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Analysis of C_{20}-C_{25} isoprenoidal diether of halophilic archaea-lipid content changes in the incubation

TANOUE, Ryo[1], Noriaki YAMAUCHI[2*]

[1]Dept. Earth and Planetari Sci., Grad. School Sci., Kyushu Univ., [2]Dept. Earth and Planetari Sci., Fac. Sci., Kyushu Univ.

Archaea lives in a relatively harsh condition such as submarine hydrothermal vents (high temperature, low pH), swamp mud (anaerobic), and salt marsh (high salt concentration). Their living condition, especially the high temperature and low pH may be thought to resemble to the environment of primitive earth. It is settled for the third domain of life for 16SrRNA phylogenic tree. So, it is important microorganisms for the tools to imagine the first living organisms on the earth. Archaea has a characteristic lipid which core is consisted from the glycerol and saturated isoprenoid with ether linkage. Among them, halophilic archaea lives in a salt marsh, saltpan and the environment at the high salt concentration. The main lipid core is the C_{20}-C_{20} isoprenoid diether (called archaeol, (**1**)). And characteristic C_{20}-C_{25} diether (**2**)), rarely seen in other archaea, is also existed at the core. 2 have a possibility of the biomarker for hyper saline environment. Thus, the easy analytical method of **1**and **2**were investigated toward the possibility of biomarker for hypersaline environment to the evaluation of living halophilic archaea around coastal region. For the reevaluation of the C_{20}-C_{25} diether as a biomarker for a hypersaline environment, analytical methods of isoprenoidal lipid-core produced by the halophilic archaea by ESI-MS was conducted. Further, the compositional change for **1** and **2**, which simultaneously produced by the microorganism were observed by the two halophilic archaea species.

The lipid content of thermophilc archaea adopt the harsh condition to make the single-layer C_{40} tetraether lipid and, further, the five membered ring structure were formed inside of the isoprenoidal lipid core with higher temperature. (This phenomenon has been applied to create a new index, TEX_{86}.) Further, cryophilic archaea, which lives in an Antarctic salt lake, changes the lipid content toward increasing the number of unsaturation in archaea with low temperature. For our preliminary experiment, lipid content changes with the incubation condition were observed from hypersaline neutral *N. pallidum* JCM 8980. Thus, content change with the incubation condition (temperature, pH, salt concentration) for other species were observed.

Alkaline hypersaline archaea Natronomonas pharaonis JCM 8858 has similar optimum condition other than optimum pH (8.5). Lipid core changes about the several pH, salt concentration, and temperature (normal condition, 300 ml medium contains casamino acids 4.5g, sodium citrate 0.9g, glutamate 0.75g, magnesium sulfate 0.75 g, potassium chloride 0.6 g, sodium chloride 60 g) were observed. The microorganism was incubated for ordinal 12 days (at the beginning of the stationary phase) and lipid were extracted, hydrolyzed, and purified to give the lipid core. The ratio of **1** and **2** were determined with the ESI-MS at the negative ion mode with the $CHCl_3$-containing solvent system for the observation of chloride-adduct ion.

The growth ratio was almost the same without the low temperature condition observed significant growth bunting. The lipid content of *N. pharaonis* previously reported was consisted to our result. Further, temperature increasing caused increasing of the content of **2** with the hypersaline archaea. For the pH change, optimal pH tends to increase **2**, and other pH decrease the content of **2**. Salt concentration is not seemed to affect to the content of the lipid core. Usually, Salt tolerance of halophilic archaea has been secured and the nature of the protein, high potassium concentration in the cytoplasm. However, a change of the contents of the lipid core with the temperature change is the same as *N. pallidum*, which is considered to the adaptation to temperature change in halophilic archaea. Similar experiment for alkaline halophilic archaea *Natronobacterium gregoryi* will be reported.

Keywords: halophilic, archaea, biomarker, hypersaline environment