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Ultra-sensitive metaproteomics (uMetaP) redefines the “dark” metaproteome, enables single-bacterium resolution, and discovers hidden functions in the gut microbiome

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Introduction

The interplay between members of microbial communities (microbiomes) is crucial for planetary health. While genomic methods have greatly expanded the taxonomic characterization of microbiomes, complementary techniques for directly quantifying biological functions are needed for deeper understanding. Metaproteomics (MetaP), analyzing microbial samples with liquid chromatography coupled mass spectrometry (LC-MS)-based proteomics, has emerged as a powerful tool for investigating host-microbiome interactions. It offers a direct view of functional changes within hundreds of microbes and their host. However, compared to single-organism LC-MS, MetaP faces significant hurdles. Microbial samples are immensely complex, harboring an estimated 100 million unique peptide species with a vast dynamic range. Current MetaP methods miss more than 60% of the species detectable by commonly used genomic-based methods (e.g., full-length 16S rRNA), limiting functional characterization. **We present uMetaP – a novel solution offering a significant leap in sensitivity, depth, throughput, and accuracy.**

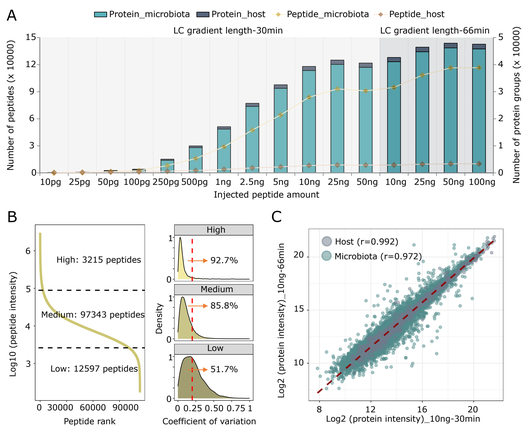

Figure 1. Proteotyping characterization of uMetaP powered by DIA-PASEF. A) Peptide and protein identifications obtained with uMetaP along a dilution series of a mouse fecal peptide sample and different LC gradient lengths (average of injection triplicates). Error bars show the standard deviations. B) Intensity rank of peptides identified using 10 ng in a 30-min gradient (left) and the coefficient of variations (CV) across different abundance regions (right) delimited by the dotted lines in the intensity rank graph. The red-dotted lines indicate CV = 0.2. C) Quantitative reliability by calculating the correlations of identified protein groups (host and microbiota) using 10 ng with two different LC gradients.

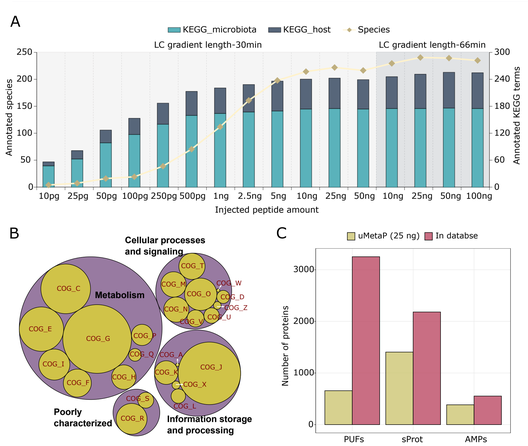

Figure 2. Taxonomic and functional profiling with uMetaP powered by DIA-PASEF. A) Taxonomic and functional coverage with varying peptide quantities and LC gradient lengths (average of injection triplicates). B) Representation of 24 COG functional categories annotated for microbial proteins identified using 25 ng with a 30-min gradient. C) Amount of PUFs, sProt, and AMPs experimentally detected using 25 ng with a 30-min gradient and present in the microbial protein database.

