Int.J.Curr.Microbiol.App.Sci (2016) *5*(10): 379-385

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 10 (2016) pp. 379-385**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.510.043

Potential of Halophilic Bacteria for Extracellular Enzymes

K.D. Kamble*

Department of Microbiology, Sant Gadge Baba Amravati University, Amravati, Maharashtra, India
**Corresponding author*

Keywords

Halophiles, bacteria, enzymes, efficiency L-asparaginase

Article Info

Accepted:
14 September 2016
Available Online:
10 October 2016

A B S T R A C T

Although protease, amylase and lipase are frequently studied; in the list L-asparaginase also finds position. Halophilic enzymes are currently preferred because these remains active at wide range of pH and temperature and also the yield of purified functional enzymes is high. Having studied around 29 bacteria for DNA degrading abilities; as extended part of the study these bacteria are undertaken to determine their potential for protease, amylase, lipase and L-asparaginase productions. The study included members of genera *Providencia, Rheinheimera, Halomonas, Alishewanella, Serratia, Marinobacter, Marinospirillum, Bacillus, Exiguobacterium* and *Vibrio* species. The enzymatic activities were observed in terms of detectable zones of hydrolysis which were measured. The zones of hydrolysis appeared after 24 h and were at its maximum towards 48 h. The results revealed enzyme activities largely varied among genus and within genus also. Amylase was efficiently produced by *Bacillus cereus* (DN3), *Vibrio species* (DN25), *Marinobacter species* (DN16) whereas excellent protease activity was exhibited by *Bacillus cereus* (DN3), *Bacillus thuringiensis* (DN2) and *Vibrio* Sp (DN25). *Exiguobacterium* (SK1 and SK2) was found to be the most efficient lipase producing bacteria followed by *Providencia rettgeri* (DN1) and *Bacillus thuringiensis* (DN2). The therapeutic enzyme L-asparaginase was produced only by two bacteria i.e. *Pseudomonas* (DN13) and *Halomonas* (DN19). Looking at industrial market of the enzymes; it is suggested that these bacteria would be promising sources of stable and robust enzymes.

Introduction

Meteorite impact craters frequently strikes on surface of moon, whereas very few are experienced by earth of which Lonar Lake impact crater situated in Buldhana district, Maharashtra State, India is one. The crater is aged approximately 50,000 years covering an area of 1.9 km and depth of 135m (Vijayan *et al.*, 2012; Antony *et al.*, 2012).

However the lake is experiencing both topological modifications and physicochemical changes as the diameter and salinity is changing because of infilling of the lake by people's activity as well as by seasonal monsoon.

The lake is of an immense field of interest harboring potential bacterial metabolites and

enzymes of industrial and medicinal use. Bacterial culture dependent studies from this lake were initiated by Joshi *et al.,* which stated predominance of Firmicutes over Proteobacteria (Joshi *et al.,* 2008). This fact was also supported by Deshmukh *et al.,* wherein seven Firmicutes and three Proteobacteria were reported (Deshmukh *et al.,* 2013). In recent years presence of haloalkaliphilic methanogenic bacteria was revealed in this lake including a novel species, namely *Methylophaga lonarensis* (Antony *et al.,* 2012). Sharma *et al.,* (2016) also isolated a novel species from Lonar lake i.e. *Streptomyces lonarsensis*. Bacterium *Bacillus lonarensis* is also among the novel species reported recently (Reddy *et al.,* 2015). Culture independent approaches reports Firmicutes 34% followed by Proteobacteria (29.5%), Actinobacteria (6.8%), Deinococcus-thermus (4.5%), Cytophages-flavobacterium-bacteroidetes (13.3%), Planctomycetes (6.8%), Cyanobacteria (4.5%) and Spirochetes (2.27%) of which 80% belonged to cultivable bacteria (Wani *et al.,* 2006). Metagenome approaches also showed presence of *Coxiella burnetii* (17%), *Fibrobacter intestinalis* (12%), *Candidatus Cloacamonas acidaminovorans* (11%) and archaebacteria like *Methanosaeta harund-inacea* (35%), *Methanoculleus chikugoe-nsis* (12%) and *Methanolinea tarda* (11%) were reported to be dominant (Dudhagara *et al.,* 2015).

Phylogenic analyses were also carried to study temporal diversity after beginning and well before beginning of monsoon. Seasonal variation in bacterial flora before and after monsoon showed a majority of *Cyanobacteria* (>80%). *Fusibacter* and *Tindallia magadiensis* were found in post-monsoon samples only, whereas species of *Planococcus rifietensis, Bordetella hinzii* and *Methylobacterium variabile* were reported in pre-monsoon samples. Presence of phototrophic bacteria in post monsoon samples was also reported by Surakasi *et al.,* (2010).

