. .Results of several animal models clearly indicate that gut derived compounds may create autistic-like behavior in the animal..Additionally, comparative studies in germfree and conventional animals have also demonstrated data indicating an important, microbial govern gut-brain axis together with a post-natal 'window for establishment' of this axis. However ,irrespectively of whether the data are of human or animal origin, most of these studies are hampered with a lack of proper biomarker(s) .The possibilities that autism is associated with alterations in the entire gut microbiome have to be taken into considerations, making evaluation of proper biomarkers even more difficult - and challenging. Various approaches for establishment of biomarkers will be evaluated, and a draft, covering molecular microbiological investigation of feces, biochemical investigations of urine and feces, functional nuclear magnetic imaging (fNMRI)of the brain together will a clinical evaluation, will be presented.

CONCLUSION

Autism is a disorder involving many organs and functions, including the gut microbiome. Increased knowledge may create diagnostic and therapeutic improvements.

DIFFERENCES IN COMPOSITION AND ABUNDANCE OF THE FECAL VIRAL COMMUNITIES BETWEEN CROHN'S DISEASE PATIENTS AND HEALTHY INDIVIDUALS

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INTRODUCTION
Complex inflammatory diseases intensively studied in order to ascertain their possible origin include the inflammatory bowel disease (IBD), one of whose major forms is Crohn's disease. This chronic and disabling disease, characterized by frequent relapses and progressing bowel injury, involves genetic risk factors linked to autophagy genes as well as environmental agents. However, since the interaction alone between bacteria and genes seems to be insufficient to explain the etiology, progress and incomplete effectiveness of drug treatments on this condition, the search of other environmental factors has to be taken into consideration. One of these factors is the role that interactions involving viruses could play and accumulating evidence shows that these interactions should be analyzed in more detail.
OBJECTIVES
Our main goal was the comparison of the viral communities between two groups of study: healthy volunteers and patients affected by Crohn’s disease to find out compositional and/or abundance variations that could be linked to the disease.
METHOD/DESIGN
We have analyzed the fecal virome and microbiome from several healthy control individuals as well as from patients affected by Crohn’s disease from Hospital Universitario La Fe in Valencia (Spain). We have applied a metagenomic approach consisting of filtrations, viral nucleic acid extraction, multiple displacement amplification and 454 pyrosequencing, followed by a customized bioinformatical analyses to process the sequences, assign the taxonomy and do statistical inferences.
RESULTS
Our results have revealed some differences in the diversity, composition and abundance patterns between both groups, such as lower diversity in Crohn’s samples or a bias in the abundance of certain viruses.
CONCLUSIONS
Even if the viral communities may not strongly differ overall, the under or overrepresentation of particular viral taxa in patients samples opens the door to the search of possible associations of those already characterized or newly identified viruses with Crohn's disease.

INEXPENSIVE, SENSITIVE AND SPECIFIC DIAGNOSTIC ASSAYS FOR EARLY DETECTION OF M. BOVIS IN CATTLE

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The present study was planned to estimate the sensitivity of the single intra dermal tuberculin test, dipstick assay, ELISA (using MMP, Cured culture filtrate ESAT-6, MBP70 and CFP10) and histopathlogical examination of the positive slaughtered animals for the detection of bovine tuberculosis. A total number of 1850 of cattle from different farms in Egypt were examined for bovine tuberculosis (TB) by tuberculin intradermal test using bovine Purified Protein Derivative (PPD) prepared from Mycobacterium bovis (M. bovis). A total of 36/1850 (1.90%) were positive reactors by single cervical test. Histopathological examination of 155 lymph nodes and 69 organ tissues revealed that 37 lymph nodes and 19 organ tissue samples showed typical granuloma for tuberculosis. The collected samples (lymph nodes and tissue) from those showing visible lesions were showed positive isolations for M. bovis from 50 out of 96 samples (52%). ELISA assay using different antigens for skin tuberculin tested cattle (n=60: 24 Negative Reactor & 36 Positive Reactor) was increased to 61.67 and 63.33% by using ESAT-6 and MPB70, respectively. The dipstick assay was covered by the same antigens of ELISA assay. The sensitivity of the dipstick was ranged from 88.8 to 100%, while recorded 100.0% for ELISA. The Specificity of the dipstick was ranged from 87.5 to 100%, while recorded 91.6-100.0% for ELISA according to antigen type. In conclusion, the dipstick of the present study is inexpensive, sensitive and specific diagnostic assays for early detection of tuberculosis in cattle.
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EVALUATION OF POMEGRANATE JUICE AS SUBSTRATE FERMENTATION FOR SINGLE CELL PROTEIN (SCP) PRODUCTION

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INTRODUCTION

Single-cell proteins are single or mixed cells of microorganism (algae, fungi, yeast and bacteria), which are used as protein supplement in human foods or animal feeds. Fermentation is the process that SCP is produced usually through the upgrade of cheap and wastes, such as agricultural (cheese whey, lignin, hemicelluloses and starch) and some industrial wastes.

OBJECTIVES

Pomegranate juice was selected as fermentation substrate for biomass production in then frame of the present research study. The reason for this choice is that many researchers enrich the international literature, regarding the beneficial effect of pomegranate consumption in human diet.

METHOD / DESIGN

Likewise, 20 fermentations were organized and conducted in triplicate. The main target of these fermentations was the utilization of diluted pomegranate juice for biomass production by baker's yeast (a commercial and easy adapted microorganism) and by the mixed kefir culture, which is considered difficult to be cultured, due to its variety of microorganism that is consisted of. Particularly, various fermentation batches of pomegranate juice were conducted in different temperatures, with and without the use of air supply, and in 3 different initial sugar concentrations.

RESULTS

The application of kefir did not show to be effective since the highest biomass yield that varied between (0,10-0,29g/g) while the highest biomass concentration was measured at 8,9g/l. However, in the case of baker's yeast utilization instead of kefir, higher biomass yields (0,40g/g) and higher biomass concentration (13,8g/L) were achieved. These results are quite promising since these values are quite close to the literature.

CONCLUSIONS

The outcome of results showed that the fermentation system designed with the use of kefir was not satisfied, because biomass yields, biomass concentrations and biomass productivities were quite low. These values are comparable to values achieved in the literature concerning other substrates exploitation and they are estimated positive.

LACTOBACILLUS RHAMNOSUS CNCM I-4036 SUPERNATANT DECREASES INFLAMMATORY RESPONSES INDUCED BY ENTEROPATHOGENIC ESCHERICHIA COLI IN HUMAN DENDRITIC CELLS

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INTRODUCTION
Dendritic cells are pivotal in maintaining immunological gut homeostasis. Innate pattern-recognition receptors, such as Toll-like receptors, play a crucial role in the host recognition of probiotics. Signalling via these receptors influences the chemokine and cytokine response of dendritic cells and provides a platform for modulation innate as well as adaptive immune responses in the host.
OBJECTIVES
The aim of this study was to determine the effect of culture supernatant of novel strain Lactobacillus rhamnosus CNCM I-4036, isolated from breast-feeding infant feces, to modulate human dendritic cells response against enteropathogenic Escherichia coli CECT 742.
METHODS
To achieve this goal, we used human dendritic cells, generated from umbilical cord CD34+ progenitor cells. In order to obtain the supernatant, L.rhamnosus was grown anaerobically at 37⁰C for 24 hours. Supernatant was neutralized and 10x concentrated. Human dendritic cells were directly challenged by addition of Lactobacillus rhamnosus CNCM I-4036 supernatant, Escherichia coli CECT 742 or both. After 4 hours incubation, the medium was replaced with a new one, containing antibiotics and cytokines. After 20 hours, culture supernatants were collected for cytokine analysis. IFN γ, IL-1β, IL-6, IL-8, IL-10, IL-12p40, IL-12p70, TNF-ALPHA, MCP-1(CCL2), MIP-1ALPHA (CCL3), RANTES (CCL5), MDC (CCL22) and IP-10 (CXCL10) were measured by immunoassay, with a MILLIplex kit using the Luminex 200 system based in the xMap technology. Differences between treatments were assesed by the U Mann Whitney test.
RESULTS
Dendritic cells decreased all the pro-inflammatory cytokines in response to stimulation with probiotic supernatant and enteropathogenic Escherichia coli.
CONCLUSIONS
It seems that L.rhamnosus CNCM I-4036 secretes metabolites or factors that are able to activate dendritic cells and modulate pathogen-induced inflammation, exhibiting immunomodulatory capacities. The use of supernatants, instead of live bacteria, has attractive advantages; such products would be very safe and have a long shelf-life.

ZOONOTIC GERMS IN GRADE-A MILK

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Keywords: EHEC, Campylobacter, Salmonella, Grade-A raw milk, consumer-expectation

INTRODUCTION

Advice is that raw milk should not to be consumed by pregnant women, young children and elderly people. However, epidemiological research showed that drinking of raw milk by pregnant mothers and young children is protecting the child for atopic diseases and asthma.

OBJECTIVES

we investigated the zoonotic quality of 'Vorzugsmilch' sold over a biodynamic farmshop. We interviewed consumers about their reasons to choose for this farm raw milk.

METHOD

routinely, monthly taken bulk tank milk (BTM) samples were investigated after the standards of the 'Vorzugsmilch'- regulation (n=60). Single cow milk (SCM) (2x37) and dung samples (1x36) were taken from all lactating animals in the herd. An open questionnaire was carried through with 16 consumers about their reasons to consume raw milk.

RESULTS

Table 1. Analysis of BTM samples of farm D, monthly in 2004-2010, mean values per ml (n=50). Somatic Cell Counts x 1.000 122 Germs x 1.000 5,9 Coliform 18 Staph.aureus (Sau) <5 Strept.agalactiae (Sag) <5 Listeria 0 Campylobacter 0 Salmonella 0 Pseudomonas 379 pH 6,79 Campylobacter and Salmonella were never found, either BTM (Table 1) or SCM (not given). Listeria was not found in BTM samples and only in 1 of the 65 SCM. Sau and Sag were always below the lowest detection level (<5). EHEC was not found in the SCM, however, was detected in 6 out of 34 single cow dung samples. Serotype O91:H21 was the main EHEC type. Main reasons to buy this milk were the biodynamic background (38%), taste (32%) and the natural untreated quality (32%).

