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New Method for Cultivating Methanogenic Microorganisms - Providing Clues to Mechanism of Biogas Generation and Application Studies –

Introduction

Researchers of JAMSTEC, Nagaoka University of Technology, The University of Tokyo and Kochi Institute for Core Sample Research of JAMSTEC, have successfully cultivated and isolated diverse subseafloor microbes including methanogens (methane-producing microbes), using a continuous-flow bioreactor that was originally developed for wastewater treatment.

In this study, Methanogens belonging to the genera Methanobacterium and Methanosarcina were detected concomitantly with methane production, along with a variety of unknown bacterial and archaeal species (*1). The results are expected to provide clues, not only to clarifying microbial activity and carbon cycling in subseafloor sediments, but also to developing "green" technology to convert atmospheric carbon dioxide (CO2) into natural gas.

Their work will be published online in The ISME Journal on June 9.

Title: Cultivation of methanogenic community from subseafloor sediments using a continuous-flow bioreactor.

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Methods and Results:

To effectively activate seafloor microbes, researchers developed a new cultivation method using a traditional water treatment reactor. The incubation was carried out with cores collected from seabeds (water depth: 1.180 meters) off Hachinohe, northern Japan, during the shakedown expedition of the Deep-Sea Drilling Vessel Chikyu in 2006,
The results saw a successful enrichment and subsequent isolation of marine subseafloor microbes, including methanogens, which are known to be extremely difficult to culture in vitro.

1) The new bioreactor (Fig.1) was developed based on a down-flow hanging sponge (DHS) (*2) system used for water treatment. The hanging sponges serve as a habitat for microbial cells and their growth. To ensure the

activation of anaerobic sedimentary microbes (including methanogens), the reactor was filled with nitrogen. In addition, synthetic seawater supplemented with glucose, yeast extract, acetate and propionate as an energy source was fed in continuously, creating conditions similar to those found in seafloor sedimentary environments.

2) Polyurethane sponge-cubes were soaked with a mixture of the sediment slurry of the cores. During the incubation, effluent water and gas were sampled for analysis. After 289 days of incubation, methane was first observed in the gas phase of the reactor. The stable carbon isotope compositions of the detected methane showed evidence of microbial methane production.

3) To identify the microbial species cultivated in the DHS reactor, molecular analysis using the 16S rRNA gene (*3) was performed. The results showed the successful cultivation of diverse anaerobic microbes, predominant with the genera Methanobacterium and Methanosarcina. Uncultured bacterial and archaeal lineages were also detected.

4) Subsequent to the cultivation, researchers successfully isolated four methanogens and six bacteria in pure culture (Fig. 2). These include microbes only having been confirmed by genetic analysis, as well as some possibly novel species taxonomically.

Future perspectives
The newly developed incubation system will allow for cultivating subseafloor microbes, which had not hitherto been obtainable due to difficulty in cultivation. The system has demonstrated its extreme effectiveness in the quest for clarifying the functions of subseafloor microbial communities and their geochemical roles in carbon cycle. The success in the enrichment and isolation of microorganisms previously cultivated with conventional methods and thus poorly understood, will now allow for characterizing microbial metabolism and genetic properties. This could lead to the discovery of microbial and genetic resources valuable for environmental and genetic engineering, as well as applied studies in geobio-engineering and technology.

The detailed characteristics of methanogens cultivated in this study are expected to contribute to the understanding of mechanisms of natural gas generation, especially from methane hydrates - a priming fuel resource. The information also holds hopes for advancing "green innovation technology." This includes the establishment of a sustainable carbon cycle in the seafloor, where conceptually anthropogenic carbon dioxide would be artificially sequestrated below the seafloor and microbiologically converted to natural gas with the help of methanogens.

***1.Achaea**
All life on Earth belongs to three domains; Archaea, Bacteria and Eucarya. Methanogens are a member of archaea. Recent studies have indicated that archaea is living in marine sediments predominantly.

***2.Down-flow hanging sponge (DHS) bioreactor**
DHS bioreactor is an innovative system that provides inexpensive treatment of urban sewage. It was developed by Hideki Harada, Nagaoka University of Technology (now Professor of Tohoku University), and Akiyoshii Ohashi, Professor of Hiroshima University. Hanging sponges are used as artificial

habitat for microbes and exposed in the gas phase. It has the highest microbial retention ability among the existing wastewater treatment methods. In recent years, DHS reactors have been widely recognized internationally, emerging as the first Japanese water treatment technology commercially used both at home and abroad.

***3.16S ribosomal RNA (16S rRNA) gene**

16S rRNA gene is a component of the subunit of ribosome that forms the particular protein molecules based on gene sequences. Ribosome and RNA exist within all life forms. Ribosome RNA is relatively slow in evolution and thus its sequences are widely used for gene-based phylogenetic classification. The use of 16S rRNA is also common in estimating biomass of specific phylotypes.

