Romanian Biotechnological Letters *Vol. 14, No. 5, 2009, pp. 4658-4664*

ORIGINAL PAPER

Extracellular hydrolytic enzymes of halophilic bacteria isolated from a subterranean rock salt crystal

Received for publication, May 11, 2009
Accepted, September 20, 2009

ROXANA COJOC[1], SIMONA MERCIU[1], GABRIELA POPESCU[1], LUCIA DUMITRU[1], MASAHIRO KAMEKURA[2] AND MĂDĂLIN ENACHE[1*]
1 – Institute of Biology of the Romanian Academy, 296 Splaiul Independentei, P.O. Box 56-53, Bucharest 060031, Romania
2 – Halophiles Research Institute, 677-1 Shimizu, Noda, Chiba 278-0043, Japan
[*]Institute of Biology of the Romanian Academy, 296 Splaiul Independentei, P.O Box 56 - 53, Sector 6, Bucharest 060031, Romania
Tel: + 40/21/221 9202
Fax: + 40/21/221 9071
Email: madalin.enache@ibiol.ro
madalin_enache@yahoo.com

Abstract

The current study describes the extracellular hydrolytic activities of halophilic bacteria isolated from subterranean rock salt. The estimated number of grown colonies was approximately 3000 per gram of a rock salt on the surface of the medium MH containing 10% NaCl. The investigated strains, randomly selected from the colonies observed on the plate, showed at least one of the tested extracellular hydrolytic activities and one strain hydrolized six out of seven tested substrates. Our investigations showed that hydrolytic activities for Tween 80 and casein are predominant among the isolated strains although the NaCl concentration varies up to 2M. The presence of combined hydrolytic activities in some isolated strains could be an advantage to use in some biotechnological applications in various fields of industry or agriculture. To the author's knowledge, this is the first study on the extracellular hydrolytic properties of halophilic microorganisms isolated from subterranean salt crystals at 206m.

Keywords: salt mine, Slanic Prahova, hydrolytic enzymes, halophilic microorganisms

Introduction

During several periods in the Earth's history, massive sedimentation of halite from hypersaline seas took place. Subterranean salt deposits are distributed all over the world (GRANT & al. 1998 [11]). The isolation of viable halophilic microorganisms from rock salts has been reported, and most of the isolated strains belong to the Haloarchaea (DENNER & al. 1994 [4]; NORTON & al. 1993 [21]; RADAX & al. 2001 [23]; STAN-LOTTER & al. 1993 [25], STAN-LOTTER & al. 1999 [26]). Most of the studies appear to support the hypothesis that various populations of halobacteria are relict populations derived from ancient hypersaline waters (McGENITY & al. 2000 [19]). The difficult access to these deposits together with the special requirements of halophilic microorganisms for survival, make the contamination with other microorganisms from surface sites to be very difficult (GRANT & al. 1998 [11]; McGENITY & al. 2000 [19]).

In Romania, there are a lot of salt massifs with special characteristics, mainly in the proximity of the Carpathian area (DRAGANESCU, 1990 [5]). Some of them are in the outcrop or very close to the surface and have a high purity and large salt resources. These

massifs are exploited from ancient times by different methods mainly by state own salt mining companies (BORDEANU C., COVACI S., 2007 [3]) currently by Salrom, but little is known about their microbiota (ENACHE & al. 2008 [8]). The salt deposit from Slanic (formed in the Neogen period) is located underground from 45.5 m to 499 m deep (DRAGANESCU, 1990 [5]). The general aspect of this deposit is a mixture between white and gray salt crystals as a consequence of turnovers that took place during precipitation processes, due to the climatic and sedimentary variations (HAR & al. 2006 [12]).

Extracellular hydrolytic enzymes such as amylases, proteases, lipases have potential in different areas of chemical industry (KAMEKURA & al. 1982 [14], MELLADO & al. 2004 [20]; SANCHEZ-PORRO & al. 2003 [24]). Industrial processes are carried out under specific conditions which cannot always be adjusted to the optimal values required for the activity of the available enzymes (KAMEKURA, 1986 [16]). Halophiles are the most likely source of thermotolerant and salt adapted enzymes (KAMEKURA M., ONISHI H., 1983 [15]), such as nuclease (ONISHI & al. 1983 [22]), protease (KAMEKURA M., ONISHI H., 1978 [13]), amylase (KHIRE, 1994 [17]; KOBAYASHI & al. 1986 [18]), β-xylanase and β-xylosidase (WAINØ M., INGVORSEN K., 2003 [31]). Some of these enzymes and biotechnological potential of halophilic microorganisms were rewieved by Ventosa et al. (VENTOSA & al. 1989 [28]; VENTOSA A., NIETO J.J., 1995 [27]).

