TITLE: TRIMETHYLAMINE AND METHANOL AS CARBON SOURCES FOR METHANOGENIC ARCHAEA FROM SEDIMENT AT THE VICINITIES OF WANDA GLACIER, ANTARCTIC PENINSULA.

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In marine environments, where sulfate is abundant, non-competitive substrates as methanol and methylamines are commonly used by methanogenic archaea. Until recently, it was believed that only members from *Methanosarcinaceae* family were able to use methanol and methylated amines as substrate, but members from the newly discovered order *Methanomassiliicoccales* were found to use methanol or trimethylamine in the presence of H_2. Marine sediments in the vicinites of Wanda Glacier (King George Island, Antarctic Peninsula) are a potential hotspot for methylotrophic methanogenesis. In the present work, cultivable diversity of methanogenic archaea was studied with focus on the use of trimethylamine and methanol in the presence of either hydrogen or nitrogen. Enrichment cultures were incubated at 20ºC in artificial seawater, added with vitamins and yeast extract and reduced with L-cysteine, under atmosphere of H_2:CO_2 (80:20) or N_2:CO_2 (70:30) and 5 or 10mM trimethylamine with or without methanol as substrates. Methane production was measured by gas chromatography (GC-FID). Cell growth and predominant morphologies were observed by contrast phase and fluorescence microscopy. Illumina MiSeq 16S rRNA sequencing was performed in selected cultures. Methane production of up to 45% (2.0456 mmols.mL-1) after 6 days incubation was observed in cultures containing trimethylamine, methanol under H_2:CO_2 atmosphere at initial stages of enrichment, indicating that methylotrophic methanogens were abundant and active in the sediment. However, further transfers to new media did not reach the same production, showing that cultivation conditions must be improved to achieve stability. Methane production was stoichiometrically related to trimethylamine concentration under both H_2 and N_2 atmosphere and methane decrease along incubations indicated that anaerobic methanotrophic process might be on course. 16S rRNA sequencing revealed predominance of *Methanoccocoides sp*. and *Methanosarcina sp*. The genus *Methanomassilicoccus* was also found, but in low abundance. Fluorescent cell morphologies were very diverse, indicating that methylotrophic substrates favor methanogen´s diversity in the area. These results show that methylamines must be important methanogenic substrates in Antarctic sediments and efforts are being made to reach culture stability, favor growth of all detected methanogenic groups and to confirm methanotrophy in the cultures.

Keywords: archaea, methylotrophic, methanogenic, marine sediment, Antarctica

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