CONCLUSIONS

Risk of transfer of zoonotic diseases is low from Grade-A milk, because hygiene standards during milking and processing are high. Consumers prefer this milk due to taste and natural quality.

MICROBIOLOGICAL MONITORING OF CORYNEBACTERIUM DIPHTHERIAE CARRIAGE IN TERRITORIAL MENTAL HOSPITAL PATIENTS

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Key words: Corynebacterium diphtheriae, carriage, monitoring.

INTRODUCTION

Epidemiological monitoring of Corynebacterium diphtheriae carriage acquires special significance in Russia within a period of decreased diphtheria incidence rate. Objectives: The objective of the examination consists in microbiological monitoring of Corynebacterium diphtheriae carriage to predict development of diphtheritic infection and epidemic.

METHODS

The research was conducted using Procedural Guidelines for Laboratory Diagnosis of Diphtheritic Infection. Toxigenic properties of C. diphtheriae were determined using Elek-test. Results: 8,776 patients were examined from December 2010 through November 2011. Toxigenic strains of C. diphtheriae were not isolated therefrom. 46 nontoxigenic strains were isolated, 38 strains thereof were ranked as C. diphtheriae mitis biovariant, 8 strainsâ€“ as C. diphtheriae gravis biovariant. The rate of C. diphtheriae isolation of eleven months made 5.2 per 1,000 people examined. The majority of strains (33) were isolated in the winter-spring season,13 strains were isolated in autumn. Thus, the rate of isolation in those seasons made 8.2 and 8.8, respectively. 21 culture strains were isolated of the same hospital department with 92.5% thereof ranked as mitis biovariant. C. diphtheriae toxigenicity is known to be a major factor of pathogenicity, which determines development of diphtheria. There are strains of C. diphtheriae with a low level of toxin production that are undetectable by Elek-test. According to literature, 25% - 40% of all isolated nontoxigenic strains do bear a â€˜silentâ€™ tox-gene. The nontoxigenic strains of C. diphtheriae isolated require additional reidentification, molecular and genetic typing.

CONCLUSIONS

Thus, mitis biovariant appears prevalent (82.6%) in the nontoxigenic strains of C. diphtheriae isolated. Final inference of toxin production, extent thereof, and epidemiological significance of the strains isolated may only be made after the additional research. Finding of the true toxigenicity of strains of C. diphtheriae defines clinical and epidemiological significance thereof.

STAPHYLOCOCCUS AUREUS AS INDICATOR OF CONDITIONS CONDUCIVE TO DEVELOPMENT OF NOSOCOMIAL STRAINS Svetlana

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Key words: Nosocomial strains, S. aureus, MRSA.

INTRODUCTION

Psychoneurological hospitals are institutions with high risk of hospital-acquired infections (HAI). This is contributed by the deterioration of self-care skills due to the deformation of a personality. In hospitals, droplet contact and airborne transmission modes are most noteworthy. One of the factors giving rise to an increase in HAI is development of nosocomial strains of microorganisms characterized by multidrug resistance. Microbiological monitoring appears to be the most important element of an epidemiological surveillance system.

OBJECTIVES

To find out the source and ways of HAI agents to develop within hospital departments. Methods: Staphylococcus aureus was chosen to be an indicator microorganism. Reasons: S. aureus is one of the predominant causative agents of nosocomial infections and an indicator microorganism of sanitary significance in respect of causative agents of droplet infections of both bacterial and viral origin (tuberculosis, diphtheria, flu). Three antibiotics were used as epidemiological markers of the strains isolated: Oxacillin, Clarithromycin, Clindamycin. Samples were taken in the middle of business hours, when microorganisms accumulate in the ambient in the maximum number. All in all, 209 people were examined (66 employees and 143 patients). The material was taken from nasal mucosa. Surface washing of 204 different items of the ambient was also examined. Results: Both patients and employees are MRSA strains carriers. Carriage of S.aureus in the nasal mucosa of patients is 3.4 times as much as that of the employees.The main reservoir of MRSA strains in the ambient is procedure units.

CONCLUSIONS

Multidrug-resistant strains of S. aureus appear to persist and accumulate in the ambient.Lack of multidrug-resistant strains of S. aureus in the biological material proves to be evidence of proper disinfection and sanitation efforts made at the hospital departments and of sterility of medical techniques.

IN VITRO MODEL TO DETERMINE THE TRANSIT TOLERANCE OF SOME MICROENCAPSULATED PROBIOTIC STRAINS IN THE HUMAN GASTROINTESTINAL TRACT

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INTRODUCTION
The consumption of probiotic cultures in adequate doses (106 – 107) positively affects the composition of gastrointestinal tract microflora and extends a range of host benefits. Microencapsulation of probiotics offers a potential way of improving the survival of probiotics during passage trough the gastrointestinal tract.
OBJECTIVES
The objective of this study was to assess the tolerance of some microencapsulated microbial strains in simulated gastrointestinal tract conditions.
DESIGN
L. acidophilus La-5, L. casei - 01, L. helveticus Lh. B 02, B. bifidum Bb-12, K. lactis NRRL Y- 8279 and Sacch. cervisiae DSMZ 70449 were microencapsulated in 3% sodium alginate matrix. The viability of free and microencapsulated of these strains in simulated gastrointestinal tract environment was determined.
RESULTS
The strains viability decreased significantly in the presence of lysozyme (100 ìg /ml) except B. bifidum and K. lactis in microencapsulated form. The viability of all strains at pH 1.5 was less than at pH 3.0 throughout 180 min except L. acidophilus and L. helveticus in microencapsulated form showed high viability. At 2% bile salt, the resistance of microencapsulated strains was significantly higher than free form. The release of viable cells from microcapsules in simulated colonic pH solution increased significantly as the exposure time increased. L. helveticus is the most sensitive strain while B. bifidum was the most tolerant strain in 0.1% phenol concentration. Only yeast strains were susceptible to neomycin.
CONCLUSIONS
The microencapsulation of strains protected them against adverse effects of gastrointestinal tract environment and enhanced their survivability, where the viable cells were still above the levels suggested to produce their claimed health benefits. Key words: Probiotics, Microencapsulation, Gastrointestinal tract and Transit tolerance

RESISTANCE TO MAMMALIAN DIGESTION AND PREBIOTIC PROPERTIES OF NOVEL GALACTO-OLIGOSACCHARIDES FROM LACTULOSE

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INTRODUCTION
Galacto-oligosaccharides are considered functional dietary compounds capable of modulating the composition and metabolic activities of intestinal microbiota. Indeed, lactulose-derived galacto-oligosaccharides are attracting increasing attention due to their prospective prebiotic applications.
OBJECTIVES
The aims of this study are to evaluate a) the resistance to digestion of novel lactulose-derived galacto-oligosaccharides and b) if their major components are fermented by the microbiota to promote the selective growth of beneficial bacteria in the large intestine.
METHOD/DESIGN
Lactulose-derived galacto-oligosaccharides were incorporated in a single dose (1%, w:w) to rats for a period of fourteen days. Chromium oxide was included in diets as an indigestible marker. Fecal samples were collected weekly whereas the intestinal contents of rats were collected at the end of the dietary intervention period. Di- and trisaccharides were identified and quantified in dietary, ileum and fecal samples by GC-MS. Different microbial groups were quantified using qPCR, and the Bifidobacteria group analysed by PCR-denaturing gradient gel electrophoresis.
RESULTS
Quantitative evaluation of carbohydrates from dietary and ileal samples demonstrated that the disaccharide fraction of lactulose-derived galacto-oligosaccharides, mostly composed of β-galactobioses and galactosyl-fructoses, was fully resistant to digestion in the small intestine. The trisaccharide fraction exhibited a limited digestion as evaluated in ileal samples of treated animals. The chromatographic profile of fecal samples of rats fed lactulose-derived galacto-oligosaccharides demonstrated the complete fermentation of galacto-oligosaccharides in the large intestine. A significant increase in the growth of both Bifidobacteria and Eubacterium rectale/Clostridium coccoides groups was observed by qPCR in response to the dietary treatment in large intestine; at specie level, a significant and selective increase of Bifidobacterium animalis was revealed.
CONCLUSIONS
The reported data demonstrate the resistance to digestion and fermentation selectivity of lactulose-derived galacto-oligosaccharides by specific bifidobacteria species and support their potential role as prebiotic ingredient in functional foods.

ANAEROBIC INTESTINAL BACTERIA GROWING AS SINGLE- OR DUAL-SPECIES BIOFILM

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INTRODUCTION

So far, biofilms of human intestinal anaerobic bacteria have been poorly investigated. The MacFarlane group reported in 2007 on the occurrence in microcolonies of bacteroides and bifidobacteria and on their distribution throughout the mucus layer, while in 2010 the Ceri group cultured multi-species biofilms from Bacteroidetes, Firmicutes and Actinobacteria recovered from colonic biopsies.

OBJECTIVES

The aim of our study was to investigate anaerobic strains isolated from clogged biliary stents and belonging to the genera Bacteroides, Clostridium, Fusobacterium, Finegoldia, Prevotella and Veillonella for their ability to form in vitro single- or dual-species biofilms.

METHODS

The ability to adhere was evaluated by the quantitative biofilm production assay. Then, strains were investigated for their ability to grow as biofilms by Field Emission Scanning Electron Microscopy (FESEM) and Confocal Laser Scanning Microscopy (CLSM). Experiments on dual-biofilm formation were planned on the basis of the anaerobic strains isolated from each clogged biliary stent, by selecting those in which a couple of anaerobic strains belonging to different species contributed to the polimicrobial biofilm development.

RESULTS

We demonstrated the ability of strains to grow in sessile mode and to synergistically interact in forming dual-species biofilm. The microscopical analysis allowed us to distinguish between the interacting bacterial species on the basis of their different features (rods or spear-shaped bacilli vs cocci) and to evaluate their relative contribution to form the mixed biofilm.

CONCLUSIONS

This is the first report on the ability of the anaerobes Bacteroides oralis, Clostridium difficile, Clostridium baratii, Clostridium fallax, Clostridium bifermentans, Finegoldia magna and Fusobacterium necrophorum to form biofilms. The in vitro development of dual-species biofilms by Fusobacterium necrophorum + Veillonella spp, Bacteroides fragilis + Finegoldia magna, and Finegoldia magna + Clostridium difficile has a particular significance, since these couples were selected on the basis of their isolation from the same clogged biliary stent.

