

Title:

Species-resolved metaproteomics sheds light on bacterial functional diversity

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Abstract (300 words maximum):

Bacteria drive Earth's biogeochemical cycles, yet linking species to their realized functions in environmental settings remains challenging. While genome-resolved metagenomics allows to reveal bacterial functional potential, it falls short in identifying activities at in situ conditions. To address this limitation, we integrated knowledge of metabolic potential as derived from genome-resolved metagenomics with functional profiles elucidated by metaproteomics, thereby achieving species-level insight into dynamic bacterial ecophysiology. We conducted a three-month high-resolution time series study in Lake Zurich, collecting epilimnetic water samples during the spring phytoplankton bloom (36 time points). This period of dynamic microbial and environmental shifts provides an ideal context to study bacterial processes in situ. Limited databases for environmental bacteria hinder accurate proteomic identification and functional assessment. We thus constructed tailored sample- and species-specific protein databases by a hybrid metagenomics strategy based on assembling and error-correcting both long-read and short-read genomic data. The subsequent metaproteomic approach employed extended peptide separation using ultra-high-performance liquid chromatography (UHPLC) coupled with data-independent acquisition (DIA) mass spectrometry. Integrating our tailored databases with recent advancement in DIA methodologies significantly improved proteomic resolution, allowing the precise identification of approximately 14,000 species-specific proteins. By combining metaproteomic data with abundance profiles of 495 bacterial species – (derived from >3,200 metagenome-assembled genomes) we captured the temporal dynamics of the functional profiles of the most abundant bacterial taxa in the pelagic microbiome of Lake Zurich. Our results on the functional properties of the bacterioplankton community of Lake Zurich offer unprecedented insight into the metabolic and energy allocation strategies of key microbial players within this complex and dynamic ecosystem. Our methodology of synergizing metaproteomics with tailored metagenomic databases facilitates species-specific functional analyses, thereby elucidating distinct metabolic responses to environmental fluctuations and adaptive strategies within microbial communities.