

Diversity of Halophilic Archaea from Ezzemoul Sabkha in Algeria

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Abstract: Sabkhas and chotts are examples of hypersaline environments inhabited by halophilic microorganisms. In the present study, a culture-dependant methodology was used to determine the archaeal diversity present within Ezzemoul sabkha located in the north-eastern of Algeria. Twenty-seven strains were isolated and subjected to a polyphasic taxonomic characterization. The isolates tested placed within the family Halobacteriaceae, a group of extremely halophilic, aerobic archaea that have a salinity tolerance of 3 to 4M and associated with six genera Halorubrum, Halobacterium, Haloterrigena, Haloferax, Halomicrobium and Haloarcula. Halorubrum species were found to be the dominant Archaea community as indicated by the presence of different strains recovered from this sabkha. In the other hand, two new species within Halobacteriaceae family; Halorubrum ezzemolulense sp. nov. and Halomicrobium katesii sp. nov. were described.

Keywords: Halophilic archaea, sabkha, diversity

1 Introduction

Halophilic Archaea have been associated with extreme environments and constitute the family Halobacteriaceae in the order of Halobacteriales. They represent a considerable fraction of the prokaryotic world in hypersaline environments in term of numbers, biomass and genetic heterogeneity [1]. Their adaptations to grow at high salinity make them interesting for fundamental research and the exploration for biotechnological process [2]. The present research focuses on archaeal diversity in a hypersaline lake of Algeria using culture-dependent molecular approaches.

2 Materials and Methods

2.1 Sampling Sites

Brines were collected from Ezzemoul in north-east, Algeria. The sabkha received their water supply from high ground water levels, precipitation or run-off from adjacent areas. Water levels as well as brine concentrations started to change in spring and the lakes were completely evaporated ephemeral salt pans during June-July. The total salt concentrations and pH were measured with refractometer (Leica) and pH-meter (Hanna).

2.2 Isolation of Halophilic Archaea

The brines were diluted and 100 µl were plated in duplicate onto HM contained (per litre of distilled water): 5 g proteose-peptone no. 5 (Difco), 5 g yeast extract (Difco), 1 g glucose with 25% (w/v) total salts. The stock of total salts at 30% (w/v) was prepared as described by Subov [3]: 234 g NaCl, 42 g MgCl₂·6H₂O, 60 g MgSO₄·7H₂O, 1 g CaCl₂·2H₂O, 6 g KCl, 0.2 g NaHCO₃, 0.7 g NaBr, 0.005 g FeCl₃ and 1000 ml distilled water. The medium was brought to pH 7.0 and incubation was carried out at 37°C. Colonies arising on the plates were selected for isolation based on gross morphological differential characteristics (size, pigmentation and shape). They were transferred to fresh media and pure cultures were obtained.

2.3 Characterization of Isolates

Phenotypic tests of isolates were performed according to the proposed minimal standards for the description of new taxa in the order Halobacteriales [4].

2.4 DNA Extraction, 16S rRNA Gene Amplification and Sequencing

Genomic DNA was extracted and purified from cells as described by Lind and Ursing [5]. The 16S rRNA gene sequences were amplified from purified genomic DNA using primers as described previously [6]. Comparative sequence analyses were performed by comparing new recent insert sequences with those available in the online databases provided by the National Centre for Biotechnology Information using the Blast search program (<http://blast.ncbi.nlm.nih.gov/>). Multiple sequence alignments were performed using Clustal W. Phylogenetic trees were constructed on the basis of neighbour-joining method using Mega 6 program package [7].

3 Results and Discussion

3.1 Sampling Site

Generally, the pH of brines is quite close to neutrality (7.5), and the salinity ranged from 20 to 30% (w/v).

3.2 Phenotypic Characterization of Isolates

Sabkhas are examples of high salty environments inhabited by halophilic microorganism. Our study is the first attempt to investigate halophilic Archaea in sabkha Ezzemoul. Throughout the course of this work, we further characterized twenty-seven isolates. They formed colonies ranged in colour from pale-pink to red. Cells were extremely pleiomorphic, rod or pleiomorphic-rod and stained Gram-negative. All the isolates were extreme halophilic, had a salt concentration for growth at least 10% (w/v) NaCl, and could tolerate salt concentration up to 25-30% (w/v) NaCl. The isolates grew best between 37 and 40°C, and pH of 6.5 to 7.5. All stained Gram negative, and were catalase- and –oxidase positive. Among the strains tested for indole production and urea hydrolysis the majority were negative and nitrate reduction was observed with some strains.

3.3 Molecular Identification and Phylogenetic Analysis

The phylogenetic analysis was done with obtained sequences and related ones obtained from the Genbank database. Most isolates shared more than 97% identity with their closest phylogenetic relative. The tree constructed by neighbour-joining method depicting the phylogenetic relationships of isolates and their closest relatives is shown in Figure 1. They are placed within the family Halobacteriaceae. We discovered six genera: Halorubrum, Halobacterium, Haloterrigena, Haloferax, Halomicrobium, and Haloarcula.

