Conclusion: The IVERPP strategy is novel and powerful approach to study endosomal trafficking events including bacterial protein toxin translocation events.

Keywords: Endosome, Toxin Translocation

P09.18 Revealing the Response of the Active Mucosal Microbiota from the Rat Colon to a Change in Diet

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Introduction and Objectives: The intestinal microbiota is a densely inhabited microbial community that provides many functions for the host including the degrading of non-digestible nutrients into useful metabolites, the synthesis of vitamins and the regulation of the immune system. The microbiota is known to evolve over the life time of the host and to respond to different environmental influences. The focus of the study was to observe the response of the mucosal microbiota from a high-fat diet rat model as well as from a chow diet fed diet rat model to a change in diet. **Methods:** To analyse the response of the gut microbiota to a switch in diet 16S rRNA gene pyrosequencing and LC-MS/MS metaproteomic analysis hyphenated with protein-based stable isotope probing (protein-SIP, ^{15}N-fully labelled diet) was performed. **Results and Discussion:** As a result, we were able to decipher the mucosal colon microbiota community structure in regard to taxonomy, enzymatic functionalities and active taxa related to nitrogen utilisation from the feed over a three day period. Microbial active taxa in regard to nitrogen utilisation belonged to the abundant phyla like Firmicutes, Proteobacteria and Bacteroidetes as well as those from low abundant phyla like Spirochaetes, Deinococcus-Thermi and Planctomycetes. In addition, we observed rapid changes in the community composition including a decline of Enterobacteriaceae and Streptococcaceae. Identified proteins were assigned to functional categories of which replication, transcription, signal transduction as well as carbohydrate and amino acid metabolism were overrepresented. **Conclusion:** The integrated data analysis opens the path to understand the complex gut microbiota in more detail using protein-SIP to identify the active taxa for specific substrate utilisation.

Keywords: Protein-SIP, metaproteomics, Gut microbiota

P09.19 Label Free Study for Control of Listeria Monocytogenes to Enhance Food Safety

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Introduction and Objectives: Listeriosis is a disease caused by Listeria monocytogenes. it is an ubiquitous foodborne pathogen extremely hazardous for human population that usually affects high risk patients such as the elderly, immunosuppressed patients and pregnant women. However, it can also affect people who do not have these risk factors. For these reasons it is mandatory to counteract the listeria growth in food, avoiding the use of antibiotics, lantibiotics or chemical compounds. the strategy used is based on the selection of specific strains of starter bacteria (Lactococcus lactis) able to counteract listeria growth. In order to highlight these mechanisms of bacterial competition, the secretome of these two microrganisms in co-culture (BHI first, milk for validation second) has been studied through a proteomic and peptidomic approach. **Methods:** The bacterial secretome of the lactococcus strain with a strong activity in the inhibition of listeria growth has been analysed through 2D electrophoresis, shotgun analysis (UPLC-MS system, Waters) and top-down peptidomics (LTQ-Orbitrap). Data have been validated through MRM analysis (Bruker HCT PLUS, skyline software) both in vitro and in milk in order to resume cheese-making conditions. **Results and Discussion:** Obtained data highlighted, during competition, the higher production by listeria of the moonlighting protein enolase, of Septation ring formation regulator EzrA, involved into cell replication and the lower secretion of Endopeptidase P60. In parallel, during competition, L. lactis produced higher amounts of Secreted 45 kDa protein and switched from lantibiotic Nisin A production to Nisin Z production. **Conclusion:** In competition with listeria, L. lactis, produced higher amounts of Secreted 45 kDa protein with peptidoglycan lytic activity and NisinZ, instead than NisinA, in order to enhance lantibiotic solubility in less acidic environment. The demonstrated properties of this L. lactis strain, using these three complementary proteomics approaches, may help in the additives-free listeriosis prevention. Work supported by Ministry of Health-CCM Milano EXPO 2015 Project

Keyword: Listeria monocytogenes, food safety, bacterial competition, proteopeptidomics

P09.20 Protein Signature during Biofilm Formation in Staphylococcus Aureus Food Isolates

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Introduction and Objectives: Human handling of food products as well as infection/colonization of livestock or farm workers have been described as mechanisms for the contamination of food with S.aureus.Moreover, S. aureus is able to produce biofilm. Low rates of antibiotic transport within biofilms, protective effects of the biofilm matrix, and low rates of metabolic activity within the biofilm interior have all been found to contribute to the persistence of these pathogen. The mechanism and/or process of biofilm formation in S. aureus is poorly understood and the studies on the expression profiles of genes involved in biofilm mechanism are still limited in number. Anyway, S.aureus biofilm on food contact surfaces poses a serious risk of food contamination. The aim of this work is to analyze proteomics of different strain of this bacteria isolated from food with different capacity to produce biofilm in order to elucidate mechanism of biofilm formation. **Methods:** The experiment was conducted with S.aureus strains such as MRSA and MSSA isolated from (food)environments . S.aureus ATCC 35556 (a known strong biofilm producer), S.aureus ATCC 29213 (a known weak biofilm producer) has been used as references strains. S.aureus strains showing ability to produce biofilms will be classified as weak, moderate or strong . Two