

Fluorescence-Based Cell Sorting and Targeted Proteomic Analysis of Active Methane-Oxidizing Syntrophic Consortia from Environmental Samples

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Abstract: Determining the links between phylogenetically identified microorganisms with their metabolic activity and function in complex natural ecosystems has been a long-standing challenge in microbial ecology. Here we propose new methodological strategies that offer direct links between microbial activity (protein expression), phylogenetic identity and genome content for uncultured syntrophic consortia involved in the anaerobic oxidation of methane. Specifically, we will employ fluorescence-activated cell sorting (FAC) to sort translationally-active microorganisms that have been fluorescently labeled using a click-chemistry based technique called BONCAT, or bioorthogonal noncanonical amino acid tagging. Individually sorted translationally active AOM consortia recovered from methane-fed sediment incubations will be used for 16S rRNA analysis and metagenomic sequencing, while parallel FAC separated consortia will be pooled for proteomic analysis and mass spectrometry imaging, with the objective of developing new insights into the details of the metabolic pathways central to AOM and methane fueled syntrophy by different archaeal and bacterial lineages and under different electron accepting conditions. Together, this research helps to address a key methodological gap in the field and will provide fundamental information regarding protein expression and biochemical mechanisms involved in this globally important biological methane sink.