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Identifying methanogenic microbial community members obtained from 2-km deep subseafloor coalbed

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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. 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. Bacterial community was predominated by the members within Actinobacteria, Bacteroidetes, Firmicutes, Chloroflexi and Gammaproteobacteria. Using the reactor enrichment as inoculum, subsequent batch-type cultivation led to the successful isolation of several anaerobic microorganisms, including the hydrogenotrophic methanogen. 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.