METAGENOMIC AND METAPROTEOMIC TAXONOMIC ANALYSES OF FECAL SAMPLE FROM HEALTHY INDIVIDUAL

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Introduction

The human gastrointestinal tract is colonized by a diverse microbial community called microbiota. Gastrointestinal microbiota has been a focus of numerous studies as it plays an important role for the host - involving the training of its immune system, helping in the fight against pathogenic microorganisms and in metabolic processes. Gene composition of the microbiota was identified using metagenomics, but functional analysis and information on produced proteins of the microbial community were obtained by metaproteomics. This paper compares the influence of protein extraction methods and the influence of different mass spectrometry instruments on the taxonomic composition of the intestinal microbiota. The results were compared with genomic results obtained by 16S rRNA gene sequencing.

Materials and methods

Metaproteomic analysis of gut microbiota sample was conducted on two mass spectrometry instruments. The first analysis was done using high resolution mass spectrometer ESI-Q-TOF SYNAPT G2-Si (Waters, Milford, MA, USA) and the latter one was carried out on 4800 Plus MALDI-TOF/TOF mass spectrometer (Applied Biosystems Inc., Foster City, USA). For analysis of data obtained by ESI-Q-TOF Synapt G2-Si newly developed a method by Section for bioinformatics of Faculty of Food Technology and Biotechnology, based on Latent Semantic Indexing (LSI) developed was used. The data obtained by MALDI-TOF/TOF was analyzed using other tool developed by Section for bioinformatics – ProteinReader.

A fecal sample was collected from a healthy donor and stored at -20 °C until analysis. The homogenized sample was used for proteomic analysis as shown in Fig. 1. Genomic DNA was extracted using the QIAamp PowerFecal DNA Kit. The pair-end sequencing of a DNA on the Illumina MiSeq device was performed as an external service at Molecular Research Lab, Texas, USA. Reads were merged using PandaSeq program, with a targeted length between 400 and 500 base pairs. Merged reads were imported into QIIME program to assign taxonomy to each read and exported as *biom* table containing information about sequences, their taxonomic assignments and abundances. The obtained *biom* table was used as a reference taxonomy of the sample, later to be used for comparison with metaproteomic methods.

Ethics approval:
The study was approved by the Children's Hospital Srebrnjak and PBF research ethics committee.

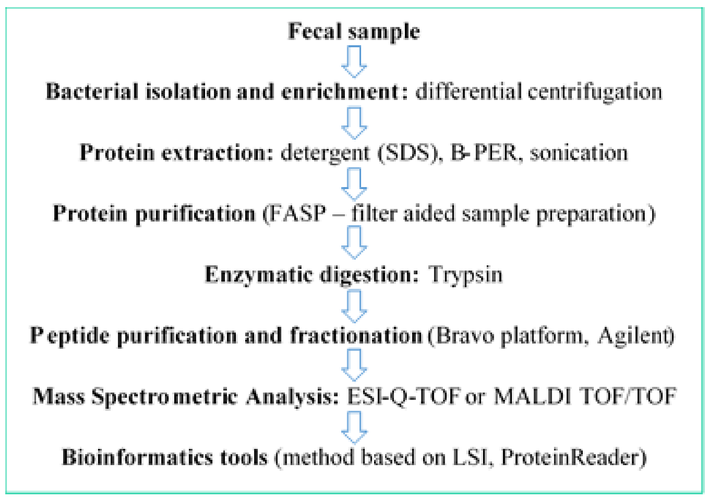

Fig. 1: Schematic overview of the experimental steps.

Results

Final results representing taxonomy assignments of obtained data using a metagenomic and two metaproteomic methods were shown using pie charts representation produced with Krona program.

