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RESEARCH ARTICLE

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Screening of hydrolytic extremozymes in haloalkaliphilic *Archaea* by culture and molecular-based methods

ABSTRACT:

Exploring of extremophilic *Archaea* and their enzymes had great significance to biocatalysis. Enzymes produced by *Archaea* allow improvement in multiple sectors of industry. They can help reduce the quantity of waste energy and material consumption, thus making the technology more environmentally–friendly. This study aimed to screen hydrolytic extremozymes in different Soda Lakes of Wadi Al-Natrun, Egypt, by enzymatic agar-plate assays and molecular-based methods. Five hundred and thirty-five haloalkaliphilic archaeal strains isolated from different Soda Lakes were screened for production of protease, amylase, pectinase, chitinase, cellulase, lipase and esterase at pH 10 and 25% NaCl (w/v). Furthermore, metagenomic DNA was extracted from water sample of Ga'ar Lake and constructed library were sequenced to identify the genes encoding target enzymes by using illumina Hiseq2000 system. By enzymatic agar-plate assay, all tested strains showed potential production of extracellular enzymes, a total of 39.4% of screened strains produced protease, 27.1% showed amylase activity, 25.9% for lipase and 7.4% displayed cellulase activity, but none of tested strains produced chitinase or pectinase. While, by shotgun metagenomic technique, all genes encoding metabolically active hydrolytic enzymes studied were detected in water sample of Ga'ar Soda Lake. Metagenome-derived DNA libraries have focused on many classes of enzymes, among these hydrolytic enzymes were prominent. The results of both methods indicated that these soda lakes are rich with commercially valuable enzymes.

KEY WORDS:

Extremozymes, Extracellular activity, Metagenome, Soda Lakes, Wadi Al-Natrun, Egypt.

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INTRODUCTION:

Extremophiles, able to live in unusual habitats, can potentially serve in a verity of industrial applications (Horikoshi, 2008). The groups of *Halobacteriaceae* that can grow under alkaline conditions in the presence of salt are referred as haloalkaliphiles. The dual extremity of halolkaliphiles make them interesting models for fundamental research and exploration of biotechnological potential (Dodia *et al*., 2008; Joshi *et al*., 2008; Bominadhan *et al*., 2009; Purohit and Singh, 2011). Haloalkaliphilic archaea have largely been studied from the concentrated hyper saline environments; Soda Lake, Solar Saltern, Salt brines, Carbonate springs and Dead Sea. Soda Lakes represent stable and extremely productive aquatic ecosystems. Most of the alkaline Soda Lakes in Africa, India, China and elsewhere with pH values of 11 and higher and salt concentrations exceeding 300 g/l are teeming with life (Oren, 2002). The enzymes from extremophilic organisms, particularly halophilic and

haloalkaliphilic archaea are relatively less explored. Among the enzymes, hydrolytic enzymes are the most important groups of industrial enzymes.

Moreover, it's an emerging approach to explore diversity and metabolic processes based on the analysis of the total genomics DNA of microbial communities in their natural environments. In the direction of metagenomics studies, isolation and analysis of environmental DNA are the key steps, which would allow mining microbial diversity and help understanding the dynamics, properties and functions of these organisms (Desai and Madamwar, 2007; Purohit and Singh, 2009). The functional diversity of a community can be quantified by annotating metagenomic sequences with functions, this usually involves identifying metagenomic reads that contain protein coding sequences and comparing the coding sequence to a data base of genes, proteins, protein families or metabolic pathways for which some functional information is known (Nacke *et al.,* 2012). The function of the coding sequence is inferred based on its similarity to sequences in the database. Doing this for all metagenomic sequences produces a profile that describes the number of distinct types of functions and their relative abundance in the metagenome (Oulas *et al*., 2015).

