Original Article

TIPS Trends in Pharmaceutical Sciences 2018: 4(2): 109-112.

Isolation and identification of culturable aerobic halophilic archaea associated with salt crystals from Urmia Lake

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Abstract

Urmia Lake is one of the largest hypersaline lakes in the world. Water evaporation and saturation cause formation of lots of salt crystals on the lake beach. In this study, extremely halophilic strains were isolated from salt crystals that were formed in distinct regions of the lake. The isolation was performed by means of a modified Marine agar medium and DNA of the isolated strains were extracted and amplified by PCR, using universal primers that amplify archaeal 16S rDNA. The amplified archeal DNA fragments were purified, and were subjected to 16S rRNA gene sequencing analysis, which was compared to known sequences by a Blast search at NCBI (National Center for Biological Information). Similarity analysis based on 16S rRNA gene sequences of all isolates indicated that the archaeal isolates belong to three different halophilic genera of euryarcheota: *Halorubrum*, *Haloarcula* and *Halobacterium*. These extreme halophilc archaea can be used as a potential source of new therapeutic metabolites and enzymes as well as antibiotic compounds along with novel biotechnological applications.

Keywords: Halophilic archaea, Salt Crystals, Urmia Lake.

1. Introduction

Even the most inhospitable places on earth harbor organisms, which convinces us to broaden the spectrum of conditions at which we suppose life exists. These conditions include extremely high or low temperatures, even above or below the limits at which water remains at its liquid state at the normal atmospheric pressure, salt concentrations near saturation point, hydrostatic pressures up to 1,000 atmospheres, so nothing can stop life (1). One of these extreme places is Urmia salt lake in Iran. Lake Urmia (or Ormiyeh) is one of the largest hypersaline lakes in the world with halite deposited on its coastal locations. It is located in the north-west of Iran. Due to drought and increased demands for agricultural water in the lake's basin, the salinity of the lake has risen to more than 300 g/L during recent years. It resembles the Great Salt Lake in the western USA (2). Hypersaline environments, with salinities far more than the normal seawater salinities, generally originate as a result of evaporation of seawater. Such environments are inhabited by halophiles. Halophilic microorganisms are salt-loving organisms with a capacity to balance the osmotic pressure of the environment and resist the denaturing effects of salts, such as salt induced co-aggregation of proteins. They include diverse groups of organisms that thrive extreme saline environments. They have been routinely isolated

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Recieved: 29/04/2018; Accepted: 29/05/2018

from marine salterns and hypersaline lakes with 3.5-4.5 M (20-30 g %) NaCl (3).

Archaea are reported from hypersaline regions, such as natural econiches, including the Dead Sea, the Great Salt Lake, and man-made salt pans (4).

Identification and classification of halophilic microorganisms is generally achieved through comparison of their 16S rDNA sequences. Although several researches have been performed to isolate and identify archaeal community of hypersalines and solar salterns water, very few reports exists about salt crystals archaeal community. So in the present study this subject has been noticed.

2. Materials and methods

2.1. Collection of samples and isolation of dominant archaea

Samples were taken from distinct regions of Urmia salt lake beaches. The suspensions were plated on modified Marine agar, as a suitable medium for halophilic organisms and were incubated at 30 ºC. Plates were checked for colony growth daily. After keeping a sharp lookout for colony observation for one month, colonies with different morphological characteristics were isolated.

2.2. Purification and storage of the isolates

Each isolate was purified by twice streak-plating. An isolate was considered purified after a visual observation of the colony and a microscope observation of the Gram stained cells. The purified strains were conserved on screw cap bottles contained 10 ml of previously mentioned medium.

2.3. Characterisation of the isolates

TThe 16S rRNA genes of the representatives were amplified using polymerase chain reaction (PCR). Template DNA was prepared by boiling method as explained in the following: A colony harvested from a fresh MA culture of the organism was suspended in 100 µl of TE buffer (10 mM Tris, 10 mM EDTA, pH 8.0) in a microcentrifuge tube and was heated at 95 ºC for 5 min, and then the extracted DNA was visualized on an ethidium bromide-stained 2% agarose gel. For some isolates that the mentioned method proved unsuccessful, a commercial kit procedure (QIAGEN, Inc.) was used. In all cases, 5 µl of the resultant template preparation was used in the subsequent 25 µl PCR reactions. The respective DNA template preparations were subjected to PCR amplification by using the two halophilic arhaea-specific 16S rDNA primers Arch F (5'-CYGGTTGATCCTGCCRG-3') and Arch R (5'-GTATTACCGCGGCGGC-3'). PCR was performed on an MJ mini thermal cycler (Bio-Rad). The program protocol were as follows: initial denaturation at 94 °C for 4 min; 30 cycles consisting of denaturating at 94 °C for 1 min, annealing at 56 °C for 1 min, extansion at 72 °C for 2 mins, and a final extension step at 72 °C for 8 mins (5). The PCR products were analyzed by electrophoresis in a 1% agarose gel.

