# Report for the Joint Use/Research of the Institute for Planetary Materials, Okayama University

**2022 fiscal year first term / second term / others**

Day/Month/Year

**Category:**International Joint Research/ General Joint Research/ Joint Use of Facility/ Workshop

**Name of the research project:** Lipidomics of archaeal growth in serpentine environments

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**Collaborator**

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**Affiliated institution and department:** Institute for Planetary Materials, Okayama University at Misasa

**Research report:**

**Research purpose**

In order for the origin of life (such as methanogenic, hydrogenotrophic archaea) to have emerged from carbon dioxide, water, and rocks on the primitive Earth, a consistent, chemically transmutable source of energy was indispensable. Currently, the process of serpentinization is being recognized as a progressively plausible candidate for this energy source. Serpentinization of ultramafic crusts would have facilitated a constant supply of bioessential components, including hydrogen, methane, formate, and ammonia and metals in off-ridge alkaline hydrothermal springs. Life in serpentinizing systems is dominantly microbial, though it should be noted that this does not preclude eukaryotes. Nonetheless, bacterial and archaeal biomass in these systems massively outweighs other classifications. While surficial serpentinizing systems host a significantly higher biomass and cell abundance than subsurface systems, it is estimated that, in aggregate, subsurface microbial communities account for nearly 90% of all bacterial and archaeal biomass on Earth, as well as nearly 15% of our planet's total biomass. Prokaryotes in serpentinizing systems are dominantly hydrogen- and methane-cycling bacteria and archaea, alongside subordinate acetate-, iron-, and sulfate-cycling groups. Relevant eukaryotes include fungi, nematodes, and rotifers, though they are orders of magnitude less abundant than the prokaryotes (Bar-On, Phillips, and Milo 2018).

The overall goal of the project is thus to gain better understanding of mineral-microbe interactions in environments with active or inactive serpentinization.

The primary objective of this project is to discern lipids derived from methanogenic archaea within

serpentine rocks, with the aim of enhancing the precision of microbial community structure analysis on a macroscopic level in such environments.

The methodology employed in this study involves a comparative analysis of viable microorganisms, utilizing established DNA extraction and analytical techniques, alongside: (1) isolated lipids obtained from serpentine rocks and (2) purified lipids obtained from cultured methanogenic archaea that were isolated from the designated site of interest.

The model species used in this study is the type strain of *Methanobacterium oryzae*, first described by Joulian *et al.* (2000) and an isolated version of a similar (98% similarity) species. The methanogen was isolated from the target area, in the Chimaera serpentine rocks north of Antalya, Turkey. It is described as a non-motile, rod-shaped, obligate anaerobic, methanogenic archaea isolated from serpentinizing rice soil near Pila, Luzon, Philippines. Cells occur individually or in chains and range from 0.3-0.4 μm width and 3-10 μm length. *M. oryzae* lacks cytochromes—proteins involved with redox catalysis among anaerobes (Fauque and Barton 2012)—which enables their growth at extremely low $H_2$ pressure. Methanogens with cytochromes grow significantly faster under optimal conditions than those without, having as much as double growth yield, although they have higher $H_2$ pressure requirements (Neubeck et al. 2016). Thus, the *M. oryzae* is an ideal methanogen for studying survival in low-temperature serpentine environments.

**Preliminary and previous results**

Earlier findings have demonstrated the proficient extraction of lipids from serpentine material obtained from an active serpentinization locality located north of Antalya, Turkey, as documented by Rattray et al. (2022). Furthermore, Neubeck et al. (2017) have extensively investigated the community structure of microorganisms in the same region. Nevertheless, archaea typically occur in significantly lower abundance and are consequently challenging to detect and classify. In light of this, the lipid data acquired through the aforementioned investigation furnish a superior resolution and accuracy in the assessment of the microbial colonization of rocks.

Preliminary findings, from experiments using olivine alteration as the primary source of $H_2$ through low temperature serpentinization, have shown that methanogens may alter the olivine phase of the ultramafic rocks and grow on the alteration products, see plots below.