Despite advances in understanding the diversity of haloarchaea, studying their hydrolytic enzymes received less attention. However, some special haloarchaeal enzymes are characterized in details (Ruiz and de Castro, 2007; de Castro *et al*., 2008; Müller-Santos *et al*., 2009). The aim of this study was to describe the potential of enzymatic production in haloalkaliphilic archaeal population including: amylase, cellulase, chitinase, lipase, pectinase and protease qualitatively by screening535 archaeal strains, and by using metagenomic library generation and shotgun sequencing.

MATERIAL AND METHODS:

Site description and sampling:

Sediment and water samples were collected from four soda lakes of the Wadi Al-Natrun in September of 2014, Lake UmRisha, a saturated hypersaline lake (30°20'48.70'' N, 30°23'08.14'' E), Lake Razoniya (30°21.748'N, 30°21.137'E), Lake Ga'ar (30°27.222' N, 30°10.83' E) and Lake Khadra (30°26.566' N, 30°13.133' E). All samples were immediately stored at 4°C upon arrival at the Suez Canal University for microbiological investigation. For DNA extraction, samples were immediately frozen at - 80°C upon arrival biotechnology institute, faculty of agriculture, Suez Canal University. Temperature, pH and electric conductivity (EC) were measured in the field. The concentration of some ions such as Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, HCO_3^{-}, CO_3^{-2}, SO_4^{-2}, p, and NO_3^{-2} in the environmental samples under study was done in Faculty of agriculture, Suez Canal University, according to standard procedures of Clesceri *et al*. (1989).

Isolation and growth conditions:

The growth haloalkaliphilic medium described by Tindall *et al*. (1980) was used for isolation procedures (Dyall-smith, 2008). The pH of the medium was adjusted on 9.5 - 10. Cultures were incubated at 40°C for one month. Pure isolates were obtained by successively cultivation on the same medium.

Screening of strains for extracellular hydrolytic activities:

To qualitative detection of producing extracellular hydrolyses, different enzymatic agar-plate assays were performed; Haloalkaliphilic minimal medium was used for screening of all enzymes produced by five hundred and thirty-five haloalkaliphilic isolates; this medium was developed by Mwatha and Grant, (1993), with the following composition, yeast extract, 1 g; KNO_3, 1 g; KH_2PO_4, 1 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 150 g; Na_2CO_3, 18 g; pH was adjusted to 10. The minimal medium was supplemented by the following substrates separately; 0.1% Tween 80 for lipase activity, 1% Starch for amylase activity, 0.1% skim milk for protease activity, 0.5% crystalline cellulose for cellulase activity, 1%chitin and pectin for chitinase and pectinase detection, respectively, were used for evaluation of hydrolytic enzyme production according to the suggested methods in Laboratory Manual for General Microbiology (Stukus, 1997).

Identification of the isolates:

Genomic DNA was extracted from fifty haloalkaliphilic strains, the best enzymatic producers, using a modified method from Experimental Techniques in Bacterial Genetics, described by Maloy (1990).The 16S rRNA gene was amplified with a set of *Archaea*-universal primers (Invitrogen, USA), the primers 5'-ATTCCGGTTGATCCTGCCGG-3' (positions 6–25 in *Escherichia coli* numbering) and 5'AGGAGGTGATCCAGCCGCAG-3'; positions 1540 – 1521 (Ventosa *et al*., 2004). The PCR conditions: 50 µl of reaction system, reaction cycles 30 times, 95°C pre-denaturation 5 min, 94°C denaturation 1 min, 60°C annealing 1 min, 72°C extension 1 min 30 s, 72°C final extension 10 min, 4°C hold. 50 ng/µl of each PCR product was used to prepare the samples which were delivered to MacroGen Company in Korea (http://www.dna.macrogen.com) following their specifications. The sequences were analyzed using BLAST (http://www.ncbi.nlm.nih.gov/BLAST) to get a preliminary identification of the strains. The cluster analysis was performed using the MEGA 6 software package.

High-throughput DNA Sequencing:

Metagenomic DNA was extracted directly from environmental samples, frozen sediment and water samples (- 80°C), using 15 ml of water (filtered through 0.2 µm membrane filters) and 10g of sediment according to Mesbah *et al.* (2007). Two DNA samples, water and sediment of lake Ga'ar, were arrived to MacroGen company www.dna.macrogen.com for library construction and sequencing following illumina Hiseq2000 manual's instructions.

