Mining novel CODHs through culture-independent functional metagenomic screening

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As biocatalysts, carbon monoxide dehydrogenases hold great potential, as they are capable of reducing CO2 to CO at high rates and under exclusion of any undesired carbon-containing by-products. In the enzyme complex with acetyl CoA synthases, CODHs function as key enzymes in the reductive acetyl-CoA pathway. Thus, CODHs can be found in physiologically versatile marine microorganisms colonizing a broad range of thermally and chemically distinct marine habitats including sediments and deep-sea hydrothermal vents. However, this natural source for novel biocatalysts is currently insufficiently utilized as the vast majority of microorganisms resist cultivation (around 91 to 96 % in marine environments). With the aim of capturing particularly active CODH enzymes from otherwise inaccessible, yet uncultured marine microbes, we have developed a function-based metagenomic CODH screening tool. We could successfully demonstrate that the activity of recombinant CODHs from phylogenetically distinct microbial species is detectable by this colorimetrical CODH screen. This proof of principle not only served to demonstrate the range of CODHs detectable by the screen, but also led to the identification of a protein critical for achieving maximum activity of heterologous *Rhodospirillum rubrum* CODH.

Screening of a hydrothermal deep-sea vent metagenomic library resulted in the identification of two fosmid clones with CO converting abilities. However, database comparisons with the whole metagenomic fosmid inserts revealed only low similarities, with the highest resemblance to prokaryotes that are not yet known to exhibit CODH activity. Sub-cloning and transposon mutagenesis are now used to identify the genes responsible for the activity.