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Enhancement of methane oxidation in methanotrophs

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Methane, a potent greenhouse gas, has garnered significant attention due to its environmental impact and economic potential. Enhancing methane catalysis poses challenges in both chemical and biological sectors. Biological methane conversion offers advantages such as higher conversion rates, improved selectivity, self-renewal properties, and economically feasible upstream processing. The complete genome of Methylosinus trichosporium OB3b was sequenced using Nanopore technology, identifying 4,877 genes on the chromosome. The integrated metabolomic profile revealed 63 metabolites, out of which 14 are screened down with regard to their commercial relevance. Bifunctional aldehyde dehydrogenase and phospholipase were annotated in the ethanolamine pathway, while fatty acid desaturase was identified as a one-step enzyme enabling the direct conversion of precursor R-decanoyl-[acp] into R-decenoic acids in the R-decenoic acid pathway. A pan-genomic study of 75 Type II methanotrophs spanning different genera revealed 256 exact core gene families. Among the 22 observed hypothetical proteins in the core genes, the functionality of 12 were identified. Notable findings included Methylocella tundrae, which possessed three copies of sMMO components, setting it apart from other methanotrophs. Despite of all observed strains had essential genes for the serine pathway, Methylooceanibacter marginalis lacked serine hydroxymethyltransferase (SHMT), Methylobacterium variabile had both isozymes of SHMT, and Methylobrevis sp. displayed a unique serine-glyoxylate transaminase isozyme. Only nine strains contained anaplerotic enzymes, indicating their utilization of the glyoxylate pathway, whereas the remaining strains followed the enoylmalonyl-CoA (EMC) pathway. Methylovirgula sp. 4MZ18 possessed genes from both pathways, and Methylocapsa sp. S129 possessed the A-form of malate synthase, distinguishing it from other strains with the G-form. The study also uncovered phylogenetic relationships and distinct clustering patterns among Type II methylotrophs, leading to the proposal of a separate genus for Methylovirgula sp. 4M-Z18 and Methylocapsa sp. S129. With an effort to enhance methane oxidation rates in OB3b, macromolecular modeling and docking were employed revealing an unexplored active site within the particulate form (pMMO). Additionally, we identified five potential mutants (B:Leu31Ser, B:Phe96Gly, B:Phe92Thr, B:Trp106Ala, and B:Tyr110Phe) that show promise in improving methane oxidation rates. The regulation of the Cu switch was observed within a narrow range of Cu concentrations, specifically between 3 and 5 μM. Notably, this regulation resulted in a significant increase in methane consumption rates, rising from 3.09 to 3.85 μM.day-1 on the 6th day. The upregulation of Mbn synthesis genes (mbnABC) and the Ton-B siderophore receptor gene (mbnT) at above 5μM Cu may be responsible for the enhanced Mbn synthesis leading to increased Cu consumption. To distinguish between cells expressing the soluble form of MMO (sMMO) and the pMMO, we developed a quantitative assay based on the Naphthalene-Molisch principle. Overall, this dissertation provided valuable insights into the genetic diversity of methylotrophs, effective strategies for enhancing methane oxidation rates, and the regulatory role of copper in MMOs.

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