RESULTS:

Physico-chemical properties of sample sites:

The studied lakes were highly alkaline and hypersaline. The temperature at the collecting sites was in the range 37 - 40°C. The pH was in the range from 8.03 to 9.05. E.C values were 158.8 and 143.2 dS m^{-1} for water samples of lakes Rosania and Ga'ar, respectively. More information about the dominance of different anions and cations of the water and sediment samples was illustrated in table 1.

Table 1. Physico-Chemical analysis of water and sediment samples of Soda Lakes in Wadi Al-Natrun, Egypt.

Sample sites		Soluble ions (gl^{-1})											
		Ph	EC (dsm^{-1})	Na^+	K^+	Ca^+	Mg^{2+}	P	CO_3^{2-}	HCO_3^-	Cl^-	SO_3^{2-}	NO_3^-
Water	Ga·ar	9.05	143.2	39.9	12.48	0.24	0.4	299	14.4	16.836	61.9	10.08	42.6
	Khadra	8.5	15.44	3.25	0.058	0.4	0.449	525	0	0.183	6.44	0.72	3.02
	Rosania	9.1	158.8	48.34	1.48	0.4	0.486	534	6.264	4.794	69.3	7.68	11.4
Sediment	Ga·ar	8.68	6.17	68.2	0.7	0.9	1.1	64.6	1.56	5.12	55	11.4	1.8
	Rosania	8.74	6.74	77.3	0.1	0.2	0.4	52.5	1.67	2.49	40	8.6	1.7
	Khadra	8.09	0.905	6.3	0.1	1.7	1.9	68.7	0.72	1.09	7	2.4	0.7
	Umrisha	8.46	6.4	72.3	0.1	0.1	0.4	40	0.48	1.09	65	7.4	0.4

Characterization of the isolated strains:

Haloalkaliphilic archaeal strains were obtained from different sediment and water from four Soda Lakes in Wadi Al-Natrun, Egypt. These microorganisms were found to be growing optimally at 20% salt concentration and their optimum pH was in the range 8 - 10. Most isolates showed the typical pink, orange red and red pigmentation. Most of them were polymorphic shapes (short rods, triangles, squares and flat disks), whereas some coccoid cells were also observed. All isolated strains were Gram negative.

Enzymatic activity of the isolated strains:

The ability of producing six different hydrolytic enzymes was tested qualitatively among haloalkaliphilic archaeal strains isolated from different four lakes in Wadi Al-Natrun. A total of 39.4% of screened strains produced protease, 27.1% showed amylase activity, 25.9% for lipase and 7.4% displayed cellulase activity, but none of the tested strains could produce chitinase or pectinase enzymes. As shown in table 2, fifty strains were morphologically different and displayed one or more hydrolase enzymes activity. Of these strains, ten strains, presented four hydrolytic enzymes; protease, amylase, lipase and cellulase, were selected for identification by 16S rRNA gene sequencing.

Table 2. Detection of enzymatic activity from screened archaeal strains.

strains	Enzymatic activities					
	Protease	Lipase	Amylase	Cellulase	Pectinase	Chitinase
WN31	+	+	+	+	-	-
WN32	+	+	+	+	-	-
WN33	+	+	+	+	-	-
WN34	+	-	-	-	-	-
WN35	+	+	+	+	-	-
WN36	+	+	+	-	-	-
WN37	+	+	-	-	-	-
WN38	-	-	+	-	-	-
WN39	-	+	+	-	-	-
WN40	+	+	+	-	-	-
WN41	+	+	+	+	-	-
WN42	+	-	+	-	-	-
WN43	+	-	+	-	-	-
WN44	+	+	+	+	-	-
WN45	+	+	+	-	-	-
WN46	+	+	+	+	-	-
WN47	+	+	+	-	-	-
WN48	-	-	+	-	-	-
WN49	+	+	+	-	-	-
WN50	-	+	+	-	-	-
WN51	+	+	+	-	-	-
WN52	+	+	+	+	-	-
WN53	-	-	+	-	-	-
WN54	+	+	+	-	-	-
WN55	+	+	+	-	-	-
WN56	-	+	+	-	-	-
WN57	+	+	+	+	-	-
WN58	+	+	+	-	-	-
WN59	+	+	+	-	-	-
WN60	-	+	+	-	-	-
WN61	+	+	+	+	-	-
WN62	+	+	+	-	-	-
WN63	+	+	+	-	-	-
WN64	+	-	+	-	-	-
WN65	+	+	+	-	-	-

