Proteomics method measures carbon uptake of marine microbes

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In a paper published April 26th in *mSystems*, a team of researchers led by microbiologists at Oregon State University, in Corvallis, describe a successful trial of a new method of identifying the carbon uptake of specific marine bacterioplankton taxa. The technique uses proteomics - the large-scale study of proteins - to observe directly the metabolic processes of communities of microorganisms.

Oregon State microbiologist Ryan Mueller, senior author on the paper, says the technique illuminates the carbon uptake process at three levels. "It shows how much is being used, by which microbes, and how they're using it to produce new proteins," he says. "It provides information about which organisms are taking up different substrates."

Marine bacterioplankton play a critical role in the carbon cycle. They recycle chemicals and decompose carbon-rich material like dissolved free amino acids (DFAA), which can result from many processes including lysing cells or phytoplankton bloom die-offs. Bacterioplankton process half of the organic carbon fixed by phytoplankton and other microbes through photosynthesis, but not all microbial communities have the same uptake rates. Linking particular taxa to metabolic responses has been an open question in the field for decades.

The researchers tested their new method, called proteomic stable isotopic probing, or proteomic-SIP, on eight seawater samples, including six collected from the ocean at Monterey Bay, California, and two from Newport, Oregon. To those samples they added DFAAs enriched with

the isotope carbon-13. Using high-resolution mass spectrometry, they extracted and analyzed proteins from the samples - half of the samples after 15 hours, and the other half after 32 hours. They used software developed by researchers at Oak Ridge National Laboratory, in Tennessee, to analyze the proteomics data.

Their analysis turned up metabolic patterns for particular taxa. The proteins associated with Rhodobacterales bacteria, for example, showed high levels of newly synthesized peptides enriched with the carbon isotope. In comparison, bacteria from Flavobacteriales and SAR11 communities had much lower enrichment.

In recent years, microbiologists have called on an arsenal of techniques to better understand the role of marine bacterioplankton in the carbon cycle. Previous efforts have used metagenomics, gene expression profiling, or fluorescence in situ hybridization, or FISH. Samuel Bryson, lead author on the new paper, notes that some previous techniques have also used stable isotope probing - though not at the same level of precision.

"Our main advantage over other SIP techniques is the direct measure of substrate incorporation into proteins," he says.

Although the current experiment uses proteomic-SIP to measure DFAA use, Bryson says it can readily be extended to other materials. He and his team have begun conducting experiments using multiple substrates that the bacterioplankton use, including glucose, lipids, and whole proteins. Mueller says their strategy was to start simple - with amino acids - and run more difficult experiments over time.

"In that way we're going to a more realistic type of environment," he says.