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# Characterization of Some Bacteria in the Waters of Chott of Aïn El-Beida in the South of Algeria

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## Abstract

Hypersaline environments, represented by the chotts, the Sebkhas and the saline soils, etc., are a typical example of extreme environment harboring a particularly interesting microbial flara. In this context, the aim of this word is to try to make a characterization of the bacteria in the waters of the chott of Aïn El-Beida in the region of Ouargla (South Algeria). The water samples taken are characterized by a pH between 6.6 and 8.39, the water is neutral to alkaline water, with a temperature between 8˚C to 10˚C, and an electrical conductivity between 2.4 to 6 ds/m reflecting extremely salty water. These characteristics enabled us to select a microflora specific to this environment. The microbiological analsis of these samples revealed fifteen isolated strains, including the following species: *Escherichia coli* 2, *Flavobacterium meningosepticum*, *Shigella sp*, *Aeromonas salmonicida*, with a bacterial density that varied between 0.27 × 104 to 2.236 × 104, which may explain why the water in this ecosystem is rich in specific important microflora. The results of NaCl tolerance have revealed to us that certain isolated strains tolerate a NaCl concentration up to 35%, these are euryhaline bacteria. With regard to the antagonist test, the strains (S8) have a strong antifungal capacity, which can be explained by the fact that latter has a significant competitive power against *Aspergillus carbonarius* and *Penicillium sp*. The microbial exploration of this specific extreme ecosystem proved successful, as we were able to detect significant specific microbial diversity.

## Keywords

Waters, Characterization, Bacteria, Chott, South of Algeria

## 1. Introduction

Many microorganisms colonize all aquatic ecosystems. These microorganisms develop in physico-chemical conditions favorable to their growth, most of which have good water availability, a temperature between 20˚C and 30˚C, atmospheric pressure, and a neutral pH [1]. On the other hand, other microorganisms qualified as extremophiles are able to colonize extreme environments in terms of temperature, pH, salinity, depth or other physico-chemical parameters. These environments are characterized by physico-chemical conditions considered hostile to the life of most organisms. From the 1980s, many techniques have been developed for the identification and determination of the composition of halophilic microbial communities in hypersaline environments. The study of these communities is essential to understand the functioning of this type of ecosystem and the interactions that exist between the microbial communities [2] [3]. Hypersaline environments are numerous in Algeria, several of them are considered as wetlands and classified as Ramsar sites. Many studies have focused on these environments highlighting their fauna and flora diversity. However, the study of these environments from the point of view of microbial diversity has been little explored. In view of current knowledge, we conducted a study to characterize some halophilic bacteria from the waters of the chott of Ain El Beida, in the Sahara of Algeria. The site is a salt depression surrounded by palm groves and crossed by several canals that drain excess water from the plantations and the nearby town. Several species of birds from the African-Eurasian flyway stop at the site; some spend the winter there like the common shelduck Tadorna ferruginea, while others nest there, notably the black stilt Himantopus himantopus and the elegant avocet Recurvirostra avosetta. The mammals of the site have not yet been the subject of in-depth studies. Saharo-Mediterranean vegetation belongs to 12 families and some, such as Chenopodiaceae, are known for their medicinal properties against gastric problems and should benefit from special protection measures to prevent overexploitation. Sewage pollution and illegal dumping of waste are the main threats to the biodiversity of the site [4].

We therefore endeavored during this work to apprehend the halophilic bacteria in this habitat. The objective of our work is to make a characterization of some bacteria in an aquatic ecosystem, water samples taken from chott (outlet of untreated water) were subjected to physico-chemical analyzes followed by identification of isolated bacteria.

