Comparative genomic and activity-based analyses reveal widespread potential for direct extracellular electron transfer among diverse methane-oxidizing ANME archaea

Chadwick, Grayson,[1]* (chadwick@caltech.edu), Connor Skennerton,[1] Hang Yu,[1] Mark Ellisman[2], Gene Tyson,[3] Roland Hatzenpichler[1], Rex Malmstrom[4], Tanja Woyke[4], and **Victoria J. Orphan**[1]

[1]California Institute of Technology, Pasadena, CA; [2]University of California, San Diego, San Diego, CA; [3]University of Queensland, Brisbane, Australia; and [4]Joint Genome Institute, Walnut Creek, CA

Project Goals: The major goals of this project are to identify novel archaea and syntrophic microorganisms involved in methane cycling, examine their genomic characteristics, and test their metabolic potential and interspecies interactions using cross-disciplinary methods across a range of spatial scales. Using a combination of experimental and modeling-based approaches, we are testing potential metabolic interactions between methane-oxidizing archaea and sulfate-reducing bacteria, with a specific focus on direct extracellular electron transfer as one of the key mechanisms for energy conservation during anaerobic oxidation of methane. More broadly, by measuring and modeling spatial patterns of activity within diverse structured consortia and biofilms at the micron scale, our goal is to determine whether these patterns can provide fundamental information about the nature of the interaction and metabolic interchange.

The anaerobic oxidation of methane coupled to sulfate-reduction is a microbially mediated process requiring a syntrophic partnership between currently uncultured archaea, known as anaerobic methanotrophs (ANME) and sulfate-reducing bacteria (SRB). These sediment-hosted organisms form multi-celled aggregates comprised of one ANME and one SRB partner; and these syntrophic consortia exhibit diverse, but reproducible, spatial organization. ANME lineages are polyphyletic, representing several family-level and one order-level clades of the *Methanomicrobia*. Based on their phylogenetic position and metabolic similarity to methanogens, it is probable that the mechanism for growth by anaerobic methane oxidation occurred through horizontal gene transfer of genes involved in extracellular electron transfer and has evolved multiple times in different clades. Here we used metagenomic sequencing to reconstruct the genomes of 31 representatives from all known ANME clades to determine what separates ANME from methanogens and what differentiates ANME clades from each other. In order to better understand the functional basis for the different consortia spatial structures, we have optimized a protocol for fluorescence *in situ* hybridization (FISH) to identify the archaeal and bacterial partners and observe aggregate morphology combined with laser micro-dissection to recover and amplify the 16S ribosomal RNA genes directly from the imaged consortia, offering a direct link between aggregate structure, strain-level phylogenetic identity, and genomic content. These genomic and microscopic observations of individual ANME clades are considered in light of recent spatial modeling efforts to better understand the adaptive rational for the varying community organizations observed in nature.

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