

## Evaluation and Isolation of Halophilic Bacteria from the Meyghan Lake in Arak, Iran

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### ABSTRACT

**Background and Objectives:** Halophilic bacteria can grow and survive in environments with a wide range of salinities. In this study, we aimed to isolate halophilic bacteria from the Meyghan Lake in Arak (Iran) and evaluate their enzymatic activity.

**Methods:** Samples were taken from four different areas of the lake. Halophilic bacteria were isolated by culture in moderate halophilic medium, sea water nutrient agar and nutrient agar containing different salt concentrations. Purification was done via consecutive culture methods, and production of extracellular hydrolytic enzymes including amylase, protease, lecithinase, DNase and lipase was evaluated.

**Results:** Among 74 bacteria isolated from the lake water samples, 24 produced amylase, 27 produced lipase (Tween 40 and 80), 68 produced protease, three produced DNase and 61 produced lecithinase.

**Conclusion:** In this study, we isolated halophilic bacteria with enzymatic activity and potential industrial applications.

**Keywords:** Biological Diversity, isolation and purification, Halobacteriales, *Meyghan Lake, Arak*.

## INTRODUCTION

The phylogenetic and metabolic diversity of halophilic microorganisms is astonishing (1). In recent years, halophilic microorganisms have been extensively studied for their biotechnological potential (2). Enzymes produced by halophilic microorganism are intrinsically stable and active at high salt concentrations, and can be used for various purposes, such as food processing, environmental bioremediation and biosynthesis (3, 4). Moreover, extracellular hydrolytic enzymes such as amylase, protease, lipase, DNase, pullulanase and xylanase have been used in the food industry, biomedical sciences and chemical industry (5).

Today, a combination of culture, molecular and chemotaxonomic methods is used to unravel information about halophilic microorganisms (5). Various microorganisms, including bacteria and archaea, are found in salty environments. These microorganisms have adapted to living in environments with high salt concentrations and high osmotic pressure (6). Enzymes found in nature have long been used in the production of food products such as cheese, juice and wine, and in the manufacture of goods such as leather and linen (7). With the advent of fermentation processes, particular attention has been paid to the production of industrial enzymes. Such enzymes can be used in the production of detergents, textiles, paper, starch, organic compounds, leather, food and drugs (8). Majority of commonly used enzymes are hydrolytic, which can decompose different natural materials. Proteases, amylases, cellulases, xylanases, pectinases, inulinases and lipases are among the most important hydrolytic enzymes used in various industries. In the absence of salt, these enzymes can maintain more than 30% of their activity (9). The Meyghan Lake is the last ecological ring in the Arak basin plain, which absorbs surface waters of seasonal meadows. The lake has an area of 100-110 Km<sup>2</sup> depending on its inlet water. The main goal of microbial ecology is to understand microbial diversity in natural habitats. This study aimed to study the biodiversity of halophilic bacteria in the Meyghan Lake in Arak, Iran.

## MATERIALS AND METHODS

In this descriptive cross-sectional study, sampling was carried out from the Meyghan Lake in February 2009. Overall, 100 samples were collected from a depth of 30-50 cm and from different areas of the lake, and then transferred to the laboratory in sterile glasses containers. In addition, salt samples of that area were collected. Other characteristics of the samples including temperature and pH as well as geographical characteristics of the relevant points were recorded. The samples were transferred to the laboratory for measuring pH and salinity. Moderate halophilic medium (12% salt) and seawater-nutrient agar (3% salt) were used for isolation of moderately halophilic bacteria (8). The medium was prepared with various salt percentages (0, 3, 5, 7.5, 10, 15 and 20%) and pH of 7. All media were autoclaved at 15 psi and 121 °C. The concentration of salt at which optimum growth was observed after 12 hours was recorded. Isolates were identified by morphological assessment, gram staining, catalase test and oxidative test (9).

The conditions of the media used in this study were selected based on climatic conditions of the Meyghan Lake and requirements of different bacterial species. Table 1 shows the components of the culture media used for isolation of halophilic bacteria.

