

CLOSE VICINITY OF AEROBIC AND ANAEROBIC METHANOTROPHS IN SURFACE SEDIMENTS OF HÅKON MOSBY MUD VOLCANO

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Håkon Mosby Mud Volcano (HMMV) is an active methane-seeping mud volcano at 1250 m water depth on the Norwegian margin of the Barents Sea (72°00'N, 14°45' E). Recently, Niemann et al. (2006) detected novel microbial communities from two surface habitats (Center, *Beggiatoa* mats), which are functioning in different methanotrophic modes: (1) aerobic methanotrophic γ -proteobacteria of the orders *Methylococcales* and *Methylophaga* at the Center, with the former clearly dominating, and (2) anaerobic methanotrophic archaea (ANME-3) closely-associated with a sulfate-reducing bacterial partner (*Desulfobulbus* spp. - DBB) at the *Beggiatoa* mats. The biomarker inventory at the Center habitat is dominated by C16:1 ω 9c and C16:1 ω 8c fatty acids indicating type I methanotrophs; the surrounding anaerobic ANME-3/DBB consortium is characterized by specific C16:1 ω 5c and C17:1 ω 6c fatty acids, irregular isoprenoids (PMI:4, PMI:5), and glycerol diether lipids (archaeol, hydroxyarchaeol). However, 16S rDNA clone sequence analyses and cell counting via fluorescence *in situ* hybridization (FISH) performed on the *Beggiatoa* mat samples show that aerobic methanotrophs of the order *Methylococcales* are also present in considerable amounts (33 out of 132 clones, cell numbers of up to $3.9 \pm 2.5 \times 10^8$ cells cm⁻³; Lösekann et al., in prep.).

Here we report substantial abundances of a suite of five steroids (three 4-methylated sterols and two 4-methylated steroid diols) and three hopanoids (diploptene, diplopterol, and a hopenol with a diplopterol structure containing one double bond in ring A or B) which we found in concert with the ANME-3/DBB consortium at the surface sediment of the *Beggiatoa* mats. All of these steroidal and hopanoidal biomarkers, some of which have not been described before, are substantially depleted in ¹³C ($\delta^{13}\text{C}$: -79 to -70‰ VPDB), proving their biosynthesis from an organism involved in methane consumption. The presence of steroids as membrane lipids is usually restricted to the kingdom of *Eukarya* but has been reported from aerobic methanotrophic bacteria such as *Methylococcus capsulatus* (Bouvier et al., 1976) and *Methylosphaera hansonii* (Schouten et al., 2000) which are both closely-related to the dominant *Methylococcales* types detected here. This finding suggest a spatially close vicinity

of aerobic and anaerobic methanotrophic communities and can be regarded as a recent analogue of what has been similarly described by Birgel et al. (2006) from an ancient Late Cretaceous methane seep environment.

The potential of aerobic methanotrophs to grow at very low oxygen levels (down to 0.1%) has been shown (Jahnke and Nichols, 1986). *In situ* oxygen penetrates ca. 1 mm at the *Beggiatoa* mats of HMMV (de Beer et al., 2006), and therefore might explain the simultaneous presence of both modes of methane oxidation. Whereas 4-methylated sterols were also detected in lesser concentrations at the Center habitat, the presence of additional and new biomarkers for aerobic methanotrophy (4-methylated steroid diols, diplopterol and hopenol) seems to indicate an adaptation of *Methylococcales* types to the enhanced environmental stress at the *Beggiatoa* mats.

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