Cont. Table 2

WN66	+	+	+	-	-	-
WN67	+	-	+	-	-	-
WN68	+	-	+	-	-	-
WN69	-	-	+	-	-	-
WN70	+	+	-	-	-	-
WN71	-	-	+	-	-	-
WN72	-	-	+	-	-	-
WN73	+	+	+	+	-	-
WN74	+	-	+	-	-	-
WN75	+	-	-	-	-	-
WN76	+	+	+	-	-	-
WN77	+	+	+	-	-	-
WN78	+	+	+	-	-	-
WN79	+	+	+	-	-	-
WN80	+	+	-	-	-	-

Identification of the strains;

The phylogenetic affiliations of the isolated strains were revealed by 16S rRNA gene sequencing. The strains were all belonged to the family *Halobacteriaceae* and closely associated with the genera: *Halobiforma, Natronococcus, Natronobacterium*, *Natrinema, Natrialba, Natronomonas and Haloterrigena* with high similarities (≥ 90%) to known species within these genera. The tree showing the phylogenetic relationships of the selected isolated strains is represented in figure 1.

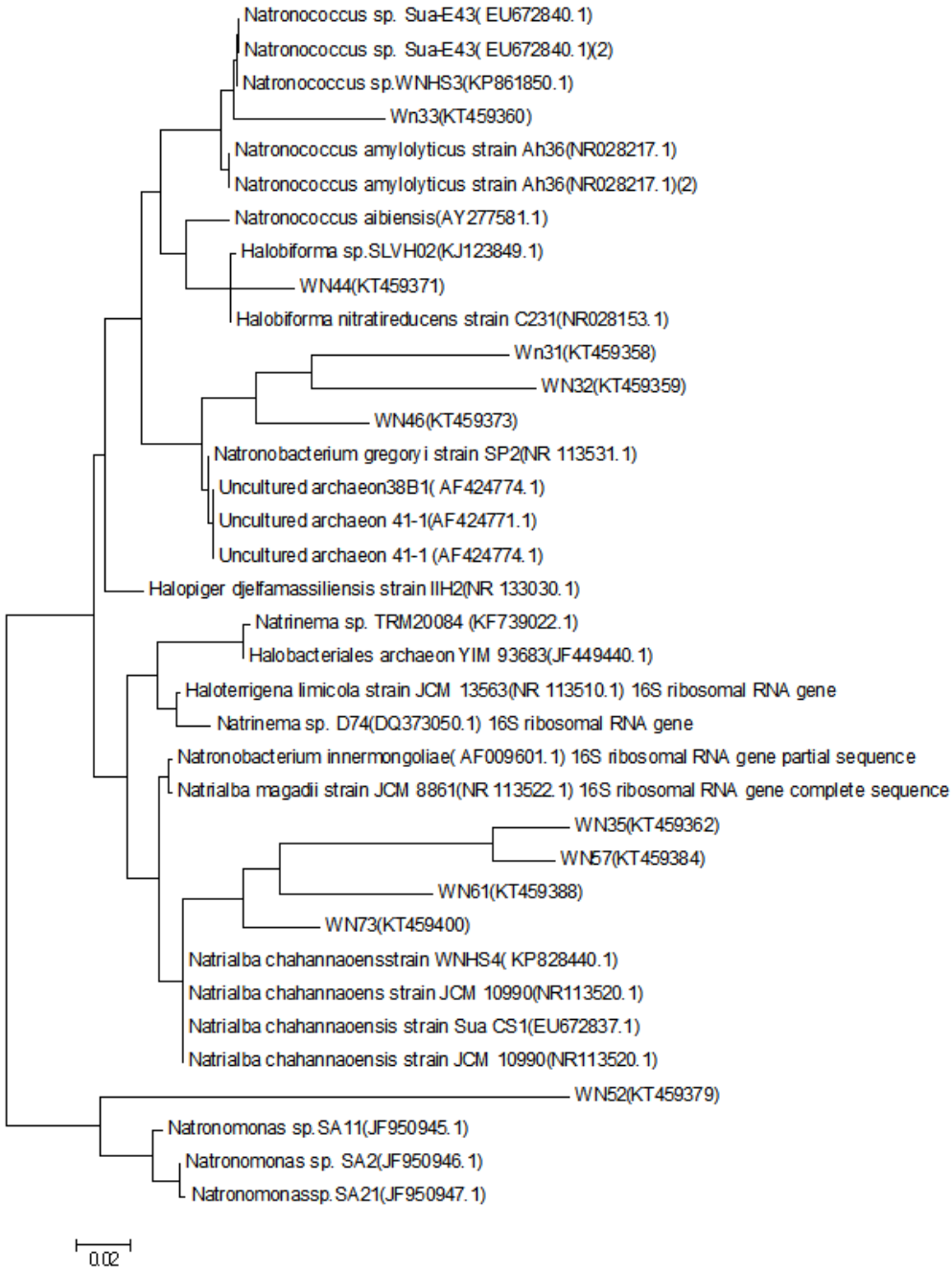

Fig. 1. Neighbor-joining tree (partial sequences ~950 bp) showing the phylogenetic relationships of archaeal 16S rRNA gene sequences of isolated strains to closely related (S ≥ 90%) sequences from the GenBank database.

Nucleotide sequence accession numbers:

The 16S rRNA gene data of the ten archaeal strains reported in this study have been deposited in the NCBI and GenBank nucleotide sequence databases under the accession numbers as shown in figure 1.

Metagenomics analysis:

The results of functional analysis for water sample of Ga'ar Soda Lake indicated that 21.4% contained predicted proteins with known functions and inter pro match. The detection of genes encoded for different functions and activities presented only 5% of genes belong to hydrolase activity. The data was annotated by EBI metagenome (www.ebi.ac.uk) under EBI Metagenomic database with an accession number PRJEEB18746.The