MODULATION OF GUT MICROBIOTA AFTER SUPPLEMENTATION WITH CITRUS FRUIT EXTRACT USING THE TIM-2 IN VITRO MODEL OF THE HUMAN COLON

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OBJECTIVE: increasing intake of polyphenols could be a useful strategy to support and enhance gut health. Citrus flavonoids such as hesperidin and naringin have previously shown beneficial effects on barrier function and intestinal inflammation. The aim of this study was to assess effects of a natural citrus fruit extract (CFE) rich in hesperidin on gut microbiota composition and activity using the *in vitro* TIM-2 model of the human colon.
METHODS: the TIM-2 units were inoculated with human fecal samples and supplemented with 250 mg CFE, 350 mg CFE, or control for 72 hours. Samples were collected at baseline, after 24 h, 48 h, and 72 h. Gut microbiota composition and activity, and short-chain fatty acid (SCFA) production were determined using 16s rRNA gene sequencing and chromatography, respectively.
RESULTS: on the genus level, a dose-dependent significantly increased abundance of *Roseburia* (q = 0.134) was observed after CFE supplementation. A similar trend was observed for *B. eggerthii* (q = 0.184) and *E.ramulus* (q = 0.134), although not significant. Moreover, the relative abundances of *Enterococcus* (q = 0.134) and *L. mucosae* (q = 0.198) were significantly increased after CFE supplementation. Cumulative production of total SCFAs was higher after CFE supplementation compared to control, which was reflected by increased production of acetate.
CONCLUSIONS: in conclusion, CFE supplementation increased abundance of microbes and SCFAs known for anti-inflammatory and anti-obesity effects. These results highlight the potential for supplementation with CFE as an enhancer for gut health.

BIOCHEMICAL AND ANTIBIOTIC INFLUENCE OF GASTROINTESTINAL TRACTS MICROFLORA IN NEONATAL

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OBJECTIVE: colonisation of the neonatal intestinal tract by microbiota may occur as early as the foetal stage, and this colonisation preforms the intestinal microbiota, which is reflected in the intestinal activity and neonate vitality. This study aimed to isolate and identify common bacteria in 19 preterm neonates spending their first weeks of life in the neonatal intensive care unit.
METHODS: first, stool samples were collected, and bacteria were isolated and purified from those samples.
Common bacterial species were investigated regarding their susceptibility or resistance to antibiotics. From 19 stool samples, 15 contained three species in common: *Enterobacter cloacae*, *Enterococcus faecalis* and *Klebsiella pneumoniae*.
RESULTS: differences in the microbial formation and density were correlated with the type of delivery and feeding as well as the administration or no administration of antibiotics to the preterm neonate. Antibiotic susceptibility testing was undertaken, and minimum inhibitory concentrations were determined. The results showed that the minimum level of isolates was affected by several of the most commonly used antibiotics from the following families: aminoglycosides, carbapenems, fluroquinolones, glycylcyclines and polymyxins.
CONCLUSIONS: in the present study, we identified the most common bacterial species present in the intestinal microflora of premature infants during their first days after birth. *Enterobacter cloacae* and *Klebsiella pneumoniae* were the most common gram-negative bacteria, while *Enterococcus faecalis* was the most prevalent gram-positive bacterium. Our microbial susceptibility testing showed that these isolates were sensitive to several of the most commonly used antibiotics from the following families: aminoglycosides, carbapenems, fluroquinolones, glycylcyclines and polymyxins. The analysis revealed a significant level of sensitive towards most tested antibiotics among certain strains isolated from neonates, which raises concern.

AKKERMANSIA MUCINIPHILA ANTIMICROBIAL SUSCEPTIBILITY PROFILE

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OBJECTIVE: this study aims to characterize the antimicrobial susceptibility profile of Akkermansia muciniphila DSM 22959, a human commensal and next-generation probiotic candidate, using phenotypic and in silico analyses.
METHODS: phenotypic antibiotic susceptibility assessment: A. muciniphila DSM 22959 was grown in PYGM medium and subcultured at least twice before use. Minimum inhibitory concentration was determined for 8 clinically relevant antimicrobials, as recommended by EFSA-FEEDAP, using broth microdilution and E-test® methods. Both assays were performed at least with three independent replicates and with technical duplicates.
In silico analysis: Antimicrobial resistance genes (ARG), virulence factors (VF), genomic islands (GI) and mobile genetic elements (MGE) were predicted in A. muciniphila DSM 22959 whole genome (accession number: NZ_CP042830.1) using several available databases and bioinformatics tools.
RESULTS: phenotypically, A. muciniphila DSM 22959 shows susceptibility to ampicillin, tetracycline, colistin and fosfomycin and is resistant to gentamycin, kanamycin, streptomycin (aminoglycosides) and ciprofloxacin. Akkermansia muciniphila contains 26 annotated ARG that support the observed resistance profile. Other ARG might not be expressed under the tested conditions. Most ARG and VF are not embedded within GI or MGE. No plasmids were reported for this strain.
CONCLUSIONS: the same susceptibility categorization was obtained in both phenotypic methods. The phenotypic resistance profile is supported by the genomic context. However, there is

no evidence of horizontal acquisition or potential transferability of the identified ARG and VF. Thus, the antimicrobial susceptibility profile of the probiotic candidate A. muciniphila DSM 22959 meets the safety criteria required to be considered for human consumption.