MOTILITY IN NON-FLAGELLATE MULTI-DRUG RESISTANT ACINETOBACTER BAUMANNII LINEAGES: THE ANSWER FOR ITS PERSISTENCE?

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INTRODUCTION
Acinetobacter baumannii is an emerging opportunistic pathogen widely distributed in hospital settings. Its ability to survive in adverse conditions and ability to acquire multiple antibiotic resistance have made this a difficult pathogen to treat. Despite the lack of flagella, some studies suggest motility as a virulence feature also allowing its long lasting survival in abiotic surfaces.
OBJECTIVES
In this work we investigated different types of motility (swimming, swarming and twitching) and the possible correlation between this ability and the persistence of specific Acinetobacter baumannii lineages. Additionally, we tried to associate possible alterations in some genes implicated in frimbriae or pili formation with each type of motility.
METHOD
The study included 30 multi-drug resistant A. baumannii clinical isolates from several sources recovered from 2001-2011. Genomic based typing methods (PFGE; 2 MLST schemes), characterization of carbapenem-hydrolyzing Class D b-lactamases, fimbriae and pili encoding genes were performed by PCR and sequencing. Motility assays for all the isolates were performed in different culture media with different agar percentages and temperatures.
RESULTS
The tested isolates included different lineages and carbapenem-hydrolyzing Class D b-lactamases (ST2/ST92- and ST2/ST118-carrying blaOXA-23, ST2/ST98-carrying blaOXA-40, and ST15/ST103 -carrying blaOXA-58). Differences were observed in what concerns to motility behavior at different temperatures and the tested genes (ORFs encoding type IV pilus, fimA, ppk, csuE, bfmR, bfmS) among isolates belonging to the different lineages.
CONCLUSIONS
Our results suggest that motility might have an important role in surface colonization and dissemination of particular lineages of Acinetobacter baumannii. A better understanding of the mechanisms underlying this capacity could contribute to the development of new strategies to restrain its spreading.
KEYWORDS: Acinetobacter baumannii, resistance, motility, dissemination

FIRST REPORT OF A NEW VIM-1 VARIANT IDENTIFIED IN A ST15 KLEBSIELLA PNEUMONIAE CLONE CO-PRODUCING SHV-12 IN PORTUGAL

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INTRODUCTION
Outbreaks of carbapenemase producing Enterobacteriaceae have been increasingly reported at global scale. In Portugal, they have been scarcely described and mainly associated with KPC enzymes.
OBJECTIVES
Our aim was to characterize a presumptive carbapenemase-producing Klebsiella pneumoniae isolate from a Portuguese patient.
METHODS
One Klebsiella pneumoniae isolate identified in an urine from a female outpatient with previous multiple hospitalizations presumptively identified as carbapenemase producer was analysed. Bacterial identification, antibiotic susceptibility testing and conjugation experiments were performed by standard methods. Presence of carbapenemases and extended-spectrum beta-lactamases (ESBL) were investigated by phenotypic tests (DDST, Hodge test, beta-lactams/beta-lactams inhibitors or EDTA) and confirmed by PCR (blaMBL, blaESBL) and sequencing. Clonal identification was performed by MLST. Linkage of blaMBL and class 1 integrons was investigated by PCR and sequencing. Fluoroquinolone resistance genes (qnrA, qnrB and qnrS) were searched by PCR.
RESULTS
A novel VIM-type enzyme differing from VIM-1 by one aminoacid mutation (A93V) was identified in a ST15 K. pneumoniae isolate co-producing SHV-12. The blaVIM-1-like gene was located within a ca. 4.0-Kb class 1 integron (blaVIM-1-like-aacA4-aphA15-aadA1-catB2), with some similarity to others (In70, In113) previously identified among VIM-1-producing P. aeruginosa and K. pneumoniae isolates in Europe. Neither blaVIM-1 nor blaSHV-12 were transferable by conjugation. This isolate was resistant to imipenem (MIC=4 g/L), but susceptible to ertapenem and meropenem (MIC=0.25-0.38 g/L), and conferred resistance to kanamycin, tobramycin, nalidixic acid, ciprofloxacin, chloramphenicol and sulphonamides. Fluoroquinolone resistance genes were not detected.
CONCLUSIONS
We describe a novel integron type containing a new blaVIM-1 variant identified in the intercontinental ST15 K. pneumoniae clone, co-producing SHV-12. The emergence and spread of multidrug resistance platforms containing blaMBL genes among Enterobacteriaceae need to be further monitored. Keywords: Klebsiella pneumoniae, VIM, Integron, SHV-12, ST15.

DIVERSITY AND BIOFILM-PRODUCTION ABILITY OF WIDESPREAD ESCHERICHIA COLI PHYLOGROUP A (ST10, ST23) AND B1 (ST155, ST359) LINEAGES

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INTRODUCTION
Besides widespread Escherichia coli clones of phylogroups B2 and D, some A and B1 uropathogenic (UPEC) clones have increasingly been identified among ESBL/AmpC producers worldwide.
OBJECTIVE
The diversity of representative A and B1 UPEC isolates from different settings, and their ability to adhere and form biofilm on abiotic surfaces is investigated.
METHODS
Fifty-one widespread A (22 ST10, 14 ST23 complexes) and B1 (8 ST155 complex, 7 ST359) from 6 EU (n=47) and 2 South American (n=4) countries were studied (1997-2008). They were ESBL (CTX-M, TEM, SHV, GES) or AmpC (CMY, DHA) producers or non-ESBL/AmpC producers from nosocomial (52%) and community (12%) outbreaks, healthy volunteers (14%) or animals (22%). Clonal relatedness was established by PFGE/MLST. Screening for 38 ExPEC virulence factors (VFs) was performed by PCR. Biofilm production was investigated by a modified quantitative assay.
RESULTS
Extra-intestinal pathogenic E. coli (ExPEC) features were identified among ST23 (42.9%), but also ST359 (28.6%), ST155 (25%) and ST10 (9.1%), although all human isolates caused extraintestinal infections. fimH, iutA, iss and iroN (38-100%) were common in all STs. ST23 exhibited a higher virulence score (median 9/range 2-14) and was enriched in traT, ompT, cvaC, fyuA and tsh (50-71%). ST10/ST359 contained frequently usp (52-71%) and traT (43-76%), and ST155 ompT and sfa/focDE (43-57%). A high clonal diversity (48 PFGE-types) was detected, although clusters containing isolates from different settings/geographic regions were identified in each clonal group. Biofilm production was detected among ST23 (n=8; 0.25<O.D.<0.48), and rarely among ST155 (n=1; O.D.=0.52) or ST10 (n=2; 0.47<O.D.<0.73) isolates, classified as moderately adherent strains.
CONCLUSION
The virulence profile of particular A and B1 widespread E. coli clones could justify their association with extraintestinal disease. Enrichment in ExPEC VFs and abiotic adhesion ability of ST23 complex isolates might favor its emergence as an extraintestinal pathogen.
Keywords: Escherichia coli; Virulence; Adhesion; Biofilm

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PILOT STUDY OF LACTOBACILLUS REUTERI DSM 17938 FOR THE TREATMENT OF ACUTE CHILDHOOD DIARRHOEA

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INTRODUCTION
Probiotics may be an effective adjunct to the management of acute diarrhoea Lactobacillus reuteri (L. Reuteri) ATCC 55730 was shown to shorten the duration of acute diarrhoea. Recently, this strain was found to carry potentially transferable resistance traits for tetracycline and lincomycin, which led to the development of a new daughter strain, L. reuteri DSM 17938 derived from L. reuteri ATCC 55730 by the natural removal of unwanted plasmid-borne resistances.
OBJECTIVE
to test the efficacy and safety of a new daughter strain, L. reuteri DSM 17938 derived from L. reuteri ATCC 55730 in children with acute diarrhoea. Primary outcomes were the rate of unresolved diarrhoea after five days of treatment and duration of diarrhoea.
METHOD/DESIGN
children (6 to 36 months old), hospitalized in three paediatric disease wards in Southern Italy for acute diarrhoea with clinical signs of dehydration were randomized to receive orally in a double blind fashion either L. reuteri (dose of 108 colony-forming units per day) or placebo.
RESULTS
out of 96 eligible children, 74 were enrolled into the study, 5 patients, were withdrawn and 69 completed the study, 35 in the L. reuteri group and 34 in the placebo group. L. reuteri significantly reduced the duration of watery diarrhoea as compared to placebo (2.1±1.7 vs. 3.3±2.1 days; $p<0.03$); on day two and three of treatment watery diarrhoea persisted in 82% and 74% of the placebo and 55% and 45% of the L. reuteri recipients respectively ($p<0.01$; $p<0.03$). Finally, children receiving L. reuteri had a significantly lower relapse rate of diarrhoea (15% vs. 42; $p<0.03$). There was not a significant difference in hospital stay between the groups. No adverse events were recorded.
CONCLUSION
Our study shows that L reuteri DSM 17938 as an adjunct to rehydration therapy is efficacy in the treatment of acute diarrhoea reducing the frequency, duration, and recrudescence rate of the disease.

