Research in Microbiology 166 (2015) 688–699

www.elsevier.com/locate/resmic

Diversity of methanogens and sulfate-reducing bacteria in the interfaces of five deep-sea anoxic brines of the Red Sea

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Received 2 March 2015; accepted 2 July 2015
Available online 17 July 2015

Abstract

Oceanic deep hypersaline anoxic basins (DHABs) are characterized by drastic changes in physico-chemical conditions in the transition from overlaying seawater to brine body. Brine-seawater interfaces (BSIs) of several DHABs across the Mediterranean Sea have been shown to possess methanogenic and sulfate-reducing activities, yet no systematic studies have been conducted to address the potential functional diversity of methanogenic and sulfate-reducing communities in the Red Sea DHABs. Here, we evaluated the relative abundance of Bacteria and Archaea using quantitative PCR and conducted phylogenetic analyses of nearly full-length 16S rRNA genes as well as functional marker genes encoding the alpha subunits of methyl-coenzyme M reductase (*mcrA*) and dissimilatory sulfite reductase (*dsrA*). Bacteria predominated over Archaea in most locations, the majority of which were affiliated with *Deltaproteobacteria*, while *Thaumarchaeota* were the most prevalent Archaea in all sampled locations. The upper convective layers of Atlantis II Deep, which bear increasingly harsh environmental conditions, were dominated by members of the class *Thermoplasmata* (Marine Benthic Group E and Mediterranean Sea Brine Lakes Group 1). Our study revealed unique microbial compositions, the presence of niche-specific groups, and collectively, a higher diversity of sulfate-reducing communities compared to methanogenic communities in all five studied locations.

Keywords: Hypersaline environments; Brine pools; Biodiversity; Methanogens; Sulfate reducers

1. Introduction

Hypersaline water bodies at the bottom of the ocean (brine pools) are present in the Mediterranean Sea, the Gulf of Mexico and the Red Sea [1]. In the Red Sea, a total of 25 such deep-sea hypersaline brine pools have been discovered at depths ranging from 1196 to 2850 m below sea level [1,2]. These environments are extremely saline (up to 26% salinity), anoxic, rich in heavy metals and characterized by drastic changes in physicochemical conditions when compared to the overlaying seawater [3].

The interface between the brine pools and the seawater (BSI) represents a highly peculiar environment that harbors a high microbial diversity and biomass [4–6]. The increase in microbial biomass can be explained by the drastic changes in density, which result in an *in situ* particle trap for debris sinking through the water column, thus increasing the concentrations of available nutrients [6,7]. In addition, the BSI is also characterized by sharp changes in physicochemical parameters including salinity, oxygen concentration, temperature and redox potential, all of which provide a large variety of environmental niches for different metabolic groups [8,9]. The microbiology of the BSIs of some of the Red Sea brine pools has been explored with a combination of cultivation-dependent [10,11] and molecular–based methods [12,13]. Previous studies based on 16S rRNA gene sequences

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http://dx.doi.org/10.1016/j.resmic.2015.07.002
0923-2508/

uncovered novel groups of Archaea and Bacteria inhabiting the BSI of Shaban Deep and Kebrit Deep of the Red Sea [6,12].

Microbial community studies in the Mediterranean DHABs revealed that diverse biogeochemical processes apparently co-occur in the BSI [8]. Other investigations reported on the importance of methanogenesis and sulfur cycling in these environments [4,14]. These findings were also corroborated by recent metagenomic studies, where pathways for methanogenesis and/or sulfate reduction were detected in brines from the Red Sea and in DHABs in the Mediterranean Sea [15–17]. Additionally, unique microbial communities were found to thrive in the sediments of two brine pools in the Red Sea and many of the reported microorganisms are hypothesized to play a dominant role in the methane and sulfur cycle, based on their phylogenetic affiliations [18].

