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New Contamination Test Revealed Microbial Activities Related to Methane Hydrate Formation in the Mogami Trough

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The formation of methane hydrates is generally controlled by methanogenic activity in marine sediments. In case of marine sediments associated with shallow methane hydrates, it is technically challenging to penetrate hard strata with methane hydrates and carbonate crusts by microbiologically suitable piston coring (PC). Alternatively, rotary core barrel (RCB) is used for deep drilling at shallow hydrate sites, whereas microbiological contamination from drilling mud is problematic and requires an accurate contamination test. Although contamination tests using perfluorocarbon and/or fluorescent microspheres are widely used, it is difficult to maintain constant tracer concentrations in drilling mud for accurate evaluation of contamination. In this study, RCB coring was conducted with a new tracer, amino G acid, to deeply penetrate a shallow hydrate site in Mogami Trough. During drilling, the concentration of amino G acid in drilling mud was successfully maintained at $\sim 8 \times 10^5$ ppb within $\pm$10%. As the detection limit of amino G acid by high performance liquid chromatography was 0.03 ppb, it is possible to detect the intrusion of 10^2 nl of drilling mud into 1 ml of porewater. Population density in drilling mud was at $\sim 5 \times 10^7$ cells/ml, and it was found that our new method is capable of detect 10 contaminated cells in 1 ml of sediment. Based on the concentration of amino G acid in porewater, the level of contamination was accurately calculated, and it was found by 16S rRNA gene sequence analysis that highly contaminated sediment samples were dominantly colonized by gammaproteobacteiral species of the genera *Shewanella*, *Listonella* and *Vibrio*. In sediment samples with low contamination, many sequences were affiliated within bacterial groups commonly found in deep PC sediments such as the phylum Chloroflexi and candidate divisions JS1 and NT-B2. The rates of methanogenesis via acetate fermentation and CO_2 reduction were measured by sediment slurry incubation with 14C-labbeled aceatate and bicarbonate, respectively. Although acetate fermentation was greatly influenced by drilling contamination, CO_2 reduction was not apparently influenced. Interestingly, the rate of CO_2 reduction was highest in a deep sediment sample win methane hydrates, which suggests that in-situ microbial activities might contribute to ongoing formation of methane hydrates in Mogami Trough.

Keywords: Methane hydrate, Marine sediment, Contamination test, Microbial community, Methanogenesis