Microbiological Research 207 (2018) 289–298

Contents lists available at ScienceDirect

Microbiological Research

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

Diversity and bioprospecting of extremely halophilic archaea isolated from Algerian arid and semi-arid wetland ecosystems for halophilic-active hydrolytic enzymes

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ARTICLE INFO

Keywords:
Wetlands
Dry salt lakes
Haloarchaea
Hydrolytic enzymes
Esterase
Protease

ABSTRACT

The diversity of haloarchaea associated with different dry salt lakes in northeastern Algeria was investigated together with their potential of hydrolytic enzyme production. A total of 68 aerobic halophilic archaea were isolated from saline sediments. Based on the 16S rRNA gene sequencing, the isolates were assigned to seven phylotypes within the class *Halobacteria*, namely *Haloarcula*, *Halococcus*, *Haloferax*, *Halogeometricum, Haloterrigena, Natrialba,* and *Natrinema*. The results showed that *Haloferax* group was found to be dominant in all samples (30 isolates) (44%) with high diversity, followed by *Halococcus* spp. (13%) (9 isolates). All phylotypes are extreme halophiles and thermotolerant with the ability to grow at temperatures up to 48 °C. In addition, the screening for extracellular halophilic enzymes showed that 89.7% of the isolates were able to produce at least two types of the screened enzymes. The strains producing esterase, gelatinase, inulinase, cellulase and protease activities were the most diverse functional group. These data showed an abundant and diverse haloarchaeal community, detected in Algerian wetland ecosystems, presenting a promising source of molecules with important biotechnological applications.

1. Introduction

Hypersaline environments host a considerable diversity of extremely halophilic archaea as well as halophilic and halotolerant bacteria (Oren, 2002). In the past few years, the microbial diversity of such hypersaline environments has been extensively explored using both culture-dependent and culture-independent techniques (Benlloch et al., 1995; Borsodi et al., 2013; Burns et al., 2004; Youssef et al., 2012), and the halophilic prokaryotes has been found in a wide range of saline environments within various geographical locations including salt lakes, marine salterns and saline soils (de la Haba et al., 2011; Lizama et al., 2001).

Halophilic archaea are traditionally associated with the members of the euryarchaeal class *Halobacteria* (Oren, 2014), which they constitute a heterogeneous group of microorganisms including species belonging to various genera (Gupta et al., 2015; Minegishi et al., 2010). Haloarchaea are characterized as extremophiles that require at least 1.5 M NaCl with optimum growth at 15–30% (2.5–5.2 M) (Litchfield, 2011). They have a unique ability to survive and grow at high salt concentration and thus, could serve as tremendous model systems to understand the molecular basis of high salt adaptation (Oren, 2002). The class *Halobacteria* accommodates 52 recognized genera, members of which inhabiting the thalassohaline, athalassohaline environments and other different ecological niches (Amoozegar et al., 2017).

Due to the ability to adapt hostile conditions, haloarchaea present specific features with biotechnological and industrial interests such as the capacity to produce biopolymers “biosurfactants, bioplastics exopolysaccharides”, pigments “bacteiorhodopsin”, antimicrobials and hydrolytic enzymes with stable and optimal activity under high temperature, salt concentration and extreme pH (DasSarma and DasSarma,

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https://doi.org/10.1016/j.micres.2017.12.011
Received 19 September 2017; Received in revised form 15 November 2017; Accepted 23 December 2017
Available online 04 January 2018
0944-5013/

2015; Delgado-García et al., 2012; Margesin and Schinner, 2001). Such halophilic tolerant or active enzymes are expected to be a very powerful tool and biocatalysts in industrial biotransformation processes performed under harsh conditions of pH, temperature, ionic strength and/or limited solubility (Delgado-García et al., 2012; Schreck and Grunden, 2014).

Algeria harbors several wetlands and hypersaline lakes, with rare typology and ecology in the world, of which 50 are classified as being of international importance as Ramsar sites (Aliat et al., 2016). They are characterized by typical endorheic systems that consist of saline lake ecosystems "Sebkhas and Chotts" with typical alternation of drought phase in summer and flooding in winter. Those located in arid and semi-arid regions constitute an important habitat in terms of biodiversity and functional role (Chenchouni et al., 2015).

Algerian wetlands have drawn the attention of many scientists because of their high diversity of flora and fauna (Chenchouni et al., 2015; Neffar et al., 2016). However, all aspects related to microbiota (diversity and bioactivity) are poorly investigated and remains unidentified (Boutaiba et al., 2011; Hacène et al., 2004, Kharroub et al., 2014; Quadri et al., 2016). In this report, we attempted to discuss the diversity of extremely halophilic archaea found in different sebkhas and chotts ecosystems located in arid and semi-arid climatic zones and bioprospecting for different salt active hydrolytic enzymes. Microbial isolation and molecular phylogenetic analysis of culturable haloarchaea were performed in order to provide information about their potential production of enzymes and their applications in future biotechnological processes.

