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## Taxonomical exploration of complex samples by proteomics-derived proteotyping

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### Abstract

The correct identification of the organisms present in a complex sample is a crucial issue for microbiological diagnostics and research. Proteotyping based on tandem mass spectrometry, a methodology derived from metaproteomics, can help establish an inventory of organisms because the origin of the detected peptides can be traced back. The methodology we developed takes into account taxon-specific peptide sequences, but also the other peptides that are shared between taxa. An unbiased search of a large generic database is followed by a peptide search restricted to the most representative organisms to better delineate taxa. We introduced the term „Taxa-to-Spectrum Matches“ (TSMs) to best describe the composition of any sample. Here, we demonstrate the sensitivity of the approach, as trace amounts of biological material equivalent to a single human cell are sufficient (Mappa et al. 2023a). We document its accuracy with an artificial reference assemblage of 24 bacterial species with several challenges such as very closely related organisms (Mappa et al., 2023b). We illustrate the potential of the methodology to describe biofilms collected from very hostile environments such as nuclear storage pools (Pible et al., 2023). For these biofilms, we report the identification of three genera, namely *Sphingomonas*, *Caulobacter*, and *Acidovorax*. Once identified, their functional characterization by metaproteomics showed that these organisms are metabolically active. The differential expression of the Gene Ontology GOslim terms between the two main microorganisms highlights their metabolic specialization. The methodology can also be applied on biological material from very old samples (Oumarou Hama et al., 2023). As the approach can be performed rapidly on a limited amount of material, we foresee a wide application in microbiological diagnostics and microbiome research (Armengaud 2023).

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