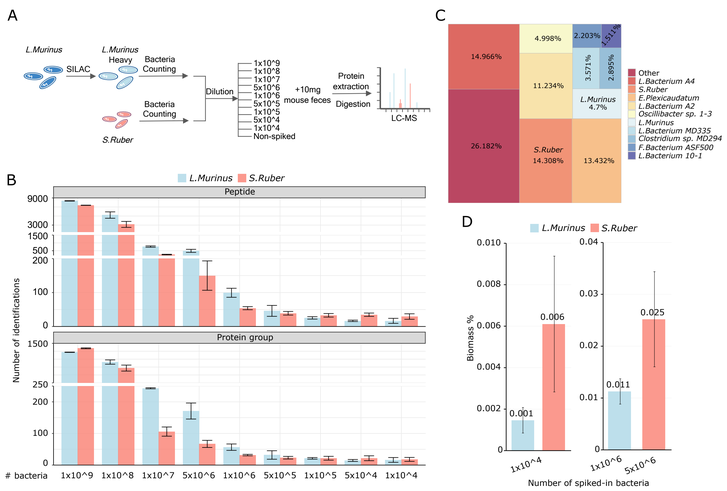

Figure 3. Unveiling the real limits of detection and quantification in the “dark” metaproteome. A) Experimental workflow of uMetaP analysis with stable isotope labelling (SILAC) of *L. Murinus* and *S. Ruber* to assess the sensitivity limits in the “dark” metaproteome. Spike-in triplicates were prepared for each spike-in bacterial amount. B) Identification summary of identified peptides and protein groups cross var ying numbers of spiked-in *L. Murinus* and *S. Ruber*. C) Example of relative biomass abundance assessment by uMetaP with 1 x 10^8 spiked-in bacteria. D) Calculation of relative biomass abundance using identified species-specific peptides considering LLoD (1 x 10^4 bacteria; left) and LLoQ (1 x 10^6 bacteria for L.Murinus and 5 x 10^6 bacteria for S.Ruber; right).

Conclusions

- ☐ The impressive performance of uMetaP establishes a new state-of-the-art in microbiome proteotyping, leading to unprecedented taxonomic profiling (similar to full-length 16S rRNA) and shedding light into untapped functional information.
- ☐ The use of a SILAC-labelled bacterium and an exogenous bacterium establishes the first reliable LLoD (10000 bacteria in 10mg feces) and LLoQ (1 million and 5 million bacteria in 10mg feces background) in a complex metaproteome.
- ☐ The first assessment of detection, quantitative accuracy, and precision at the single-bacterium resolution (500 fg).

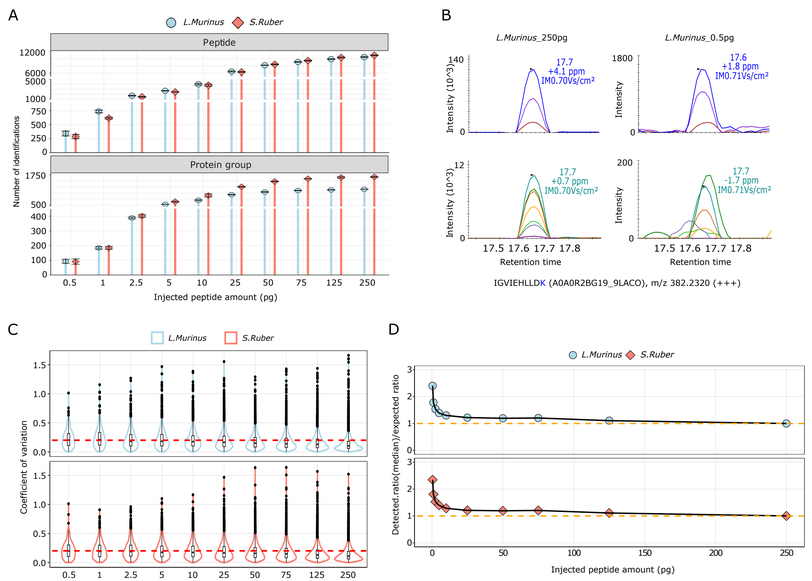

Figure 4. Pushing the sensitivity limits to quantify biology at single-bacterium resolution. A) Number of identified peptides and protein groups in a dilution series (ranging from 0.5 to 250 pg) of a two-proteome mix (SILAC-labeled *L. Murinus* and non-labeled *S. Ruber*). B) Representative extracted precursors (upper panel) and ion fragments (lower panel) obtained from uMetaP’s analysis of 500 fg in comparison with 250 pg injected peptide for *L. Murinus*. C) Quantitative precision across the dilution series measured by the coefficient of variation (CV) of all peptides detected among triplicates. The red-dotted lines indicate the CV = 0.2. D) Quantitative accuracy across the dilution series, measured as the median ratio of commonly identified peptides across the whole dilution series (Detected ratio; reference intensity at 250 pg) to the theoretical dilution factor (expected ratio). The yellow-dotted lines indicate the perfect accuracy of 1.

Conflict of interest statement: F.X., M.B., R.K.R.K, D.G.V., and M.S. declare no competing financial interest. C.K., J.K., and T.M. are current employees of Bruker Daltonics GmbH & Co. KG.