In the present study, we further describe twenty-seven isolates from Ezzemoul sabkha. All were placed within Halobacteriaceae family belonging to Euryarchaeota phylum and including members related to species of the genera Halorubrum, Haloterrigena, Haloferax, Halomicrobium, Haloarcula, and Halobacterium. Two new species [8, 9] were described. The sequence similarities of isolates ranged between 95.8 and 99.8% to closely related species (data not shown). The majority of the strains from this study were more closely related to Halorubrum chaoviator Halo-G^T isolated from sea salt in Baja California, Mexico, Western Australia and the Greek island of Naxos. Such dominance of the genus Haloubrum was in agreement with the previous cultivation-based study on archaeal communities inhabiting hypersaline environments [11].

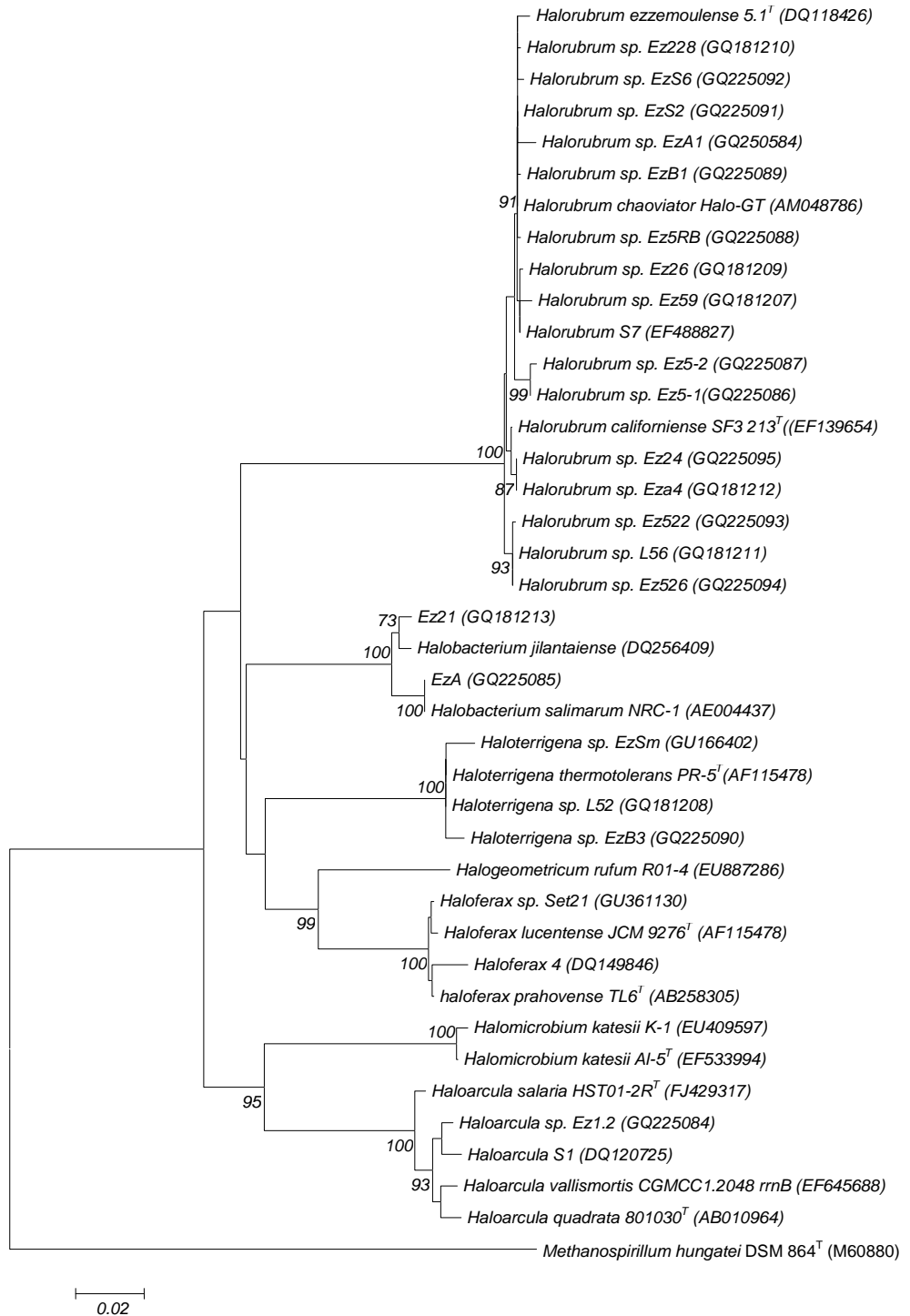


Figure 1

Neighbour-joining phylogenetic tree based on 16SrRNA gene sequence showing the position of isolates with respect to other species of Halobacteriaceae. Numbers at nodes indicate bootstrap values (based on 1000 replications). Bar, 0.02 substitutions per nucleotide position.

Conclusion

Ezzemoul sabkha is a hypersaline salt lake located in semi-arid climate from which salt is extracted for human consumption. Ezzemoul is subjected to rainfall season, high solar radiation and larger temperature fluctuations between winter and summer. The archaeal community hosted this habitat include members of Halobacteriaceae family that are abundant and ubiquitous in hypersaline lakes but the general conclusion that emerged from this study is that the diversity of halophilic Archaea described above is undoubtedly only a small fraction of the true diversity of haloarchaea in this sabkha.

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