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Methanotroph diversity in natural and anthropogenic habitats as an inexhaustible source of novel biotechnologically relevant strains

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Motivation and Aim: Aerobic methanotrophs are metabolically unique bacteria that are able to utilize methane as a sole source of energy. A defining characteristic of these organisms is the use of methane monooxygenase enzymes to catalyze the oxidation of methane to methanol. This metabolic capability makes methanotrophs an attractive target for various biotechnological applications for the conversion of methane to a microbial single cell protein or other value-added products. However, despite the long history of studying aerobic methanotrophs, the cultured diversity of these bacteria and, in particular, the number of biotechnologically relevant strains with high growth characteristics remains limited. The project acknowledged in this report was initiated in order to expand the pool of currently available fast-growing methanotrophs by isolating novel strains of these bacteria from various natural and anthropogenic habitats where both methane and oxygen are available.
Methods: The samples of activated sludge collected from several wastewater treatment plants, sediments from freshwater ponds and rivers of various location, as well as landfill cover soils were used as sources for isolation of new methanotroph strains. The cultivation approach was adjusted for isolating methanotrophs growing in the temperature range 35-45°C. The obtained isolates were characterized phenotypically and used for the experiments on continuous cultivation in 1.5 l bioreactors. The genome sequences of novel strains were determined by the combination of MiSeq and Nanopore techniques and used for comparative genome analyses.
Results: As revealed by the high-throughput 16S rRNA gene sequence analysis, methane-oxidizing enrichment cultures obtained from various samples contained representatives of the genera *Methylococcus*, *Methylomonas*, *Methylocaldum*, *Methylosinus* and *Methylocystis*. Further attempts were focused on isolating novel strains of fast-growing *Methylococcus* species, since the latter have been extensively exploited for large-scale commercial production of microbial proteins. Four novel isolates of these bacteria were isolated from activated sludge, landfill cover soil and freshwater sediments. The determined genome sizes of novel isolates varied between 3.2 and 4.0 Mb. As revealed by the phylogenomic analysis, strains IO1, BH and KN2 affiliated with *Mc. capsulatus*, while strain Mc7 represented a novel species. Highest temperature optima (45–50°C) and highest growth rates in bioreactor cultures (up to 0.3 h^{-1}) were recorded for strains obtained from activated sludge. The comparative analysis of all complete genomes of *Methylococcus* species revealed surprisingly high genome synteny and a striking similarity in gene content in all strains of *Mc. capsulatus*,

which also displayed high growth rates on methane. Another research direction was focused on obtaining new strains of fast-growing methanotrophs that produce carotenoids, since the latter increase the value of feed protein. Five novel isolates of yellow- to red-pigmented *Methylomonas* species have been obtained and characterized with regard to their growth characteristics and the composition of produced carotenoids. One of the novel isolates was shown to be especially promising with regard to high growth rates on methane and methanol. This isolate was also assigned to the novel species of the genus *Methylomonas, 'M. rapida'*.

Conclusion: In summary, methanotroph diversity in natural and anthropogenic habitats remains an inexhaustible source of novel biotechnologically relevant strains.

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