## 2. Presentation of the Chott Aïn El-Beida

Salt lakes in Algeria

Algeria has a large number of aquatic ecosystems of the natural wetland type in the Mediterranean region. These areas are represented in the form of fresh or marine water marshes, Oued, dams and reservoirs, of which more than 50% of these sites are salt lakes covering approximately two million hectares [5] [6]. The majority of these water bodies are made up of huge continental salt lakes, gener-

ally bordering in arid to semi-arid areas. These lakes stretch from the northern Algerian coast to the Sahara crossing the High Plateaus, formed by vast continental endorheic depressions, synonymous with the Chotts and Sebkhas [7]. The Chott is defined as a salty zone surrounding the Sebkhas, which is the humid bottom of the depression [8]. Hypersaline environments are those whose dissolved salt content is higher than that of seawater (35 g/L) [9] (Satayarayana *et al.*, 2005). When we exceed 100 g/l in salts, the environments become extreme and inhibit the growth of a large majority of microorganisms [10].

The chott of Aïn El-Beida constitutes the low point of the city of Ouargla **Figure 1**. Lying in a North-West, South-East direction over a length of 5.3 km, its width varies from 1 to 1.5 km, not exceeding 1.50 m in depth [11]. The water supply of the chott comes from the water table, the level of which varies according to the season and the actions of man (drainage of the palm grove and irrigation).

## 2.1. Material and Methods

Before choosing the sampling station, a visual recognition such as the color of the water, the direction of the wind or any other relevant observations (bad odours, etc.). Water samples from the chott are taken at three different points and from a depth ranging from 1 cm to 50 cm (**Figure 2**).

