

Cultivation of microbial dark matters from marine sediments: novel bacteria affiliated with uncultured groups of the phylum *Chloroflexi*

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Subseafloor sediments are known to be a critical component of marine biogeochemical cycles across Earth. A number of previous biochemical and molecular biological studies have demonstrated that subseafloor sedimentary environments possess an enormous microbial biomass, comprised of microorganisms with unique function and phylogenetic diversity. However, the detailed physiological and metabolic properties of individual species are largely unknown because most subseafloor sedimentary microbes have evaded conventional cultivation methods. As a novel approach to cultivation of uncharacterized subseafloor microorganisms, we have implemented a down-flow hanging sponge (DHS) continuous-flow bioreactor system to initiate *in vitro* enrichment and successfully obtained cultures of target organisms [1, 2, 3]. In this presentation, we report cultivation, partial physiological and genomic characterization, of two new bacterial strains belonging to uncultivated lineages of the phylum *Chloroflexi* with high prevalence across subseafloor environments and yet undefined ecological function.

One of the *Chloroflexi* bacteria is affiliated with an uncultured group of the class *Anaerolineae*, and it has been in pure culture (strain MO-CFX2). The strain was isolated from a methanogenic community enriched in a DHS reactor, which was originally obtained from subseafloor sediments off Shimokita Peninsula, Japan. Strain MO-CFX2 grew in an anaerobic medium containing yeast extract and peptone. The genome reveals that strain MO-CFX2 may degrade glucose and lactate and also respire halogenated compounds. Currently, we are going to characterize these and other physiological traits in detail.

The other *Chloroflexi* bacterium belongs to an uncultured lineage of the class *Dehalococcoidia*, and it is being purified (strain MK-GIF9). This strain was obtained from an anaerobic methane-oxidizing DHS reactor inoculated with biomass collected from Nankai Trough methane seep sediments. The genomic analysis of strain MK-GIF9 indicates that the bacterium can use only several amino acids as energy sources. We also found a glycine fermentation pathway that produces formate as an end product. Based on the genomic analysis, we suspected that the growth of strain MK-GIF9 can be promoted if the metabolic byproduct (formate) is continuously removed by a formate-scavenging partner (*e.g.*, methanogens). Indeed, co-cultivation of MK-GIF9 with a formate-utilizing methanogen successfully stimulated methanogenic glycine degradation and promoted growth of strain MK-GIF9. The specialized ability to cooperatively degrade amino acids sheds light on the poorly understood fate of amino acids in subseafloor sediments. Taken together with the ubiquity and predominance of *Chloroflexi* bacteria in subseafloor environments, our results implicate that amino acids including glycine may be one of the important precursors for methane production in subseafloor sediments.

References; [1] Imachi et al., (2011) *ISME.J.* 5., 1923 –1925. [2] Aoki et al., (2014) *PLOS ONE.* 9, (8), e105356 [3] Inagaki et al., (2015) *Science.* 349, (6246), 420 –424.

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