On the quest for novel CODHs: Delving into the uncultured microbial life and its hidden enzymatic potential

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Given the need to reduce global CO_2 emissions and advancing sustainable industrial approaches, microbes and their versatile biocatalysts offer enormous potential that can be used on the way to a sustainable future. However, this natural source for novel biocatalysts is currently insufficiently utilized as the vast majority of microorganisms resist cultivation. This is particularly true for deep-sea habitats, where it is estimated that 91 to 96 % of microbes cannot be cultivated. Using functional metagenomics, we gain access to this oceanic black box filled with numerous hypothetical proteins of unknown function. Here we report on a functional-screen that we have successfully developed to target carbon monoxide dehydrogenases (CODHs, EC 1.2.7.4) from the environment without relying on the cultivability of the native host.

As biocatalysts, CODHs hold great potential, as they are capable of reducing CO_2 to CO at high rates and under exclusion of any undesired carbon-containing by-products. As key enzyme of the reductive acetyl-CoA pathway 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. With the aim of capturing particularly active CODH enzymes from otherwise inaccessible, yet uncultured marine microbes we have developed the function-based colorimetrical CODH screening tool. We could successfully demonstrate that the activity of recombinant CODHs from phylogenetically distinct microbial species is detectable, reflecting the screen's scope. Screening of a hydrothermal deep-sea vent metagenomic library resulted only recently in the identification of two active clones. However, sequencing of the fosmid insert ends shows highest similarity to microbial species that are not yet known to exhibit CODH activities. PacBio sequencing of the whole fosmid inserts in combination with transposon mutagenesis is now being used to identify the genes responsible for the activity.