

# The Impact of Oxygen Concentration on Methane-Oxidizing Bacteria and the Characterization of Novel Bacteria from SURF

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Methane-oxidizing bacteria (MOB) play a crucial role in the global methane cycle, serving as significant actors in biogeochemical cycling. However, their physiological response to changing oxygen concentrations remains incompletely understood. This study, in part, demonstrates how two MOB species, *Methylosinus trichosporium* OB3b and *Methylomonas* sp. WSC-7, respond to changing oxygen concentrations and the addition of catalase, a hydrogen peroxide scavenger, in growth media. Through transcriptomics analysis, we showed that under high oxygen conditions, *M. trichosporium* OB3b upregulates genes involved in reactive oxygen species (ROS) defense, including cytochrome c peroxidase and superoxide dismutase, suggesting a need to deal with elevated ROS levels. Conversely, *Methylomonas* sp. WSC-7 exhibits cell clustering behavior, potentially as a defense mechanism against ROS toxicity. Differential expression of flagellar biosynthesis genes and chemotaxis response genes further supports this adaptive response. Additionally, rates of methane oxidation for both strains are impacted by the concentration of oxygen and amending the growth media with catalase. Our findings underscore the importance of oxygen concentration in modulating MOB physiology and suggest potential strategies for optimizing their growth conditions, especially for the effective isolation of novel MOB. This work also describes the characterization of two novel strains of MOB, SURF-1 and SURF-2. The strains were isolated from a microbial mat and submerged sediments, respectively, inside a tunnel at the Sanford Underground Research Facility. Specifically, they were isolated from the “17 Ledge” mining area approximately 4,850 ft below the surface. Analysis of the strains using phylogenetic and chemotaxonomic analyses coupled with analysis using genome-wide estimates of similarity indicate the strains are novel members of the genus *Methylomonas*.

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