INFANT FORMULA SUPPLEMENTED WITH ALPHA-LACTALBUMIN AND NUCLEOTIDES INDUCES CHANGES IN THE INTESTINAL MICROBIOTA OF INFANTS

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INTRODUCTION
It has been stated that indigenous microbiota plays an important role providing defense mechanism against colonization of pathogenic bacteria. Type of feeding is one of the main factors influencing the establishment of the intestinal microbiota. As breast feeding is considered as ideal food for infants, infant formulas are designed to resemble the composition of human milk. To this end, infant formulas have been supplemented with ?-lactalbumin and nucleotides, compounds naturally present in breast milk, due to its beneficial properties described, including growth, repair and differentiation of gastrointestinal tract in infants. However, there are scarce studies on its effects on intestinal microbiota.
OBJECTIVES
To determine the influence of infant formula supplemented with ?-lactalbumin and nucleotides and non-supplemented infant formula on intestinal microbiota comparing it with the microbiota in breastfed infants.
METHOD / DESIGN
61 Healthy full-term infants were recruited, 33 of them were fed exclusively with human milk. Formula fed infants were randomized to a control formula (n=14) and a formula supplemented with ?-lactalbumin and nucleotides (n=14). Faecal samples were collected at 2, 4, 8 and 12 weeks of life to study different microbial populations by quantitative PCR
RESULTS
Infants fed with human milk showed the lower levels of the microbial groups; Atopobium, Clostridium leptum, Enterobacteriaceae and Enterococcaceae respect to formula-fed infants. However, Bifidobacterium and lactobacillus populations followed similar levels. At the first sampling, Bacteroides group and Clostridium leptum group showed significant differences between the two formulas assessed, being lower in the group receiving the supplemented formula. Overall comparison between both infant formulas showed significant differences in Atopobium, Bacteroides y Clostridia IV groups, showing the supplemented formula the lowest values.
CONCLUSION
Our results demostrate that infant formula supplemented with ?-lactalbumin and nucleotides induce beneficial changes on the intestinal microbiota composition, reducing some of the potentially pathogenic microbial groups.

ADHESION OF PROBIOTIC PREPARATION ‘LATOPIC’ TO THE HUMAN COLON ADENOCARCINOMA CELL LINE CACO-2

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KEY WORDS: probiotic bacteria, Caco-2 cells, adhesion

INTRODUCTION

The adhesive ability of bacteria to intestinal cells is considered as one of the selection criteria for probiotic strains. The aim of the study was to determine the adhesion of commercial probiotic preparation ‘Latopic’ to Caco-2 cells in culture in vitro.

OBJECTIVES

The probiotic preparation contains 3 probiotic strains Lactobacillus casei £OCK 0900, Lactobacillus casei £OCK 0908 and Lactobacillus paracasei £OCK 0919. They were obtained from the collection of the Institute of Fermentation Technology and Microbiology (£OCK 105), Technical University of Lodz, Poland. The strains possess full probiotic documentation, they are licensed (P-382760, P-382761, P-382762) and they are deposited in the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences in Wroclaw (no.: B/00019, B/00020; B/00021). The Caco-2 cell line (ATCC HTB 37) was purchased from the American Type Culture Collection (ATCC, USA, Lot number: 58844056).

METHOD

Caco-2 cells were seeded with 1 ml culture medium containing 106 cells/well in 24-well tissue culture plate. Tested ‘Latopic’ was resuspended in non-suplemented DMEM and 1 ml of the suspension was added to each well and incubated at 37 oC for 1 h in 5% CO2. Unattached bacteria were removed, and Caco-2 cells were lysed. Adhering bacteria were enumerated by plate counting with MRS agar and then incubated at 37 oC for 48 h. Adhesion assay was performed in four replicates.

RESULTS

The adherence percentage of ‘Latopic’ to Caco-2 cells was compared by counting between initial and adhered bacteria (log CFU/ml). The adherence for ‘Latopic’ was 86,6%.

CONCLUSIONS

Probiotic preparation ‘Latopic’ possess high ability to adhere to Caco-2 cell line. This work was supported by grant number 12010110 from The National Center for Research and Development, Poland.

GENO- AND CYTOTOXICITY OF FAECAL WATER AFTER INCUBATION OF 2-AMINO-1-METHYL-6-PHENYL-1H-IMIDAZO[4,5-B]PYRIDINE (PHIP) WITH FAECAL MICROORGANISMS AND PROBIOTIC LACTOBACILLUS CASEI DN 114-001

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KEY WORDS: probiotics, carcinogens, diet

INTRODUCTION

Aromatic amines (HCA) are substances with high mutagenic potential. The aim of the study was to evaluate cyto- and genotoxicity of faecal water (FW) after incubation of heterocyclic amine PhIP with faecal microorganisms and/or probiotic Lactobacillus casei DN 114-001.

OBJECTIVES

The commercial probiotic strain of Lactobacillus casei DN 114-001 (Actimel, Danone) was engaged. The stool samples derived from 15 healthy persons (3 groups, 5 persons in each group): group A (breast-feed children up to 24th month of life); group B (persons at the age of 30-40); group C (persons at the age of 75-85).

METHOD

To faeces were added: (1) PhIP (at the final concentration of 50 µg/mL), (2) the tested probiotic strain (at the final concentration of 1010 cfu/mL), (3) PhIP and probiotic strain. The negative control was sample of faeces in sterile PBS without additions. The samples were incubated for 72 h at 37 oC in anaerobic conditions. Genotoxicity of faecal water was evaluated with the comet assay, cytotoxicity was measured with neutral red uptake (NRU) assay with usage of Caco-2 cell line.

RESULTS

The most genotoxic (10.9 %±2.6) was FW of old persons (75-85 years old) and the least of children (4.2%±1.0). Lb. casei DN 114-001 decreased genotoxicity of FW after incubation with PhIP from 18.2%±3.00 to 6.03%±0.72 in case of the group of adults; from 16.0±1.72 to 6.2±0.72 for elderly, and from 13.08±1.59 to 8.64±2.60 in case of children. The cytotoxicity of FW was changed after incubation of probiotic strain, but it depended on the person's age.

CONCLUSIONS

It was observed that cyto- and genototoxicity of FW depended on the person's age and its individual intestinal microbiota.

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SELECTIVELY STIMULATED GROWTH OF INTESTINAL MICROBIOTA BY THE NEW ENZYME-RESISTANT DEXTRIN.

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KEY WORDS: intestinal microbiota, resistant dextrin

INTRODUCTION

A prebiotic is a non-digestible food ingredient that beneficially affects that the host by selectively stimulating the growth and/or activity of one or, a limited number, of bacteria in the colon. Fermentation of same oligosaccharides is not as selective. There is, therefore, a need for new prebiotic status therefore of distinct, selective stimulation of growth of lactic acid bacteria and non-fermented or slightly fermented by other, sometimes pathogenic intestinal bacteria. Promising sources of prebiotics are starch products, especially resistant starch.

OBJECTIVES

The objective of the present study was to apply enzyme-resistant dextrin, prepared by heating of potato starch in the presence of citric or tartaric acid, as a product with potential prebiotic properties, selectively stimulated growth of intestinal microbiota isolated from faeces of 70-year old men.

METHOD

Resistant dextrin was prepared by simultaneous thermolysis and chemical modification of potato starch in the presence of hydrochloric acid as catalyst of thermolysis and tartaric or citric acid as derivatizing agent. The bacteria (Lactobacillus, Bifidobacterium, Clostridium, E. coli and Fusobacterium) were isolated from faecal of three 70-year old men volunteers. All cultures were conducted in both containing 1% of dextrin as the only carbon source. Control cultures contained glucose.

RESULTS

The tested strains of intestinal bacteria are able to make use of resistant dextrins as the only carbon source, but in varying degrees. Most intensive growth characterised by the bacteria of the genus Bifidobacterium and Lactobacillus, for all strains the maximum multiplication of the cells was approximately 108 CFU/ml. Other bacteria - Clostridium and Fusobacterium less utilize dextrin. The results of this study may indicate the value of the studied prebiotic dextrin.

This work was supported by grant number N N312 335339 from National Center for Research and Development, Poland.

ß-GLUCURONIDASE AND ß-GLUCOSIDASE ACTIVITY IN HUMAN FAECAL WATER IN THE PRESENCE OF CARCINOGEN PHIP AND LACTOBACILLUS CASEI DN 114-001 IN VITRO

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KEY WORDS: probiotics, carcinogens, â-glucuronidase, â-glucosidase

INTRODUCTION

High faecal enzymes activity is recognised as a biomarker of harmful action of the gut microbiota and colon cancer. 2-amino-1-methyl-6-phenyl-1H-imidazo[4,5-b]pyridine (PhIP) is mutagenic aromatic amine ingested with diet, which can be a substrate for intestinal microbiota. The aim of the study was to determinate activity of â-glucuronidase and â-glucosidase in human faecal water after incubation of PhIP with faecal microorganisms and Lactobacillus casei DN 114-001.

OBJECTIVES

The probiotic strain of Lactobacillus casei DN 114-001 (Danone) was engaged. The stool samples derived from 15 healthy persons (3 groups, 5 persons in each group): group A (breast-feed children up to 24th month of life); group B (persons at the age of 30-40); group C (persons at the age of 75-85).

METHOD

To faeces were added: (1) PhIP (at the final concentration of 50 µg/mL), (2) the tested probiotic strain (at the final concentration of 1010 cfu/mL), (3) PhIP and probiotic strain. The control was sample of faeces without additions. The samples were incubated for 72 h at 37 oC in anaerobic conditions.

RESULTS

PhIP increased activity of tested enzymes in faecal water of all persons. The average â-glucosidase activity was 0.19±0.02 U/mg for children, 0.77±0.26 U/mg for adults, 1.18±0.27 U/mg for elderly. â-glucuronidase activity for children was 0.48±0.04 U/mg, for adults 0.75±0.27 U/mg, for elderly 1.55±0.06 U/mg. Lb. casei DN 114-001 decreased the enzyme activity comparing with the sample of faeces with PhIP: in elderly it was 81% lower; in children 59% and in adults 50% lower.

CONCLUSIONS

Lb. casei DN 114-001 can inhibit activity of â-glucuronidase and â-glucosidase in the presence of PhIP, but the degree depends on person’s age and human individual microbiota.

Scientific work financed from The National Center for Research and Development, Poland, as a research project number N N312 203836.

METABIOTICS. MITH OR REALITY

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Traditionally, probiotics on the base of live microorganisms consider to be safe and beneficial. Unfortunately, their effects may have short-lived success or are absent or uncertain. Some probiotic bacteria even belonging to Lactobacillus or Bifidobacterium may cause opportunistic infections, increase incidence of allergic sensitization and autoimmune disorders, produce microecological imbalance, modify gene expression, transfer antibiotic resistant and virulence genes, cause disorders in epigenomic genome integrity and induce chromosomal DNA damages. The commercially available probiotics should be considered as a first generation of means correcting microecological disorders. Further their development will include the selection of natural metabiotics and/or working out the synthetic (or semi-synthetic) metabiotics that will be analogies or improved copies of natural boiactives produced by probiotic microorganisms. Metabiotics are structural components of probiotic microorganisms and/or their metabolites and/or signaling molecules with determined (known) chemical structure that can optimize host specific physiological functions, regulator, metabolic and/or behavior reactions connected with activity of host indigenous microbiota. Metabiotics on the base of LMW molecules of microbial origin for nutrition and medicine has already distributed in some countries (E. coli glycoprotein with anorexic activity; lactobacilli polysaccharide - glycopeptide with antihypertension effect and so on). Metabiotics have some advantages because of exact chemical structure, well dosed, very safety and long shelf-life. Thus, now metabiotics should not consider Myth; they are result of natural evolution of Probiotic conception.

