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Recent advances in proteotyping pathogens and environmental microorganisms by tandem mass spectrometry

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Question. Tandem mass spectrometry-based proteotyping of microorganisms has key advantages over other approaches. Due to a greater number of signals recorded, the methodology allows the identification of microorganisms at highly resolved taxonomic levels and can manage complex samples (Grenga et al., 2019). Here, we document its application to pathogens and environmental microorganisms, test its sensitivity and explore the possibility of high-throughput for culturomics.

Methods. Peptides generated with trypsin were analyzed by means of several high resolution instruments (LTQ-Orbitrap XL, Q-Exactive HF, Exploris480 mass spectrometers) operated in data-dependent acquisition mode. MS/MS spectra were identified using a cascade of searches conceived for maximizing metaproteomics results against a giant database comprising all sequenced genomes to date. The taxonomy of the microorganisms was established on the basis of the taxon-specific peptides and the taxon-assigned MS/MS spectra.

Results. The distribution of assigned Taxon-Spectrum Matches according to the taxa present in the database was modeled, leading to the discovery of the “Phylopeptidomic” signature (Pible et al., 2020). This signature makes it possible to quantify the biomass of each microorganism present in a mixture. Sample preparation for proteotyping has been improved to analyze hundreds of isolates (Hayoun et al., 2019 & 2020), making this methodology truly amenable to culturomics. The proteotyping approach can be applied to clinical pathogens as well as poorly documented environmental microorganisms, whatever the branch of the Tree of Life. We further documented the methodology showing that the sensitivity of the method is 10E4 bacterial cells required for typing at the species level. Finally, a multiplexing methodology was developed to be able to analyze dozens of isolates per analytical run, and identify their potential antibiotic resistance at the same time.

Conclusions. Overall, tandem mass spectrometry-based proteotyping is an interesting application of proteomics for rapid microbiological taxonomy. The taxonomic identification without a priori and the characterization of resistance to antibiotics pave the way for a new revolution in the clinical microbiology laboratory.

References.

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