

Investigation microbial community for gas-hydrate site off-Sakhalin Island

*Mizuki Abe¹, Akihiro Hachikubo¹, Hirotsugu Minami¹, Hirotohi Sakagami¹, Satoshi Yamashita¹, Nobuo Takahashi¹, Hitoshi Shoji¹, Jin K Young³, Boris Baranov⁴, Anatoly Obzhirov², Masaaki Konishi¹

1.Kitami Institute of Technology, 2.V.I. Il'ichev Pacific Oceanological Institute FEB RAS, Russia, 3.Korea Polar Research Institute, Korea, 4.P.P. Shirshov Institute of Oceanology, RAS, Russia

Gas hydrates (GH) are widely spread in the sediments under the subsea floor and form at high pressure and low temperature. GH oriented gas were grouped to thermogenic and microbial gas according to composition carbohydrate gas and isotopic molecular weight of methane. The role of microbial communities in the GH sites have been already investigated by several research groups. Isotopic composition of gas hydrate is often use to determine gas derivation such as thermogenic or microbial gases. However, combination study both isotopic gas analysis and microbial diversity have not been performed at all. Recently we successfully obtained mix gas-derived GH core sediment in the western Sakhalin slope off Sakhalin Island by exploration using ultrasonic wave sonar and gravity coring, in SSGH 15 project by using Russian research vessel *Akademik M. A. Lavrentyev*. We will demonstrate that results of investigating molecular and isotropic composition of the sediment gas, sulfate and sodium compositions, and microbial composition of a GH-bearing sediment core obtained in SSGH15.

Molecular composition ratio C_1/C_2+C_3 below SMI were in the range between 116 and 225, while $d^{13}C$ and dD values of methane were in the range of -48.9 and -45.7 permil, and of -165 and -149 permil, respectively. These results indicated that the gas compose of large amount of thermogenic gas and small amount of microbial gas.

Forty two individual clones have successfully analyzed, until we submit this article. Three *Aciduliprofundum* related clones, and three *Methanobrevibacter* related clones were detected. These sequences were clustered into oceanic methanogen in the phylum *Euriarcheota*. This result implied that these archaeon generate microbial methane in the core, and may correspond to decrease isotopic ^{13}C ratio of methane and increase the C_1/C_2+C_3 ratio. Slight amount of sequence in *Crenarcheota*, which may be involved anaerobic methane oxidation (annamox). Interestingly, heterotrophic bacteria in cluster of *Dehalococcoidetes*-related *Chloroflexi*, of *Candidatus artibacteria* (named as division JS1/OP9), and of *Planctomycetes* were frequently widespread in the core. The phylum *Chloroflexi* is a lineage for which the class '*Dehalococcoidetes*' was proposed to accommodate the tetrachloroethane respiring coccus *Dehalococcoides* (1). These bacteria may contribute to decomposition of difficultly degradable organic matters accumulated on deep sea floor. *Planctomycetes* have been often detected, and widespread in methane-seep (2), but the functional characters have been unknown. Recently, *Candidatus artibacteria* have been revealed to play significant role as symbiotrophic scavenger in artificial methanogenic bioreactor, by using single cell genome analysis (3). According to the study, *artibacteria* may support methanogen and *chloroflexi* through the by-product generation such as acetate, butyrate, and H_2 . These results implied that thermogenic and microbial mixed-derived gas composition may be formed by symbiotic metabolism of those species, but not simply generated from inorganic gases such as CO_2 and H_2 by methanogen.

1)Maymo-Gatell, X., Chien Y., Gossett J. M., Zinder S. H. (1997) Isolation of bacterium that reductively dechlorinates tetrachloroethane to ethane. *Science* 276: 1568-1571.

2)Yanagawa K., Kouduka M., Hachikubo A., Tomaru H., Suzuki Y. (2014) Distinct microbial communities thriving in gas hydrate-associated sediments from the eastern Japan Sea. *J Asia. Ear. Sci.* 90: 243-249.

3)Nobu M. K., Narihiro T., Rinke C., Kamagata Y., Tringe S. G., Woyke T. Liu W-T (2015) Microbial

dark matter ecogenomics reveals complex synergistic networks in a methanogenic bioreactor. ISME J. 9: 1710-1722.