Title: Optimizing bacterial protein extraction from coronary artery samples for in-depth proteome analysis

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Abstract

We have previously detected DNA of oral bacteria in coronary artery samples. To study the phenomenon, we conducted a pilot study to optimize the protein extraction method and proteomics data-analysis.

We extracted proteins using four methods from homogenized right coronary artery (RCA) samples collected from 10 people post-mortem. The methods were described in 1) Hendy et al. 2018, 2) Palmer et al. 2021, 3) Herrington et al. 2018, and 4) Nehme et al. 2019. The samples were then processed using protein aggregation capture (PAC) -based sample preparation before LC-MS/MS analysis in data-independent acquisition (DIA)-mode.

Raw data files were submitted to DIA-NN for protein identification. The bacterial genuses were chosen based on 16S-data and they were: Fusobacterium, Streptococcus, Veillonella, Prevotella, Lacticaseibacillus, Lactobacillus, Limolactobacillus and Clostrium. Human proteome database was included in the searches.

We identified altogether 95 bacterial proteins and 4 243 human proteins. Methods 3 and 4 yielded the largest number of proteins identifying 64 and 63 proteins respectively. Bacterial proteins encompassed about 0.5% of all detected peptides. Fusobacterium proteins contributed to most identified bacterial proteins (70%). Methods 1 and 2 were unsuitable due to low yield which made protein quantification impossible.

Methods 3 and 4 seemed efficient methods and we identified numerous bacterial proteins.