The isolates were screened based on the ability to produce extracellular enzymes including amylase, DNase, lipase and protease.

Regression analysis was performed to obtain the correlation between variables previously verifying normality and variance homogeneity. Statistical analysis was done using SPSS (version 18).

## RESULTS

Presence of the cations in water was evaluated using atomic absorption spectrophotometer (AAAnalyst 100, PerkinElmer). The amount of chlorine anions, sulfate anion and carbonate was measured by titration. Table 2 shows the concentration of some important anions and cations in the Meyghan Lake.

Table 1- Components of the culture media used for isolation of halophilic bacteria

Medium	pH	Components/ingredients of the culture media (g/L)	
Moderate halophilic medium	7.5	NaCl	101
		MgCl <sub>2</sub> .6H <sub>2</sub> O	7
		MgSO <sub>4</sub> .7H <sub>2</sub> O	9.6
		CaCl <sub>2</sub> .2H <sub>2</sub> O	0.36
		KCl	2
		NaHCO <sub>3</sub>	0.06
		NaBr	0.026
		Yeast Extract	10
		Peptone	5
		Glucose	1
Modified growth medium	7.5	Agar	15
		NaCl	183.9
		MgCl <sub>2</sub> .6H <sub>2</sub> O	22.9
		MgSO <sub>4</sub> .7H <sub>2</sub> O	26.8
		CaCl <sub>2</sub> .2H <sub>2</sub> O	2.95
		KCl	5.36
		Yeast Extract	2
		Peptone	10
		Agar	15
		Sea water nutrient agar	7.5
Yeast Extract	1		
Meat Extract	2		
NaCl	20		
MgSO <sub>4</sub> .7H <sub>2</sub> O	5		
MgCl <sub>2</sub> .6H <sub>2</sub> O	3		
KCl	0.5		
CaCl <sub>2</sub>	0.05		
Agar	15		

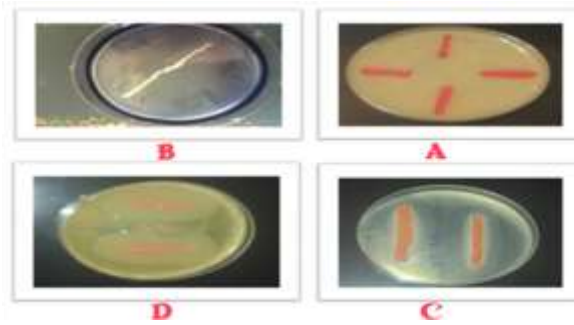
Table 2- Concentration of some anions and cations in Mevghan Lake

Ion	Concentration (mg /L)
Na <sup>+</sup>	80000
Ca <sup>++</sup>	425
Mg <sup>++</sup>	5304.1
K <sup>+</sup>	1650
Cl <sup>-</sup>	171046.25
HCO <sub>3</sub> <sup>-</sup>	762.5
SO <sub>4</sub> <sup>-6</sup>	1344
Fe <sup>++</sup>	1.54

Table 3- Activity of lecithinase, DNase, protease, lipase (tween 40 and 80) and amylase in the 74 bacteria isolated from the water samples