In this study we describe the screening of the extracellular hydrolytic enzymes produced by halophilic microorganisms isolated from underground rock salt and evaluated the biotechnological potential of the microorganisms isolated from these subterranean samples. To the author's knowledge, this is the first study on the evaluation of extracellular hydrolytic activities of microorganisms isolated from subterranean salt rock.

Materials and methods

Isolation of halophilic microorganisms

The rock salt samples were collected from the subterranean wall of the salt mine Unirea, located in Slanic Prahova area. In this study the samples were taken at a depth of 206 – 208 m where the air temperature is 12^0C throughout the year, and the humidity is about 10% lower than that of the surface.

Salt crystals were taken from the surface of the mine wall, at the height of ~ 2 m from the floor. One gram of salt crystal with no apparent contamination by clay or soil was immersed and washed three times in sterile 10% NaCl solution for few minutes to wash the outside surface and the crystal was then dissolved in 50 ml of sterile 10% NaCl and aliquots of 1 ml solution was mixed with 20 ml of autoclaved molten agar medium (MH) heated at 55^0C. The MH medium containing (g/L): yeast extract (10), proteose peptone (5), glucose (1), NaCl (100), $MgCl_2$ x $6H_2O$ (7), $MgSO_4$ x$7H_2O$ (9.6), $CaCl_2$ x $2H_2O$ (0.36), KCl (2), $NaHCO_3$ (0.06), NaBr (0,026) (VENTOSA & al. 1989 [29]). After solidification, the Petri dishes with samples were incubated for several days at 28^0C and the number of grown colonies was counted. For further investigation we randomly selected 16 strains which were tested for their growth in the presence of chloramphenicol (20 μg/ml) and sodium deoxycholate (40 μg/ml), as well as their susceptibility to several antibiotics (50 μg/ml, Table 1), that were added to the above medium as described previously (ENACHE & al. 2007 [7]). The tested strains were incubated for 72 hours at 30^0C. The range of salt concentration that allows bacterial growth was tested in media with various concentrations of NaCl (0, 1, 2, 3, 4 and 5 M).

Detection of extracellular enzymatic activities

The MH medium without proteose peptone and glucose was designated as basal MH and used in the following experiments. The NaCl concentrations in the basal MH media varied until to 4M.

Amylolytic activity

In order to test the amylolytic activity, starch (2 g/L) was added to the basal MH as previously described by Gonzales et al. (GONZALEZ & al. 1978 [10]). The selected strains were incubated at 30^0C for 48 hours, and plates were flooded with I_2-KI solution (0,1% I_2 – 0,2% KI). The presence of a clear zone around the colony indicates the starch hydrolysis and strains was recorded positive for amylase production.

Gelatin hydrolyzing activity

Gelatin (150 g/L) was supplemented to the basal MH medium, and 2 milliliters were transferred to small testing tubes that were inoculated with the tested strains and incubated at 30^0C. For the control, after incubation, the cultures were maintained for 10 minutes at 4^0C. The liquefaction of gelatin that indicates production of enzyme gelatinase was recorded.

Lipolytic activity

First, the basal medium was supplemented with 0,01% $CaCl_2$ and Tween 80 (GONZALEZ & al. 1978 [10]) and inoculated with selected strains then incubated at 30^0C for 48h. The colonies surrounded by a precipitate were considered positive. In another experiment, the sterilized basal MH medium was supplemented with 2,5% olive oil (w/v) at 60^0C. The mixture was homogenized and 0,001% rhodamine B was added at temperature of 50 - 55^{o}C, before pouring into the plates. The tested strains were inoculated in spots on the surface of the medium and incubated at 28^{o}C for 48h. Positive strains were identified by the presence of an orange-red halo under UV light (BHATNAGAR & al. 2005 [2]).

