Molecular fingerprinting of methanogens in eastern Mediterranean Sea mud volcanoes

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The occurrence of methanogenic microorganisms was investigated at the Amsterdam and Kazan mud volcanoes, eastern Mediterranean Sea, by PCR amplification of the methyl co-enzyme reductase A (*mcrA*) gene. Subcores were obtained from box cores recovered with the R/V AEGAEO in May 2003. Sediment was sampled from the surface down to 40 cm below sea floor (cmbsf) with a resolution of 5 cm. The concentrations of methane, sulfate, and sulfide were determined at the same sediment layers. Although hydrate was only observed at Kazan MV (very tiny crystals), the geochemical composition of both cores showed evidence for gas hydrate occurrence (salinity as low as 12 per mill due to the dissociation upon recovery). The vertical profiles of methane and sulfate showed that anoxic oxidation of methane takes place between 5 and 15 cmbsf but *mcrA* was found only in Kazan MV at 15 and 20 cmbsf, making possible the existence of biogenic methane in this mud volcano. Sequencing of the amplified *mcrA* revealed that the closest relatives of these sequences were related to uncultured methanogenic Archaea from other methane hydrate bearing sediments from the Cascadia Margin and the Kuroshima Knoll of the orders Methanosarcinales and Methanobacteriales.

Carbon isotopic characterization of *Archaea* inhabiting deeply buried sulfate/methane transition zones

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The deep marine biosphere is thought to contain abundant microbial inhabitants, estimated to be a tenth of the Earth's total biomass. Sediments from this environment were recovered during Ocean Drilling Program (ODP) Leg 201 (D'Hondt et al., 2003), and were analyzed by both molecular biological and organic geochemical techniques. Of particular interest in these sediments were four sulfate/methane transition zones (SMTZ) seen at ODP Sites 1227, 1229 and 1230, two of which coincided with strongly elevated cell counts (D'Hondt et al., 2003). Archaeal cells in these zones were analyzed for abundance and $\delta^{13}C$ composition by both whole cell analysis (FISH-SIMS) and intact membrane lipids (HPLC-ESI-MS^n). Fluorescent in-situ hybridization cell counts showed more archaeal cells than bacterial, which was reflected by intact membrane lipid abundance. Isotopic compositions by both techniques (often around -20‰) suggest that methane is not an important carbon source for these cells. Autotrophic carbon fixation appears to be an unlikely metabolism given the relationship between the isotopic composition of DIC and archaeal biomass. The isotopic evidence suggests that the bulk archaeal community is heterotrophic, possibly mediating the oxidation of methane without consuming it as a carbon source. This novel information about the metabolism of uncultivated deeply buried *Archaea* raises interesting new questions regarding the significance of the elevated cell counts and archaeal activity at SMTZs in deep marine sediments.

Reference

D'Hondt, S., Jørgensen, B.B., et al. 2003. *Proc. ODP, Init. Repts. 201*. ODP, College Station, TX.