

# Aerobic methanotrophy in North Sea sediment and Baltic Sea water column as revealed by bacteriohopanepolyol lipids and genomic approaches

D. Rush, L. Villanueva, M.T.J. van der Meer, E.C. Hopmans, J.S. Sinninghe Damsté

Aerobic methane oxidation (AMO) removes methane (CH<sub>4</sub>), a potent greenhouse gas, from the marine system. Global estimates show that aerobic methanotrophs oxidize a significant amount of CH<sub>4</sub> in the world's oceans. AMO communities have been observed responding to rapid surges in CH<sub>4</sub> (Kessler et al., 2011) as well as maintaining constant CH<sub>4</sub> oxidation in environments where the presence of CH<sub>4</sub> is more stable (Loesekann et al., 2007). This highlights their role in regulating CH<sub>4</sub> emissions. It is of interest to trace AMO during times in Earth's history when CH<sub>4</sub> cycling was more pronounced than today in order to better predict how methanotrophy will respond to future shifts in CH<sub>4</sub> concentration.

It is, however, unclear which microbes are responsible for marine AMO, and what lipid biomarkers we can use as tracers for past events. The structure of the AMO community in modern marine systems remains relatively unknown; few marine aerobic methanotrophs have been isolated in pure culture. Additionally, our recent investigation of bacteriohopanepolyol (BHP) lipids in CH<sub>4</sub>-influence marine systems showed that aminopentol, once considered source-specific to all Type I methanotrophs, is not a universal biomarker for marine AMO. Novel methylcarbamate bacteriohopanepolyols appear to be better biomarker candidates for marine AMO (Rush et al., 2016).

Here, we studied the water column of the Baltic Sea at two sites (Arkona Basin and Gotland Deep) and the sediment of a cold CH<sub>4</sub> seep in the North Sea (Doggerbank) to elucidate the lipid fingerprint of the local aerobic methanotrophic communities. There is a natural flux of CH<sub>4</sub> in the North Sea sediment, whereas the seasonal anoxia in the Baltic Sea has intensified over the Anthropocene, causing a positive flux of CH<sub>4</sub> to the atmosphere. This makes these two locations ideal to study methanotroph community shifts.

Water column suspended particulate matter collected at 42 m water depth at Arkona Basin, and at 70 m at Gotland Deep, respectively, was used to set up microcosm experiments with variable temperature, pH, and methane ( $\delta^{13}\text{CH}_4$ ) concentration. The same experimental set-up was applied to the oxic top 2 cm of Doggerbank sediment. We monitored CH<sub>4</sub> concentration in these microcosms as an indication of CH<sub>4</sub> consumption by methanotrophs. Once CH<sub>4</sub> concentration dropped below 0.1%, incubations were terminated. Genomic analysis of the communities that grew under the different microcosm conditions revealed that in the North Sea microorganisms belonging to the Marine Methylotrophic Group 2 thrived at high pH (pH

9) and high temperature (30°C). In the Baltic microcosm experiments, we observed increased abundance of Marine Methylotrophic Group 1 at low CH<sub>4</sub> concentration (0.5 and 1% amended). The lipid inventories of these microcosms were screened using ultra high performance liquid chromatography (UHPLC)-high resolution MS, focusing specifically on amino-BHPs. In order to link potential biomarker lipids to methane oxidizer groups, we will trace isotopically heavy <sup>13</sup>C both in the DNA and lipids of the organisms oxidising CH<sub>4</sub>.

## References

- Kessler JD, Valentine DL, Redmond MC, Du M, Chan EW, Mendes SD, et al. (2011) A persistent oxygen anomaly reveals the fate of spilled methane in the deep Gulf of Mexico. *Science* 331: 312±315. doi: 10.1126/science.1199697 PMID: 21212320
- Loesekann T, Knittel K, Nadalig T, Fuchs B, Niemann H, Boetius A, et al. (2007) Diversity and abundance of aerobic and anaerobic methane oxidizers at the Haakon Mosby mud volcano, Barents Sea. *Appl Environ Microbiol* 73: 3348±3362. doi: 10.1128/AEM.00016-07 PMID: 17369343
- Rush D, Osborne KA, Birgel D, Kappler A, Hirayama H, Peckmann J, et al. (2016) The Bacteriohopanepolyol Inventory of Novel Aerobic Methane Oxidising Bacteria Reveals New Biomarker Signatures of Aerobic Methanotrophy in Marine Systems. *PLoS ONE* 11(11): e0165635. doi:10.1371/journal.pone.0165635