hydrolytic enzymes detected by Shotgun metagenomics sequences were different types of lipases (phospholipase D, lipase maturation factor, phospholipase A1 and phospholipase D,C-terminal), amylases (alpha amylase, glucoamylase), proteases (protease prsw, tricorn protease c1 domain), cellulases (putative cellulase), pectinases (pectin lyase fold, pectinesterase), chitinases (glycoside hydrolase chitinase active site), xylanase as well as DNase.

DISCUSSION:

Enzymes from microorganisms that can survive under extreme conditions could be particularly useful for applications under unusual conditions including extremities of temperature, pH, salt, pressure, etc. Major interests have so far focused on the enzymes of thermophiles that can function at higher temperatures. The enzymes from halophilic and haloalkaliphilic archaea that can function in extreme pH and salinity have been less explored, but are now generating interest from this point of view (Horikoshi, 2008; Makhdoumi-Kakhki *et al.*, 2012). With the advancement in molecular tools, it would be possible to get insights into the biocatalytic mechanisms for greater applications (Santos and Sato, 2009).

In this study, by investigating 535 haloalkaliphilic archaeal strains, wide range of hydrolytic enzymes could be detected in the isolated strains which supported by the results of Makhdoumi-Kakhki *et al.* (2012), who surveyed hydrolytic enzymes activity in haloarchaeal strains isolated from hyper saline environment Aran-Bidgol Lake, Iran. Patil and Bajikal (2013) reported that the isolation and studying of the hydrolytic enzymatic diversity of three extremely haloalkaliphilic strains *Natrinema, Natrialba and Natronobacterium* from Lonar Lake, India.

