

## Evidence of microbial methane generation in the deep aquifer of accretionary prism in southern Okinawa Island, Japan

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The accretionary prism situated the Pacific Ocean side of Southwest Japan forms thick sediment that accretes onto the nonsubducting tectonic plate at a convergent plate boundary. The sediments contain a deep aquifer that a large amount of anaerobic groundwater and methane are accumulated. The methane is mainly generated by methanogenic archaea (biogenic origin) or thermal degradation of organic matter (thermogenic origin). Anaerobic groundwater and natural gases are observed from in the deep aquifers of the accretionary prism in Shizuoka Prefecture, Miyazaki Prefecture and Okinawa Island, Southwest Japan. The methane, which was collected from deep aquifer in Shizuoka and Miyazaki Prefectures, is composed biogenic origin and thermogenic origin. The methane of biogenic origin is mainly generated by syntrophic consortium of  $H_2/CO_2$ -producing fermentative bacteria and  $H_2/CO_2$ -using methanogenic archaea in deep aquifer of accretionary prism. However, methane generation process in the deep aquifer of accretionary prism in southern Okinawa Island has not been investigated. Therefore, the aim of this study was to identify the methane generation mechanism in the deep aquifer accretionary prism in southern Okinawa Island. In this study, we measured environmental parameters and dissolved ion concentrations of the deep groundwater. To identify the origin of methane, we performed stable carbon isotopic analysis. Furthermore, we also determined the potential of microbial methane production by anaerobic culture experiments and 16S rRNA gene analysis.

The groundwater and natural gas samples were collected from 4 deep wells constructed in southern Okinawa Island. The chemical analysis of the groundwater suggested that the deep aquifers are affected by seawater or seawater and rainwater. Analysis of the natural gas showed that methane is more than 93 vol% in all gas samples. Stable carbon isotopic analysis of methane in the natural gas and dissolved inorganic carbon (mainly bicarbonate) in the groundwater suggested that the methane was derieved from both thermogenic and biogenic origin. Analysis of 16S rRNA gene showed that the dominance of  $H_2/CO_2$ -producing fermentative bacteria and  $H_2/CO_2$ -using methanogenic archaea in the groundwater. Anaerobic cultivation targeting syntrophic consortium of the fermentative bacteria and the methanogenic archaea revealed high potential of microbial methane generation was obserbed in all groundwater samples.

From these results, it was shown that the methane generation is conducted by syntrophic consortium of  $H_2/CO_2$ -producing fermentative bacteria and  $H_2/CO_2$ -using methanogenic archaea in the deep aquifer of accretionary prism in southern Okinawa Island. Furthermore, our results suggested that methane generation has been conducted by subterranean microbial community in deep aquifer of accretionary prism in wide range of the Pacific Ocean side of Southwest Japan.

Keywords: accretionary prism, deep aquifer, methane production, fermentative bacteria, methanogenic archaea, syntrophic consortium