Isolation and physiological characterization of an archaeon at the prokaryote-eukaryote interface

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The origin of the eukaryotes remains a major enigma in biology. Current data suggest that eukaryotes may have emerged from an archaeal lineage known as “Asgard” archaea. Despite the eukaryote-like genomic features found in these archaea, the evolutionary transition from archaea to eukaryotes remains unclear due to the lack of cultured representatives and corresponding physiological insight. In this presentation, we will introduce the isolation procedure and unique physiological properties of the first cultured representative of Asgard archaea. To effectively cultivate deep marine sediment microorganisms including Asgard archaea, we employed a continuous-flow bioreactor with polyurethane sponges, called down-flow hanging sponge (DHS) bioreactor. We anaerobically incubated deep-sea methane-seep sediment collected from the Nankai Trough, Japan, and fed with methane as the main energy source for more than 2,000 days at 10˚C. During the bioreactor cultivation, the microbial community composition was monitored using 16S rRNA gene-based techniques such as cloning/iTAG, qPCR and fluorescence *in situ* hybridization (FISH). As phylogenetically diverse uncultured microorganisms including Asgard archaea members were increasingly enriched over time, we transitioned towards further enrichment and isolation in glass tubes/vials with simple substrates and selective compounds (*e.g.*, antibiotics). Cultures were incubated for at least 12 months and analyzed for microbial community composition even when no turbidity was observed, as growth was expected to be very slow. One culture amended with casamino acids and antibiotics contained a small population of a novel archaeon (designated strain MK-D1) belonging to the “*Candidatus* phylum Lokiarchaeota” of the Asgard archaea. Through successive *in vitro* cultivation combined with qPCR- and iTag-based cell density and community composition monitoring, we obtained a pure co-culture of the target archaeon and methanogenic archaeon *Methanogenium*– 12 years after the first sampling of deep-sea sediment. Strain MK-D1 is a strictly anaerobic archaeon that degrades amino acids syntrophically with sulfate-reducing bacteria and methanogenic archaea via interspecies hydrogen (and/or formate) transfer. The archaeon has an extremely slow growth rate and low cell yield, i.e., the doubling time was approximately 14–25 days and maximum cell density 10^5 cells/ml, which are thousand-fold lower than a model microorganism *Escherichia coli*. The MK-D1 cells are small cocci (approximately 550 nm in diameter) and generally form aggregates surrounded extracellular polymer substances. Electron microscopic observations revealed that the cells contain no visible organelle-like inclusions, although eukaryote-like intracellular complexes have been proposed for Asgard archaea in previous metagenomic-based studies. Instead, MK-D1 is morphologically complex and unique protrusions that are long and often branching. The MK-D1 cells also produce many membrane vesicles. Based on these unique physiological properties and genetic features (details in the subsequent presentation by Masaru K. Nobu). We proposed the name “*Candidatus* Prometheoarchaeum syntrophicum” for strain MK-D1.

References

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Keywords: Archaea, deep-sea sediment, Asgard archaea, origin of eukaryotes, syntrophy

