Identifying 2 km-deep methanogenic community members using a long-term bioreactor cultivation

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During the Integrated Ocean Drilling Program (IODP) Expedition 337, we successfully enriched a methanogenic microbial community from 2 km-deep lignite coalbed samples using a down-flow hanging sponge (DHS) reactor [1, 2]. During the DHS reactor operation for 932 days at near the *in-situ* temperature of 40°C, we observed a continuous methane production since the 7th day, even without adding any organic substrates in the seawater-based medium after 721 days. The carbon isotopic composition of methane gradually decreased with the operational time from -42.9‰ to -94.0‰, suggesting the significant contribution of microbial methanogenesis. Interestingly, the effluent contained acetate (up to 0.6 mM), which is most likely a major end-product of the heterotrophic microbial activity. Electron microscopic observation of the lignite particles showed that remarkably abundant and morphologically diverse microbial cells tightly attached to the particles. 16S rRNA gene-tag sequence analysis revealed that archaeal community was consisted mainly of a hydrogenotrophic CO_2-reducing methanogen related to *Methanobacterium*, whereas no aceticlastic methanogens were detected. Bacterial community was predominated by the members within *Firmicutes* and *Gammaproteobacteria*. These data suggest that the enriched microbial community represents a heterotrophic microbial ecosystem that largely relies on coaly organic matter, and its activity produces both acetate and methane via the degradation of lignite. On-going efforts on metagenomic and metatranscriptomic analyses will reveal genetic and functional networks of the 2 km-deep microbial community, as well as the comparative genomics of isolates obtained from the methanogenic community.

[1] Imachi *et al*. (2011) *ISME J*., **5**, 1913-1925. [2] Inagaki *et al*. (2015) *Science*, **349**, 420-424.