2. Materials and methods

2.1. Study area

The sampling study was carried out in seven typical wetlands; Five sites of the Hauts Plateaux in Northeastern Algeria, a region with a typical semi-arid Mediterranean climate, characterized by hot-dry summers (30–35 °C) followed by cool-wet winters (3–8 °C) and two others located in hot hyper-arid lands (Sahara desert, northeastern) with very hot dry summer and absolute maxima exceeding 50 °C in July-August (Fig. 1). The water supply is highly dependent on rainfall and the majority of these wetlands run dry during the dry season from June to August (Aliate et al., 2016). Three of these wetlands are Ramsar sites; Chott Tinsilt (**CTI**), Chott El Beïdha–Hammam Essoukhna (**CHS**), Sebkha El Hamiet (**SE**) with the exception of Sebkha Djendli (**SDJ**), Sebkha Medghacen (**SMD**), Sebkha Oumache (**SOM**) and Chott Kralla (**KAR**).

2.2. Sample collection and isolation of halophilic archaea

Soil samples (100 g) were collected aseptically during dry periods between June and August 2016. Soil sampling was performed according to the four cardinal points in each of the seven studied sites. Two plating techniques, liquid enrichment and dilution plating, were used to isolate Haloarchaea in two saline nutrient-rich media: (i) Halophilic medium (HM) (Ventosa et al., 1982) supplemented with different total salt concentrations (15, 20, 25 and 30%), (ii) Medium adapted from the Oren formula (Oren, 1983). The stock solution was prepared as described by Subov (1931), containing per liter: NaCl, 234 g; $MgCl_2{\cdot}6H_2O$, 42 g; $MgSO_4{\cdot}7H_2O$, 60 g; $CaCl_2{\cdot}2H_2O$, 1 g; KCl, 6 g; $NaCO_3H$, 0.2 g; NaBr, 0.7 g; $FeCl_3$, 0.005 g. All media were adjusted to pH 7.3 before autoclaving and 2% of Bacto-Agar (Difco) was added for solidification. The plates were incubated for four weeks at 35 °C. Colonies were re-isolated by streaking on fresh plates with the same medium. Pure cultures were subcultured and stored on HM with 20% of total salt at 4 °C.

2.3. Phenotypic features: growth temperature, pH range, and sodium chloride tolerance

Gram staining of cells was carried out as described by Dussault (1955). Growth of haloarchaea isolates in different selected temperatures of (4, 22, 30, 35, 40, 45 and 50 °C), pH (4–9) with 0.5 intervals and in various salt concentration from (0–30%) with 2.5% intervals, was determined on HM agar for 3 weeks according to the protocols (Lizama et al., 2001; Rodriguez-Valera et al., 1980). The growth was carried out by spreading 20 µl of a culture suspension of each strain onto the surface of the respective media.

2.4. Molecular identification by 16S rRNA sequencing and phylogenetic analysis

2.4.1. Genomic DNA extraction

Representative halophilic archaea isolates were selected for molecular characterization (Table 1). The cultures initially isolated were grown on 20 ml HM broth supplemented with 20% total salt for 15–20 day. The cells were harvested at approximately the late exponential growth phase by centrifugation (3800xg for 20 min), and the Genomic DNA of haloarcheal isolates was extracted using Genomic DNA Purification Kit (Qiagen DNeasy kit, Germany) according to the manufacturer's protocol.

2.4.2. PCR amplification

The 16S rRNA gene amplification was done using HotStarTaq®MasterMix (Qiagen) (premixed solution contains HotStarTaq DNA Polymerase, PCR Buffer, and dNTPs with a final concentration of 1.5 mM $MgCl_2$ and 200 µM each dNTP) with the two primers D30 (5′-ATTCCGGTTGATCCTGC −3′) and D56 (5′-GYTACCTTGTTACGAC-3′). The final 25 µl volume of reaction mixture contains 12.5 µl HotStarTaq®MasterMix and 12.5 µl of a solution containing 200 nM of each primer, 1.5 mM of additional $MgCl_2$ (Promega, Madison, WI, USA) and template DNA diluted in PCR grade water. The PCR amplification consisted of initial denaturation at 97 °C for 5 min, followed by 30 cycles of denaturation at 97 °C for 45s, annealing at 55 °C for 60s, extension at 72 °C for 2 min, and a final polymerization step of 72 °C for 2 min.

2.4.3. Sequencing and phylogenetic analysis

PCR products were purified using Amicon®Ultra0.5 centrifugal filter devices (Merck, Irland) and direct sequencing was performed by using a PRISM TaqDye Deoxy DNA sequencer "Applied Biosystems". Phylogenetic analyses were carried out using MEGA software version 6 and the 16S rDNA sequences of haloarchaea were aligned using the CLUSTALW program (Thompson et al., 1994) against corresponding nucleotide sequences of representative's genera recovered from GenBank databases and EzTaxon-server (Kim et al., 2012). Evolutionary distance matrices were generated as described by Jukes and Cantor (1969) and phylogenetic trees were constructed using the neighbor-joining method Saitou and Nei (1987). The topologies were evaluated by bootstrap sampling expressed as a percentage of 1000 replicates (Felsenstein, 1985). The 16S rRNA gene sequences were submitted to NCBI GenBank and the assigned accession numbers were MG758706-MG758773

2.5. Screening of extracellular hydrolytic enzymes

For the qualitative detection of extracellular enzymes, the relevant assays were performed on agar plates using a drop spot technique after incubation at 35 °C for 2–4 weeks. Starter cultures used for the enzymatic screening were obtained by growing the haloarchaea microorganisms in 10 ml HM broth supplemented with 20% total salt with shaking at 120 rpm for about one week. Aliquot (10 µl) of broth culture of each test archaeon was spotted onto appropriate media. All