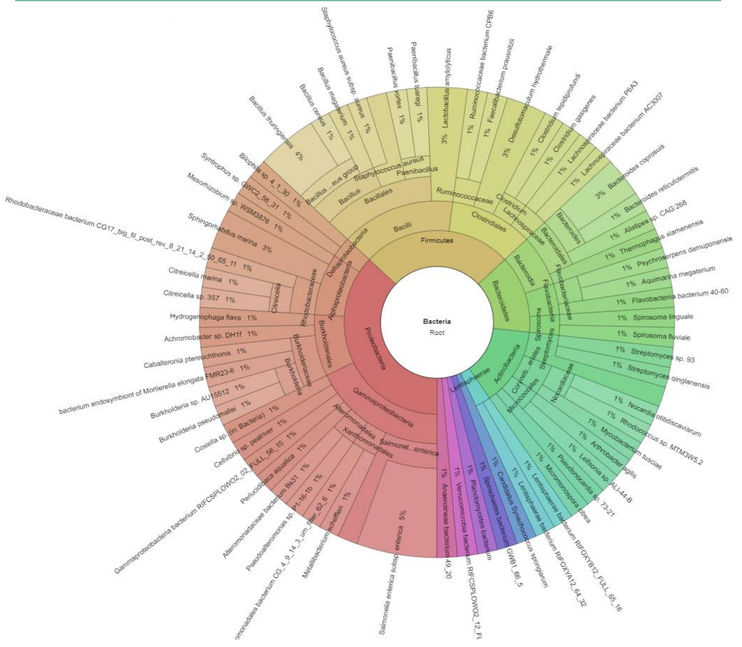

Fig. 2: Taxonomic representation of microorganisms in a sample D7. The preparation included B-PER, sonication and 30 kDa filters. The analysis was performed on the ESI-Q-TOF mass spectrometer and the LSI based bioinformatics tool developed on PBF.

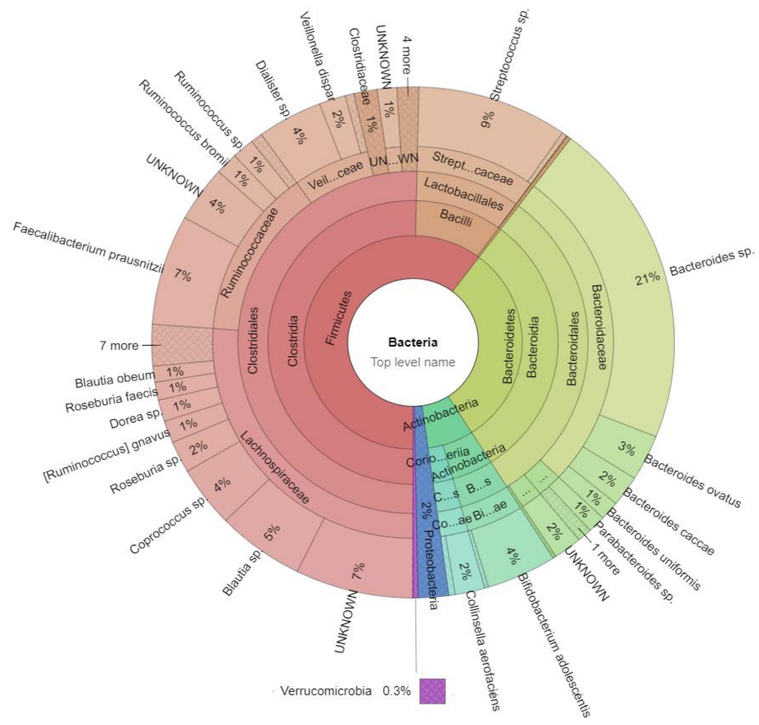

Fig. 3: Taxonomic composition of the sample determined with 16S rRNA gene sequencing

Discussion

Sample preparation is a critical step in successful protein analysis. Due to differences in bacterial cell wall structure, sonication or other mechanical disruption method and lysis buffer are necessary for effective analysis (Tanca et al., 2014). The largest number of bacterial species (73) was identified from analysis D7 on ESI-Q-TOF mass spectrometer. During the preparation of the sample, commercial detergent B-PER, 30 kDa cut-off filters and sonication were used in protein extraction. The most abundant bacterial phylum in the sample were *Proteobacteria*, *Firmicutes* and *Bacteroidetes*. Samples in which sonication or other mechanical disruption was not used resulted in significantly fewer bacterial species, only 10 species were determined by analysis on the MALDI-TOF/TOF mass spectrometer. These results show a high probability of human protein presence and of bacteria *Faecalibacterium prausnitzii*. This bacterium is abundant in gut microbiota along with genus *Ruminococcus* and has a high protein expression (Kolmedar et al., 2012). 16S rRNA sequencing results show the highest representation of the *Firmicutes* and *Bacteroidetes*, while the phylum *Proteobacteria* in comparison to the metaproteom is found in a significantly smaller number.

Conclusions

1.) Of all tested methods, 4% SDS, B-PER and sonication during protein isolation from bacterial biomass in feces resulted in the greatest number of identified peptides and bacterial species.

2.) The bioinformatics tool developed at the Faculty of Food Technology and Biotechnology coupled with the ESP-Q-TOF Synapt G2-Si mass spectrometer identified more peptides and more bacterial species than the MALDI-TOF/TOF mass spectrometer.

3.) The most abundant phylum in the sample is *Firmicutes*. The most proteins from the phylum *Firmicutes* were identified from the species *Faecalibacterium prausnitzii* and the genus *Ruminococcus*.

4.) Metagenomic results mostly match proteomic results for phylum *Firmicutes*, *Bacteroidetes* and *Actinobacteria*, while phylum *Proteobacteria* differ significantly.

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Literature:

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