2.4. 16S rRNA sequence analysis

The PCR products were sequenced and compared with the sequences deposited in databases by Blast search of NCBI (6). The sequences were aligned and phylogenetic trees were constructed by the neighbor-joining method using the MEGA7 program.

3. Results

A total of 14 distinct colonies were isolated on modified Marine Agar. The majority of the grown colonies had a red or pink color and their microscopic morphology were rod or cocci. The PCR result showed that the isolates are from archaeal genera, and sequencing of their PCR bands revealed that ten of the isolates belong to Halorubrum species, three of them are associated to Haloarcula species, and one is related to Halobacterium species. The 16S rRNA sequences from isolates were aligned and compared with the sequences of related bacteria. The phylogenetic tree was constructed by the neighbor-joining method (figure 1). On the basis of the 16S rRNA gene *sequence analysis*, the isolates showed high similarity to the species.

4. Discussion

The aerobic halophilic Archaea of the family Halobacteriaceae, order Halobacteriales are the halophiles par excellence. Most of the red colourations in saltern crystallizer ponds and hypersaline

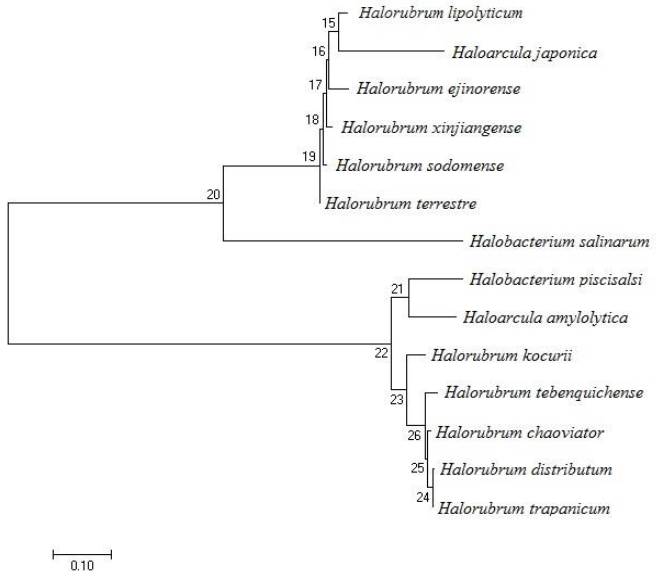

Figure 1. Dendrogram estimated phylogenetic relationships on the basis of 16S rRNA gene sequence data of the isolates using the neighbor-joining method.

lakes is due to the C_{50} carotenoid pigments found in large concentrations in the membranes of majority of this family members (7). The halophilic Archaea of the family *Halobacteriaceae* contain 36 genera, such as *Halobacterium, Haloarcula, Halococcus, Haloferax, Halorubrum*, and *Halobaculum*, with 129 species. The genus *Halorubrum* is the genus with the largest number of species within the family (8). Extremely halophilic archaea have been isolated and characterized from different saline environment in different parts of Turkey, Chile, and China (3). Many species of halophilic archaea have been isolated from crystallizer ponds, the NaCl-saturated ponds in which halite are deposited. From the saltern ponds near Alicante, Spain, species of *Haloarcula, Haloferax, Halorubrum*, and *Halobacterium* have been recovered at a high frequency (9).

They exhibit features characteristic of archaea. Pigmented halophilic archaea and microalgae absorb the energy of light in saltern ponds, thereby raising the water temperature, increasing the rate of evaporation and hastening the deposition of salt. Halophiles posses many hydrolytic enzymes, such as DNase, lipase, amylase, gelatinase, and proteases capable of functioning under conditions that lead to precipitation or denaturation of most proteins. Halophilic proteins compete effectively with salts for hydration, a property that may result in the resistance to other low water activities such as the presence of organic solvents (10).

5. Conclusion

This study investigated the microbial community of the salt crystals from Urmia Lake. The isolates belong to three archaeal genera, Halorubrum, Haloarcula, and Halobacterium. These extreme halophilc archaea can be used as potential sources of new metabolites and enzymes for biotechnological applications.

Acknowledgements

This study was part of project supported by Shiraz University of Medical Sciences. (Grant number: 93-01-058284).

Conflict of Interest

None declared.

6. References

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