Isolate	Amylase	Lipase (tween 80)	Lipase (tween 40)	Protease	DNase	Lecithinase
N1	-	-	-	+	-	+
N2	-	-	-	+	-	+
N3	-	-	-	+	-	+
N4	+	-	-	+	-	+
N5	-	-	-	+	-	+
N6	-	-	-	+	-	+
N7	-	-	-	+	-	+
N8	-	-	-	+	-	+
N9	+	+	+	+	-	+
N10	-	-	-	+	-	+
N11	-	-	-	+	-	+
N12	-	-	-	+	-	+
N13	+	-	-	+	-	+
N14	-	-	-	-	-	-
N15	-	-	-	+	-	+
N16	-	-	-	+	-	+
N17	-	-	-	+	-	+
N18	+	+	+	+	-	+
N19	+	+	-	+	-	+
N20	+	+	+	+	-	+
N21	-	-	-	+	-	+
N22	+	-	-	+	+	+
N23	+	+	+	+	-	+
N24	-	-	-	-	-	-
N25	-	-	-	+	-	+
N26	+	-	-	+	-	+
N27	-	-	-	-	-	-
N28	-	-	-	-	-	-
N29	+	+	-	+	-	+
N30	-	-	-	-	-	-
N31	-	-	-	+	-	+
N32	-	-	-	+	-	+
N33	+	-	-	+	+	+
N34	-	-	-	-	-	-
N35	-	-	-	+	-	+
N36	-	-	-	+	-	+
N37	+	+	+	+	-	+
N38	+	+	+	+	-	+
N39	-	-	-	+	-	+
N40	-	-	-	+	-	+
N41	+	+	-	+	-	+
N42	-	-	-	+	-	+
N43	+	+	-	+	-	+
N44	+	+	+	+	-	+
N45	-	-	-	+	-	+
N46	-	-	-	+	-	+
N47	-	-	-	+	-	+
N48	+	+	+	+	-	+
N49	-	-	-	+	-	+
N50	-	-	-	+	-	+
N51	-	-	-	+	-	+
N52	+	+	-	+	-	+
N53	-	-	-	+	-	+
N54	-	-	-	+	-	+
N55	-	-	-	+	-	+
N56	-	-	-	+	-	-
N57	-	-	-	+	-	+
N58	+	+	-	+	+	-
N59	-	-	-	+	-	+
N60	-	-	-	+	-	+
N61	+	+	-	+	-	+
N62	-	-	-	+	-	-
N63	-	-	-	+	-	+
N64	-	-	-	+	-	+
N65	-	-	-	+	-	+
N66	-	-	-	+	-	-
N67	+	+	-	+	-	+
N68	-	-	-	+	-	+
N69	+	+	-	+	-	-
N70	-	-	-	+	-	+
N71	+	+	-	+	-	-
N72	-	-	-	+	-	+
N73	+	+	-	+	-	+
N74	-	-	-	+	-	-

Figure 1- Image of culture plates showing protease activity (A), amylase activity (B), lipase activity (C) and DNase activity (D)



## DISCUSSION

Our results showed that the Meyghan Lake might be a source of halophilic bacteria that can be used for production of new drugs and other bioactive compounds. Due to the ability to grow in hypersaline environments, halophilic microorganisms have great biotechnological potential (9-11). For example, bacteriorhodopsin is used in holography. In the Far East, halophilic microorganisms are also used for producing traditional brews, such as fish sauce and soy sauce (6). New enzymes and biocatalysts are continuously being developed to be used in paper, textile and food industries (13, 14). Protein engineering lead to increasing the enzymes efficiency and will deploy their department because increases the compatibility capacities in existing case of processes (15). As mentioned previously, hydrolase enzymes are an important group of microbial enzymes with various industrial applications (16-18). Proteases, amylase, lipase and cellulose are important for production of laundry detergents. Proteases are also used in the production of milk and fruit juice (17). Chitins enzyme can be utilize for biological control of fungal pathogens. Moreover, DNase enzyme can be used to detect contamination of hospital settings with certain pathogenic bacteria, and for quality control of food.

Halophilic microorganisms were first investigated as contaminants of salty foods, but later studies on their structure and

physiology elucidated the great potential of these microorganisms in biotechnology (19).

Since industrial processes create unique and extreme physical and chemical conditions, identification of enzymes capable of tolerating or adapting to such conditions is of utmost importance (18, 20). The majority of microorganisms that are able to produce such enzymes has simple nutritional requirements and is able to use various compounds as sources of energy (16, 20).

## CONCLUSION

The Meyghan Lake in Arak has a more unique environment compared to other salty wetlands of Iran. Unlike other salty lakes in Iran, the main salts in the Meyghan Lake are sodium chloride and sodium sulfate. In this study, we isolated halophilic bacteria with enzymatic activity and potential industrial applications. Among the 74 bacteria isolated from the lake water samples, 24 produced amylase, 27 produced lipase (Tween 40 and 80), 68 produced protease, three produced DNase and 61 produced lecithinase.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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