Casein hydrolyzing activity

The basal medium was supplemented with 1% casein. After inoculation the tested strains were incubated at 30^0C for 48 hours. Positive strains were detected based on the presence of a clear halo that indicates the casein hydrolysis by enzyme caseinase.

Cellulase activity

The basal medium was supplemented with 0,5% carboxymethylcellulose (CMC). The similar sized wells were cut in the solidified medium and a volume of 200 µl of bacterial culture was placed in each well. The strains were incubated for 24h at 28^{o}C. After that, distilled water was used to rinse out the content of the wells and then, the Petri dishes were flooded with a 0,1% Congo Red solution for 15 minutes. The dyeing agent was removed by rinsing the dishes with a 1M NaCl solution for 5-10 minutes as described by Farkas & al. (1985 [9]).

Xylanase activity

The basal medium was supplemented with 1% Remazol Brilliant Blue (RBB)-Xylan (Sigma Aldrich) (FARKAS & al. 1985 [9]). After inoculation at 30^0C for 48 hours, positive strains were detected by the presence of a halo under UV light.

Estimation of percentages of amylase and proteinase positive strains from rock salt samples

In order to estimate the percentage of strains with amylolytic or proteolytic activities, the salt solution resulted after salt crystal dissolving was diluted in sterile solution of 10% NaCl and spread on agar plates containing either starch or casein as described above. The total number of colonies and amylase or protease positive colonies were counted.

Table 1. Characterization of the investigated strains

Strains	Morphology	Gram staining	Growth in the presence of antibiotics*				Maximum concentration of NaCl (M)
			Neo	Pen	Ans	Ert	
1/1	Rods	+	+	-	-	-	4
1/2	Rods	-	-	-	-	-	3
1/4	Cocci	+	+	+	+	-	3
1/6	Rods	-	-	-	-	-	2
1/9	Rods	-	-	-	-	-	3
1/10	Cocci	+	+	+	+	-	3
1/12	Rods	-	+	-	+	-	3
1/13	Cocci	+	+	+	+	-	2
1/14	Cocci	+	+	+	+	-	2
1/15	Cocci	+	-	+	+	+	3
1/16	Cocci	+	+	-	+	-	3
1/17	Cocci	+	+	+	+	+	3
1/18	Cocci	+	+	+	+	-	2

* Neo = neomicin, Pen = penicillin, Ans = anysomycin, Ert = erithromycin

Results

Isolation of halophilic bacteria

The number of colonies obtained on the surface of the MH agar medium with 10% NaCl was approximately 3000 per gram of rock salt, taking into consideration that 60 colony forming units were observed on the plate. Since the most organic matter could be conserved in sedimentary halite as polymeric structures which could be hydrolyzed under hydrolases actions, the percentages of strains isolated from rock salt that showed proteolytic or amylolytic activities were estimated. On agar medium plates without salt, approximately 13% and 15% of colonies showed proteolytic activity and amylolytic activity, respectively. At the concentration of 1M NaCl, the number of proteinase positive colonies remained relatively constant (13%), while colonies with amylolytic activity increased up to 32%.

For further investigations on the production of hydrolytic enzymes, 16 representative strains were isolated and judged by round colonies, translucent or mat, covex or flat, with right or sinuous margins and having white, cream or orange colours. Thirteen strains have grown in the presence of sodium deoxycholate but not in the presence of chloramphenicol, bacitracine and rifampicin, while only three strains have grown in the presence of chloramphenicol but not in the presence of sodium deoxycholate. We selected 13 strains which showed growth in the presence of sodium deoxycholate, which most probably are bacteria. Morphology, Gram staining, antibiotics sensitivity and the range of NaCl concentrations that permitted growth are summarized in Table 1. All the strains did not lyse in distilled water. The strain 1/1 has grown on the medium with NaCl until the concentration of 4M NaCl. Moreover, eight strains have grown up to 3M NaCl and four isolates on media with 2M NaCl. The temperature of the Unirea salt mine is approximately 12^0C throughout the year and we choose to test the capacity to grow at this temperature (Table 2).

Hydrolytic activities

The strains were tested for their capacity to hydrolyze using extracellular enzymes substrates such as starch, gelatine, casein, Tween 80, olive oil, carboxymethyl cellulose and RBB-xylan.