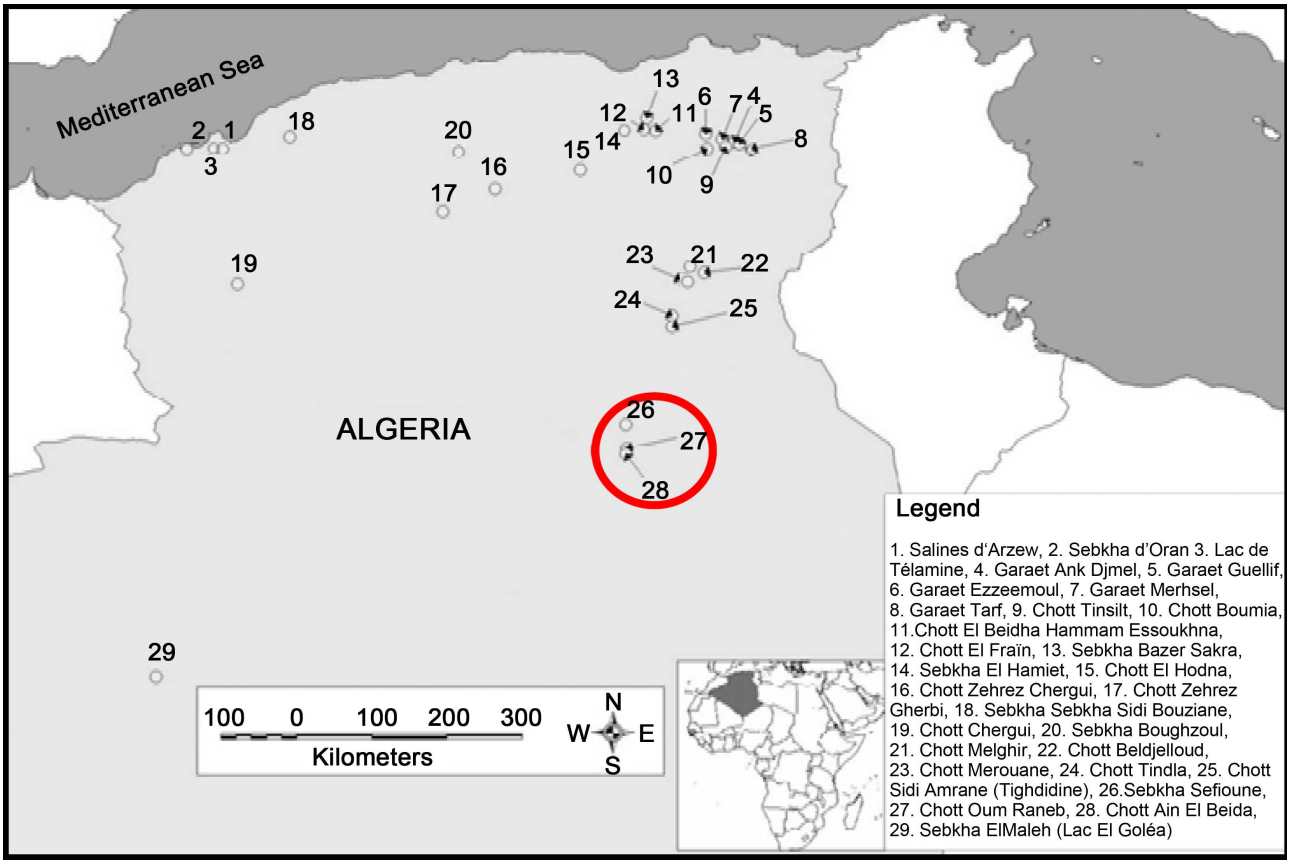


**Figure 1.** Geographical location of chotts and sebkhas in Algeria (non-exhaustive list) (Demnati *et al.*, 2017) [12].

Figure 2. Sampling site.

### 2.2. Measurement of Physicochemical Parameters of Samples

Measurement of the physico-chemical parameters of the samples: pH, temperature and electrical conductivity are measured in situ. The pH in the aquatic environment is essentially a function of the chemical composition of the aqueous solution and the activity of the organisms [13]. Electrical conductivity makes it possible to assess the quantity of salts dissolved in water [14]. Microbiological analyzes are carried out in the bacteriology laboratory of the Mohamed Boudiaf hospital, Isolated strains are identified through analysis based on standard microbiological techniques. Tolerance of characterized strains to NaCl: we carried out this test to determine the optimum growth of the isolated strains and to see the influence of different NaCl concentrations according to their behavior. The isolated strains were tested for their tolerance to NaCl. Different concentrations (7.5%; 10%; 12.5%; 15%; 17.5%; 20% and 35%). And we also carried out the test of antagonistic activity of bacterial strains with two referenced fungi, to study an important ecological, biotic factor which is the competition.

## 3. Results and Discussion

### 3.1. Results of Physical and Chemical Analyses

The samples of water taken from different sampling points show a T˚C varied between 8˚C and 10˚C. The evolution of the temperature of a salt lake or chott remains linked to local conditions such as the regional climate, the topography, the duration of the sunshine, the flow and the depth [15].

The recorded pH of sample 3 is neutral varied between 6.6 to 7.2 while the pH of sample 1 and sample 2 tends towards alkalinity with values 7.55 and 8.39 successively. The electrical conductivity (EC) of the water sample is between 2.4 and 6 ds/m, these results which allow us to qualify the water of the chott of Aïn El-Beida from salty to very salty (hyper salty) [16]. Identification involves a series of steps, most often following one another in a specific order. It is carried out based on standard microbiological techniques such as morphological and biochemical analysis (gram staining, catalase and oxidase) and finally by the use of an API 20 E identification system. Concerning the bacterial density the values vary between 0.27 × 104 and 2.236 × 104 for the isolated strains. It is concluded that the microbial density of the chott water of Aïn El-Beida is abundant and diversified. For the results of the strains of the water samples of the Chott of Ain

Elbaida (Table 1).

### 3.2. Tolerance of Bacterial Strains to NaCl

In the context of the characterization of isolated bacteria, we tested NaCl tolerance (Table 2). The strains that have a high tolerance to NaCl are halophilic bacteria, which explains their presence in the chott of Aïn El-Beida. Salinity is considered an abiotic factor and may even be a limiting factor for the growth of these halophilic bacteria, as well as certain environmental conditions such as physico-chemical parameters such as pH, electrical conductivity and temperature influence the growth of microrganisms.