REAL-TIME QUANTIFICATION OF BACTERIAL BIOFILM FORMATION

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INTRODUCTION
Several methods are available to study biofilms in vitro, but their applications are limited by a low sensitivity, high labor intensity and/or long time lags to obtaining a result. Therefore, sensitive, reproducible and fast methods are desirable for real time biofilm monitoring.
OBJECTIVES
To present and evaluate a new reliable, labeling-free and rapid assay for monitoring biofilm formation in real time.
METHOD
Impedance-based technology measurements in microtiter plates with gold electrodes.
RESULTS
Species biofilm growth curves showed an S-shape as cells grew attached to the wells and disrupted the electrical current. The strains tested were variable in the slopes and reached different cell index values, in agreement with their biofilm capacity as assessed by attachment to standard microtiter well plates and safranin staining. Biofilms of Staphylococcus aureus and S. epidermidis were enhanced by adding sugars to the culture medium, and were heavily reduced in mutants of the biofilm regulatory gene sarA or by protease treatment in protein-based biofilm matrixes. We show the power of this technique by measuring biofilm growth of clinical isolates of S. aureus and S. epidermidis in the presence of 10 different antibiotics. The patterns of antibiotic resistance in biofilms were very different to those obtained by traditional methods, and show that the effect of antibiotic treatment once the biofilm is formed gets dramatically reduced. In addition, real-time cell index measurements showed that several antibiotics stimulated biofilm growth, stressing the importance of evaluating antibiotic efficacy under biofilm growth conditions.
CONCLUSIONS
We show for the first time RTCA is also able to measure the growth of bacterial biofilms quantitatively and reproducibly in real time. We propose the use of real-time measurements for shedding light on the biology of biofilm formation and for clinical applications like selection of efficient antibiotic therapy in biofilm-forming pathogens.

ORAL ADMINISTRATION OF B. INFANTIS REDUCES BACTERIAL DNA TRANSLOCATION RATE IN MICE DURING INDUCTION OF EXPERIMENTAL CIRRHOSIS

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INTRODUCTION
bacterial-DNA translocation from gut is a frequent event arising in cirrhosis and induces severe immunological complications in this setting. Primary prophylactic strategies, mainly based on the use of antibiotics, are under consideration to prevent these episodes. However, these approaches are not free of adverse effects.
OBJECTIVE
To evaluate alternative strategies to prevent bacterial-DNA translocation during induction of experimental cirrhosis.
METHODS/DESIGN
Female Balb/c mice were included in a 16-week study protocol. Animals received two weekly, weight-controlled doses of carbon tetrachloride (CCl4) that were intragastrically administered. Protocol 1: One week prior to laparotomy, different subgroups of animals orally received Norfloxacin, Interleukin 10 Chimera, B infantis, or vehicle. Protocol 2: Animals were treated as in Protocol 1 and received an intraperitoneal dose of E coli 24 hours before laparotomy, Laparotomies were performed at weeks 7, 10, 13 and 16 (n=10/week/condition) in both protocols. Bacterial-DNA translocation into mesenteric lymph nodes was evaluated by broad-range PCR of 16SrRNA prokaryotic gene followed by partial sequencing analysis.
RESULTS
Rate of bacterial-DNA translocation into mesenteric lymph nodes along the study protocol was 30% until week 10 and 60% in weeks 13 and 16. In Protocol I, the administration of Interleukin 10, norfloxacin and B. infantis significantly reduced this late rate to 20% of treated animals. In Protocol 2, intraperitoneal administration of E. coli induced a 100% of bacterial-DNA translocation in CCl4-treated animals. However, animals pretreated with norfloxacin reduced this rate to 30-40%, those pretreated with IL-10 reduced it to 30-40% and those pretreated with B. infantis significantly did it to 10% of animals. B. infantis significantly reduced bacterial-DNA translocation rates observed in animals pretreated with norfloxacin at weeks 13 and 16.
CONCLUSIONS
oral administration of B. infantis significantly downregulates bacterial-DNA translocation episodes in mice challenged with E. coli during induction of experimental cirrhosis.

CLOSTRIDIUM DIFFICILE INFECTIONS IN HUMANS AND ANIMALS: AN UPDATE

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Clostridium difficile is an important emerging pathogen in both humans and animals. Characteristically, Clostridium difficile infection (CDI) has been considered nosocomial but in recent years a remarkable rise in the occurrence of community associated CDIs has been observed , the source of which is not clearly defined. The similarity of various PCR ribotypes in C.difficile strains recovered from humans and domestic animals suggests a possible animal reservoir for human CDI. Epidemiological research on this potential relationship, however, is limited. Studies from several countries showed that some strains belonging to humans or other mammals are indistinguishable by molecular analysis suggesting a common source, i.e human-to-animal transmission or zoonotic transmission. The current epidemic strain, NAP1/027/BI, has caused outbreaks of human disease in North America and Europe for nearly a decade and is a cause of sporadic disease worldwide. This strain has been isolated also from both food and companion animals. Other C. difficile strain types known to cause disease in humans, including ribotype 017 and ribotype 078 have been isolated also from animals.Clearly, C.difficile is an established human and animal pathogen and there is considerable overlap among some animal and human strains. It is unclear, however, what this implies for the epidemiology of CDI, specifically, whether and how zoonotic transmission occurs. No study has demonstrated the occurrence of a human C. difficile infection as a result of animal contact, although animal acquisition of C. difficile from humans has been suggested by some investigations. Further epidemiological studies are needed to assess the role of animal contact in transmission of C. difficile to humans and vice versa, to determine whether animal-to-human transmission occurs and to document the risks attributable to this mode of transmission.

MODULATION OF THE IMMUNE RESPONSE BY LACTOBACILLUS RHAMNOSUS IN A MOUSE MODEL OF GLUTEN-DEPENDENT ENTEROPATHY.

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INTRODUCTION
Celiac disease (CD) is a very common food-sensitive enteropathy that is triggered by gluten ingestion and is mediated by intestinal CD4+ T cells. Alterations of the intestinal microbiota seem to play a role in CD pathogenesis.
OBJECTIVES
We analyzed in vitro and in vivo the modulatory effects of L. rhamnosus in a transgenic mouse model of gliadin-induced enteropathy.
METHODS
L. rhamnosus OLL2838 (Meiji Dairies Corporation, Odawara, Japan) was grown in MRS at 37°C in microaerophilic conditions. Bone marrow derived dendritic cells (DCs) were generated in vitro from DQ8 mice and incubated with OLL2838 (1:10, DCs:bacteria) for two days. To induce the enteropathy DQ8 transgenic mice were intragastrically administered with 4 doses of gliadin (500 microg/dose) and indomethacin (1.5 mg/100ml drinking water), a cyclooxygenase inhibitor, for 10 days. In some experiments L. rhamnosus was administered during this treatment. Mice were sacrificed to collect mesenteric lymph nodes (MLN) and small intestine. DCs and MLN cells were analysed by FACS, for cytokine expression (ELISA) and NO2 release. Intestinal specimens were evaluated by morphology and for levels of total glutathione (GSHtot), glutathione S-transferase (GST) and caspase-3 activities.
RESULTS
OLL2838 increased the expression of CD80,CD11c and CD40 maturation markers on DCs. Secretion of IL-12, TNF-alpha, IL-10 and NO2 release were strongly induced by the probiotic strain ($P<0.05$). In enteropathic mice, OLL2838 administration failed to recover villus blunting but further increased the secretion of IFN-gamma($P<0.05$) by MLN cells. Notably, OLL2838 reduced the intestinal toxicity associated with the enteropathy, as GSHtot and GST activity were found enhanced, whereas caspase-3 activity was reduced ($p<0.001$).
CONCLUSIONS
OLL2838 showed immunostimulatory properties both in vitro and in vivo without altering the phenotype of the response. In the mouse model of gliadin-induced enteropathy OLL2838 recovered the associated cytotoxic status but not the mucosal architecture.

INULIN AND PECTIN CAN AFFECT THE GROWTH, BIOCHEMICAL FEATURES AND SURVIVAL UNDER SIMULATED GASTROINTESTINAL CONDITIONS OF THE PROBIOTIC LACTOBACILLUS ACIDOPHILUS.

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The viability of L. acidophilus DSM 20079, after its passage through the simulated gastric and pancreatic juices, was evaluated as function of its pre-growth in a medium containing the known prebiotics pectin or inulin, and compared to glucose, used as control. The presence of pectin or inulin did not affect the growth (12.11log10 cfu/mL and 12.08 log10 cfu/mL for pectin and inulin respectively versus 12.22log10 cfu/mL obtained for glucose). Pectin and inulin, in contrast to of glucose, induced cell stress resistance against gastrointestinal juices (ƒ´log10 1 and 2 cfu/ml respectively, versus ƒ´ƒnlog10 4.5ƒn for glucose). The data were confirmed by the analysis of the protein pattern following stress treatments. An impressive metabolic change, as function of the growth conditions, was demonstrated by analyzing the proteomic profile with a £g-2DE system. The analysis revealed a different pH protein distribution, mostly acidic in the presence of pectin and neutral-alkaline in the presence of inulin. Both prebiotics stimulated the production of butyrate, not detectable in the presence of glucose, 14.5 fold higher after growth in the presence of inulin than of pectin. Three specific proteins were detected at pH 6 after growth in the presence of pectin or inulin. They could be correlated to the stress resistance and/or to the production of butyrate. The dynamic picture of the growth, viability, proteome and production of some SCFAs exhibited by the strain grown on pectin, inulin or glucose, provided interesting insights into probiotic-prebiotic interactions, and is important for an understanding, also at molecular level, of how a prebiotic can benefit a probiotic strain, leading not only to a selective stimulation of its growth and enhancement of its resistance against stress conditions, such the passage through gastric and pancreatic juices, but also affecting its subsequent protein expression and the production of some healthy bio-molecules, like butyrate.

