

Quantification of novel methanogens and anaerobic methanotrophs in marine sediments of the Helgoland mud area, North Sea

AJINKYA C. KULKARNI^{1*}, OLUWATOBI E. ONI¹, ANNIKA SCHNAKENBERG¹, INES HESSE¹, HALELUYA WAMI¹, AND MICHAEL W. FRIEDRICH¹

¹Microbial Ecophysiology group, University of Bremen, Leobener Str. 3, 28359, Bremen, Germany.

(*correspondence: s_m48zy9@uni-bremen.de)

Methanogens and anaerobic methanotrophs (ANME) have been widely studied in marine sediments with an emphasis on their role in methane cycling and anaerobic oxidation of methane (AOM) respectively. Next generation sequencing approaches have provided insights into the presence of new species within the *Euryarchaeota*, such as the *Methanomassiliicoccus* like group (MLG) [1] and the ANME-1 related/ group g-h [2]. Existing *mcrA* gene (encoding methyl coenzyme M reductase alpha subunit, a key enzyme involved in methane cycling) primers either lack coverage of these new groups or yield shorter length amplicons for the same. Here, we designed new cloning and quantitative PCR (qPCR) primers targeting the *mcrA* gene, which allow us to quantify MLG and ANME-1 related groups previously detected in marine sediments of the Helgoland mud area (HMA) in the North Sea [3]. MLG were dominant in and around the sulphate-methane transition zone (SMTZ, 26-156 cmbsf) wherein their numbers were $\sim 0.15\text{-}1.32 \times 10^6$ gene copies/g wet sediment. On the other hand, the ANME-1 related members showed high gene copy numbers throughout the sediment, ranging from 1.21 to 3.87×10^6 gene copies/g wet sediment. Thus, with the newly developed primers and qPCR assays, quantification of these novel groups of archaea will allow obtaining detailed insights into their abundance and thus their relevance for biogeochemical processes such as methanogenesis and AOM in sedimentary environments.

[1] Zhou *et al.* (2014), *Frontiers in Microbiology* 5: 789. [2] Takeuchi *et al.* (2011), *Environmental Microbiology* 13(12), 3206-3218. [3] Oni *et al.* (2015a), *Frontiers in Microbiology* 6: 365