Quantification of methanogenic potential in environemtnal samples

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A Ni porphinoid, coenzyme F430 is the prosthetic group of methyl-coenzyme M reductase, which catalyse a final step of methanogenic reactions in all methanogenic pathways including CO_2 resuction, acetate fermentation and methylotrophic pathyways[1]. F430 is unstable, hence native F430 will not be persisting in sediments through a geologic time scale. Given its unique structure, functionality and lability, F430 can be a robust biomarker for a quantitative estimation of *in situ* activities of living methanogens.

Conventionally F430 was analyzed photometrically using HPLC or off-line MS [2]. Recently we have developed a high sensitive detection of F430 by a HPLC-MS/MS. This sensitivity (femto mol level) is 10^4 to 10^5 times higher than the conventional photometric dection and only 10^2 to 10^4 methanogen cells are required for quantification of F430 in environmental samples. Our analyses indicated that concentration in paddy field ranges between 9870 and 253 fmol/g which is 1-2 orders of magnitude higher than those in marne sediments off Japan, ranging between 100 and 7 fmol/g, and 1910 fmol/g from Peru Margin (68 mbsf).

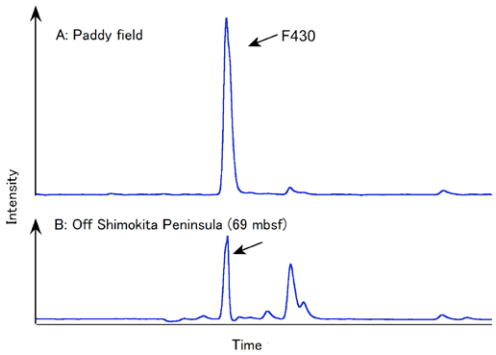

Fig. 1. HPLC-MS/MS chromatograms of derivatized F430 from a paddy field and marine sediment.

[1] Thauer (1998), *Microbiology* **144**, 2377-2406. [2] Takano, Kaneko, Kahnt, Imachi, Shima & Ohkouchi (2013), *Org. Geochem.*, in press.

Biomineralization of Strontianite ($SrCO_3$) by *Proteus mirabilis*

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Microbially induced mineralization of carbonate minerals has drawn much attention in recent decades because of its practical applications such as atmospheric CO_2 fixation through mineral carbonation and solid phase capture of toxic radionuclide or metal contaminants (i.e., ^{90}Sr, ^{60}Co and Cd). The objectives of this study were to investigate the potential for microbially induced precipitation of strontianite ($SrCO_3$) using microorganisms enriched from rhodoliths and to identify mineralogical characteristics of the bio-precipitates.

Carbonate forming microorganism (CFM) was enriched from rhodoliths and aerobically cultured at 25°C in D-1 medium containing 30 mM Sr-acetate. The microorganisms were analyzed by 16S rRNA gene DGGE analysis to confirm microbial diversity. Mineralogical characteristics of the bio-precipitates were determined by XRD, TEM/SEM-EDS analyses.

A 16S rRNA sequence analysis showed the CFM was mainly *Proteus micrailis* [1]. The growth of CFMs gradually increased for 16 days (OD_{600} = 2.613) and then decreased until 22 days (OD_{600} = 2.016) of incubation. Medium pH in the biotic group increased from acidic at first (pH = 5.3) to alkaline condition (pH = 8.6). The pH changes might induce favorable geochemical condition for Sr-carbonate precipitation. In the abiotic group, neither growth of microorganism nor changes in pH were detected.

The enriched microorganisms mediated the precipitates in D-1 medium containing 30 mM Sr-acetate. The bio-precipitates were identified strontianite ($SrCO_3$) by XRD analysis. SEM-EDS analyses showed that the precipitates were round in shape, and around 60 ㎛ in size, and composed of C, O and Sr. TEM-EDS analyses showed that the Sr-carbonate minerals were irregular in shape, 60~70 nm in size, and composed of C, O, and Sr.

These results indicate that the CFM induce the precipitation of strontianite ($SrCO_3$) by biological processes. Therefore, CFM such as *Proteus micrailis* may play one of important roles in Sr immobilization in Sr-contaminated water and CO_2 fixation in natural environments.

[1] Chimetto et al. (2011) Int. J. Syst. Evol. Microbiol. **60(1)**, 60-64.

DOI:10.1180/minmag.2013.077.5.11