**RELATIVE CONTRIBUTION ESTIMATIONS OF METABOLIC PROCESSES IN MICROBIAL ECOSYSTEMS USING MULTIPLE ISOTOPIC ANALYSIS.** M. Nakagawa[1], G. Alexis[2], M. Julien[2], K. Yamada[3], and N. Yoshida[2,3], [1] Earth Life Science Institute, Tokyo Institute of Technology, Tokyo, Japan (mailing address; IE-09, 2-12-1 Ookayama, Meguro-ku, Tokyo, 152-8550 Japan, e-mail: nakagawa.m.ae@m.titech.ac.jp), [2] Earth Life Science Institute, Tokyo Institute of Technology, Tokyo, Japan, [3]School of Materials and Chemical Technology, Tokyo Institute of Technology, Kanagawa, Japan

**Introduction:** Lipid biomarkers have been used to estimate microbial community structures from sedimentary records [1,2]. In particular, the chemical and isotopic composition of fatty acids (FAs) provide a signature for the physiology and/or metabolism of organisms. The carbon (C) isotopic composition of FAs is linked to the biological pathways involved in carbon fixation and lipid synthesis because they are biosynthesized from acetyl coenzyme-A (acetyl CoA) monomers, that follows different pathways and fractionation factors for each carbon fixation pathway [3]. Hydrogen (H) isotopic compositions have been reported recently as a useful tool to identify central metabolic pathways and show different characteristics between microbial communities [4,5]. Moreover, Zhang et al. (2009) [6] reported that hydrogen isotopic fractionations between FAs and water revealed the primary nutritional group (heterotrophs, chemoautotrophs, or photoautotrophs). It follows that combined hydrogen and carbon isotopic analyses can lead to an identification of the metabolic activities associated with microbial communities.

Modern microbial mats at hot springs have been studied to get a better understanding of their prokaryote communities and evolution in the Precambrian era [7]. A phylogenetic analysis has revealed that thermophilic organisms found in thermal water environments appear on the deepest branches of the bacterial and archaeal domains [8]. We focused on different microbial community in hot spring microbial mats and estimated relative contributions of carbon metabolic processes with using C-H isotopic values of FAs.

We will also present progress of method development on lipid compounds which provide position specific analysis and perspectives for constructing new isotopic signatures of FAs PSIA.

**Samples and Mehods:**

*Compound isotopic analysis of FAs in hot spring-microbial mats.* Six microbial mats were sampled from different water temperature from 50℃ to 87℃. Microbial mat samples were freeze dried and saponified. After separation of neutral and acid fractions, the latter was esterified to convert FAs to FA methyl esters. FAs compositions and isotopic values were measured by gas chromatography (GC) and GC-combussion (C) or pyrolysis(Py)-isotope ratio mass spectorometer (IRMS)

*Method development of position specific isotopic analysis of FAs* Several FAME standards were measured by Py-GC-C-IRMS. The pyrolysis condition and efficiency on FAs were investigated.

**Results and Discussions:** Various C number FAs from C14:0 to C21:0 were found in microbial mats. The carbon and hydrogen isotopic delta values ($\delta^{13}C$ and $\delta^{2}H$) were varied from −25.5‰ to +5.6‰ and from −460‰ to −92‰, respectively. These isotopic variations were provided by mixing of some autotrophic C processes (reductive acetyl CoA pathway, reverse TCA cycle. 3-hydroxy propionate cycle, and Calvin cycle) and heterotrophic processes. Isotopic mass balance calculation with the regulation of characteristic FAs information provide relative contributions of each metabolic processes on total FAs. The results showed the main autotrophic C processes gradually shift from chemoautotrophic, anoxygenic photosynthetic, and oxygenic photosynthetic processes as the temperature decrease.

For the PSIA method development proceeded to be able to measure different positions in FAME estimated by the pyrolyzed peaks of methane, $CO_2$, ethane, and so on.

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