Goldschmidt 2022 Abstract
https://doi.org/10.46427/gold2022.11503

Methanotrophic Activity in the Deep Environment: Enhancement of Methane Catalysis Rates

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Methane is a powerful greenhouse gas, second to carbon dioxide in abundance and yet over 25-times as effective in trapping heat. The impact of methane fluxes from the deep biosphere of non-coal mines on microorganisms and the associated microbiome involved in methane oxidation has not been previously studied. There are still several intriguing questions about methanotrophs and their key methane monooxygenase (MMO) enzymes. There is a controversy regarding the active site(s), specifically whether the active site(s) are in the *pmoB* (α subunit), *pmoA* (β subunit), or *pmoC* (Î³ subunit). Also, MMOs have lower affinity towards methane.

With our years of exploration in the deep biosphere (300 to 5,000 ft. levels) of the Sanford Underground Research Facility (SURF) at Homestake Gold Mine (Lead, SD, USA), we have confirmed the synergy among more than 44 different bacterial and archaea phyla in water, soil, sediments, and rock samples. Our results indicated the domination of *Rhodobacteraceae, Alcaligenaceae, Bradyrhizobiaceae, Mycobacteriaceae, and Pseudonocardiaceae* families in deep biosphere of SURF. Certain microbial members of these families had soluble methane monooxygenases (sMMO). The relative importance of *Rhodobacteraceae* in SURF and our results with *Rhodobacter* sp. showing their ability to carry out methanotrophy suggest that evolutionary groups other than conventional methanotrophs are involved in methane oxidation. These findings suggest that there may be more novel methanotrophs that are unidentified and virtually unstudied.

Using in silico approaches, we unveiled whether the active site(s) of pMMO is located within the copper center or within the vicinity of the copper center using *Methylosinus trichosporium* OB3b as a model organism. To increase the catalysis rates of methane in pMMO of OB3b, selected amino acid residues interacting at the binding site of ethylbenzene, toluene, 1,3-dibutadiene, and trichloroethylene were mutated. Based on screening the strain energy, docking energy, and physiochemical properties, five mutants were down selected, B:Leu31Ser, B:Phe96Gly, B:Phe92Thr, B:Trp106Ala and B:Tyr110Phe, which showed docking energies of -6.3, -6.7, -6.3, -6.5 and -6.5 kcal/mol, respectively as compared to the wild type (-5.2 kcal/mol) with ethylbenzene. These results suggest that these five mutants would likely increase methane oxidation rates compared to the wild-type pMMO.