Lipid biomarkers for methanogens in hypersaline cyanobacterial mats from Guerrero Negro, Baja California Sur

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Analyses of sediments from the vicinity of active methane seeps have uncovered a particular suite of lipid biomarker patterns that characterize methane consuming archaea and their syntrophic, sulfate reducing partners. These isoprenoid biomarkers, largely identified by their anomalously light carbon isotopic signatures, have been a topic of intense research activity and are recorded in numerous methane-rich environments from Holocene to Cenozoic. This phenomenon has implications for depleted kerogens at 2.7 Ga on early Earth (Hinrichs 2002). In contrast, the lipid biosignatures of methane producing archaea are not readily identified through distinct isotopic labels and have received comparably little attention in analyses of archaea in environmental samples. Indeed, environmental analyses generally detect only free archaeal lipids, not the intact, polar molecules found in the membrane of living organisms. As part of the Ames NAI, the ‘Early Microbial Ecosystem Research Group’ (EMERG) is working to understand microbial processes in the hypersaline cyanobacterial mats growing in the salt evaporation ponds of the Exportadora de Sal at Guerrero Negro, Baja California Sur, Mexico. The aim of this study was to develop methods by which we could identify the organisms responsible for methane generation in this environment. While the ester-bound fatty acids, hopanoids and wax esters provide a means to identify most of the bacterial components of these mats, the archaea which are evidently present through genomic
assays and the fact of intense methane production (Hoehler et al. 2001), have not been identified through their corresponding lipid signatures.

Archaeal core lipids present a number of analytical challenges. The core lipids of methanogens comprise C_{20}, C_{40} and sometimes C_{25} isoprenoid chains, linked through ether bonds to glycerol. As well as archaeol (C_{20}), sn-2- and sn-3-hydroxyarchaeol are associated particularly with methylotrophic methanogens. Recently, we have also identified a dihydroxyarchaeol in a hyperthermophilic methanogen (Summons et al. 2002). Additional structural diversity is encoded into the polar head groups that are attached to the glycerol ether cores. The C_{20} core lipids are readily analyzed by GC-MS as their volatile trimethylsilyl derivatives while compounds with intact polar head groups can only be detected using LC-MS approaches. Our approach was to utilize the alternative of an ether cleavage reagent (BBr_{3} vs. HI) and a hydride reducing agent to convert all ether lipids to hydrocarbon in order to provide a vertical profile of quantitative information that might be matched to methane fluxes. We have found that while conventional acid hydrolysis and HI treatment will destroy hydroxyarchaeols, molecular information remains intact through use of BBr_{3} for ether cleavage. This method revealed the presence of traces of biphytane and various ether alkyls associated with some sulfate reducing bacteria within the mat structure. An interesting, and potentially valuable, byproduct of the method utilizing HI was the identification of abundant homohopanoids after superhydride reduction. Evidently present as sulfur-bound diagenetic products these hopanoids are likely cyanobacterial biomarkers in the early stages of diagenetic preservation.

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References: