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SALT TOLERANCE OF BACTERIAL STRAINS ISOLATED FROM HYPERSALINE WATER LOCATED IN LOPATARI, ROMANIA

Irinel Gabriel PROCA, Florentina MATEI, Camelia Filofteia DIGUȚĂ, Ștefana JURCOANE

University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Biotechnologies, 59 Mărăști Blvd., District 1, Bucharest, Romania

Corresponding author email: irinelproca@yahoo.com

Abstract

Saline waters are environments largely unexplored in which organisms are able to survive/withstand in the extreme conditions. The aim of this research was to explore saline water located in Lopătari, Buzău County, România. Several physico-chemical properties were assessed such as pH, density, concentration of cations and anions. The pH of water sample was slightly acidic (5.2).The ions of sodium, chloride and total dissolved solids were detected dominant 129.9 $g.L^{-1}$, 209.83 $g.L^{-1}$and 113 gL^{-1}, respectively. A total of fourty four (44) aerobic bacteria were isolated on culture media supplemented with 5%, 10 %, and 15% NaCl, respectively. The isolates were screened by morphological criteria, Gram stain. All bacterial isolates were tested for their tolerance to different concentrations of salt in solid media. Two bacterial isolates (4.5%) has grown over a wide range of salt concentrations ranging from 0% to 12.5%, 17 bacterial isolates (38.6%) under salt concentration between 0% and 7.5%. Other 19 bacterial isolates (41%) demonstrated a large salt tolerance ranging from 5 to 25%. Halophiles can offer important opportunities in biotechnological applications such as food, pharmaceutical, detergents, environmental bioremediation and biosynthetic processes, being one of the main reasons for the future research of them.

Key words*: bacterial isolates, hypersaline water, biochemical analysis, salt tolerance.*

INTRODUCTION

In the last years, the research has been focused towards the detection of halophilic microorganisms with the ability to grow in saline environments and useful in different industrial applications (O'Brien et al., 2004; Enache et al., 2007; Enache et al., 2009; Oren, 2010; Neagu et al., 2014).

Hypersaline environments are typical extreme habitats distributed worldwide as hypersaline waters (natural or man-made salt lakes, the Dead Sea), salt mines, saltern pond brines or salted soils (Rodriguez-Valera, 1988; Ventosa, 2006; de la Haba et al., 2011).

Although, a lot of salted environments are located in Romania, only several works have been focused on the investigation of some halophilic microorganisms (especially bacteria and Archaea) isolated from few saline areas: Lake Telega (Enache et al., 2007), Salina Unirea (Cojoc et al., 2009), Balta Albă (Neagu et al., 2014) and Techirghiol (Enache et al., 2009).

The salt concentration of these ecosystems can influence the diversity of microbial communities. Non-halophiles grow optimally at less than 2% NaCl, slight halophiles grow optimally at 2–5% NaCl; moderate halophiles grow optimally at 5–20% NaCl; and extreme halophiles grow optimally above 20–30% NaCl (DasSarma and Arora, 2001).

The purpose of this research was to briefly explore the bacterial diversity from hypersaline water located in Lopătari, Buzău County, România and to assess salt tolerance of the bacterial isolates.

MATERIALS AND METHODS

Sample sources: Lopătari region is located in the Subcarpathians Curvature, in the Buzău County, România (45° 29'14.7 "N 26° 37'24.8" E), in the basin of Slănic river. The study area is 56-60 km far away from Buzău and is part of the massive Sările-Bisoca, which covers an area of about 57 hectares (Figure 1). About 1000 ml of surface water were collected during June 2016 in clean sterile bottles and stored at 4°C until use.

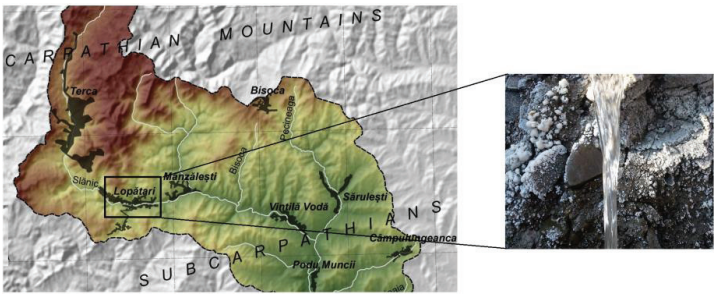

Figure 1 – Map site – hypersaline water streams located in the village Lopătari, Buzău County, România

Physico-chemical analysis of the samples included: pH using digital pH meter, Na^{+}, K^{+} by atomic absorption spectrometry (ISO 9964-1/1993), Ca^{2+}, Mg^{2+} by atomic absorption spectrometry (SR ISO 7980/1997), Cu^{2+}, Zn^{2+} by atomic absorption spectrometry (SR ISO 8288/2001), Fe^{2+} by atomic absorption spectrometry (SR 13315/1996) Mn^{2+} by atomic absorption spectrometry (SR8662-2/1997), TDS by electrochemical method (internal procedure) $SO4^{2-}$ by turbidimetry method (EPA 9038), Cl^{-} by volumetric method (SR ISO 9297/2001).

Isolation and identification procedure: To isolate **halophilic bacteria**, 50-100 mL were filtered using Millipore membrane filters (0.22-µm pore), and the membranes were placed on Nutrient Agar (Merck, Gemany) supplemented with 5%, 10 %, and 15% NaCl, respectively. After incubation for several days at 36±2°C, different colonies were selected and further purified on nutrient agar. The isolates were screened by classical methods (including cultural characteristics, microscopic morphology (cell shape and grouping, motility, Gram-stain reaction).

For the **halotolerance test**, a loop of bacterial cells from 24-48 h cultures was streaked in line on the Nutrient Agar plate supplemented with different NaCl concentrations: 0 – 30%. After incubation at 36±2°C for 7 days, the ability of the bacterial isolates to grow in the presence of salt was monitored. All experiments were carried out independent, in duplicate.

RESULTS

Hypersaline water streams located in Lopatari, Buzău County, România were taken in account for this study This salt water is used frequently in home-preserved vegetables and fruits by local people.

As illustrated in Table 1, the pH of water sample was slightly acidic (5.2) in contrast to alkaline lakes, such as Balta Albă (pH 8.6) or Wadi Natrun Lake (pH 11).

The ions of sodium (129.9 $g.L^{-1}$) and chloride (209.83 $g.L^{-1}$) were detected at higher concentrations, with chloride value similar to the concentration of the Dead Sea. The total dissolved solids detected were 113 $g.L^{-1}$.

Also, the potassium, magnesium and calcium ions were detected, but at low concentrations. Concentration of Fe^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+} ions were under LoQ (quantification limit=0.1 mg/L).

Table 1 – Comparative physico-chemical properties of hypersaline waters

Source	pH	Density	Na^{+}	K^{+}	Ca^{2+}	Mg^{2+}	Cl^{-}	SO_4^{2-}	Reference
Lopătari, România	5.2	1.19	129.9	0.17	1.7	0.28	209.83	3.89	In this study
Balta Albă, România	8.6	1.1	137	26	2	6	44	nd	Neagu et al., 2014
Great Salt Lake, SUA	7.7	nd	105	6.7	0.3	11.1	181	27	Grant, 2004
Dead Sea	6.3	nd	39.7	7.6	17.2	42.4	219	0.4	
Lake Magadi	11	nd	161	2.3	0	0	111.8	16.8	
Wadi Natrun, Zugm	11	nd	142	2.3	0	0	154.6	22.6	

Ions are represented as g per liter, density as cm^3 per g, n.d.- not determined

A total of 44 aerobic bacterial strains were isolated, from which 19 strains on culture media supplemented with 5% NaCl; 14 strains on culture media supplemented with 10 %, and 11 strains on culture media supplemented with 15% NaCl, respectively. 12 bacterial species were identified by classical methods. In addition, it was observed that the bacterial diversity decreases with the adaptability to high salt concentrations.

Table 2 – Salt tolerance of bacterial isolates

No.	Strains	NaCl concentrations									
		0%	2.5%	5%	7.5%	10%	12.5%	15%	20%	25%	30%
1.	10.5	+	+	+	+	-	-	-	-	-	-
2.	11.5	+	+	+	+	-	-	-	-	-	-
3.	13.5	+	+	+	+	-	-	-	-	-	-
4.	14.5	+	+	+	+	-	-	-	-	-	-
5.	24.5	+	+	+	+	-	-	-	-	-	-
6.	45.5	+	+	+	+	-	-	-	-	-	-
7.	46.5	+	+	+	+	-	-	-	-	-	-
8.	**47.5**	+	+	+	+	+	+	-	-	-	-
9.	**48.5**	+	+	+	+	+	+	-	-	-	-
10.	50.5	+	+	+	+	-	-	-	-	-	-
11.	51.5	+	+	+	+	-	-	-	-	-	-
12.	54.5	+	+	+	+	-	-	-	-	-	-
13.	55.5	+	+	+	+	-	-	-	-	-	-
14.	57.5	+	+	+	+	-	-	-	-	-	-
15.	58.5	+	+	+	+	-	-	-	-	-	-
16.	59.5	+	+	+	+	-	-	-	-	-	-
17.	60.5	+	+	+	+	-	-	-	-	-	-
18.	62.5	+	+	+	+	-	-	-	-	-	-
19.	63.5	+	+	+	+	-	-	-	-	-	-
20.	26.10	-	+	+	+	+	+	+	+	+	-
21.	27.10	-	-	+	+	+	+	+	+	+	-
22.	28.10	-	+	+	+	+	+	+	+	+	-
23.	29.10	-	-	+	+	+	+	+	+	+	-
24.	31.10	-	-	+	+	+	+	+	+	+	-
25.	**32.10**	-	+	+	+	+	+	+	+	+	+
26.	33.10	-	-	+	+	+	+	+	+	+	-
27.	**35.10**	-	+	+	+	+	+	+	+	+	+
28.	36.10	-	-	+	+	+	+	+	+	+	-
29.	37.10	-	-	+	+	+	+	+	+	+	-
30.	38.10	-	-	+	+	+	+	+	+	+	-
31.	**39.10**	-	+	+	+	+	+	+	+	+	+
32.	41.10	-	-	+	+	+	+	+	+	+	-
33.	65.10	-	-	+	+	+	+	+	+	+	-
34.	15.15	-	-	+	+	+	+	+	+	+	-
35.	16.15	-	-	+	+	+	+	+	+	+	-
36.	17.15	-	-	+	+	+	+	+	+	+	-
37.	18.15	-	-	+	+	+	+	+	+	+	-
38.	19.15	-	-	+	+	+	+	+	+	+	-
39.	20.15	-	-	+	+	+	+	+	+	+	-
40.	21.15	-	-	+	+	+	+	+	+	+	-
41.	22.15	-	-	+	+	+	+	+	+	+	-
42.	23.15	-	-	-	+	+	+	+	+	+	-
43.	25.15	-	+	+	+	+	+	+	+	+	-
44.	26.15	-	-	+	+	+	+	+	+	+	-
+/- (detection or not of bacterial growth on nutrient agar media supplemented with NaCl)											

Salt tolerance of the bacterial isolates was tested on solid media with NaCl content ranging from 0 to 30%. All the bacterial isolates were able to grow in the presence of 5-7.5% of salt (table 2). Among these strains, 17 bacterial isolates (38.6%) grow at salt

concentration up to 7.5% NaCl. In particular, 2 bacterial isolates (4.5%) showed tolerance up to 12.5% NaCl.

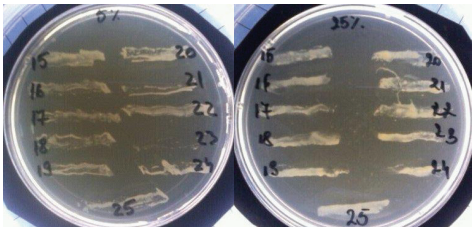

Figure 2 – Bacterial growth on culture media supplemented with 5%, and 25% NaCl

For 19 bacterial isolates (41%), the growth occured over a wide range of salt concentrations ranging from 5 to 25% NaCl (Figure 2 and table 2). Others 3 bacterial isolates (approximate 7%) shown a large salt tolerance between 2.5% to 30% NaCl.

CONCLUSIONS

Extremes properties of the hypersaline waters located in Lopătari, România (e.g. pH low, high sodium and chloride concentrations) determined an especially interesting bacterial diversity. Of a total 44 bacterial strains isolated in this study, 4.5% demonstrated a salt large tolerance ranging from 0% to 12.5%; 7% ranging from 2.5% to 30%; 41% ranging from 5 to 25%, respectively. The ability of several bacterial isolates to grow in a very wide salt concentration can be of great importance for biotechnological future applications. Several bacterial isolates will be identified by molecular methods and tested for their antagonistic or enzymatic activity.

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