Lipolytic enzymes, comprising esterases and lipases, are extensively distributed in microorganisms, plants, and animals. These enzymes were catalyzed the hydrolysis, synthesis, or transesterification of ester bonds. At present, these enzymes represent about 20% of commercialized enzymes in industry (López-López *et al.,* 2015), as they have great potential in several industrial processes such as production of biodegradable polymers, detergents, food flavoring, oil biodegradation, or waste treatment (Anobom *et al.,* 2014). In this study, 25.9% of the screened strains displayed lipase activity, this percentage was similar to previous study of Lizama *et al*. (2001) who stated that the 20% of isolated haloarchaeal strains from Salt Lake in Chile produced amylase and lipase. Moreover, protease enzyme was the most common enzyme of screened strains (represented 39.4%), and produced by species with various genera, *Halobiforma, Natronococcus, Natronobacterium*, *Natrinema, Natrialba, Natronomonas and Haloterrigena*. Ozcan *et al.* (2006) demonstrated the production of amylase, protease, lipase from haloarchaeal strains isolated from hypersaline environment, while Birbir *et al*. (2007) showed amylase, protease, lipase, cellulase, nuclease activities in the same environment.

The analytical study of functional genes in saline and alkaline environment was evaluated previously by Keshri *et al*. (2013). To our knowledge, this is the first study for surveying hydrolytic enzymes by Shotgun Metagenomic technique in Soda Lakes of Wadi Al-Natrun, Egypt. In this investigation, metagenomic analysis of water sample of Ga'ar Lake revealed that 5% of genes encoded for different functions and activities belonged to hydrolytic activities, including protease, lipase, amylase, cellulase, pectinase, chitinase, xylanase and DNase.

CONCLUSION:

Haloalkaliphilic archaeal hydrolytic enzymes seem to be very good candidate for industrial application which are not only require salt, but also may have excellent activity at high temperature, low water activity and high pH value. Therefore, this study aimed to search for these enzymes either by enzymatic agar-plate assay or functional metagenomics for further cloning, enzyme purification and characterization. By investigating 535 haloalkaliphilic archaeal strains wide range of hydrolytic enzymes could be detected in the isolated strains, 39.4% of screened strains produced protease, 27.1% showed amylase activity, 25.9% for lipase and 7.4% displayed cellulase activity. By functional metagenomics, 5% of genes encoded for different functions and activities belonged to hydrolase activities. The results of both previous methods indicated that these soda lakes are prosperous with commercially valuable enzymes.

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REFERENCES:

Anobom CD, Pinheiro AS, Aguieiras ECG, -Andrade GC, Moura MV, Almeida RV, Freire DM. 2014. From structure to catalysis: recent developments in the biotechnological applications of lipases. Biomed Res. Int., 2014: 1-14.

Birbir M, Calli B, Mertoglu B, Bardavid R, Oren A, Ogmen M, Ogan A. 2007. Extremely halophilic Archaea from Tuz Lake, Turkey, and the adjacent Kaldirim and Kayaciksalterns. World. J. Microbiol. Biotech., 23(3): 309-316.

Bominadhan U, Rajakumar R, Sivakumaar PKV, Melvin M. 2009. Optimization of protease enzyme production using *Bacillus* sp. isolated from different wastes. Bot. Res. Int., 2(2): 83-87.

Clesceri LS, Greenberg AE, Trussell RR. 1989. Standard Methods for the Examination of Water and Wastewater", 17th ed. American Public Health Association, American Water Works Association.

de Castro RE, Ruiz DM, Giménez MI, Silveyra MX, Paggi RA, Maupin-Furlow LA. 2008. Gene cloning and heterologous synthesis of a haloalkaliphilic extracellular protease of *Natrialba magadi*. Extremophiles, 12 (5): 677–687.

