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Tapping into the unknown: Functional screening for CO_2 reducing enzymes gives unprecedented insights into the uncultured microbial majority

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Most of the microbes assumed to colonize the seafloor and its subsurface resist cultivation. Thus, their catalytic capabilities and contribution to element cycling remain enigmatic. Moreover, within this as-yet uncultured microbial majority, many useful biocatalysts for CO_2 sequestration, conversion to valuable chemicals, and energy storage might be hidden. We are using functional metagenomics to access this oceanic black box filled with numerus hypothetical proteins of unknown function. Here we report on two of the functional screens we have successfully established for seeking CO_2-reducing enzymes from metagenomic libraries.

Our first screen has been designed to detect the key enzyme of the Calvin-Benson-Bassham cycle, the ribulose-1,5-bisphosphate carboxylase (RubisCO), which links CO_2 to ribulose-1,5-bisphosphate to produce 3-D phosphoglycerate. Using this HPLC-based RubisCO to screen through 8000 fosmid clones with deep-sea hydrothermal vent origin resulted in the recovery of 49 RuBP converting fosmid clones whose inserts show highest similarity to mainly Gamma- but also Alphaproteobacteria and Archaea. One clone was particularly noticeable, as it converts RuBP exceptionally fast, i.e. up to 20 mM is used up in a few seconds in the RubisCO assay (0.0013 mg µl-1 crude protein extract), but 3-PGA is not produced in measurable concentrations. Indeed, biochemical characterization and crystallization of the overexpressed novel enzyme confirms the detection of a novel enzyme previously not associated with RuBP conversion. This expands our current understanding of RuBP associated metabolism, as it possesses a yet unknown function in a previously unrecognized potential RuBP pathway.

Our second screen is a colorimetric one allowing to detect the reduction of CO_2 to CO, a reaction catalyzed by the key enzyme of the reductive acetyl-CoA pathway, the carbon monoxide dehydrogenases (CODH). The activity of recombinant CODHs from phylogenetically distinct microbial species was successfully demonstrated, reflecting the screen's scope. Currently, the screen is used through a metagenomic fosmid library constructed from anoxic marine sediments from the Eckernförde Bight (SW Baltic Sea, Germany). The aim is to mine particularly active CODHs to be used as biocatalysts for CO_2 reduction from flue gas, for energy storage, and production of commodity chemicals in electrochemical cells.