TRANSCRIPTOME AND MITOCHONDRIAL ANALYSIS OF ASD CHILDREN COMPARED TO HEALTHY CONTROLS

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OBJECTIVE: Autism Spectrum Disorder (ASD), like many modern society pathologic conditions, is an epigenetically initiated disease. The purpose of this study was to determine the differences in the oral microbiome and mitochondrial health between children from a "blue zone" in Colombia, healthy and A.S.D. children in the U.S.A.
METHODS: the A.S.D. section included 30 children and young adults, ages 6 to 21, who were sampled at three different intervals. The sampling consisted of buccal swabs for Mitoswab testing, and saliva for full transcriptomics, in order to determine the entire virus, bacteria, archaea, and fungus range of species. Dietary and health information was obtained, as were consents and assents per I.R.B. requirements. The Colombia component included 30 children, ages 6-16, who were healthy and within normal behavior standards. Buccal swabbing and salivary sampling was performed only once with this group, as with the typical healthy USA controls. The USA Healthy Control group consisted of children 6-16 who had no history of any medications.
RESULTS: significant differences between each subject and intervention group were demonstrated by the Richness and Shannon Diversity plots. The USA healthy children group had a greater Richness than the other two groups but the Shannon diversity was not significantly different.
CONCLUSIONS: the microbiomes of individuals diagnosed with A.S.D. is significantly different from healthy children in a developing country and healthy children from the USA. Microbiome shifts may have strong epigenetic consequences that may be involved in ASD development.

EVALUATION OF NOMADIC AND NICHE-SPECIALIST LACTOBACILLI AS POTENTIAL VAGINAL PROBIOTICS

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OBJECTIVE: the aim of this study is the development of a multi strain probiotic gel to promote lactobacilli-dominated vaginal microbiota in pregnant women and to establish a proper eubiosis on the new-born. Mainly nomadic lactobacilli, isolated from food sources, were screened for functional characteristics and the capability to inhibit *Streptococcus agalactiae, Staphylococcus aureus* and *Candida albicans*, which may lead to adverse pregnancy-related outcomes.
METHODS: one hundred fourteen strains were screened for hydrophobicity, auto-aggregation, peptide hydrolysis, hydrogen peroxide production, and lactic acid isomers quantification. Cell-free supernatants (CFSs) of the candidate strains were co-inoculated with vaginal pathogens for high-throughput inhibition screening. Aiming to evaluate the reduction of the expression of genes involved in the inflammatory cascade the best performing strains were investigated *in vitro* alone and in combination.
RESULTS: fifteen *Lactiplantibacillus plantarum* strains showed outstanding hydrophobicity traits. The auto-aggregation capacity was specie-independent, while the peptide concentration distribution was quite similar among lactobacilli. The production of hydrogen peroxide was strain dependent, with the highest concentrations found for *Lacticaseibacillus paracasei*. *Lb. plantarum* produced both isomers of lactic acid, while *Lb. paracasei* produced only L-isomer. *S. aureus* and *S. agalactiae* were strongly inhibited by a wide range of CFS in different modes of action, whereas *C. albicans* inhibition was less frequent.
CONCLUSIONS: overall, *L. plantarum* had the highest pathogen inhibition score and the best functional traits. Two of the best performing strains showed a reduction on the expression of genes involved in the inflammatory cascade in human keranocytes.

INVESTIGASTIONS OF THE POTENTIAL MECHANISM OF ACTION OF A MULTI-STRAIN PROBIOTIC COMPOSITION AGAINST URO-GENITAL PATHOGENS BY *EX-VIVO* STUDIES

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OBJECTIVE: the urogenital microbiota is dominated by lactobacilli able to counteract pathogens growth. Vaginal infections occur when the urogenital microbiota is unbalanced. The aim of the study was to evaluate the efficacy of SynBalance® Femme, a product containing *L. plantarum* PBS067, *B. animalis* subsp. *lactis* BL050 and *L. rhamnosus* LRH020, to inhibit the adhesion and the growth of pathogens involved in uro-vaginal infections.
METHODS: the antimicrobial and preventive effects of the three probiotic strains and their combination SynBalance® Femme, have been evaluated on a reconstructed bladder epithelium (HBE), infected with *E. coli* and on a reconstructed vaginal human epithelium (VHE, A431 modified) infected with *C. glabrata and C. albicans*, *G. vaginalis*, *N. gonorrheae* and *T. vaginalis*, respectively. In addition, the effects on the viability and the integrity of reconstructed tissues after TEER treatment were also assessed.
RESULTS: a strong antimicrobial activity was observed for *B. lactis* BL050, *L. plantarum* PBS067 and *L. rhamnosus* LRH020, on HBE previously colonized by *E. coli*. For *L. rhamnosus* LRH020 a preventive activity was also observed by SEM analysis. TEER results