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# Characterization and antimicrobial potential of extremely halophilic archaea isolated from hypersaline environments of the Algerian Sahara

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ABSTRACT

Halophilic archaea were isolated from different chotts and sebkha, dry salt lakes and salt flat respectively, of the Algerian Sahara and characterized using phenotypic and phylogenetic approaches. From 102 extremely halophilic strains isolated, forty three were selected and studied. These strains were also screened for their antagonistic potential and the production of hydrolytic enzymes. Sequencing of the 16S rRNA genes and phylogenetic analysis allowed the identification of 10 archaeal genera within the class *Halobacteria*: *Natrinema* (13 strains), *Natrialba* (12 strains), *Haloarcula* (4 strains), *Halopiger* (4 strains), *Haloterrigena* (3 strains), *Halorubrum* (2 strains), *Halostagnicola* (2 strains), *Natronococcus*, *Halogeometricum* and *Haloferax* (1 strain each). The most common producers of antimicrobial compounds belong to the genus *Natrinema* while the most hydrolytic isolates, with combined production of several enzymes, belong to the genus *Natrialba*. The strain affiliated to *Halopiger djelfamassilliensis* was found to produce some substances of interest (halocins, anti-*Candida*, enzymes). After partial purification and characterization of one of the strains *Natrinema gari* QJ1, we found similarities between the antimicrobial compound and the halocin C8. Therefore, the gene encoding halocin C8 was amplified and sequenced.



## 1. Introduction

Hypersaline habitats occur worldwide and are typical of extreme environments including saline lakes, salterns, and saline and hypersaline soils (Oren, 2002a,b). The Algerian Sahara consists of numerous extreme ecosystems including those with hypersaline soils, such as the chotts and sebkhas, which are respectively, dry salt lakes and salt flats occurring on arid coastline.

Microorganisms that thrive in these habitats are considered as halophiles and extremophiles that need not only to be able to withstand the high ionic composition but also other environmental stressors such as alkaline pH values, low oxygen availability, high pressure, high or low temperatures, presence of heavy metals and/or other toxic compounds (Oren, 2002a,b; Ventosa 2006). The two classical groups of halophiles from hypersaline environments are the extremely aerobic halophilic archaea (Haloarchaea) and the halophilic bacteria, represented by only small number of species.

Halophilic archaea are the dominant microorganisms in hypersaline environments worldwide and can be found where salt concentrations exceed 150–200 (w/v) (Oren, 2006). The class *Halobacteria* is one of the largest groups within the domain *Archaea* which contains a single order, *Halobacteriales*, containing a single family, *Halobacteriaceae* (Grant et al., 2001).

More recently, Gupta et al. (2015) proposed two new orders within the class *Halobacteria*, *Natrialbales* ord. nov. and *Haloferacales* ord. nov., containing the novel families *Natrialbaceae* fam. nov. and *Haloferacaceae* fam. Nov. However this new classification

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is not recognized formally in Subcommittee on the taxonomy of *Halobacteriaceae*.

Halophilic archaea like other extremophiles, have many important and fascinating aspects: they can show us the limits of life "as we know it", and enlarge our understanding of the biodiversity on Earth. Elucidation of the mechanisms that enable extreme halophiles to withstand otherwise hostile conditions provides a more profound insight into the functioning of living cells and may also lead to interesting biotechnological applications and commercial uses. Halophiles may provide organic compounds like antimicrobials, enzymes, pigments, compatible solutes, and lipids that are highly stable and active under extreme conditions and which might have a biotechnological potential (Laundry industry, food coloring, pharmaceuticals, biosurfactants) (Tango and Islam, 2002; Litchfield, 2011).

Haloarchaea were the first members of archaea found to produce bacteriocins, named halocins. They are peptide or protein antibiotics secreted into the environment to kill or inhibit the sensitive haloarchaeal strains that occupy the same niche. Halocin production is a universal feature of *Halobacteria* (Rodriguez-Valera et al., 1982; Sun et al., 2005), and, although it seems that hundreds of different types exist, only a few halocins have been studied in detail. To date, little attention has been paid to this potentially interesting group of peptidic antibiotics (Atanasova et al., 2013; Litchfield, 2011). Halocins can be divided into two groups, high molecular mass and low molecular mass halocins (that have been sometimes termed microhalocins), depending on their molecular masses and main properties, essentially their heat-stability, and the NaCl concentration needed to remain active (Shand and Leyva 2008; Besse et al., 2015).

Halocin C8 (HalC8) is a stable microhalocin exhibiting strong antimicrobial activity against a wide range of haloarchaea. It was purified and characterized in *Natrinema* sp. strain AS7092 (formerly *Halobacterium* sp. Strain AS7092) (Li et al., 2003; Mei et al., 2008).

Extremozymes from halophilic archaea are not only extremely high salt tolerant but also thermotolerant because of the specific environment in which they live. They seem to be very good candidate for industrial application because, besides being salt loving, they may have excellent activity at high temperature, low water activity and high pH (Makhdoumi Kakhki et al., 2011).

Until now, only a few reports have existed on the characterization of halophilic archaea isolated from Algerian saline ecosystem, these studies focused mainly on saline lakes (Hacène et al., 2004; Boutaiba et al., 2011; Imadalou-Idres et al., 2013).