Table 2. Growth at 12^0C in the dark on the MH medium with 10 % NaCl

Strains	72 hours	3 weeks	5 weeks	7 weeks	9 weeks	11 weeks
1/1	+	+	+	+	+	+
1/2	-	+	+	+	+	+
1/4	-	-	+	+	+	+
1/6	-	+	+	+	+	+
1/9	-	-	-	+	+	+
1/10	-	-	-	-	-	-
1/12	+	+	+	+	+	+
1/13	-	-	-	-	-	+
1/14	-	-	-	-	-	+
1/15	-	-	-	-	-	+
1/16	-	-	-	-	-	+
1/17	-	-	-	-	-	+
1/18	-	-	-	-	-	-

+ = colonies were observed; - = colonies were not observed

The isolates showed at least one of the above hydrolytic activities while one strain (named 1/9) was detected positive for six substrates. The results summarized in the table 3 indicated that Tween 80 and casein hydrolytic activities were predominant. However, three strains showed amylase activity. The strains that which produce amylase were able to hydrolyze carboxymethyl cellulose, with one exception strain 1/6. On the other hand, increasing the NaCl concentration in the culture medium there was observed that the hydrolytic activity is influenced, even if strains showed a good growth rate up to 2M NaCl.

We noticed that the capacity to hydrolyze starch was not associated with the Tween 80 hydrolysis, exceptions strains 1/2 and 1/9 in the presence of 1M NaCl. At this salt concentration the hydrolysis of Tween 80 was not associated with olive oil hydrolysis, with one exception strain named 1/1. The other hydrolytic activities were associated in various ways depending on NaCl concentrations as it is presented in table 3. At the concentration of 3 and 4M NaCl some strains were able to grow but no hydrolytic activity was detected.

Table 3. Extracellular hydrolytic activities in media with NaCl content of 1M and 2M

Strains	Starch		Gelatin		Casein		Tween80/ Olive oil		CMC		RBB-Xylan	
	1M	2M	1M	2M	1M	2M	1M	2M	1M	2M	1M	2M
1/1	-	-	-	-	-	-	+/+	-/+	-	-	-	-
1/2	+	+	-	-	+	-	+/-	-/-	+	+	-	-
1/4	-	-	-	-	+	-	+/-	-/-	-	-	-	-
1/6	+	+	-	-	-	-	-/-	-/-	-	-	-	-
1/9	+	+	+	+	+	-	+/-	+/-	+	+	+	+
1/10	-	-	-	-	-	-	+/-	-/-	-	-	-	-
1/12	-	-	-	-	-	-	-/+	-/+	-	-	-	-
1/13	-	-	-	-	-	-	+/-	-/-	-	-	-	-
1/14	-	-	-	-	+	-	+/-	-/-	-	-	-	-
1/15	-	-	-	-	+	-	+/-	-/-	-	-	-	-
1/16	-	-	-	-	-	-	+/-	-/-	-	-	-	-
1/17	-	-	+	+	+	-	+/-	-/-	-	-	-	-
1/18	-	-	-	-	-	-	+/-	-/-	-	-	-	-

CMC = carboxymethylcellulose; RBB-Xylan = Remazol Brilliant Blue Xylan; + = enzymatic activity was present; - = absence of enzymatic activity

Discussion

There is a report which showed the absence of microorganisms on the surface of subterranean salt deposits, former mining area, open for the public (DEAK & al. 2007 [6]). We washed the outside surface of the salt crystal by rinsing with sterile 10% NaCl solution in order to eliminate some microorganisms present on the surface of investigated salt crystals, although the weight of investigated crystal was diminished not more than five, maximum seven percent. Ancient evaporite deposits are inhabited mainly by halobacteria even if a variety of claims of isolation of eubacteria from salts are reported (GRANT & al. 1998 [11]). The most acceptable studies are of bacteria isolated by Vreeland et al. (VREELAND & al. 2000 [30]).
Hypersaline environments represent a valuable source of extracellular hydrolytic enzymes with potential in different economical fields. Although a lot of investigations have been done on characterization of enzymes from halophilic microorganisms, little is known about extremozymes produced by rock salt microbiota. Our investigation showed that salt rock samples were characterized by a relatively low number of halophilic microorganisms that belong to *Bacteria*. Similar results regarding the number of colony forming units from brines and rock salt were reported in a previous paper (NORTON & al. 1993 [21]). The relatively low number of microorganisms in the investigated environment can be correlated with the low temperature that characterizes the investigated area.
Extracelluar cell-wall degrading enzymes are important to utilize organic and inorganic materials in environments. Our preliminary isolates revealed some beneficial industrial applications of their extracellular enzymes for bioconversion of organic materials to useful products in hypersaline solide form environments or in polluted environments with high ionical strength, such wastewater or chemical or pharmaceutical processing areas.
One of the isolated strains hydrolyzed six tested substrates. This could be an advantage to be used in some biotechnological applications in various fields of industry or agriculture. Six investigated strains had only one hydrolytic activity at 1M NaCl. At 2M NaCl, many strains appeared to lose their hydrolytic properties, and the predominant activity at 2M NaCl was the lipase hydrolyzing Tween 80 or olive oil, suggesting that strains having this activity can be studied for further investigations regarding lipid degradation in saline conditions or some oil degradation in seawater and wastewater environments (AL-DARBI & al. 2005 [1]).