ANALYSIS OF GASTRIC MICROBIOTA OF THE PATIENTS WITH CHRONIC GASTRITIS WITH OR WITHOUT HELICOBACTER PYLORI INFECTION

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INTRODUCTION
Helicobacter pylori is associated with the occurrence of acute/chronic gastritis, peptic ulcer disease and gastric cancer/MALT lymphoma. Many reports indicated that various probiotic bacteria including Lactobacillus, Bifidobacterium and Clostridium inhibit the growth and colonization of H. pylori, suggesting that microbial ecology between gastric bacteria and H. pylori is important for the establishment of persistent infection with H. pylori.
OBJECTIVES
In the present study, gastric microbiota of the patients with chronic gastritis with or without H. pylori infection was analysed by cultivation and PCR techniques.
METHOD/DESIGN
Gastric biopsy specimens were endoscopically taken from 8 patients with chronic gastritis. The presence of H. pylori was detected by microaerophilic cultivation and PCR using H. pylori-specific primers. For analysis of gastric microbiota, 11 kinds of paired primers targeted 16S rRNA genes were used.
RESULTS
H. pylori was positive by both culture and PCR in 4 out of 8 patients. Streptococcus was dominantly detected in all the patients, and there was no significant difference in the number of Streptococcus between H. pylori positive and negative patients. Atopobium, Enterococcus, Lactobacillus and Veillonella were detected in only H. pylori positive patients. By culture method, streptococci including S. salivarius, S. parasanguis, S. mitis and S. oralis were dominantly isolated. Other microbiota such as Neisseria, Actinomyces, Rothia were isolated, but no significant difference in the number was observed between H. pylori positive and negative patients.
CONCLUSIONS
These results imply that H. pylori infection may affect the colonization of gastric microbiota. It is suggested that H. pylori colonization in gastric mucosa increase gastric pH by suppressing gastric acid secretion, resulting in the outgrowth of gastric microbiota including Streptococcus, Atopobacterium, Lactobacillus and Veillonella.

THE ACTIVITY OF B-GLUCURONIDASE AND PARTICIPATION OF INDIVIDUAL INTESTINAL BACTERIA IN RAT FECES AFTER ADMINISTRATION OF A PROBIOTIC PREPARATION OF LACTOABCILLUS RHAMNOSUS

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INTRODUCTION

In the synthesis of carcinogenic compounds and other toxic substances in the large intestine there are involved fecal bacterial enzymes, which mostly belong to the class of reductases and hydrolases. Among them, the highest activity is shown by â-glucuronidase.

AIM

The aim of this study was to determine the number of the dominant fecal bacteria and the activity of â-glucuronidase in the fecal content coming from 16 experimental animals that were given probiotic Lactobacillus rhamnosus with low â-glucuronidase activity.

MATERIALS AND METHODS

12-moth-old rats were given a freeze-dried probiotic preparation with the density of 3x109 cells of bacteria per day, with their diet for 2 months. The feces was collected at the intervals of 2 weeks. The control group were given a placebo (starch). The number of fecal bacteria was determined on selective media. The â-glucuronidase activity was determined spectrophotometrically using phenolophtalein-â-D-glucuronide as the substrate. The number of bacteria and their activity were monitored before, during and 5 weeks after discontinuation of administering the probiotic preparation. Results and

CONCLUSION

The administration of probiotic Lactobacillus rhamnosus did not affect significantly the numbers of Enterococcus, Bifidobacterium, Bacteroides, Clostridium, and the total number of bacteria in the feces of the rats. The number of Lactobacillus before the administration of the probiotic ranged from 1*107 to 4*109 cfu/g, and during the administration and after its discontinuation it decreased by an order of magnitude. The number of Escherichia coli before the probiotic administration ranged from 1*105 to 2*108 cfu/g of the feces. After the administration discontinuation, the number of the cells dropped to 6*105cfu/g of the feces (the confidence interval of 0.05). Average â-glucuronidase activity in rat fecal content during the administration of probiotic decreased by 5.3% compared to the control group, the effect was maintained for 5 weeks after discontinuation of administration.

THE ACTIVITY OF B-GLUCOSIDASE AND PARTICIPATION OF INDIVIDUAL INTESTINAL BACTERIA IN RAT FECES AFTER ADMINISTRATION OF A PROBIOTIC PREPARATION OF ESCHERICHIA COLI NISSLE 1917

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INTRODUCTION
Bacteria with high â-glucosidase activity in the large intestine may pose a risk of cancer development. Improvement of the quality of the intestinal ecosystem as well as protection against excessive growth of pathogenic microorganisms, or those responsible for the synthesis of carcinogenic substances, can be achieved through the use of probiotic bacteria.
AIM
The aim of this study was to determine the number of the dominant fecal bacteria and the activity of â-glucosidase in the fecal content coming from 16 experimental animals that were given probiotic Escherichia coli Nissle with low â-glucosidase activity.
MATERIALS AND METHODS
12-moth-old rats (8 animals) were given a freeze-dried probiotic preparation with the density of 4x108 cells of bacteria per day, with their diet for 2 months.The control group (8 subjects) were given a placebo (starch). The number of bacteria (Lactobacillus, Bifidobacterium, Enterococcus, Bacteroides, Clostridium, Escherichia coli) was determined on selective media. The â-glucosidase activity was determined spectrophotometrically using p-nitrophenyl-â-D-glucopiranoside as the substrate. The number of bacteria and their activity were monitored before, during and 5 weeks after discontinuation of administering the probiotic preparation.
RESULTS AND CONCLUSION
The administration of probiotic Escherichia coli Nissle did not affect significantly the numbers of Bifidobacterium, Bacteroides and the total number of bacteria in the feces of the rats. The number of Escherichia coli before the administration of the probiotic ranged from 6*105 to 6*107 cfu/g, and during the administration and after its discontinuation it decreased by two of magnitude. The number of Lactobacillus, Enterococcus, Clostridium before the probiotic administration ranged from 1*107 to 2*109 cfu/g of the feces. These changes were statistically significant for the confidence interval of 0.05. Average â-glucosidase activity in the fecal contents of rats during the administration of probiotic Escherichia coli Nissle increased by 6% compared to the control group.

IMPORTANCE OF BIOFILM FORMATION BY UROPATHOGENIC E. COLI DR+ STRAINS IN URINARY TRACT INFECTIONS

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INTRODUCTION

One of the most frequent diseases, which harass people, are bacterial urinary tract infections, which can be caused by uropathogenic Escherichia coli Dr+ strains. They have Dr fimbriae on the cell surface and DraD protein, situated on the top of Dr fimbriae, as a tip subunit or surface-exposed as an unbound subunit. These structures enable the bacteria to adhere to the host tissues and invade into the tissue cells.

OBJECTIVES

The very important pathogenic mechanism which allows the uropathogenic E. coli Dr+ strains to infect urinary tracts is a biofilm formation. This mechanism enables the bacteria to colonize urinary route system. In case of the E. coli Dr+, biofilm formation is dependent on two classes of genes. One class encodes the fimbriae and the second encodes the family of autotransporter proteins, such as Antigen 43 (Ag43).

METHOD/DESIGN

To determine the role of Ag43 in bioflim formation by E. coli Dr+ we have made the agn43 gene knockout. This experiment we have done on clinical E. coli IH11128 strain using TargeTron Gene Knockout System from Sigma. Thanks to it we could analyze how the E. coli Dr+ biofilm formation is dependent on Dr fimbiae production.

RESULTS

We have investigated that the Ag43 protein does not have an important role in biofilm formation but it can be essential in survival of the bacteria within the urinary tract. The most important proteins involved in this process are DraD invasin and DraE adhesin, the component of the Dr fimbriae.

CONCLUSIONS

The development of biofilm structures protects the bacteria inside the aggregate and helps them to survive in unfavorable changing conditions of the urinary environment. That is why, the analysis of biofilm formation by E. coli Dr+ is very important, because the urinary tract infections caused by these bacteia could be treated and eliminated.

THE BETA-LACTAM RESISTANCE IN ENVIRONMENTAL ESCHERICHIA COLI .

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KEYWORDS CTX-M1, CMY-2, E. coli, PCR, Microarray

INTRODUCTION

Antibiotic-resistant organisms enter into the environment from human and animal sources. These bacteria are able to spread their genes into water-indigenous microbes, which also contain resistance genes (Baquero et al. 2008).

OBJECTIVES

To compare the occurrence of ESBLs among environmental E. coli isolated from animal and urban waste water stations.

METHODS/DESIGN

39 isolates of E. coli isolated from urban and 47 E.coli from animal waste water stations, were analysed for the presence of ESBLs and CTX-M by intepretative readings of MICs (ampicillins, cephalosporins and ertapenem). The presence of CTX-M gene groups (Woodford et al. 2006), bla CMY (Perez-Perez et al. 2002), integron 1 were determined by PCR and additional resistance genes by DNA microarray.

RESULTS

MICs of ceftriaxone were 18,4 mg/L in E. coli from urban waste water stations, while in animal water stations were only 4,8 mg/L. MICs of ertapenem were only 0.1mg/L in both groups. The ESBL phenotypes were present in 40 strains and multiresistance in 28 strains. PCR analysis revealed the presence of CTX-M1 group genes and CMY-2 genes which were associated with a class 1 integron. Additional resistance genes tetA, tetB, sul2, dfrA13, dfrA17, dfr A19, aadA2, strA, strB and catA were detected by DNA microarray in various strains.

CONCLUSIONS

CTX-M1 group and CMY-2 genes associated with integron 1 were detected in environmental E. coli frequently. Ertapenem resistance was not found. The results confirmed that environmental Escherichia coli could constitute a reservoir of ESBLs for human population. This study was supported by slovak APVV grant No. 0009-10 and VEGA 2/0005/11

REDUCTION OF VIABLE CAMPYLOBACTER COUNTS ON CHICKEN CARCASSES IN THE SLAUGHTERHOUSE BY IMMERSION IN MONOCAPRIN EMULSION INSERTED IN THE PROCESSING LINE.