Desai C, Madamwar D. 2007. Extraction of inhibitor-free metagenomic DNA from polluted sediments, compatible with molecular diversity analysis using adsorption and ion-exchange treatments, Bioresour Technol., 98(4): 761-768.

Dodia MS, Bhimani HG, Rawal CM, Joshi RH, Singh SP. 2008. Salt dependent resistance against chemical denaturation of alkaline protease from a newly isolated Haloalkaliphilic *Bacillus* sp. Bioresource Technol., 99(14): 6223-6227.

Dyall-Smith M. 2008. The Halohandbook: Protocols for halobacterial genetics. Version 3.0. University of Melbourne, Australia.

Horikoshi K. 2008. Past, present and future of extremophiles, Extremophiles,12: 1-2.

Joshi RH, Dodia MS, Singh SP. 2008. Production and optimization of a commercially viable alkaline protease from a haloalkaliphilic bacterium. Biotechnol. Bioprocess Eng., 13(5): 552-559.

Keshri j, Mishra A, Jha B. 2013. Microbial population index and community structure in saline alkaline soil using gene targeted metagenomics. Microbiol. Res., 168(3): 165–173.

Lizama C, Monteoliva-Sánchez M, Prado B, Ramos-Cormenzana A, Weckesser J, Campos V. 2001. Taxonomic study of extreme halophilicarchaea isolated from the "Salar de Atacama", Chile. Syst. Appl. Microbiol., 24 (3): 464-474.

López-López O, Knapik K, Cerdán ME, González-Siso MI. 2015. Metagenomics of an alkaline hot spring in Galicia (Spain): microbial diversity analysis and screening for novel lipolytic enzymes. Front. Microbiol., 6: 1291.

Makhdoumi-Kakhki A, Amoozegar MA, Ventosa A. 2012. *Halovenus aranensis* gen. nov., sp. nov., an extremely halophilic archaeon from Aran-Bidgol Salt Lake. Int. J. Syst. Evol. Microbiol., 62(Pt 6): 1331–1336.

Maloy SR. 1990. 1990. Experimental techniques in bacterial genetics. Jones and Bartlet Publisher Inc., pp. 125-139.

Mesbah NM, Abou-El-Ela SH, Wiegel J. 2007. Novel and Unexpected Prokaryotic Diversity in Water and Sediments of the Alkaline, Hypersaline Lakes of the Wadi An Natrun, Egypt. Microb. Ecol., 54(4): 598–617.

Müller-Santos M, Souza EMd, Pedrosa FdO, Mitchell DA, Longhi S, Carrière F, Canaan S, Krieger N. 2009. First evidence for the salt-dependent folding and activity of an esterase from the halophilic archaeon *Haloarcula marismortui*. Biochim. Biophys. Acta, 1791: 719-729.

Mwatha WE, Grant WD. 1993. *Natronobacter iumvacuolata* sp. Nov., a haloalkaliphilic archaeon isolated from Lake Magadi, Kenya. Int. J. Syst. Evol. Microbiol., 43: 401-404.

Nacke H, Engelhaupt M, Brady S, Fischer C, Tautzt J, Daniel R. 2012. Identification and characterization of novel cellulolytic and hemicellulolytic genes and enzymes derived from german grass land soil metagenomes. Biotechnol. Lett., 34(4): 663–675.

Oren A. 2002. Halophilic microorganisms and their environments. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Oulas A, Pavloudi C, Polymenakou P, Georgios A, Papanikolaou N, Kotoulas G, Arvanitidis C, Iliopoulos I. 2015. Metagenomics: Tools and Insights for Analyzing Next-Generation Sequencing Data Derived from Biodiversity Studies. Bioinform. Biol. Insights, 9: 75–88**.**

Ozcan B, Cokmus C, Coleri A, Caliskan M. 2006. Characterization of extremely halophilic archaea isolated from saline environment in different parts of Turkey. Microbiologia, 75(6): 739-746.