Halophilic enzymes are known to be active at a wide range of pH and temperature; preferably those also resist changes in purification steps. The enzymes like amylase, lipase, caseinase and cellulase were studied by Joshi *et al.,* (2008). Arsenic oxidizing and arsenic reducing bacteria have been recently reported from Lonar Lake (Bagade *et al.,* 2016). Alkaline enzymes like protease have been optimized by Rathod and Pathak (2016). Lipase enzyme from *Lysinibacillus mangiferihumi* is characterized by Tambekar *et al.,* (2016). Cyclodextrin glycosyl transferase is one more important enzyme to mention that convert starch and related glycans into cyclodextrins (Vinod and More, 2016). In order to find out few more alternatives to existing enzymes it would be important to report potent bacteria from alkaline Lonar Lake which is focus of present work.

Materials and Methods

Bacteria under study

Samples were collected from Lonar Lake in winter season. Alkaliphilic bacteria from this Lake were previously studied for DNA degradation (unpublished data). Having studied around 29 bacteria for DNA degrading abilities these were further studied for production of amylase, protease, lipase and L-asparaginase.

Amylase production

The amylase production was detected on a media containing 1% soluble starch. Plates were incubated for 24 hr and halos were measured by conventional method of iodine addition (Khanderparker *et al.,* 2011).

Protease production

A media containing casein was employed for protease production. The zones of hydrolysis were measured after overnight incubation (Gessesse and Gashe, 1997).

Lipase Production

One percent egg yolk was added to the nutrient media and zone of clearance were noted by spot inoculation after 24 hr incubation (Walavalkar & Bapat, 2002).

L-asparaginase production

A mineral base agar supplemented with glucose as carbon source and L-asparaginase as nitrogen source plus phenol red as indicator system was used for this purpose. After breakdown of L-asparagine to ammonia the color of medium changes from yellow to pink. Plates were incubated for two days at 37^0C (Prakasham *et al.*, 2007).

Results and Discussions

The zone of starch hydrolysis was maximum i.e. 20 mm in case of *Bacillus cereus* (DN3) followed by *Vibrio species* (DN25), *Marinobacter species* (DN16) with hydrolysis zones of 18 mm and 15 mm respectively (Fig1B).

