Journal of Arid Environments 115 (2015) 81–89

Contents lists available at ScienceDirect

Journal of Arid Environments

journal homepage: www.elsevier.com/locate/jaridenv

Bacterial diversity and fatty acid composition of hypersaline cyanobacterial mats from an inland desert wadi

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ARTICLE INFO

Article history:
Received 11 July 2014
Received in revised form
8 December 2014
Accepted 14 January 2015
Available online 19 January 2015

Keywords:
Desert
Wadi
Cyanobacterial mats
Fatty acids
Pyrosequencing
Cyanobacteria

ABSTRACT

Hypersaline cyanobacterial mats are infrequently reported in desert streams and information on such mats is very scarce. We investigated bacterial diversity and fatty acid composition in hypersaline cyanobacterial mats from Wadi Muqshin, located inland near the Empty Quarter desert in Oman. Most of the detected cyanobacteria belonged to known halotolerant, thermotolerant and UV resistant types that were typically reported in other hypersaline mats. A total of 84,834 ribosomal sequences were obtained, with 62–79% of the sequences affiliated to Cyanobacteria, *Proteobacteria*, *Bacteroidetes*, *Clostridia* and *Chloroflexi*. Cluster analysis showed that Mat 5 with the highest salinity was profoundly different from the other mats and shared species were ≤72% between all mats. While most *Deltaproteobacteria* in the wadi mats belonged to sulfate-reducing bacteria, a number of sequences related to purple sulfur, purple non-sulfur as well as green no-sulfur bacteria were also detected. Different saturated, branched and mono- and di-unsaturated fatty acids were detected in all mats, with the saturated 16:0 and 18:0 and the monounsaturated 16:1 and 18:1 fatty acids accounting for relative amounts of 70–77% of total fatty acids. We conclude that microbial diversity and fatty acids composition in the desert wadi hypersaline cyanobacterial mats resemble their counterparts from other hypersaline environments.

1. Introduction

Microbial mats are accretionary, cohesive, macroscopic accumulations of microbial communities, which are often laminated and grow mostly on submerged or moist surfaces (Pierson, 1992). They are distributed worldwide in a surprisingly wide range of environments (Pierson, 1992). Under hypersaline conditions, microbial mats develop well, mainly because of the restricted abundance and activity of animal grazers and the absence of competition from macrophytes (Farmer, 1992). Most studied hypersaline mats originated from intertidal flats, closed basins (e.g. solar salterns and evaporation ponds), hypersaline lakes and hot springs, but few from arid and semiarid inland saline lakes. Hypersaline mats from inland saline lakes were mainly reported from Australia, Spain, lakes of the Great Rift Valley (Africa) and the deserts of America (Bauld, 1981, 1986; Guerrero and de Wit, 1992; Jonkers et al., 2003). So far, there have been very few reports on hypersaline microbial mats from inland saline lakes or streams in the arid deserts of the Arabian Peninsula (Jupp et al., 2008), although such mats were described in intertidal flats of the region (Abed et al., 2007, 2008). It is of interest to find out whether hypersaline mats from arid deserts harbor similar/different microbial communities to their counterparts in other hypersaline environments.

The tropical Arabian Desert is the fourth largest desert in the world and it occupies most of the Arabian Peninsula. It is characterized by extreme environmental conditions, with intense temperatures reaching as high as 60 °C in hot summers, high humidity and a rainfall average less than 4 inches (100 mm) a year throughout the desert. During torrential rains, drainage basins become flooded and wadis are formed in mountain valleys. Wadis, which is an Arabic term used to describe corridors for fluvial run-off that may contain perennial, intermittent or ephemeral surface flow, are widespread in the Arabian region. Many wadis can be found across Oman, due to its special geology, and large areas of these wadis are covered by laminated cyanobacterial mats. These mats experience extreme conditions of temperature, desiccation and UV

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http://dx.doi.org/10.1016/j.jaridenv.2015.01.010
0140-1963/

and light intensity, thus are expected to attract well-adapted extremophilic microorganisms. Unusually, few wadis in Oman were found to contain hypersaline cyanobacterial mats, instead of the typical freshwater mats, due to the high evaporation rates and the recharge regimes from deep groundwater aquifers (Jupp et al., 2008). An example is Wadi Muqshin, located inland, ca. 200 km from the Arabian Sea coast, close to the Empty Quarter, and consists of a series of pools with different salinities ranging from 5 to 25% (Jupp et al., 2008). Hypersaline cyanobacterial mats in desert wadis have been infrequently reported and rarely examined in any detail.

In this study, we investigated bacterial communities (using direct microscopy and pyrosequencing) and fatty acid composition in five cyanobacterial mats from Wadi Muqshin, Oman. In particular, we asked the question, "how comparable are the hypersaline mats from wadi ecosystems to their counterparts from other habitats?". This study will provide the first detailed insights into microbial communities and lipid biomarkers in hypersaline mats from inland saline streams in the Arabian Desert.