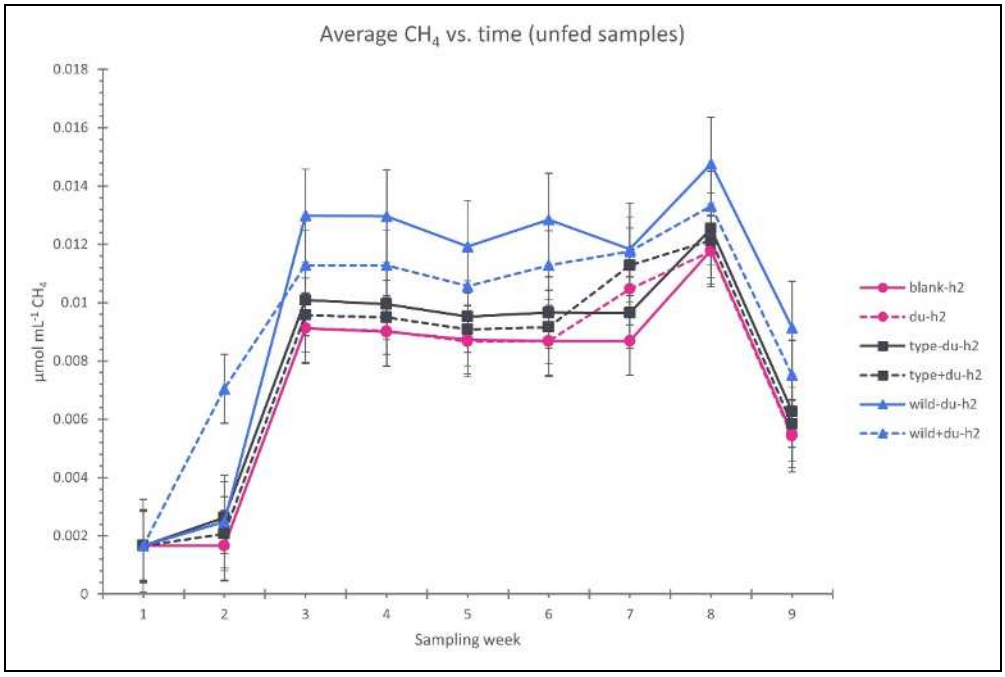
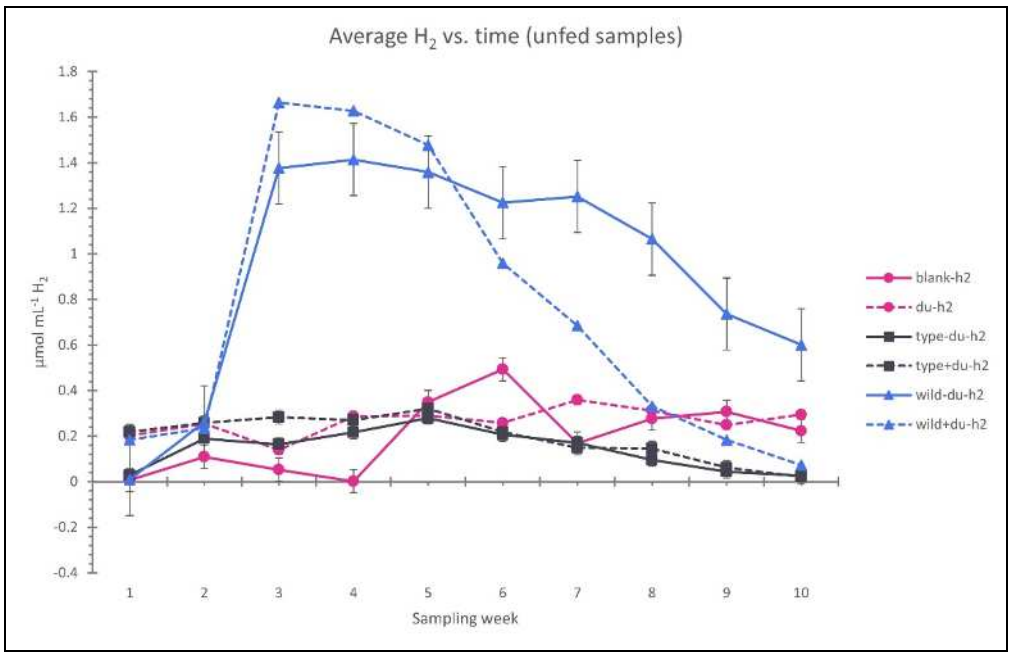



*Fig. 1.* Average (of triplicates) $CH_4$ gain in "unfed" samples (i.e., without added $H_2/CO_2$) over the experimental period. For treatment abbreviations, see Table 1.