Patil j, Bajekal S. 2013. Diversity of hydrolytic enzymes in Haloalkaliphilic archaea isolated from Lonar Lake. Int. J. Sci. Res., 2(7): 2277-8179.

Purohit MK, Singh SP. 2009. Assessment of various methods for extraction of metagenomic DNA from saline habitats of coastal Gujarat (India) to explore molecular diversity. Lett. Appl. Microbiol., 49(3): 338-344**.**

Purohit MK, Singh SP. 2011. Comparative analysis of enzymatic stability and amino acid sequences of thermostable alkaline proteases from two haloalkaliphilic bacteria isolated from coastal region of Gujarat, India. Int. J. Bio. Mac. 49(1): 103-112.

Ruiz DM, de Castro RE. 2007. Effect of organicsolvents on the activity and stability of an extracellular protease secreted by the haloalkaliphilic archae on *Natrial bamagadii*. J. Ind. Microbiol. Biotech., 34(2), 111–115.

Santos LF, Sato HH. 2009. Production of Alkaline Protease from *Cellulosimicrobium cellulans*. Braz. J. Microbiol., 40(1): 54-60.

Stukus PE. 1997. Antimicobial testing. The Kirby-Bauer method (filter-paper disk method). In: Investigating microbiology: a laboratory manual for general microbiology. (Stukus PE. Ed.)". Orlando: Harcourt Brace & Company, 1997. cap.44, pp. 243-247.

Tindall BJ, Mills AA, Grant WD. 1980. An alkaliphilic red halophilic bacterium with a low magnesium requirement from a kenyan soda lake. J. Gen. Microbiol., 116: 257-260.

Ventosa A, Gutierrez MC, Kamekura M, Zvyagintseva IS, Oren A. 2004. Taxonomic study of *Halorubrumdistributum* and proposal of *Halorubrumterrestre* sp. nov. Int. J. Syst. Evolution. Microbiol., 54(Pt 2): 389–392.

مسح للإنزيمات المائية المتحملة للظروف البيئية القاسية من بكتيريا الاركيا المحبة للملوحة والقلوية على اسس تزريعية وجزيئية

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تهدف هذه الدراسة الي انتاج الانزيمات من بكتيريا الاركيا المتحملة للظروف القاسية من الملوحة والقلوية والتي تعتبر ذات اهمية كبيرة في تحسين قطاعات عديدة في المجال الصناعي. كما يمكن أن تساعد على التقليل من استهلاك الطاقة مما يجعلها تكنولوجيا صديقة للبيئة. تم دراسة الانزيمات المتحملة للظروف البيئية القاسية في العديد من الاماكن للبحيرات المصرية في وادي النطرون على اساس دراستها بطريقة اطباق الاجار وعلى اسس جزيئية. تم فحص خمسمائة وخمسة وثلاثين سلالة معزولة من البحيرات لبكتيريا الاركيا لإنتاج أنزيمات البروتيز، الأميليز، البكتينيز، الكيتينيز، السيليوليز، والليبيز والاستريز في درجة الحموضة 10 و 25٪ كلوريد الصوديوم (وزن/حجم).

وعلاوة على ذلك، تم استخراج الحمض النووي من عينة المياه من بحيرة الجعار لتحديد الجينات التي تكود الانزيمات وأظهرت جميع السلالات قابلية لإنتاج الإنزيمات خارج الخلية، اعطت39,4٪ من السلالات ايجابية لإنتاج أنزيم البروتيز، و 27,1٪ نشاطا لانزيم الأميليز، 25,9٪ لليبيز و 7,4٪ عرض نشاطا للسليوليز، ولكن أيا من السلالات المختبرة لم تعطي نشاطا لانتاج الكيتينيز و البكتينيز. وقد ثبت باستخدام تقنية shotgun metagenomic. وجود كل الجينات الممثلة لهذه الانزيمات داخل عينة المياة المأخوذة من بحيرة الجعار. وأشارت نتائج كلتا الطريقتين أن هذه البحيرات الصوداوية غنية بالانزيمات ذات القيمة التجارية.

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