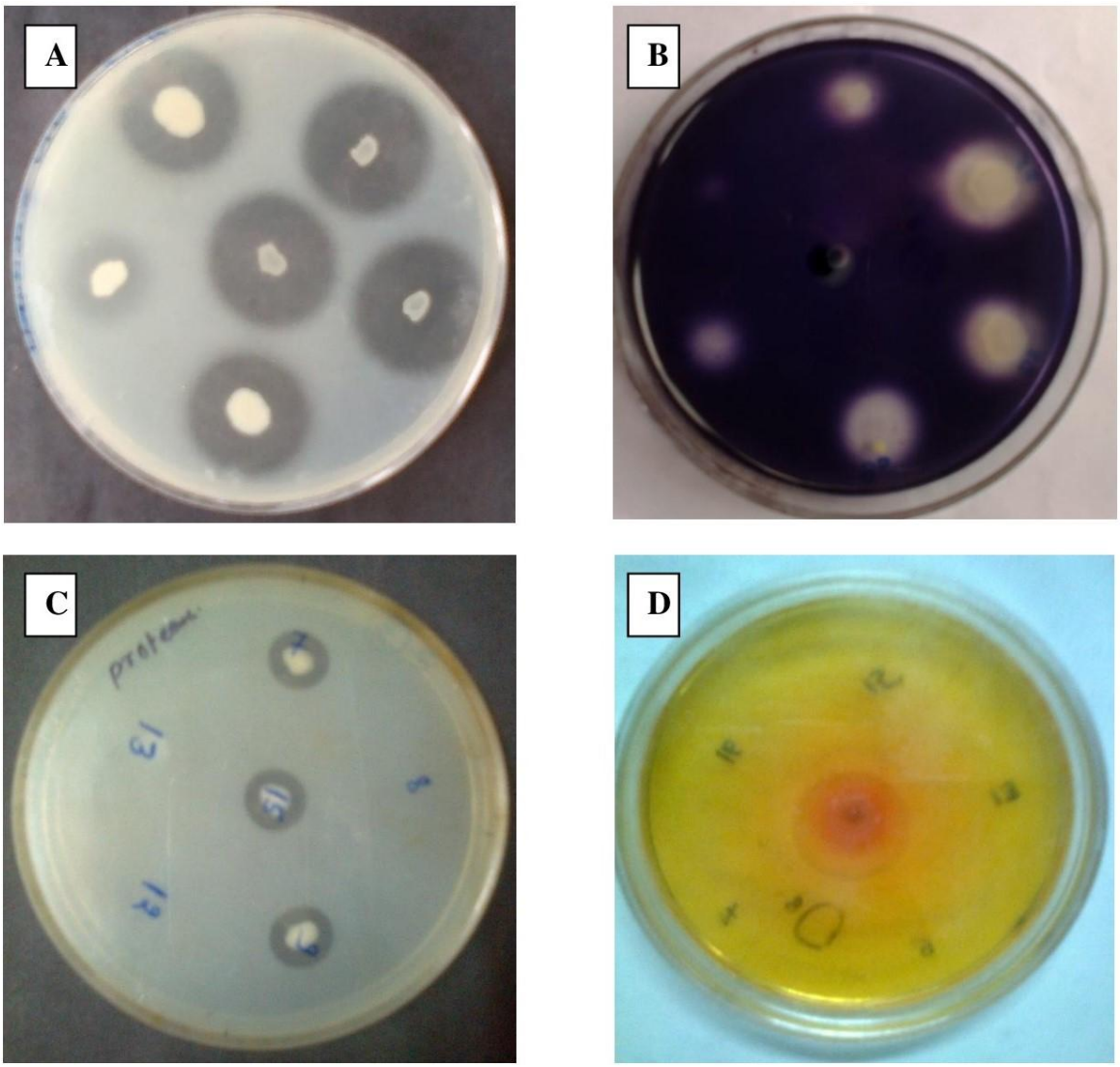
The proteolytic zone of *Bacillus cereus* (DN3) was maximum i.e. 34 mm and that of *Bacillus thuringiensis* (DN2) and *Vibrio* Sp (DN25) was 28 and 24 mm respectively (Fig1C). Excellent lipolytic activity was observed in case of *Exiguobacterium* sp (SK1 and SK2) with similar zone of hydrolysis i.e. 30 mm each whereas that of *Providencia rettgeri* (DN4) and *Bacillus thuringiensis* (DN2) was 25 mm each (Fig1A). The L-asparaginase activity was observed on the basis of pink coloration around the colony because of formation of ammonia due to L- asparagine break down. The therapeutic enzyme L-asparaginase was produced only by two bacteria i.e. *Pseudomonas* (DN13) and *Halomonas* (DN19) (Fig1D). Some of the species utilized L-asparagine but there was no detectable zone of hydrolysis. This might suggest the substrate is absorbed and utilized within the cell indicating the enzyme L-asparaginase is produced intracellularly. Many cultures could not utilize L-asparagine (Table1).

Members of *Bacillus* and *Pseudomonas* were frequently reported from Lonar Lake. In recent years protease enzyme is purified from species of *Bacillus* from Lonar Lake (Pathak and Deshmukh, 2012). We isolated species of *Vibrio* viz. *Vibrio natriengens* which is one of the fastest growing bacterium and is also a model organism for study of promoter. The bacterium is also an efficient producer of poly-beta-hydroxybutyrate (PHB) studied from marine sediments leading to faster production of PHB within short period of time (Chien, 2007). Species of *Marinobacter*, *Exiguobacterium, Alishewanella* and *Rheinheimera* and *Marinospirillum* are studied for various enzyme productions which are among the rare flora present in this lake. The spiral shaped gamma proteobacteria were designated as *Marinospirilllum* by Satomi *et al.,* (1998) which previously were close to *Oceanospirillum*. Sufficient enzymatic data of *Marinospirillum* is unavailable especially with regards to purification.

