Short-Term Protein Stable Isotope Probing of Microbial Communities to Associate Functions with Taxa

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Determining which taxa in a community perform which functions and respond to changing environmental factors is essential for understanding metabolic interactions among community members. Central to this understanding is the monitoring of qualitative and quantitative protein expression patterns in the individual taxa as a response to external stimuli. Protein stable isotope probing (Pro-SIP) has strong potential for revealing key metabolizing taxa in complex microbial communities, though these techniques have not been applied to short term *in situ* studies due to the small degree of partial labeling of the proteins. We Pro-SIP to three different biological systems to determine active taxa and protein expression patterns over time. Importantly, these *in situ* studies utilized very short incubation times (3 to 24 hours). In the first system, phototrophic microbial communities in alkaline siliceous hot springs of Yellowstone National Park, labeled bicarbonate addition highlighted the carbon assimilation pathways in different taxa. At Hot Lake, a hypersaline lake, we added different carbon-based substrates to the resident microbial mat over time and observed that incorporation of the substrates into metabolites occurred through different pathways both by direct assimilation and by breakdown of the primary substrate and assimilation through single carbon units. The third system, a simplified cyanobacteria consortium isolated from Hot Lake, illustrated that the assimilation of labeled bicarbonate was primarily through the dominant cyanobacteria and that label was only transferred to the resident heterotrophs after 24 hours of incubation. Pro-SIP experiments with short-term incubations thus have the potential to capture a snapshot in time of community functional activity, elucidating which organisms are actively expressing proteins, which pathways are being expressed, and how the taxa acclimate to environmental stimuli.