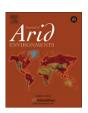
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Microbial diversity of the hypersaline Sidi Ameur and Himalatt Salt Lakes of the Algerian Sahara

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ABSTRACT

Microbial populations within hypersaline lakes often exhibit high activities of photosynthesis, dissimilatory sulphate reduction and other processes and, thus, can have profound impacts on biogeochemical cycles of carbon, nitrogen, sulphur and other important elements within arid lands. To further understand these types of ecosystems, the physicochemical and biological properties of Sidi Ameur and Himalatt Salt Lakes in the Algerian Sahara were examined and compared. Both lakes were relatively neutral in pH (7.2–7.4) and high in salt, at 12% and 20% (w/v) salinity for Himalatt and Sidi Ameur Lakes, respectively, with dominant ions of sodium and chloride. The community compositions of microbes from all three domains (*Bacteria*, *Archaea* and *Eukarya*) were surveyed through the use of 16S and 18S ribosomal gene amplification and clone library clustering using amplified ribosomal DNA restriction analysis (ARDRA) in conjunction with DNA sequencing and analysis. A high level of microbial diversity, particularly among the bacteria of the Himalatt Salt Lake and archaea of Sidi Ameur Lake, was found within these environments. Representatives from all known halophilic bacterial phyla as well as 6 different genera of halophilic archaea were identified. Moreover, several apparently novel phylotypes among both archaea and bacteria were revealed.

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1. Introduction

The use of culture-independent molecular methodology has become an essential and reliable tool for surveying the diversity of microbial life in any given habitat. Extreme environments, namely those subjected to conditions uninhabitable by most living organisms, offer unique opportunities to assess the types of microbes able to withstand the harsh conditions within a given ecosystem and further our understanding of the types of growth parameters and/or conditions under which these organisms are able to survive.

One group of extreme environments is that of hypersaline, or high-salt lakes. These habitats are dominated by halophilic archaea, or haloarchaea, which require a minimum of 9% (w/v) NaCl for growth with most displaying optimal growth at 20-26% (w/v) NaCl and some growing well in saturated salt conditions of >30% (w/v)

lakes (Arahal et al., 1996; Maturrano et al., 2006; Mutlu et al., 2008), hypersaline microbial mats (Sorensen et al., 2005) and man-made solar salterns (Benlloch et al., 1996, 2001; Bidle et al., 2005; Ochsenreiter et al., 2002).

Until recently, it was thought that haloarchaea were the sole colonizers of extreme hypersaline habitats. However, a survey performed by Antón et al. (2000) revealed the surprising presence of extremely halophilic bacteria in a saltern crystallizer pond in

NaCl (Oren, 2000). Over the last decade, the diversity of haloarchaea in various hypersaline environments has been examined and more

fully characterized, aided largely through the use of culture-

independent molecular methodology (Rodriguez-Valera et al.,

1999). Included among these studies are naturally occurring salt

colonizers of extreme hypersaline habitats. However, a survey performed by Antón et al. (2000) revealed the surprising presence of extremely halophilic bacteria in a saltern crystallizer pond in Alicante, Spain. This new halobacterial genus and species, *Salinibacter ruber* has since been found ubiquitously in numerous hypersaline environments (Antón et al., 2008). In addition to the extreme halophile *S. ruber*, moderate halophilic bacteria within phyla such as *Cyanobacteria*, *Firmicutes*, *Proteobacteria* and *Bacteroidetes* are also found in hypersaline habitats (Oren, 2008). However, haloarchaea, uniquely adapted for life in high salt, appear to remain the dominant members of these types of habitats.

Abbreviations: ARDRA, amplified ribosomal DNA restriction analysis; PCR, polymerase chain 29 reaction.

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Bou Saâda (meaning "place of happiness") is located in north-central Algeria approximately at 280 km south of the coast of Algeria between the el-Hodna Depression and the mountains of the Saharan Atlas (Fig. 1). This oasis town at the edge of the Algerian Sahara lies in stark contrast to the surrounding Saharan desert, nearby barren Ouled Naïl Mountains and adjacent hypersaline lakes including Sidi Ameur and Himalatt Salt Lakes. The biology of these two hypersaline lakes, which are related yet separated by 80 km, is of interest not only in furthering our understanding of the microbial diversity of extreme environments but also in assessing the impact of human activity on these types of water ecosystems in arid environments. Hypersalinity and pollution are likely to be two of the major parameters selecting for the microbial biodiversity of these Algerian lakes and, thus, may facilitate the identification of new genera and/or species.

In this paper, culture-independent 16S rDNA and physicochemical analyses were performed to survey the microbial diversity and further our understanding of the hypersaline Sidi Ameur and Himalatt Salt Lakes in the Algerian Sahara. Ribosomal gene amplification combined with amplified ribosomal DNA restriction analysis (ARDRA) was performed and revealed the diversity of both bacterial and archaeal DNA sequences within these environments was widespread, dependent on the properties of the site (*e.g.*, salinity, pollution) and, in several cases, novel.

2. Materials and methods

2.1. Sampling site description

Sidi Ameur and Himalatt Lakes are shallow salt lakes, or seb-khas, 55 km to the northwest and 25 km to the southeast of Bou Saâda station, respectively (Fig. 1). The waters are considered athalassohaline because their salinity derives from the dissolution of salts of continental origin. Both lakes are influenced by human activity. Sidi Ameur Lake, in particular, is closest to the city of Sidi Ameur and is bordered on one side by an open field oriented towards the city and enclosed by sand dunes on the other side. It is filled with the drainage water of local palm groves and some rainwater (198 mm/year). A high evaporation rate (1827 mm/year) during the dry season increases the salt concentrations of this lake. The average annual temperature of the Bou Saâda region, where both lakes are located, is 30 °C with minimum and maximum-

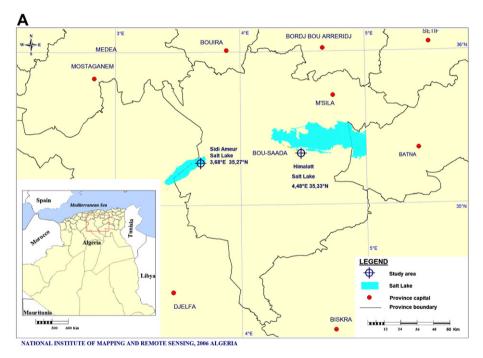






Fig. 1. Sidi Ameur and Himalatt sites of the Algerian Sahara. A. The hypersaline Sidi Ameur and Himalatt sites are respectively located 55 km to the northwest and 25 km to the southeast of Bou Saâda, Algeria (latitude of 35°13′, longitude of 4°11′ and altitude of 663 m). B. Representative images of Sidi Ameur Lake depicting the barren landscape and high salt concentrations.

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