Table.1 Comparative study of enzymes represented by zone of hydrolysis

Culture Code	Name of Bacterium	Accession Number	Zone of enzymatic hydrolysis			
			Amylase	Protease	Lipase	L-asparginase
DN1	*Providencia* sp.	JX298813	-	-	-	-
DN2	*Bacillus thuringiensis*	JX298814	14	28	25	-
DN3	*Bacillus cereus*	JX298815	20	34	-	-
DN4	*Providencia rettgeri*	JX298816	13	27	25	-
DN5	*Vibrio metschnikovii*	JX298817	-	-	-	-
DN6	*Halomonas venusta*	JX298818	-	-	-	-
DN7	*Alishewanella* Sp.	JX298819	-	18	-	-
DN8	*Pseudomonas putida*	JX298820	-	-	-	-
DN9	*Alishewanella* Sp.	JX298821	-	19	24	-
DN12	*Serratia rubidaea*	JX298822	-	-	-	-
DN13	*Pseudomonas* Sp.	JX298823	-	-	-	18
DN15	*Alishewanella* Sp.	JX298838	-	18	-	-
DN16	*Marinobacter* Sp.	JX298824	15	13	-	-
DN19	*Halomonas* Sp.	JX298825	-	8	18	16
DN20	*Halomonas campisalis*	JX298826	-	-	-	-
DN21	*Marinospirillum* Sp	JX298827	11	-	-	-
DN22	*Marinospirillum* Sp.	JX298828	-	-	-	-
DN24	*Rheinheimera chironomi*	JX298829	-	7	-	-
DN25	*Vibrio* Sp.	JX298830	18	24	25	-
DN26	*Vibrio natriegens*	JX298831	-	-	-	-
DN27	*Vibrio metschnikovii*	JX298832	-	-	-	-
DN28	*Vibrio metschnikovii*	JX298833	-	-	-	-
DN29	*Vibrio natriegens*	JX298834	-	14	11	-
DN30	*Vibrio natriegens*	JX298835	-	-	-	-
DT1	*Serratia marcescens*	KC130919	-	21	18	-
DT2	*Serratia marcescens*	KC130920	-	21	18	-
DT3	*Staphylococcus* sp.	KC130921	-	-	-	-
SK2	*Exiguobacterium* sp.	KC130922	12	23	30	-
SK3	*Exiguobacterium* sp.	KC130923	12	23	30	-

Fig.1 Representative media plates A] Lipase B] Amylase C] Protease D] L-asparaginase



Bacteria of the genus *Exiguobacterium* were found both in psychrophilic and thermophilic environments. The genus was well studied for nucleolytic enzymes by Vishnivetskaya *et al.*, (2009). The species reported in this study are promising sources of industrial and therapeutic enzymes. During study it was noted that the enzymatic activities largely varied within the genera and also within species.

In conclusion, as the industrial and therapeutic applications of enzymes are increasing there is continuous need to search efficient bacteria that has the ability to produce the enzyme extracellularly. The bacteria reported in this study would be promising sources of these enzymes. The most promising bacterium in the study was *Bacillus cereus* (DN3) which could synthesize both amylase and protease efficiently. *Exiguobacterium* sp (SK1 and SK2) is also a candidate for lipase production. *Pseudomonas* (DN13) and *Halomonas* (DN19) have not been previously studied for L-asparaginase production from this lake.

References

Antony, C.P., Doronina, N.V., Boden, R., Trotsenko, Y.A., Shouche, Y.S. and Murrell, J.C. 2012. *Methylophaga lonarensis*, A Novel Moderately Haloalkaliphilic Methylotroph Isolated From the Soda Lake Sediments of A Meteorite Impact Crater. *Int. J. Syst. Evol. Microbiol.*, vol. 62, pp. 1613-1618.

Bagade, A.V., Bachate, S.P., Dholakia, B.B., Giri, A.P., & Kodam, K.M. 2016. Characterization of Roseomonas and Nocardioides spp. for arsenic transformation. *J. Hazardous Materials*, doi:10.1016/j.jhazmat.2016.07.062

Chien, C.C., Chen, C.C., Choi, M.H., Kung, S.S. and Wei, Y.H., Production of Poly-beta-hydroxybutyrate (PHB) by *Vibrio* spp. Isolated from Marine Environment. *J. Biotechnol.*, vol. 132, pp. 259-263.

Deshmukh, K.B., Pathak, A.P. and Karuppayil, M.S. 2011. Bacterial diversity of lonar soda lake of India. *Ind. J. Microbiol.*, vol. 51, pp. 107-111.

Dudhagara, P., Ghelani, A., Patel, R., Chaudhari, R., & Bhatt, S. 2015. Bacterial tag encoded FLX titanium amplicon pyrosequencing (bTEFAP) based assessment of prokaryotic diversity in metagenome of Lonar soda lake, India. *Genomics data, 4*: 8-11.

Gessesse, A., Gashe, B.A. 1997. Production of alkaline protease by an alkaliphilic bacteria isolated from an alkaline soda lake. *Biotechnol. Lett., 19*(5): 479-481.

Joshi, A.A., Kanekar, P.P., Kelkar, A.S., Shouche, Y.S., Vani, A.A., Borgave, S.B. and Sarnaik, S.S. 2008. Cultivable Bacterial Diversity of Alkaline Lonar Lake, India. *Microb. Ecol.*, vol. 55, pp. 163-172.