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INTRODUCTION
Foodborne infections are an important public health problem worldwide. Campylobacter is considered the most common cause of foodborne illness, with over 200 thousand confirmed cases of campylobacteriosis reported in the European Union in 2007. Several foodborne pathogens, particularly Campylobacter, are associated with poultry products and much effort has been made to prevent their spread to humans by contaminated chicken products. Thus, a number of chemicals have been tested in an attempt to reduce contamination on broiler carcasses in the slaughterhouse, but with varying results. Lipids are known to have microbicidal activities against bacteria and 1-monoglyceride of capric acid, monocaprin (MC), is particularly active in killing Campylobacter. Thus, emulsions of MC in water have been found to reduce the number of viable Campylobacter on chicken carcasses in the laboratory.
OBJECTIVES
To test whether viable Campylobacter counts on contaminated chicken carcasses can be reduced in the slaughterhouse by dipping into MC emulsions.
METHOD/DESIGN
A 13 m long stainless steel trough was inserted in the processing line after evisceration and cutting of the neck skins and before spraying with ice-cold water for 2 h. It took about 1 min for a carcass to move from one end of the trough to the other. The trough was filled with 0.5% MC emulsion (20oC) from a 1000 liter tank, with a constant flow of emulsion through the trough. After cooling, each carcass was hand-massaged in a plastic bag with buffered peptone water (BPW), samples tested for Campylobacter and the viable number calculated per gm carcass.
RESULTS
A significant reduction ($P<0.05$) in mean Campylobacter counts was observed in monocaprin treated carcasses compared with controls.
CONCLUSIONS
This preliminary study shows that immersion in bactericidal monocaprin emulsion inserted in the processing line in a slaughterhouse may be a useful method to reduce Campylobacter contamination on chicken carcasses.

THE MICROBIOME GETS POPULAR

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OBJECTIVES
Analyses of the microbiota of Mother-and-Infant Pairs using omic-technologies and a population genetics approach.
METHOD
Fecal samples were obtained at different time points from thirteen healthy infants and their mothers. Metagenomic 16S sequences were obtained by 454 pyrosequencing and species-level datasets were defined according to the standard DSMZ reference types and using a 97% similarity cutoff value. Only those datasets including a minimum of 200 reads and a total length over 200bp were kept for further population-genetics analyses.
RESULTS
A total of ~200,000 reads were included in the analyses, which corresponded to 153 species-level datasets. The most abundant species included Faecalibacterium prausnitzii (11608 reads) and Bacteroides dorei (6794 reads). After excluding five Clostridium species, which showed a single haplotype in all samples, haplotype diversity values ranged from 0.29 to 0.98, with a mean value of 0.78 +/- 0.17. Tajima's D values ranged from -2.62 to 2.89, with a mean value of -0.27 +/- 1.45. Extreme values of the Tajima's D statistic, which are indicative of demographical or selective processes, were found mostly in Clostridium species (extreme negative values) and Enterobacter species (extreme positive values).
CONCLUSIONS
454 pyrosequencing allowed us to obtain species-level datasets, providing a more detailed picture of the human microbiota. In spite that at the genus level the most abundant are Bacteroides, Enterobacter and Clostridium, our results show that at the species level, Faecalibacterium prausnitzii is the most abundant. Recent medical research has suggested that low levels of F. prausnitzii may be associated with Crohn's Disease, and having a large F. prausnitzii population may be indicative of a healthy gut. Our results provide for the first time a global picture of genetic diversity levels within bacterial species forming the human microbiota and open the possibility for further research on population-level processes.

ANALYSIS OF THE EFFICIENCY OF ASSEMBLY AND ALTERNATIVE METHODS FOR VIRUS TAXONOMIC ASSIGNMENT IN THE ANALYSIS OF HUMAN INTESTINAL METAGENOMES.

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INTRODUCTION
The growing importance in understanding the impact of viruses in the human microbiome has led to several metagenomics studies focused on these pathogens. Due to the viral sequences high genetic variability, however, several studies have been unable to identify taxonomic or functional anotation when comparing the sequences to databases.
OBJECTIVES
To compare different assemblers and taxonomic assignment programs in evaluation of their performance in simulated viral sequences.
METHOD / DESIGN
Two 454 runs were simulated with taxonomical species frequencies taken from real data. The metagenomes were assembled using three different algorithms (Overlapping layout consensus, deBruin and generative probabilistic model) with different parameters. Different rates of assembly quality were used to evaluate the accuracy of the contigs.
The specificity and sensibility for the programs phymmbl, tBlastx and R script for K-mers frequencies were determined based on a data set of 200 454-simulated reads against the Viral Genome Database of the NCBI.
RESULTS
Phymmbl was the most sensitive and therefore the best candidate to be used.
The results show that assemblers works only for allocating functional information, since the number of chimeras in contigs prevents reliable taxonomic assignments. Genovo was the best assembler for low complexity metagenomes while Celera obtained the best results in highly complexity data.
CONCLUSIONS
Assemblies generate clusters of sequences of homologous proteins rather than the assembly of individual genomes. That is why it is preferable not to join the readings to give taxonomic assignments.
Although phymmbl is the most sensitive program, it has the disadvantage of giving a higher number of false positives. That is why it is necessary to combine the results of different methods to be certain of its annotations.

LONG SEQUENCE METAGENOMICS FROM HUMAN FAECES

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INTRODUCTION
Natural environments contain an incredible genetic diversity. Metagenomics aims to obtain genomic information directly from the environment of provenance without any previous knowledge about the organisms to whom it belongs. In this way, it provides a bird-eye overview about the genomic potentiality of a given sample, e.g. sea water, extreme environments, soil, organism-associated environment, etc. The collective genomes of our resident microbes represent a gene set which is estimated to be 150-fold greater than the human genetic complement.
OBJECTIVES
Modern high-throughput metagenomics is based on direct shotgun sequencing. However, it produces short reads, and coverage and assembly strictly depend on sequencing effort. Conversely, clone-libraries-based metagenomics performed in this study permits to isolate and store genome fragments allowing a-posteriori analysis.
METHOD / DESIGN
In this work we constructed a medium-large (up to 7 - 15 Kbp length) clone library from a faecal sample of a healthy volunteer. Clones have been previously end-sequenced by Sanger method. Plasmids from 364 clones have been extracted and pooled for sequencing by 454 technology. Obtained sequences were assembled and annotated.
RESULTS
We were able to perform an easy assembly of large fragments with an average coverage of 14X (N50 contig size: 8241bp). Annotation of 324 contigs revealed that most of the ORFs were connected to bacterial metabolism (418), followed by genetic information processing (114) and environmental information processing (113). The sequences contained also 43 domains of unknown function and proteins with already reported potential medical or industrial applications.
CONCLUSIONS
The used method permit to reduce enormously the sequencing efforts increasing the contigs length improving the annotation. Moreover, the method maintains the link between the living clones and contigs, allowing to return to the original clone for successive biochemical assays in case of spotting an interesting feature.

ECOLOGY OF ANTIBIOTIC RESISTANCE IN ENTEROBACTERIA OF HUMAN GUT

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INTRODUCTION
The infection of multi-resistant pathogens is a major medical issue. Many of the problems associated to antibiotic resistance depend on the spread among natural human intestinal populations of particular “high-risk” bacteria able to recruit genetic elements encoding antibiotic resistance, and possibly with high host-to-host transmission rates.
OBJECTIVES
To evaluate the effect of a unique source of antibiotic on the human gut microbiota and its relation with the acquisition of resistance.
METHOD / DESIGN
It is difficult to study the increase of of different resistance genes in the human gut microbiota due to the antibiotic therapy in human population that are subject to a high antibiotic pressure . We have a unique opportunity to avoid this problem by studying an Amerindian population, located in a small village in the Amazon rainforest (French Guyana) where the unique source of antibiotics is the dispensary of the hamlet.
We have analysed fecal samples from individuals that have acquired enterobacteria resistant to third generation cephalosporin. We studied, by applying metagenomic and metatranscriptomic approaches, the composition of intestinal active microbiota and the functional activities of the resistant enterobacterial community from eight subjects as well as their respective non-colonised controls.
RESULTS
Preliminary results points toward a lower diversity and evenness of the active microbiota as well as the different functional categories in individuals with antibiotic resistance.
CONCLUSIONS
From the results obtained, it seems that further studies are needed on the in vivo expression of microbial genes when the microorganisms are growing in their natural ecosystems.

CellENA®: A VERSATILE MICROENCAPSULATION TOOL FOR RAPID MICROBIAL DETECTION AND ANALYSIS.

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INTRODUCTION

Classical procedures for microbial detection and characterization involve colony isolation and can thereby be impractical when applied to organisms which require fast analysis of growth, or which are refractory to axenic cultivation when extracted from their natural environments.To overcome these drawbacks, new technologies based on single-cell encapsulation have been developed that facilitate isolation and analysis of microscopic colonies. In addition, these technologies can speed up assessment of antibiotic resistance/sensitivity in clinical isolates of infectious bacteria, and therefore accelerate decision making on patient treatment. In this work, we have successfully tested the Flow-Focusing encapsulation technology applied to a variety of organisms, and monitored cell viability and proliferation by flow cytometry.

OBJECTIVES

The main goal of this work was to develop applications for single cell-containing monodisperse gel microspheres with the mechanical, chemical and optical properties required for contained microcolony formation and detection.

METHOD / DESIGN

A CellENA® Flow Focusing microencapsulator was used to generate alginate gel microcapsules containing individual bacteria, yeast and human stem cells. Cell proliferation resulting in microcolony formation was monitored by flow cytometry.

RESULTS

Size and shape monodisperse alginate microcapsules, containing individual cells, were reproducibly obtained ranging from less than 100 µm to over 600 µm, by selecting the appropriate nozzle. Microcapsule morphology and optical transparency allowed efficient monitoring of microcolony formation.

CONCLUSIONS

Results show that the CellENA® microencapsulation technology could represent a robust tool for basic and applied research or monitoring in different fields of life science, including environmental microbiology and clinical diagnosis.

