

# Heterogeneity Of Anaerobic Methane-Oxidizing Archaeal Communities in the Mediterranean Inferred from Lipid Distributions and Carbon Isotopic Compositions

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Controls on methane production and consumption are important concerns in the evaluation of past and future climate change. Of particular interest are marine sediments, in which methane can be generated but only small percentages actually flux into the water column. To study dynamics and consequences of methane release from marine settings, we initiated a multi-disciplinary study of mud volcanism of the Eastern Mediterranean Ridge. This program was conducted during two cruises, one of which utilized the French submersible, *Nautilie* to sample diverse settings. Observations from the *Nautilie* revealed on all mud volcanoes active biological communities which consisted of microbial mats and macrofauna such as clams, mussels, tube worms, sponges, urchins, crabs, and fishes. These communities are likely supported by microbial production maintained by the flow of reduced compounds - especially methane - from mud flows. Indeed, pore water profiles suggest that both sulfide oxidation and anaerobic methane oxidation coupled to sulfate reduction occur in these sediments.

However, it is difficult to correlate geochemical data with biological processes, and we performed a molecular biogeochemical investigation to determine the nature of methane-consuming micro-organisms in these settings. Our work confirmed that anaerobic oxidation of methane in mud volcano sediments is mediated by consortia of archaea and bacteria (Pancost et al., 2000). Even though methane diffuses upward from underlying mud volcano sediments, the dominant biomarkers in seeps are specific for nominally methanogenic archaea rather than methanotrophic bacteria. Moreover, methanogen biomarkers are highly depleted in <sup>13</sup>C indicating that methane is the source of carbon for these organisms. Both observations are consistent with proposals that anaerobic methane oxidation is mediated by methanogens operating in reverse. And similarly low  $\delta^{13}\text{C}$  values of co-occurring biomarkers for sulfate-reducing bacteria and chemo-organotrophs confirm that a consortium of prokaryotes is responsible for anaerobic methane oxidation (Hoehler et al., 1994). These observations support and expand on previous observations (Hinrichs et al., 1999; Elvert et al., 1999; Thiel et al., 1999).

Because of sampling via submersible, we could compare lipid distributions amongst diverse settings (mud flows, seeps, brines). Archaeal lipids are all depleted in <sup>13</sup>C but are much more diverse than previously reported, with the following compound

classes represented: isoprenoid diethers (archaeol, hydroxyarchaeol); diverse dibiphytane tetraethers; and saturated and unsaturated irregular isoprenoids (pentamethylcosane [PMI] and crocetane). Some of the compounds observed are novel while others have been previously reported in such settings. At every site archaeol, PMI, and three specific dibiphytane tetraethers are present and this pattern could serve as a useful tool for identifying anaerobic methane oxidation in other settings.

However, the relative abundances of these and other compounds is highly variable. For example, a Milano mud volcano seep predominantly contains unsaturated PMI compounds and no hydroxyarchaeol, while in a Napoli mud volcano seep, hydroxyarchaeol is the second most abundant compound and only a small quantity of a single tetraunsaturated PMI is present. Other sources of variation include the distributions of unsaturated PMIs, the relative abundances of *sn*-3- and *sn*-2-hydroxyarchaeols, and the distributions of dibiphytane tetraethers.

Further insight into the heterogeneity of archaeal communities is provided by archaeal biomarker  $\delta^{13}\text{C}$  values. Although negative  $\delta^{13}\text{C}$  values indicate that <sup>13</sup>C-depleted methane served as the ultimate carbon source for archaeal lipids, the values were highly variable. For example, at the Amsterdam seep, archaeal lipid  $\delta^{13}\text{C}$  values range from -61.7 to -107 per mil and at the Milano seep, they vary from -66.9 to -92.6 per mil. Although the controls on archaeal lipid  $\delta^{13}\text{C}$  values are poorly understood, such large differences suggest that the lipids derive from distinct organisms. The variability in archaeal lipid distributions and  $\delta^{13}\text{C}$  values indicate that methane-oxidation is mediated by multiple archaea species.

Our observations provide insights into the study of life in extreme environments. The diversity and heterogeneity of archaea assemblages indicates that the capacity for anaerobic methane oxidation is not limited to a single species and helps explain why this process is observed in diverse settings. The variable distributions of archaeal membrane lipids also provide a mechanism for these organisms to adapt to a wide range of extreme conditions. Finally, the diversity of lipids present in these settings and the occurrence of novel lipids support the contention that medically or industrially useful compounds could be discovered in extreme settings.