Methanogens from deep sedimentary aquifers in northern Japan

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Microorganisms that generate methane were enriched and isolated from a methane-rich artesian spring and three deep aquifers that were situated nearby, as well as from a distant deep aquifer in a coal field, which was located on the northern island of Hokkido, Japan. The water samples were directly enriched in two basal media

;one containing methanol and auxotrophic substrates of yeast extract and peptone with a gas mixture of N_2:CO_2:H_2 (8:1:1 at 100 kPa); and the other containing acetate and auxotrophic substrates (yeast extract and peptone) with a gas mixture of CO_2:H_2 (1:4 at 200 kPa). These enrichment media were inoculated with undiluted water samples and incubated at 30oC and 37oC. Methane generation was observed in 12 positive enrichments out of a total of 20 trials (5 samples x 2 media x 2 temperatures). Based on 16S rRNA gene sequence analysis, the phylotypes were characterized and showed 97% similarities with four methanogenic genera, namely, *Methanobacterium*, *Methanocorpusculum*, *Methanoculleus*, and *Methanosarcina*. From the positive enrichments, a total of four methanogenic strains were isolated and purified from deep aquifers from which we have characterized 16S rRNA gene populations in a previous study(Shimizu *et al*. 2006). Based on the 16S rRNA gene sequence analysis, the isolates showed 98% similarity to three genera, namely, *Methanobacterium*, *Methanoculleus* and *Methanosarcina*. Three isolates (out of the total of four) were almost identical (99.9% similar) to the phylotypes that were previously characterized in the same deep aquifers (Shimizu *et al*. 2006), and these methanogens are likely to be responsible for in situ methanogenesis.

Reference

Shimizu S, Akiyama M, Ishijima Y, Hama K, Kunimaru T, Naganuma T (2006) Molecular characterization of microbial communities in fault-bordered aquifers in the Miocene formation of northernmost Japan, Geobiology **4**, 203-213.