DEVELOPING A NEW SCREENING METHOD TO DETECT NOVEL BACTERIOHOPANEPOLYOL (BHP) BIOMARKERS FOR AEROBIC METHANE OXIDATION IN MARINE ENVIRONMENTS

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The greenhouse gas methane (CH_4) is an important contributor to natural and anthropogenic global climate changes in present and past environments (Bernstein et al., 2008). Several natural marine sources of methane are known, e.g. biogenic methane produced in oxygen depleted areas of the ocean and seafloor, as well as cold seeps and mud volcanos which are closely connected to gas hydrates and/or deep thermogenic methane (Sassen et al., 1999; Valentine et al., 2001). A better understanding of the sources, sinks and chemical reaction pathways in methane cycling is important to constrain the impact of methane on global warming. However, applicable tools for determining past methane concentrations in the atmosphere and the intensity of methane cycling are currently lacking. Several important biological pathways to reduce released methane into the environment are known each using a specific subset of electron acceptors. In oxygen or nitrite dependent methane oxidation, bacteria convert CH_4 to carbon dioxide by methane monooxygenase (Murrell and Jetten, 2009). With nitrate, iron and sulfate as e acceptor archaea convert methane using the reverse methanogenesis pathway with methyl-coenzyme M reductase (Welte et al., 2016). Therefore, we are planning to investigate and develop new lipid biomarker proxies for an-/aerobic methane oxidizers in present and past marine environments.

In this study we will mainly focus on the unique methane oxidizing bacteria *Methylomirabilis oxyfera*, which produces internal oxygen from nitrite in order to oxidize methane under anaerobic conditions (Ettwig et al., 2010). In this way, this intra-aerobic methane oxidizer (*M.oxyfera*) potentially influences both the carbon and nitrogen cycles in natural environments (Ettwig et al., 2010). However, only a few studies have addressed the presence and influence of *M. oxyfera* in marine settings (Chen et al., 2014; Padilla et al., 2016), especially in the geologic past. Such an understanding would help to better comprehend the methane and nitrogen cycle with its underlying reaction pathways.

M. oxyfera is known to synthesize bacteriohopanepolyol (BHP) lipids (Welander and Summons, 2012). BHPs are known for their high structural diversity and potential selectivity for bacterial AMO (Kool et al., 2014; Welander and Summons, 2012). Preliminary findings suggest that *M. oxyfera* synthesizes characteristic BHPs, including 3-methyl BHPs which are potentially produced in anoxic environments (Kool et al., 2014). Furthermore, carbon isotope measurements showed that *M. oxyfera* lipids are not depleted in ^{13}C due to the utilization of CO_2 for lipid and biomass production rather than methane (Kool et al., 2014). Hence, the canonical tool for detecting past AMO, the presence of ^{13}C-depleted hopanoids, is not applicable for *M. oxyfera* and thus this type of AMO may have gone undetected.

In order to determine the structural diversity of BHPs in the biomass of *M. oxyfera* and to compare the results with other methanotrophic cultivations the currently used analytical method (Talbot et al., 2016) will be improved upon for a more rapid screening procedure. The existing method first derivatizes BHPs where instead the new developed method will be analyzing intact BHPs directly using HPLC-high resolution mass spectrometry (HRMS). In case of unknown or poorly characterized BHPs, semi-preparative HPLC will be used in combination with NMR to identify these unknown compounds. In addition to BHPs we will investigate other, high molecular weight, lipids in biomass of *M. oxyfera* to examine potential candidate biomarker lipids.

We will present the first results of the new developed method in analyzing BHPs as well as the detailed analysis of *M. oxyfera* and its BHP composition. Once developed, these (BHP) biomarkers for AMO will be applied to paleo-environments where methanotrophy is thought to have been an important process (e.g. gas hydrate destabilization zones) in marine environments to better understand the impact on the carbon and nitrogen cycle.

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