- All the strains studied tolerate a concentration of NaCl up to 15% except the strain (S1), six strains tolerate a concentration of 35% of NaCl. The tolerance of the strains is different for the 17.5% and 20% concentrations. The strains (S3, S4, S5, S6, S10, S11, S15) relatively tolerate high salt concentration of 20% - 35%, and can be classified as euryhaline species, they are halophilic bacteria, as has been suggested by some authors [17] [18].
- By comparing our results with the work of including the identification of the LMB3981 strain in Lake El Goléa, where the NaCl concentration is equal to 25% (w/v). Regarding strains S2 to S15, the latter developed normally at NaCl concentrations of 5% to 17.5%, similar results are found in the hypersaline lake of Bakhtegan, located in southern Iran where they were able to identify the strain AF-2004 [19].

**Table 1.** Results of the identification of strains isolated from the chott of Aïn El-Beida.

| Isolats numbers | Nam of bacteria |
|---|---|
| 1 | - |
| 2 | - |
| 3 | - |
| 4 | - |
| 5 | *Escherichia coli* 2 |
| 6 | *Flavobacterium meningosepticum* |
| 7 | *Shigella spp* |
| 8 | - |
| 9 | - |
| 10 | - |
| 11 | - |
| 12 | *Escherichia coli* 2 |
| 13 | *Aeromonas salmonicida* |
| 14 | - |
| 15 | - |

-: Not identified.

**Table 2.** The results of the strains at different NaCl concentrations.

| | NACL concentration | | | | | | |
|---|---|---|---|---|---|---|---|
| Isolat | 7.5% | 10% | 12.5% | 15% | 17.5% | 20% | 35% |
| S1 | + | - | - | - | - | - | - |
| S2 | + | + | + | + | + | - | - |
| S3 | + | + | + | + | + | + | + |
| S4 | + | + | + | + | + | + | + |
| S5 | + | + | + | + | + | + | + |
| S6 | + | + | + | + | + | + | + |
| S7 | + | + | + | + | - | - | - |
| S8 | + | + | + | + | + | - | - |
| S9 | + | + | + | + | + | - | - |
| S10 | + | + | + | + | + | + | + |
| S11 | + | + | + | + | + | +f | - |
| S12 | + | + | + | +f | - | - | - |
| S13 | + | + | +f | +f | - | - | - |
| S14 | + | + | + | + | + | + | - |
| S15 | + | + | + | + | + | + | + |

+: Presence of growth; -: Absence of growth; +f: Low growth.

- The tolerance of S2 to S15 strains shows that they can grow in environments where the NaCl concentration varies between 5% and 15%. The work of [20], at the Sfax-Tunisia salt station, revealed 40 halotolerant strains, the majority of which developed optimally between 5% - 15% salt, at 37˚C and at pH 7.

### 3.3. Test for the Antagonistic Activity of Bacterial Strains

Among our objectives to highlight the ecological factors of our bacteria selected in the waters of the chott of Aïn El-Beida, to see their interactions with the biocenosis the environment, we chose two fungi in order to evaluate the microbial activity of our strains which have been tested by the cross-streak method.

The fungis chosen for the antifungal test are referenced:

- *Aspergillus carbonarius* (Ac) which is a telluric fungus that produces toxic substances (mycotoxins).
- *Penicillium sp* (p).

The results obtained after an incubation period of 48 hours for the fungi, by measuring the zones of inhibition in millimeters between the border of the characterized strain and that of the target strain (Fungi-test) (**Figure 3**).

- To the left Ac: *Aspergillus carbonarius*
- To the right P: *Penicillium sp*
- The S3 strain inhibits the growth *of Aspergillus carbonarius* (Ac) and *Penicillium sp* (P) as shown in **Figure 3**.

The results of the antifungal activity of 15 isolated strains are presented (Table 3).

The results obtained clearly show that the antifungal action differs from one strain to another depending on the test fungi (Table 3).