Taken together, methanogenesis and sulfate reduction could thus be considered very important biogeochemical processes in deep-sea brines [4,19]. However, the composition of the microbial communities involved in both processes is largely unknown for the Red Sea brine pools. Considering the extreme conditions of these environments and the unique combination of physicochemical features in each individual brine pool [1], we postulated the existence of novel, niche-adapted groups of methanogens and sulfate reducers in the BSIs. Moreover, despite the micro-oxic conditions present in the BSI [20], members of both groups are capable of tolerating minute amounts of oxygen [21–23] and could thus play an important role in these environments. Previous 454 amplicon data [20] uncovered interesting microbial communities in the sampled sites, but as many of the relatively short sequences stem from poorly characterized groups, we decided that nearly full-length 16S rRNA gene sequences would be important to provide better phylogenetic detail and resolution on members of these groups. Therefore, we analyzed the microbial communities in the BSIs of geochemically distinct brine pools of the Red Sea, using the canonical 16S rRNA gene, as well as functional marker genes encoding for the alpha subunits of methyl-coenzyme M reductase (*mcrA*) and dissimilatory sulfite reductase (*dsrA*) to uncover the main methanogenic and sulfate-reducing communities.

2. Materials and methods

2.1. Sample collection

Water samples from the brine–seawater interfaces and the upper convective layers of the deep-sea brines were collected from the R/V *Aegaeo* during the 3rd KAUST Red Sea Expedition in November 2011 using a rosette sampler equipped with 10-l Niskin bottles and a CTD unit for monitoring salinity, temperature, transmission, oxygen and pressure (Idronaut, Italy). Large volumes (ca. 200 l) of sample were collected from Atlantis II Deep BSI (Ai); first, second and third upper-convective layer of Atlantis II Deep (labeled as A-UCL1, A-UCL2, A-UCL3, respectively), Discovery Deep BSI (Di), Erba Deep BSI (Ei), Kebrit Deep BSI (Ki) and Nereus Deep BSI (Ni) (Table 1). During sampling, we have avoided mixing between the seawater and the brine samples by carefully controlling the depth of the CTD and sampler when triggering the closure of each Niskin bottle to ensure sampling of desired layers. Furthermore, prior to sample collection on deck, we measured the salinities at the top and bottom of each individual Niskin bottle using a handheld refractometer (MASTER Refractometer, Atago, Japan) to confirm that the salinities of the samples matched the expected values of the targeted layers. Samples were then concentrated using a Tangential Flow Filtration (TFF) as described elsewhere [20]. Methane and carbon dioxide concentrations in the samples were determined via a commercial service provided by GEOMAR Helmholtz Centre for Ocean Research (Kiel, Germany, http://www.geomar.de).

2.2. DNA extraction, amplification and sequencing of 16S rRNA genes

Nucleic acids were extracted as previously described [24] and the concentrations of the DNA were measured in a NanoDrop (Thermo Scientific, USA). Partial 16S rRNA genes were amplified by PCR by using combinations of the archaeal-specific primer 4F (5′-TCCGGTTGATCCTGCCRG-3′) [25], or the bacteria-specific primer 27F (5′-AGAGTTT-GATCMTGGCTCAG-3′) paired with the universal primer 1492R (5′-GGTTACCTTGTTACGACTT-3′) [26]. The primers were chosen to produce sequences with maximum length. *In silico* testing using Silva-TestPrime (http://www.arb-silva.de/search/testprime/ [27]) with one allowed mismatch indicated a coverage for Bacteria of 65.5% and a coverage for Archaea of 46.6%, while three allowed mismatches indicated a coverage for Bacteria of 71% and a coverage for Archaea of 53.1%. In addition, the above primers have a good coverage of major taxa reported in a previous study using 454 amplicon data [20]. The PCR conditions for archaeal 16S rRNA genes were: an initial denaturation of 5 min at 94 °C, 30 cycles of 1 min at 94 °C, 1 min at 55 °C and 1.5 min at 72 °C and then a final extension step of 7 min at 72 °C. The conditions for bacterial PCR were 3 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at 53 °C and 1.5 min at 72 °C and then 7 min at 72 °C. Purified PCR products were cloned into PCR®2.1 TOPO vectors (Invitrogen) according to the manufacturer's instructions. All clones with inserts from each library (856 and 1040 for archaeal and bacterial libraries, respectively) were selected for plasmid extraction and bi-directional sequencing on an ABI 3730 × l Capillary Sequencer at the Biosciences Core Laboratory at KAUST. Raw 16S rRNA gene sequences were quality checked, trimmed and assembled using Sequencher v.4.9 (Gene Codes Corporation).

2.3. Diversity and phylogenetic analysis

Assembled archaeal and bacterial 16S rRNA sequences were aligned and analyzed using mothur v.1.31, yielding operational taxonomic units (OTUs) grouped at 97% sequence identity level [28]. Potential chimeric sequences were removed