References

1. AL-DARBI, M.M., SAEED, N.O., ISLAM, M.R., LEE, K., 2005, Biodegradation of natural oils in seawater. *Energy Sources.,* **27**, 19-34.
2. BHATNAGAR, T., BOUTAIBA, S., HACENE, H., CAYOL, J., FARDEAU, M., OLLIVIER, B., BARATTI, J., 2005, Lipolytic activity from Halobacteria: Screening and hydrolase production. *FEMS Microbiol. Lett.,* **248**, 133-140.
3. BORDEANU, C., COVACI, S., 2007. Ecomining solutions of Slănic Prahova salt rock deposit exploitation. *Buletin Resurse Minerale.,* **1**, 31-36.
4. DENNER, E.B.M., MCGENITY, T.J., BUSSE, H.J., GRANT, W.D., WANNER, G., STAN-LOTTER, H., 1994. *Halococcus salifodinae* sp. nov., an archaeal isolate from an Austrian salt mine. *Int. J. Syst. Bacteriol.,* **44**, 774–780.
5. DRĂGĂNESCU, L., 1990. Date din istoricul exploatarii sării la Slănic-Prahova. *Rev. Muzeelor.,* **27**, 68-71.
6. DEÁK, E., DEÁK, G., FLORIAN, A., MIHAI, S., *Tehnici de control si prevenire a poluarii mediului inconjurator pentru salinele din Romania*. Ed. Universitas, Petrosani, Romania, 2007.
7. ENACHE, M., ITOH, T., KAMEKURA, M., TEODOSIU, G., DUMITRU, L., 2007, *Haloferax prahovense* sp. nov., an extremely halophilic archaeon isolated from a Romanian salt lake. *Intl. J. Syst. Evol. Microbiol.,* **57**, 393-397.