- For the antifungal activity of isolate S6 is considerable with the fungus *Aspergillus carbonarius* (Ac), on the other hand we note no antifungal activity against *Penicillium sp* (p).
- The inhibition of fungal growth is mainly due to competition between the strains tested and the fungi used. *Aspergillus* produces a large number of spores which makes it more competitive, as has been suggested by some authors [21]. Competition between microorganisms also takes place for nutrients and according to who showed that *Aspergillus* contains the element of competition for the acquisition of iron [22].
- For strain S3 and strain S9 showed resistance against *Aspergillus carbonarius* with an inhibition zone of 05 mm and 02 mm successively.
- S6 and S9 inhibited only *Aspergillus carbonarius* with an inhibition zone of 40 mm and 02 mm successively.

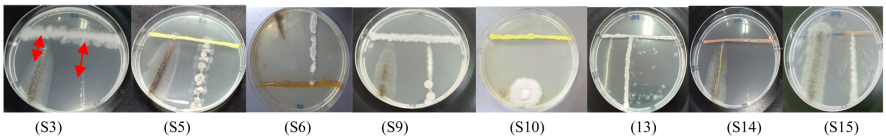


**Figure 3.** Antifungal activity of strains (S3, S5, S6, S9, S10, S13, S14, S15).

**Table 3.** Antifungal action of 15 selected isolates against 2 target fungi.

| | Muting distance (mm) | |
|---|---|---|
| **Isolate** | ***Aspergillus carbonarius* (Ac)** | ***Pénicillium sp* (p)** |
| S1 | 30 | 22 |
| S2 | 35 | 35 |
| S3 | 05 | 20 |
| S4 | 20 | 27 |
| **S5** | **00** | **00** |
| **S6** | **40** | **00** |
| S7 | 27 | 27 |
| S8 | 50 | 40 |
| **S9** | **02** | **00** |
| S10 | 30 | 20 |
| S11 | 45 | 35 |
| S12 | 40 | 35 |
| **S13** | **00** | **00** |
| **S14** | **00** | **-** |
| **S15** | **00** | **00** |

-: Absence.

- The result of the antifungal activity showed the ability of S8 to strongly inhibit the growth of *Aspergillus carbonarius* (Ac) and *Penicillium sp* (p) with an inhibition zone of 50 mm and 40 mm successively.

Strains that show good resistance against fungi can be used in biological control against fungal contamination of dates caused by *Aspergillus carbonarius (*Ac) and *Penicillium sp* (p), the latter two were the most frequently isolated on dates [23].

## 4. Conclusions

Through the results obtained, the Chott of Ain El Baida is an ecosystem, which is part of the wetlands, the isolated bacteria have a strong ecological valence, they are euryhalines, the NaCl tolerance test and the antagonism test confirm that the bacteria selected from the Chott of Ain Elbaida are tolerant, competitive and resistant. This work allowed us to have interesting data on some bacteria of our ecological niche (the chott of Aïn El-Beida) in the region of Ouargla.

We carried out various physico-chemical and microbiological analyzes to better explain the biotic and abiotic factors directly and indirectly influencing the characterization of these bacteria which play an important role in the ecological field to see the interactions between their biotope and their biocenosis.

From the physico-chemical analyzes (T˚, pH and EC), we distinguished that the water of our samples had a temperature which varies between 8˚C to 10˚C.

During the various tests carried out on the strains which are selected and purified, we have noticed that our isolates relatively tolerate a high concentration of salt, and can be classified among the euryhaline species, it should be noted that some of our strains have a power of tolerance in NaCl up to 35%.

The results of the antagonistic activity reveal that certain target fungi are inhibited by certain strains studied. Strain 8 has a strong inhibitory capacity with high values against target fungi. On the other hand, S6 and S9 inhibited only *Aspergillus carbonarius*.

This work opens up multiple perspectives in these little-explored ecosystems in order to better understand the interactions of microorganisms and their habitats.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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