

## Hydrocarbon biomarkers in a thermophilic methanogenic archaea

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Previous studies on microbial lipids have shown that archaea synthesize only isoprenoids as structural components of their biomembranes. In particular, some species of methanogens are known to synthesize C<sub>25</sub> acyclic isoprenoid 2,6,10,15,19-pentamethylcosane (PMI). This compound has been regarded as a biomarker specific to methanogens, likely recording their activity in the host sediments. Occurrence of the compound in methanogens, however, has not been well understood. For example, Holzer *et al.* (1979) found unsaturated PMIs in *Methanothermobacter thermoautotrophicum* (unknown strain), a thermophilic methanogen. Risatti *et al.* (1984) reported that this methanogen (strain ΔH) indeed biosynthesizes C<sub>25</sub> isoprenoid alkenes with one to two double bonds but possesses the 2,6,10,14,18-pentamethyl-icosane carbon skeleton. To investigate the reason for these contradictory findings, we grew cells of the same strain (ΔH), and analyzed the lipid extract for acyclic isoprenoids.

The methanogen was grown at 55°C with H<sub>2</sub> and CO<sub>2</sub> (80:20, v/v) as energy and carbon sources. Whole cells were harvested by continuous centrifugation, and were immediately freeze-dried. Dry cells (60mg) were extracted ultrasonically with 50ml of methanol/dichloromethane (2:1, v/v), 50ml of methanol/dichloromethane (1:2, v/v), and twice with 50ml of dichloromethane. The extracts were combined and separated into apolar and polar fractions using column chromatography (silica gel) with *n*-hexane and methanol/chloroform (1:2, v/v) as eluents. The apolar fraction was analyzed by gas chromatography-mass spectrometry for identification.

In contrast to previous reports, neither PMI nor its unsaturated homologues were detected from the cell extract. The major hydrocarbons were 2,6,10,15,19,23-hexamethyl-tetracosenes (squalenes) with two to four double bonds. This result suggests that synthesis of PMIs by methanogens may depend on the culture conditions. To further examine this point, we will perform the experiments with the cells of methanogen being grown under different conditions, and/or harvested at different stages.

### References

- Holzer G., Oró J. and Tornabene T.G., (1979), *J. Chromatogr.* **186**, 795-809.  
Risatti J.B., Rowland S.J., Yon D.A. and Maxwell J.R., (1984), *Org. Geochem.* **6**, 93-103.

## Paleoceanographic change off central Japan since the last 150 ka

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The northwest Pacific Ocean, where the cold Oyashio Current and the warm Kuroshio Current meet, is an ideal area in the North Pacific for monitoring the migration of the western boundary currents in response to the past climatic changes. A giant piston core (MD012421, 45.82 m length) was recovered from 2,224 m water depths off central Japan during the IMAGES VII cruise in 2001. The oxygen isotope curves of benthic (*Uvigerina* and *Bulimina*) and a sub-surface dwelling planktonic foraminifera (*Globorotalia inflata*) are similar to the standard oxygen isotope curve for the last 150 ka. Whereas the oxygen isotope curve of a surface dwelling planktonic foraminifera (*Globigerina bulloides*) shows large fluctuations; lighter values during warm periods (MIS 1 and 5) and heavier values during cold periods (MIS 2 and 6) than those of *G. inflata*. This suggests that *G. bulloides* precipitated their tests in water depths shallower than 50 m in May during bloom seasons, whereas *G. inflata* precipitated their tests in a constant water temperature.

The past sea surface temperatures (SSTs) were calculated from the relationship between the present SSTs in May and the oxygen isotope differences of benthic-planktonic foraminiferal species (*Uvigerina* – *G. bulloides*). The calculated SSTs during the MIS 2 and 6 are about 10 degrees lower than present-day. This suggests that the water mass similar to the present surface water in the Oyashio Current region shifted southward by about 4.5 degrees in latitude during the MIS 2 and 6. Such a water mass movement is also supported by the following two phenomena; 1) parallel shifts in the oxygen and carbon isotopes of *G. bulloides* for the last 150 ka and 2) close carbon isotope values between *G. bulloides* and *G. inflata* during the MIS 2 and 6.