8. ENACHE, M., ITOH, T., KAMEKURA, M., POPESCU, G., DUMITRU, L., 2008. Halophilic archaea isolated from man-made young (200 years) salt lakes in Slănic, Prahova, Romania. *Centr. Eur. J. Biol.,* **3**, 388-395
9. FARKAS, V., LISKOVA, M., BIELY, P., 1985, Novel media for detection of microbial producers of cellulase and xylanase. *FEMS Microbiol. Lett.,* **28**, 137-140.
10. GONZALEZ, C., GUTIERREZ, C., RAMIREZ, C., 1978. *Halobacterium vallismortis* sp. nov. an amylolytic and carbohydrate-metabolizing, extremely halophilic bacterium. *Can. J. Microbiol.,* **24**, 710-715.
11. GRANT, W.D., GEMMELL, R.T., MCGENITY, T.J., 1998. Halobacteria – the evidence for longevity. *Extremophiles.,* **2**, 279–288.
12. HAR, N., BARBU, O., CODREA, V., PETRESCU, I., 2006. New data on the mineralogy of the salt deposit from Slănic Prahova (Romania). *Studia UBB, Geologia,* **51**, 29-33.
13. KAMEKURA, M., ONISHI, H., 1978. Properties of the halophilic nuclease of a moderate halophile, *Micrococcus varians* subsp. *halophilus*. *J. Bacteriol.,* **133**, 59–65.
14. KAMEKURA, M., HAMAKAWA, T., ONISHI, H., 1982. Application of halophilic nuclease H of *Micrococcus varians* subsp. *halophilus* to commercial production of flavoring agent 5'-GMP. *Appl. Environ. Microbiol.,* **44**, 994–995.
15. KAMEKURA, M., ONISHI, H., 1983. Inactivation of nuclease H of the moderate halophile *Micrococcus varians* subsp. *halophilus* during cultivation in the presence of salting-in type salt. *Can. J. Microbiol.,* **29**, 46–51.
16. KAMEKURA, M., 1986. Production and function of enzymes of eubacterial halophiles. *FEMS Microbiol. Rev.,* **39**, 145–150.
17. KHIRE, J.M., 1994. Production of moderately halophilic amylase by newly isolated *Micrococcus* sp. 4 from a salt pan. *Lett. Appl. Microbiol.,* **19**, 210–212.
18. KOBAYASHI, T., KAMEKURA, M., KANLAYAKRIT, W., ONISHI, H., 1986. Production, purification and characterization of an amylase of the moderate halophile *Micrococcus varians* subsp. *halophilus*. *Microbios.,* **46**, 165–170.
19. McGENITY, T.J., GEMMELL, R.T., GRANT, W.D., STAN-LOTTER, H., 2000. Origins of halophilic microorganisms in ancient salt deposits. *Environ. Microbiol., 2*, 243–250.
20. MELLADO, E., SANCHEZ-PORRO, C., MARTIN, S., VENTOSA, A., *Extracellular hydrolytic enzymes produced by moderately halophilic bacteria*. In: VENTOSA, A., (ed.) Halophilic Microorganisms, Springer-Verlag, Berlin Heidelberg, 2004, pp 285–295.
21. NORTON, C.F, McGENITY, T.J., GRANT, W.D., 1993. Archaeal halophiles (halobacteria) from two British salt mines. *J. Gen. Microbiol.,* **139**, 1077–1081.
22. ONISHI, H., MORI, T., TAKEUCHI, S., TANI, K., KOBAYASHI, T., KAMEKURA, M., 1983. Halophilic nuclease of a moderately halophilic *Bacillus* sp.: production, purification, and characterization. *Appl. Environ. Microbiol.,* **45**, 24–30.
23. RADAX, C., GRUBER, C., STAN-LOTTER, H., 2001. Novel haloarchaeal 16S rRNA gene sequences from Alpine Permo-Triassic rock salt. *Extremophiles.,* **5**, 221–228.
24. SANCHEZ-PORRO, C., MARTIN, S., MELLADO, E., VENTOSA, A., 2003. Diversity of moderately halophilic bacteria producing extracellular hydrolytic enzymes. *J. Appl. Microbiol.,* **94**, 295–300.
25. STAN-LOTTER, H., SULZNER, M., EGELSEER, E., NORTON, C., HOCHSTEIN, L.I., 1993. Comparison of membrane ATPases from extreme halophiles isolated from ancient salt deposits. *Orig. Life Evol. Biosph.,* **23**, 53–64.
26. STAN-LOTTER, H., MCGENITY, T.J., LEGAT, A., DENNER, E.B.M., GLASER, K., STETTER, K.O., WANNER, G., 1999. Closely related strains of *Halococcus salifodinae* are found in geographically separated Permo-Triassic salt deposits. *Microbiology*, **145**, 3565–3574.
27. VENTOSA, A., NIETO, J.J., 1995. Biotechnological applications and potentialities of halophilic microorganisms. *World J. Microbiol. Biotechnol.*, **11**, 85-94.
28. VENTOSA, A., NIETO, J.J., OREN, A., 1989. Biology of moderately halophilic aerobic bacteria. *Microbiol. Mol. Biol. Rev.,* **62**, 504–544.
29. VENTOSA, A., GARCIA, M.T., KAMEKURA, M., ONISHI, H., RUIZ-BERRAQUERO, F., 1989. *Bacillus halophilus* sp. nov., a moderately halophilic *Bacillus* species. *Syst. Appl. Microbiol.,* **12**, 162–166.
30. VREELAND, R.H., ROSENZWEIG, W.D., POWERS, D.W., 2000. Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. *Nature.,* **407**, 897–900.
31. WAINØ, M., INGVORSEN, K., 2003, Production of β-xylanase and β-xylosidase by the extremely halophilic archaeon *Halorhabdus utahensis*. *Extremophiles.,* **7**, 87-93.