



A novel association of methanotrophic archaea and bacteria in a cold seepage location: significance of aerobic methane utilization

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The Nile deep-sea fan (NDSF) is a recently discovered gas seepage area representing a thick, up to 10 km, sedimentary body formed since the Late Miocene due to the activity of the Nile River (Loncke and Mascle, 2004). Besides hydrocarbon gases, the area is known for brine fluid emissions originated from almost 2 km thick Messinian evaporates deposited below the NDSF.

A novel association of lipid biomarkers derived from methanotrophic archaea and bacteria was found within the “iron” and “sulfur” mats discovered in the Chefren mud volcano (MV) during the NAUTINIL cruise in 2003 of the R/V L’Atlante. The Chefren MV is located in the Western sector of the NDSF in interior of the Menes Caldera structure at a water depth of 3000 meters. This setting is known to discharge methane and brine fluids at a temperature of ca. 50°C. We examined vertical profiles of lipid biomarker distributions from sediments collected from the “iron” and “sulfur” mat sites to learn more about microbes in these extreme ecosystems and their location within the uppermost 20 cm. Both “iron” and “sulfur” mats show the abundance of ¹³C-depleted lipid biomarkers related to archaea (hydroxyarchaeol, glycerol dibiphytanyl glycerol tetraethers) performing anaerobic oxidation of methane (AOM). Together with archaeal lipid biomarkers, the uppermost 8 cm possess ¹³C-depleted diplopterol, a marker for aerobic methanotrophy. The presence of methylotrophs was revealed by the occurrence of specific ¹³C-depleted sterols previously found in cultures of aerobic methanotrophic bacteria *Methylococcus capsulatus* (Bird et al., 1971) and *Methylosphaera hansonii* (Schouten, et al., 2000). The presence of

bacteria closely related to these methanotrophs was confirmed by analysis of bacterial 16S rDNA from the same intervals. In fact, sterols and diplopterol were detected above the layer of sediment displaying considerable AOM. This indicates that methane reaches oxygen-bearing sedimentary intervals favorable for anaerobic methanotrophs fueling aerobic methane consumption.

The bottom water in the Eastern Mediterranean contains enough oxygen to nourish aerobic respiration of methane. Marine aerobic methanotrophs are known to consume methane at oxygen concentrations as low as 6.3×10^{-3} mM, to inhabit oxic-anoxic transitions zone, and to outlast anoxic periods or deficiency of methane. Hence, our data show that in addition to anaerobic methanotrophs, aerobic methanotrophs can also act as a “microbial barrier” preventing methane escape into the water column.

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