The main purpose of the present research was to isolate and characterize archaeal strains using cultivation approaches on hypersaline soils collected from different sites of the Algerian Sahara. The study of such ecosystems could significantly contribute to expanding our knowledge of halophilic archaea. In addition the isolates were screened for their antagonisitic activities and the production of different hydrolytic enzymes.

Partial purification of the antimicrobial compound produced by one of the strains showed similarities with halocin C8. Therefore the gene encoding halocin C8 was amplified and sequenced.

## 2. Materials and methods

### 2.1. Study sites and samples collection

Soils samples from seven chotts and sebkhas in different locations of Algerian Sahara were collected aseptically. The samplings were made at the surface in center of each site in sterile plastic containers over the period 2010–2012 (Table 1).

The samples were collected from Chott Melghir (Biskra) (34°12′37.89″N, 6°21′54.565″E) in northeastern of the Algeria Sahara, Sebkha Ezzmoul (Ain M'lila) (35°55′30″N, 6°32′50″E) in northeastern Algeria, Sidi Ameur (Bousaâda) (35°12′36.97″N, 40°10′46.08″E) and Chott Zahrez Gharbi (Djelfa) (34°53′47.527″N, 2°47′52.443″E) in the north Algerian Sahara, Chott Ouargla (31°57′43.99″N, 5°23′20″E) in mid-eastern Algeria, Sebkha EL Golea (El Menia) (30°34′59.99″N, 2°52′59.998″E) in the center of the Algerian Sahara, and Sebkha Adrar (27°52′0.001″N, 0°16′59.998″W) in southwestern Algeria (Fig. 1).

pH and T° were measured in all samples using a HANNA multiparameters (HI 9829), conductivity was measured with a WTW Measuring Instrument (Multi 340i) which automatically calculates salinity.

### 2.2. Isolation of halophilic archaea

Each sample was enriched in two media: modified DSMZ medium 97 and Sehgal-Gibbons (SG) medium (Sehgal and Gibbons 1960). The modified DSMZ medium 97 contained, per liter: NaCl 250 g, $MgSO_4 \cdot 7H_2O$ 20 g, KCl 2 g, $FeSO_4 \cdot 7H_2O$ 0.05 g, $MnSO_4 \cdot H_2O$ 0.20 mg, trisodium citrate 3 g, casamino acids (Difco) 7.5 g, and yeast extract (Sigma-Aldrich, Y0875) 1 g. The SG medium contained: NaCl 250 g, $MgSO_4 \cdot 7H_2O$ 1 g, KCl 2 g, $Na_2CO_3$ 10 g, trisodium citrate 3 g, casamino acids 7.5 g, and yeast extract 10 g. The pH was adjusted to 7.0 with 1 M KOH before autoclaving. For solid medium, 20 g $l^{-1}$ Bacto-agar (Difco) was added. The enrichment procedure was conducted as follows: 1 g of each sample was added to 20 ml of the two media supplemented with ampicillin (100 μg/ml) and incubated in a shaking incubator (5 *g*) at 40 °C for 7 days. After enrichment aliquots were plated in media supplemented with sterilized sediment extracts and incubated at 40 °C in sealed plastic bags to avoid desiccation. After eight weeks of incubation, pure cultures were obtained by subsequent transfer of representative colonies to agar plates of appropriate medium.

### 2.3. Characterization of the isolates

#### 2.3.1. Morphological, cultural, physiological and biochemical tests

Colony morphology was observed under optimal growth conditions on solid media after incubation in aerobic conditions at 40° C for 7 days. Gram staining was performed using acetic acid-fixed cells as described by Dussault (1955). Cell lysis was observed by diluting dense cell suspensions with the diluted medium or distilled water. Growth and optimum conditions were determined at different temperatures (4, 25, 30, 37, 40, 55, and 60 °C), pH (4.0, 5.0, 6.0, 8.0, 9.0, 10.0, and 12.0) and NaCl concentrations (5.0%, 10.0%, 12.0%, 15.0%, 20.0%, and 30%). Tests for catalase and oxidase activities, formation of indole were performed according to the standard modified procedures by Oren et al. (1997). Production of acid from different sugars (glucose, lactose, mannitol, sucrose, xylose, fructose, arabinose, rhamnose, ribose, sorbitol, and sorbose), sterilized by filtration, added to a final concentration of 1%, was tested in medium without sodium citrate, supplemented with 0.001% of red phenol.

Antibiotic sensitivity was determined using modified DSMZ medium 97 and (SG) medium. Sterile disks containing different antibiotics were placed onto the surface of an inoculated Petri plate. The plates were incubated at 40 °C for 7 days. The antibiotics tested were: novobiocin (30 μg/disc), bacitracin (10 μg/disc), ampicilin (10 μg/disc), penicillin (10 μg/disc), nalidixic acid (30 μg/disc), gentamycin (10 μg/disc), oxacilin (10 μg/disc), kanamycin (30 μg/disc), chloramphenicol (30 μg/disc), cefotaxim (30 μg/disc), and streptomycin (10 μg/disc). Sensitivity was defined by the appearance of a zone of inhibition around the antibiotic disc.