Analysis of archaeal intact polar membrane lipids in hydrothermal chimneys from Okinawa Trough

*Shunsuke Horai¹, Hiroshi Naraoka¹, Junichi Miyazaki²

1. Kyushu University, 2. JAMSTEC

Intact polar membrane lipids (IPLs) are suitable biomarkers for viable microorganisms because the polar head groups are hydrolyzed and removed from core lipids (CLs) soon after the cell death. Although IPLs analysis has been successfully applied in several environments, previous studies in deep-sea hydrothermal vents are sparse and have been restricted to a few hydrothermal fields. In this study we have performed the analysis of lipid compositions of active chimney fragments collected from the Yokosuka hydrothermal field on the Southern Okinawa Trough back-arc basin. We have newly developed a HPLC-high resolution mass spectrometry protocol for the simultaneous screening of IPLs and those degradation products (i.e. CLs) that enabled the detection of a wide range of lipid biomarkers in small and complex mixtures of samples.

A previous DNA sequence survey reported two archaeal strains, *Methanococcales* and *Thermoplasmatales* from the same samples from the Yokosuka hydrothermal field [1]. However, most IPLs detected in this study could be produced by (hyper-) thermophilic methanogens including *Methanococcales*. The IPLs originated from *Thermoplasmatales* were not detected but the CLs including cyclopentane-containing GDGTs were present. In addition, the detection of archaeol with phosphatidylcholine head group (PC-AR, Fig.1) in this study suggests the presence of another strain of hyperthermophilic methanogen *Methanopyrus kandleri* in the chimney.

These results indicate that the utility of IPL approach using the new simultaneous screening protocol is an effective technique to evaluate microbial activity in various samples.

[1] Miyazaki J., Kawagucci S., Makabe A., Kitada K., Torimoto J., Matsui Y., Tasumi E., Shibuya T., Nakamura K., Horai S., Sato S., Ishibashi J., Kanezaki H., Nakagawa S., Hirai M., Takaki Y., Okino K., Kayama H.W., Kumagai H., Chong C. (2017) Deepest and hottest hydrothermal activity in the Okinawa Trough: the Yokosuka site at Yaeyama Knoll. *R. Soc open sci.* **4**: 171570.

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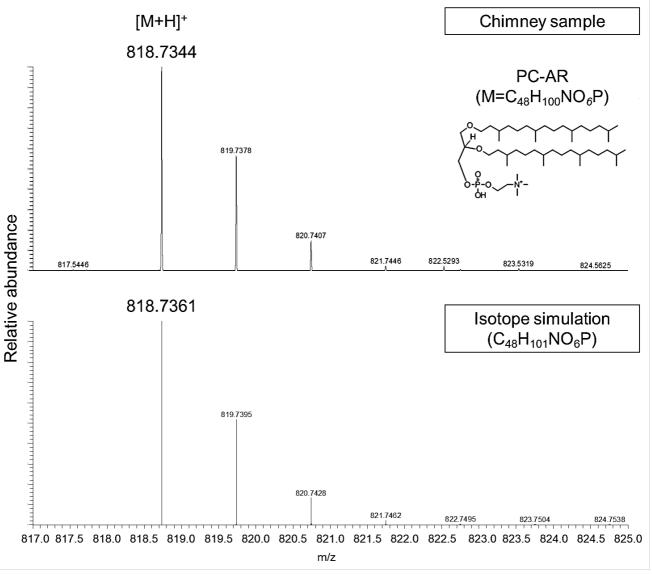


Fig. 1 Positive mode mass spectra of PC-AR detected in the chimney sample and simulated isotopic distribution pattern for $C_{48}H_{101}NO_6P$.