Appendix

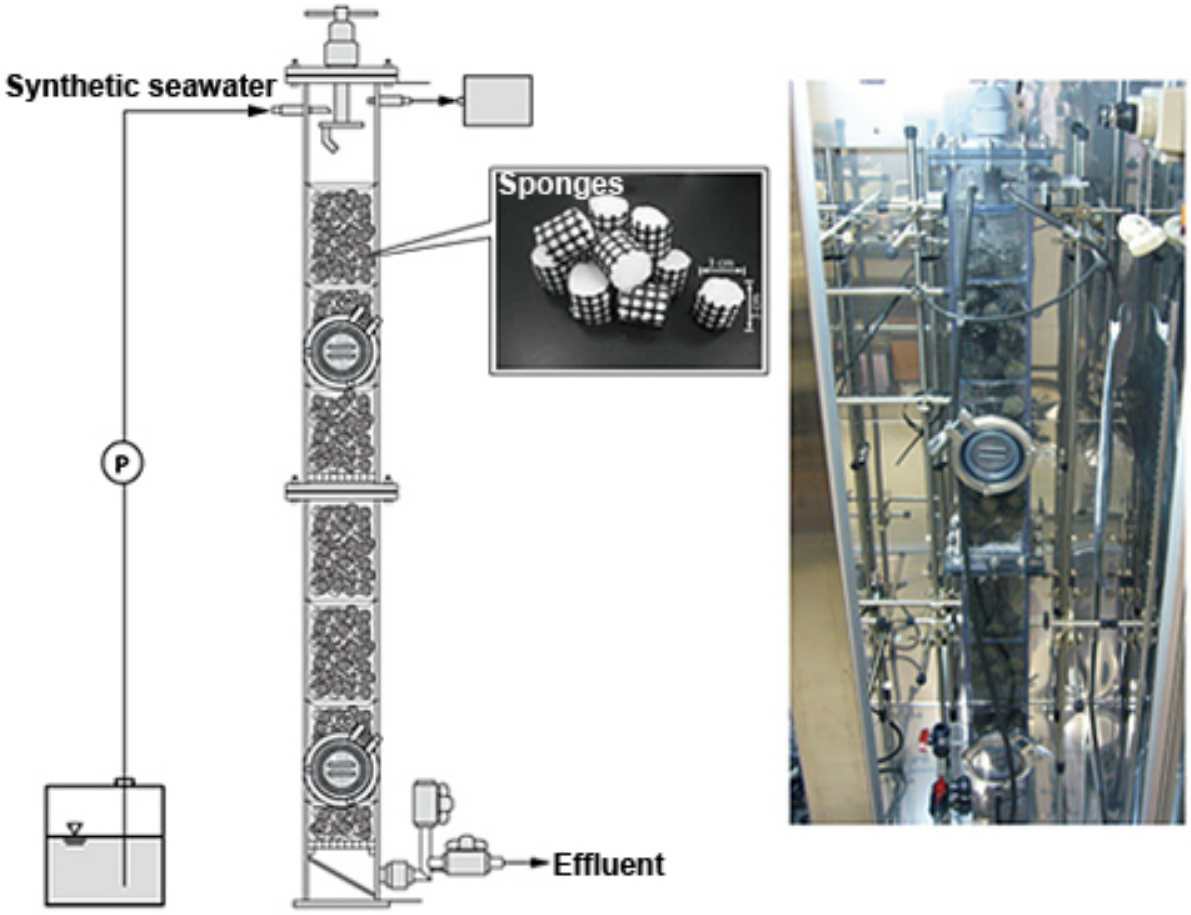

Figure 1. Newly developed DHS bioreactor

Schematic diagram (left) and a photo (right) of the system. Microbes are attached to the sponges, and synthetic seawater supplemented with nutrients is drained through them. This can create an environment similar to that of the subseafloor. Excrement of the microbes, which are an obstacle to microbial growth, can be removed with the effluent water.

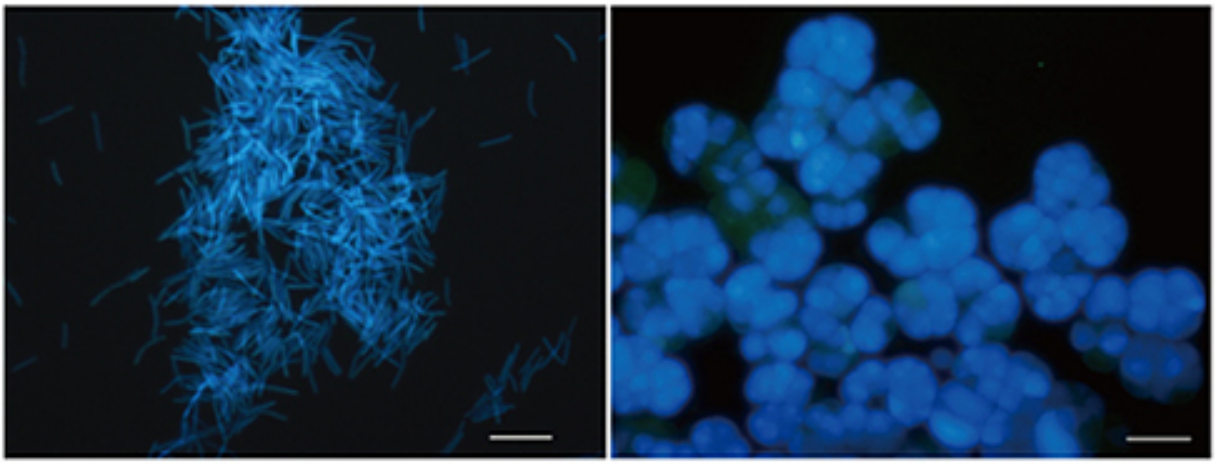

Figure 2: Fluorescence micrograph of methanogens obtained from the seafloor off Hachinohe: Mathanobacterium sp.(left) and Methanosarcina (right). Methanogens contain coenzyme F_{420}, which exhibits a blue-white fluorescence under near ultra violet light. The scale on the bottom right-hand corner is 10 micrometers.

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