

## MEMBRANE LIPIDS OF THE *THERMOTOGA* SPECIES AS INDICATORS FOR BACTERIAL ANAEROBIC OXIDATION OF METHANE

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In the marine environment, anaerobic oxidation of methane (AOM) is the most important sink for methane. Consortia of two archaeal lineages (ANME-1 and ANME-2), and sulphate-reducing bacteria of the *Desulfosarcina/Desulfococcus* branch of the  $\delta$ -proteobacteria are known to be involved in AOM (Boetius *et al.*, 2000, Orphan *et al.*, 2002). Recently, two *Thermotoga* species from the order *Thermotogales*, have been found to mediate AOM coupled to sulphate reduction in cultures (Balk *et al.*, unpublished results). The order *Thermotogales* can be found in various environments, such as continental solfatara springs of low salinity, shallow and deep-sea hydrothermal systems and high-temperature continental and marine oil reservoirs. As revealed by 16s rRNA phylogeny, they occur at the root of the tree of life, and represent a very deep branching lineage in Bacterial Kingdom (Woese, 1987). The aim of this study is to identify specific lipids of the order *Thermotogales* that might serve as biomarkers and to investigate lipid <sup>13</sup>C content in order to relate it to AOM.

GC and GC-MS analysis of the lipid extracts of *Thermotoga* cultures revealed the presence of the long chain dicarboxylic acids (15,16-dimethyltriacontanedioic acid and 13,14-dimethyloctacosanedioic acid), glycerol monoesters with both lower molecular weight chains (1-O-hexadecyl glycerol and 1-O-tetradecyl glycerol) and higher molecular weight chains (15,16-dimethyl-30-glyceryloxytriacontanoic acid and 13,14-dimethyl-28-glyceryloxy-octacosanoic acid), glycerol dialkyl diesters and glycerol mixed ester/ethers in the extracts. Using the method developed for analysis of archaeal glycerol dialkyl glycerol tetraethers by high performance liquid chromatography coupled with atmospheric pressure chemical ionisation mass spectrometry (HPLC-APCI/MS) (Hopmans *et al.*, 2000), unique, membrane-spanning glycerol dialkyl glycerol mixed tetraethers/esters of the *Thermotoga* strains were identified (see Fig. 1). After base hydrolysis of the cell residue after lipid extraction, long chain diacids and complex monoethers were also identified in substantial amounts. This suggests that they are building blocks for glycerol dialkyl glycerol mixed tetraethers/esters. Membrane-spanning lipids are considered to be an adaptation for high-temperature environments, and, as the branched glycerol dialkyl glycerol mixed tetraethers/esters seem to

be unique for *Thermotoga* species, they can be used as specific biomarkers.

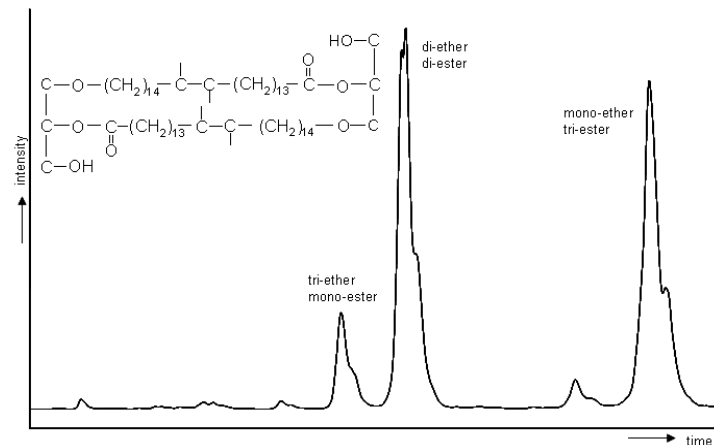


Figure 1. Base peak ion chromatogram of HPLC/APCI-MS analysis of the lipid extract of *Thermotoga maritima*, showing its characteristic glycerol dialkyl glycerol mixed tetraethers/esters.

We will now examine *Thermotoga* cultures grown with specific substrates, such as labelled methane, and different sugars, as potential carbon sources, for the presence of the membrane-spanning lipids using HPLC-APCI/MS. These lipids will then be analysed for their  $^{13}\text{C}$  content. So far, methanotrophs, both aerobic and anaerobic, could be detected by the presence of specific, carbon isotopically depleted lipids. Pilot studies using labelled  $^{13}\text{CH}_4$ , however, have shown no incorporation of the labelled  $^{13}\text{C}$  in the lipids of the *Thermotoga* species, in contrast to strongly labelled carbon dioxide, produced by the oxidation of methane, indicating that these bacteria need another source of carbon during AOM. Isolated lipids will be tested for their  $^{13}\text{C}$  content to confirm these preliminary observations. Results obtained from these experiments will shed some light on AOM mediated by bacteria in culture and in the natural environment.

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