ISOLATION AND IDENTIFICATION OF HUMAN PATHOGENIC AEROBIC ACTINOMYCETES AND FUNGI FROM LAKE VISTONIDA IN GREECE

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INTRODUCTION
Actinomycetes and fungi are typically known as inhabitants of soils. Investigations of aquatic microbial communities during recent years shown that members of the actinomycetes and fungi occur in fresh water habitants.
OBJECTIVES
The aim of this study was to identify the prevalence and distribution of human pathogenic aerobic actinomycetes and fungi in the water from different parts of the Lake Bistonida in Greece.
METHOD / DESIGN
14 water samples were collected from different and geographically significative areas of the lake. The study material was obtained in the period of winter. The specimens were collected over a period of one month, from the bottom of the lake, were numbered and put in a sterilized plastic tube and transferred to the Laboratory. Each water sample was examined by two different techniques: a) the cultured methods and b) the mouse procedure, to detect the possible spectrum of pathogenic actinomycetes and fungi.
RESULTS
20 species had been isolated belonging to 12 genera.
The isolated microorganisms belonging to the genus: Nocardia, Streptomyces, Micromonospora, Candida, Cryptococcus, zygomycetes, Acremonium , Fusarium, Microsporum , Chrysosporium, Rhodotorula, Tricosporum . Chrysosporium, Candida and Streptomyces have been the genera most represented.
CONCLUSIONS
The successful isolation of these important etiologic agents of mycoses in man, encourages for further investigations in the lakes of Greece, because of climate conditions and geographical position, could represent an ideal reservoir for other significant pathogenic fungi and aerobic actinomycetes. The recovery of nocardia in the lakes of Greece, even in this small proportion, acquires importance, because potentially it is able to explain individual cases of human nocardiosis, that are observed at certain times in our country.

LACTOBACILLUS PLANTARUM STRAINS REGULATE ACTIVATION OF INNATE IMMUNITY BY CLOSTRIDIUM DIFFICILE IN T84 CELLS

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OBJECTIVE
To evaluate the effects of C. difficile on the activation of innate immunity and the role of Lactobacillus plantarum strains in the regulation of this immune response in T82 cells.
METODS
Four C. difficile strains belonging different toxinotype were studied (HM1:TcdA+/TcdB+/CDT-; HM2:TcdA-/TcdB+/CDT-; HM3:TcdA-/TcdB-/CDT- and HM4:TcdA+/TcdB+/CDT+) by their ability to induce: (i) production of innate immunity cytokines (TNF-ALPHA, IL-1β, IL-6 and IL8), (ii) chemotaxis, and (ii) Toll-like receptor (TLR) gene expression, during T84 infection. The ability of L. plantarum CECT 7315 and L. plantarum CECT 7316 to regulate the immune responses triggered by C. difficile on T84 cells was also investigated.
RESULTS
Cells of C. difficile strains and their supernatants (Bact/SN) induced IL-8 production (pg/ml) at levels of 521.8±3.3 (strain HM1); 501.2±2.4 (strain HM2); 215.5±1.2 (strain HM3) and 150.2±5.8 (strain HM4) on T84 cell cultures at an infection rate of 10 (nº bacteria per cell), which increased as the infective dose increased. Only the strain HM1 at an infection rate of 100 exerted cytotoxic effects on T84 cells. C. difficile strains were able to increase expression of TLR4 and TLR9 but not that of TLR5. Pre-incubation of T84 cells with L. plantarum CECT 7316 or the combination of L. plantarum CECT 7315 plus L. plantarum CECT 7316 decreased IL-8 production by the infected cells. The toxigenic strain C. difficile HM1 induced a higher chemotactic effect on neutrophils, monocytes and lymphocytes than the non-toxigenic strain C. difficile HM3. High neutrophil chemotaxis correlated with high IL-8 production. L. plantarum CECT 7316 decreased chemotactic effects induced by C. difficile HM1.
CONCLUSIONS
C difficile activate innate immunity, inducing pro-inflammatory cytokine production, TLR expression and chemotaxis of immune cells. L. plantarum CECT 7316 attenuates C. difficile immune activation by decreasing IL-8 production and chemotaxis.

GUT MICROBIOTA AND INFLAMMATORY MARKERS IN ADOLESCENTS WITH DYSLIPIDEMIA

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INTRODUCTION
Dyslipidemia triggers an inflammatory process and constitutes a risk factor for cardiovascular disease (CVD). Observational studies have reported associations between the intestinal microbiota and obesity, but the specific relationship of the microbiota with lipid metabolism is unknown.
OBJECTIVE
To determine the relationships between dyslipidemia, inflammatory markers and gut microbiota structure in normal weight adolescents.
METHODS/DESIGN
Subjects included in the study (n=44; aged 9-15 years) were normal-weight according to the BMI z-score and classified as dyslipidemic (n=23) or not (n=21) according to serum triglycerides and cholesterol levels. The intestinal microbiota composition was determined in stool samples by quantitative PCR and pyrosequencing of 16S rRNA gene amplicons using a 454 Life Sciences Genome Sequencer FLX (Roche). To evaluate the immune properties of the microbiota, stool samples were used to stimulate peripheral blood mononuclear cell (PBMC) cultures obtained from healthy human subjects. Cytokines (TNF-ALPHA, IL-6 and IL-10) were quantified in supernatants of PBMC cultures and in the serum of participants by ELISA.
RESULTS
Bacteroides spp., Lactobacillus group and Clostridium coccoides group numbers were decreased in the dyslipidemic group in comparison with the control group (P= 0.037, P<0.010, P<0.010, respectively), while C. leptum group numbers were increased (P<0.010). The dyslipidemic group had also lower proportions of the genus Eubacterium (P=0.040) as determined by 16S rDNA sequencing. The dyslipidemic group had higher serum levels of TNF-ALPHA than the control group (1.17±0.41 vs. 0.48±0.21). The stool samples of the dyslipidemic group induced higher production of TNF-ALPHA (218.4 ± 45 vs. 91± 6.5) and IL-6 (63.8±15.3 vs. 30.3±5.0) by PBMCs in comparison with those of controls.
CONCLUSIONS
Dyslipidemia was associated with changes in the composition of the intestinal microbiota and its inflammatory properties, which could be related to parallel increases in the serum inflammatory cytokine TNF-ALPHA.

THE INHIBITORY ACTIVITY OF ORGANIC ACIDS PRODUCED BY PROBIOTIC CULTURE FILTRATE ON PSEUDOMONAS AERUGINOSA AND STAPHYLOCOCCUS AUREUS VIRULENCE FACTORS EXPRESSION

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INTRODUCTION
Probiotic culture filtrates (PCF) contain soluble molecules with inhibitory activity on the Quorum sensing (QS) genes expression level. The identification of these molecules is a compulsory step for their use in the development of new antipathogenic strategies.
OBJECTIVES
The study aimed to identify and quantify the antimicrobial compounds from Lactobacillus paracasei subsp. paracasei culture filtrates, with inhibitory activity on the expression of virulence factors in Pseudomonas aeruginosa and Staphylococcus aureus multidrug resistant strains.
METHODS
A capillary electrophoresis – diode array detection (CE-DAD) was used for the identification and quantification of the PCF small-molecules. We used various concentration levels of analytical standards of the identified organic acids (i.e. acetic, lactic, gamma-aminobutyric acid) for testing their biological activity against the investigated pathogenic strains. Serial broth microdilution method was used for the minimal inhibitory concentration (MIC) assay of each organic acid. The phenotypic study of the influence of sub-inhibitory concentrations of lactic and acetic acid on the bacterial strains enzymatic virulence factors expression (i.e. lechitinase, lipase, amylase, caseinase, DN-ase, hemolysins) was investigated by specific enzymatic assays.
RESULTS
The CE-DAD assay of the PCF showed that acetic acid was present in the largest amount, i.e. 42.3 mg/ml, being followed by the lactic acid, 34.2 mg/ml. The MIC was 5,287 mg/ml for acetic acid, 4,275 mg/ml for lactic acid respectively. The acetic acid showed the highest inhibitory activity on the expression of enzymatic virulence factors of both S. aureus and P. aeruginosa strains, as well as the combination of the studied acids in the PCF ratio.
CONCLUSION
The CE-DAD method enabled sensitive and reproducible analysis of organic acids present in the PCF. The acetic and lactic acid showed an antipathogenic effect at phenotypical level, representing an interesting way for the attenuation of the virulence features in two of the most important opportunistic pathogens.

CHANGES IN GUT MICROBIOTA DUE TO SUPPLEMENTED FATTY ACIDS IN DIET-INDUCED OBESE MICE

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INTRODUCTION
Consumption of a high-fat diet (HFD), which is associated with chronic "low-grade" systemic inflammation, alters the gut microbiota (GM), pointing to a possible role in the development of obesity.
OBJECTIVES
The aim of the present study was to investigate the ability of an oleic acid derived compound (S1) and a combination of omega-3 fatty acids (S2) to modulate both the body weight and the GM in HFD-induced obese mice.
METHOD/DESIGN
Female ICR outbred mice were fed either a control diet (CD) or a HFD, non-supplemented or supplemented with S1/S2. At week 19, faeces were collected in order to analyse the GM. Since the majority of the GM cannot be cultured by conventional techniques, molecular approaches were applied. Group-specific primers for accurate quantification of several major bacterial groups from faecal samples were assayed using quantitative PCR.
RESULTS
The HFD induced an increase of body weight, which was lost by supplementation with S1. Furthermore, S1 supplementation markedly increased total bacterial density and restored the proportions of bacteria that were increased (i.e. Clostridial Cluster XIVa and Enterobacteriales) or decreased (i.e. Bifidobacterium spp.) during HFD-feeding. S2 supplementation significantly increased the quantities of Firmicutes (specially the group of Lactobacillus). Correlation analysis revealed that body weight correlated positively with the phylum Firmicutes and the Clostridial Cluster XIVa, and negatively with the phylum Bacteroidetes.
CONCLUSIONS
Consumption of a HFD induced changes in the faecal microbiota, which were associated with the appearance of an obese phenotype. Supplementation of the HFD with S1 counteracted HFD-induced gut dysbiosis, together with an improvement of body weight. These data support a role for certain fatty acids as interesting nutrients related to obesity prevention.