2. Materials and methods

2.1. Sampling sites and nutrient analysis

Five cyanobacterial mats were sampled from Wadi Muqshin (48 km long) in the southwest of the Sultanate of Oman, bordering the dunes of the Empty Quarter desert. Detailed description of the site and information on its hydrogeology and mineralogy are given in Jupp et al. (2008). The wadi contains pools that are flooded during raining season, but then evaporated through intense summer (temperature can reach as high as 55 °C), resulting in extremely hypersaline conditions (salinity is between 5% and 25%). Large areas of these pools are covered with benthic, well developed, laminated cyanobacterial mats. Five mat samples were collected from different pools of the wadi; two from a downstream pool (Mat 1 and 2), two from a middle stream pool (Mat 3 and 4) and one sample from an upstream pool (Mat 5). The pools were ca. 100–200 m apart from each other and had the salinities 6.5%, 7.3 and 12% at the time of sampling, respectively. The collected mats differed in appearance, texture and salinity. The mats were cut carefully using a sterile scalpel and stored in Petri plates. A map of the sampling location, photographs of the mats and the wadi pools and detailed physical and chemical parameters of the sampling sites can be found in Jupp et al. (2008) and Abed et al. (2011a).

2.2. Microscopy and morphotype quantification of cyanobacteria

The depth of the oxygenic photosynthetic layer in each mat was determined by microsensor measurements (not shown). The top cyanobacterial layer of the mats (1–3 mm) was excised under a dissecting microscope with a clean scalpel blade and sterile forceps. Samples were torn apart, mounted in water on a microscope glass slide and observed using transmitted light, phase contrast and fluorescence microscopy. Different morphotypes were identified and photographed. Three cores from each mat sample were observed microscopically to ensure a good overall representation of resident morphotypes. Morphological identification was carried out in accordance with traditional phycological (Komárek and Anagnostidis, 2005) and bacteriological (Castenholz et al., 2001) systems.

2.3. Pyrosequencing and sequence analyses

The mat samples (ca. 300–500 mg each) were subjected to DNA extraction using the Power Biofilm DNA isolation kit (MOBIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Purified DNA extracts were submitted to Molecular Research MR DNA Laboratory (Shallowater, TX, USA) for tag-pyrosequencing. Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed as described before (Dowd et al., 2008) using the GS FLX titanium sequencing kit XLR70. One-step PCR was performed using a mixture of hot start and hot start high fidelity taq polymerases resulting in amplicons that extend 350–450 bp from the 27F region (*Escherichia coli* rRNA numbering). The bTEFAP sequencing was performed according to the MR DNA protocols (www.mrdnalab.com).

All sequences were analyzed and taxonomically classified using the NGS analysis pipeline of the SILVA rRNA gene database project (SILVAngs) (Quast et al., 2013). Each read was aligned using the SILVA Incremental Aligner (SINA) (Pruesse et al., 2012) against the SILVA SSU rRNA SEED and quality controlled (Quast et al., 2013). Reads shorter than 50 aligned nucleotides and reads with more than 2% of ambiguities, or 2% of homopolymers, respectively, were excluded from further processing. Identical reads were then identified (dereplication), the unique reads were clustered (OTUs), and the reference read of each OTU was classified using cd-hit-est (version 3.1.2; http://www.bioinformatics.org/cd-hit). The classification was performed by a local nucleotide BLAST search against the non-redundant version of the SILVA SSU Ref dataset (release 111; http://www.arb-silva.de) using blastn (version 2.2.22+; http://blast.ncbi.nlm.nih.gov/Blast.cgi) with standard settings. The classification of each OTU reference read was mapped onto all reads that were assigned to the respective OTU, yielding the number of individual reads per taxonomic path. Reads without any BLAST hits or reads with weak BLAST hits were assigned to the meta group "No Relative" in the SILVAngs fingerprint.

2.4. Fatty acid analysis

Mat materials were gently dried at 65 °C for 10 h and weighed. Lipids were extracted from dry homogenized mat two times using 100 ml chloroform/methanol (2:1, v/v) for 3 h at laboratory temperature with occasional stirring (Čertík et al., 1996). After extraction, the mixture was filtered and the extracts were collected. 0.9% KCl (1.2-fold of total extract volume) was then added, the mixture was stirred vigorously for 1 min and centrifuged to effect phase separation. The chloroform–lipid containing layer was filtered through anhydrous Na_2SO_4 and evaporated under vacuum. Total lipids were determined gravimetrically (triplicate standards of dry yeast cells were used to assess reproducibility) and used for further analysis.

Fatty acids from total lipids were converted to their methyl-esters by methanolic solution of sodium methoxide and methanolic HCl and analyzed by gas chromatography (GC-6890 N, Agilent Technologies) using a capillary column DB-23 (60 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies) and a FID detector (constant flow, hydrogen 40 ml/min, air 450 ml/min, 250 °C) under a temperature gradient (150 °C held for 3 min; 150–175 °C at a program rate 7.0 °C/min; 175 °C held for 5 min; 175–195 °C at a program rate 5.0 °C/min; 195–225 °C at a program rate 4.5 °C/min; 225 °C held for 0.5 min; 225–215 °C at a program rate 10 °C/min; 215 °C held for 7 min; 215–240 °C at a program rate 10 °C/min; 240 °C held for 7 min) with hydrogen as carrier gas (flow 2.5 ml/min, velocity 57 cm/s, pressure 220 kPa) and a split ratio of 1/20 (Inlets: heater 230 °C; hydrogen flow 51 ml/min for 2 min, then hydrogen flow 20 ml/min; pressure 220 kPa) (Čertík et al., 1996). The fatty acid methylester peaks were identified by authentic standards of C_4–C_{24} fatty acid methylesters mixture (Supelco, USA) and evaluated by ChemStation B 01 03 (Agilent Technologies).