Khanderparker, R., Verna, P., Meena, R.M. and Deobagkar, D.D. 2011. Phylogenetic Diversity of Carbohydrate Degrading Culturable Bacteria from Mandovi and Zuari Estuaries, Goa, West Coast of India. *Estuarine Coastal and Shelf Sci.*, vol. 95, pp. 359-366.

Pathak, A.P. and Deshmukh, K.B. 2012. Alkaline Protease Production, Extraction and Characterization from Alkaliphilic *Bacillus licheniformis* KBDL4: A Lonar Soda Lake Isolate. *Ind. J. Expt. Biol.*, vol. 50, pp. 569-576.

Prakasham, R.S., Rao, C.S., Rao, R.S., Lakshmi, G.S. and Sarma, P.N. 2007. L-asparaginase production by isolated *Staphylococcus* sp.-6A: design of experiment considering interaction effect for process parameter optimization. *J. Appl. Microbiol.*, vol. 102, pp. 1382-1391.

Rathod, M.G., Pathak, A.P. 2016. Optimized production, characterization and application of alkaline proteases from taxonomically assessed microbial isolates from Lonar soda lake, India. *Biocatalysis and Agri. Biotechnol.*, 7: 164- 173.

Reddy, S.V., Thirumala, M., Farooq, M., Sasikala, C., & Ramana, C.V. 2015. Bacillus lonarensis sp. nov., an alkalitolerant bacterium isolated from a soda lake. *Arch. Microbiol., 197*(1): 27-34.

Sahay, H., Mahfooz, S., Singh, A.K., Singh, S., Kaushik, R., Saxena, A.K. and Arora, D.K. 2012. Exploration and Characterization of Agriculturally and Industrially Important Haloalkaliphilic Bacteria from Environmental Samples of Hypersaline Sambhar Lake, India. *World J. Microbiol. Biotechnol.*, vol. 28, pp. 3207–3217.

Satomi, M., Kimura, B., Hayashi, M., Okuzumi, M. and Fujii, T. 1998. *Marinospirillum* gen. nov., with Descriptions of *Marinospirillum megaterium* sp. nov., Isolated from Kusaya Gravym and Transfer of *Oceanospirillium minutulum* to *Marinospirillum minutulum* copb. nov. *Int. J. Syst. Bacteriol.*, vol. 48, pp. 1341-1348.

Sharma, T.K., Mawlankar, R., Sonalkar, V.V., Shinde, V.K., Zhan, J., Li, W.J., Kumar, L.S. 2016. Streptomyces lonarensis sp. nov., isolated from Lonar Lake, a meteorite salt water lake in India. *Antonie van Leeuwenhoek, 109*(2): 225-235.

Surakasi, V.P., Antony, C.P., Sharma, S., Patole, M.S. & Shouche, Y.S. 2010. Temporal bacterial diversity and detection of putative methanotrophs in surface mats of Lonar Crater Lake. *J. Basic Microbiol.*, 50: 465–474.

Tambekar, D.H., Tambekar, S.D., Jadhav, A.S., & Sharma, N.H. 2016. Thermophilic Lipase from Lysinibacillus mangiferihumi: Screening and Partial Characterization. *Indian J. Appl. Res.*, *6*(6): 432-434.

Vijayan, S., Vani, K. and Sanjeevi, S. 2012. Topographical Analysis of Lonar Crater Using Cartosat-1 DEM. *J. Ind. Soc. Remote Sens.*, DOI 10.1007/s12524-012-0216-5

Vinod, A.S., & More, S.M. 2016. Purification and Characterization of a Novel CGTase from Alkaliphilic *Bacillus flexus* Isolated from Lonar lake, India. *Int. J. Curr. Microbiol. App. Sci.*, *5*(7): 164-170.

Vishnivetskaya, T.A., Kathariou, S. and Tiedje, J.M. 2009. The *Exiguobacterium* genus: Biodiversity and Biogeography. *Extremophiles*, vol. 13, pp. 541-555.

Walavalkar, G.S., Bapat, M.M. 2002. Staphylococcus warneri BW 94-A new source of lipase. *Indian J. Experimental Biol.*, *40*(11): 1280-1284.

Wani, A.A., Surakasi, V.P., Siddharth, J., Raghavan, R.G., Patole, M.S., Ranade, D. and Shouche, Y.S. 2006. Molecular Analyses of Microbial Diversity Associated with the Lonar Soda Lake in India: an Impact Crater in a Basalt Area. *Res. Microbiol.*, 157: pp. 928-937.

How to cite this article:

Kamble, K.D. 2016. Potential of Halophilic Bacteria for Extracellular Enzymes. *Int.J.Curr.Microbiol.App.Sci.* 5(10): 379-385. doi: http://dx.doi.org/10.20546/ijcmas.2016.510.043