**Actually Conducted Research**

So far, a method has been developed to extract standard lipid compounds using a modified bligh dyer extraction (Bligh & Dyer 1959; Boumann et al., 2006; Sturt et al., 2004). The standards have also been used to confirm the validity of an orbitrap based method to detect them (Rattray et al., 2022). In the future, the isolated culture of *M. oryzae* will be extracted and run to obtain the required data to complete the project. The data are expected to be obtained in the coming financial year and after data analysis will result in a publication.

**Expected research outcome**

The expected research outcome is an extensive understanding of the lipid structure and composition of the *M. oryzae* wild type and type strain, by using the methods and techniques described previously in Rattray et al. 2022. Archaea often have what is called an S-layer which is made of proteins that form a paracrystalline structure that is hydrophobic and is often referred to as a 'molecular sieve' (Schultze-Lam et al., 1996) (Figure 3). Most archaea seemingly have an S-layer, but a few are missing one. Several anionic groups on this layer render the surface charge negative and allows for interaction with cations. A few archaea, like some types of methanogens, may have additional cell wall structures that are similar to the peptidoglycan found in bacteria like, for example, pseudomurein (Meyer and Albers, 2020). It is often these cell wall structures that determine the extent, if any, of mineral precipitation onto the surface of the microorganism which directly affects their preservation potential in the rock record. Thus, we hope to identify the lipid composition of these both methanogens, which would aid in future identification of these species in the serpentine geological rock record.

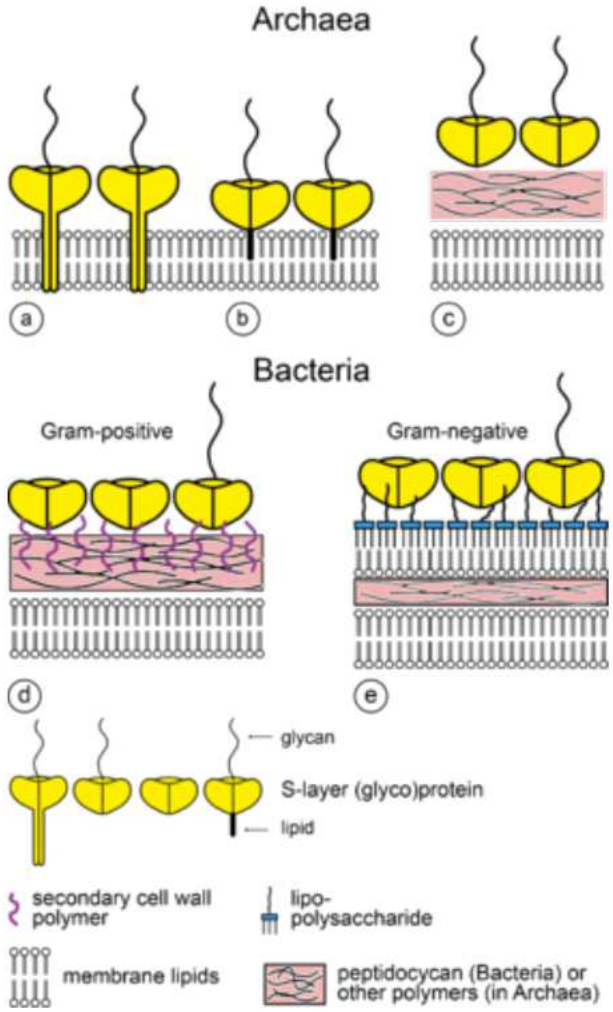



Figure 1. Cell wall structural differences between archaea (a-c), Gram-positive (d), and Gram-negative bacteria (e). The proteinaceous S-layer is often but not always present in archaea and bacteria, whereas a peptidoglycan layer is always found in bacteria, either thick and external (Gram-positive) to the membrane or thin and sandwiched between two membranes (Gram-negative).

Some archaea also have a pseudopeptidoglycan layer between the membrane and the external S-layer (Sleytr et al. 2014).

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