**Functional investigation of marine methylotrophic communities by protein based stable isotope probing**

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One-carbon compounds, i.e. compounds that only contain one carbon atom, or no carbon-carbon bonds, play key roles in Earth’s major biogeochemical cycles. Some of these compounds, like methane and dimethylsulfide (DMS), have climatic activity in the atmosphere (1), and thus are of direct relevance for the processes of global climate change. Marine habitats cover the majority of Earth’s surface and show a surprising abundance of one-carbon compounds. These environments have the potential to act both as sink and source, and consequently have a huge impact on atmospheric concentrations of carbon-one compounds. Microorganism that can metabolise one-carbon compounds are ubiquitous in the environment. Thus, these so called methylotrophs potentially play a major role in modulation of transfer processes between ocean and atmosphere.

Stable isotope probing (SIP), the metabolic labelling of microbial communities using substrates enriched with heavy, nonradioactive isotopes, is a powerful tool in environmental microbiology (2). Using SIP, the microorganisms that actively contribute to the metabolic processes of interest can be identified against the background of a complex microbial community, directly within environmental samples. A combination of the SIP approach with metaproteomic analysis, named protein-SIP, has been introduced recently, and uses mass spectrometry to identify peptides and simultaneously assess their enrichment in heavy isotopes (3).

Here we report on the application of protein-SIP on methylotrophic communities from two marine habitats. Different one-carbon compounds (methane, methanol and methylamine) labelled with carbon-13 or nitrogen-15 were employed as substrates, allowing the investigation of carbon and nitrogen cycling in these communities. Active methylotrophs were identified by detection of heavy isotope incorporation in their peptides, and the identification of functional proteins involved in methylotrophy enabled conclusions about the active biochemical pathways. The use of methylotrophs as model systems enabled us to explore the unique capabilities of the protein-SIP method for an in-depth functional analysis of microbial communities, and highlight its potential to answer key questions about microbial carbon cycling in marine ecosystems.

(1) Carpenter et al., Chem. Soc. Rev., 2012, 41, 6473–6506

(2) Neufeld et al., ISME J., 2007, 1(2), 103-110

(3) Taubert et al., ISME J., 2012, 6(12), 2291-2301