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Research article

PRODUCTION OF EXTRACELLULAR ENZYMES BY HALOPHILIC BACTERIA ISOLATED FROM SOLAR SALTERNS

Jhuma Biswas and A. K. Paul*

Microbiology Laboratory, Department of Botany, University of Calcutta
Kolkata-700019, India
*Corresponding author E. mail: amalk_paul@yahoo.co.in

ABSTRACT: During the course of survey of halophilic microorganisms, a total of sixteen bacterial isolates were obtained from coastal solar salterns of Orissa and West Bengal, India. Morphological, physiological and biochemical characteristics of these isolates indicate that majority of them belong to the genus *Halomonas*, however, members belonging to *Cobetia* and *Halococcus* were not uncommon. These isolates were screened for the production of extracellular enzymes such as amylase, glutaminase, asparaginase, xylanase, cellulase, gelatinase, inulinase, caseinase, pectinase, urease and lipase. Among these hydrolytic enzymes, glutamine and asparagine hydrolytic activities were predominant, although lipid and casein degrading activities were not inferior. However, amylase and gelatinase production were rare. None of these halophiles was able to degrade cellulose, inulin, pectin and xylan and only one isolate was capable of hydrolyzing urea.

Keywords: Halophilic bacteria, solar salterns, extracellular enzymes, *Halomonas, Halococcus*.

INTRODUCTION

Halotolerant and halophilic microorganisms grow in environments with high salinity (Rodriguez-Valera, 1988). Multipond solar salterns situated along the coasts of India, are used for commercial production of halite (NaCl) from sea water and represent an ideal environment for studying halophilic microorganisms (Ventosa et al., 1995, Grant et al., 1998). They are also found in environments like saltlakes, saline soils and even in salted foods. Studies of these halophilic microorganisms are of special importance as they are capable of producing compounds of diverse industrial, pharmaceutical and biomedical potentials. One of the most important biotechnological applications of these halophilic bacteria is centered on the production of diverse extracellular enzymes like amylase, lipase, caseinase, xylanase, inulinase, pectinase, cellulase, pullulanase, gelatinase, urease, glutaminase and asparaginase (Mellado et al., 2004). These enzymes have diverse potential uses in biomedical science (Kulkarni et al., 1999; Niehaus et al.; 1999, Rao et al., 1998; Pandey et al., 1999, 2000) and chemical industries (Margesin and Schinner, 2001). They are not only salt tolerant but also are highly active even at high temperatures and pH values. In view of these unusual properties, recently, considerable attention has been paid to these extracellular hydrolytic enzymes produced by halophilic microorganisms. Systematic screening of moderately halophilic bacteria producing extracellular hydrolytic enzymes of diverse types has been made by Sanchez-Porro et al. (2003) and Rohban et al. (2009). It revealed that moderately halophilic bacteria are potential sources of extracellular hydrolytic enzymes such as amylases, DNases, lipases, proteases etc. The well known sources of thermotolerant and salt adapted halophilic bacteria produced hydrolases (Kamekura and Onishi, 1978) include nuclease from *Bacillus* sp. (Onishi et al., 1983), protease from *Micrococcus varians* (Kamekura and Onishi, 1978), lipase by a extreme halococcus *Natronococcus* sp. (Bhatnagar et al., 2005), amylase by *Micrococcus* sp. (Khire, 1994; Kobayashi et al., 1986) and β- xylanase by extremely halophilic archeon *Halorhabdus utahensis* (Waino and Ingvorsen, 2003). During the course of survey of halophilic bacteria, a total of sixteen halophilic bacterial cultures were isolated from multipond solar salterns spreaded along the coasts of Orissa and West Bengal, India. Here we report the characteristic features, tentative identity and potency of these halophilic bacterial isolates to produce extracellular hydrolytic enzymes on solid media.

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MATERIALS AND METHODS

Source of bacterial isolates

Soil and water samples were collected from multipond solar salterns distributed along the coasts of Orissa and West Bengal, India. These samples were used to isolate halophilic bacteria following serial dilution and plating in MH medium (Ventosa et al., 1989) supplemented with NaCl. Bacterial colonies differing in morphology were isolated in pure form and maintained on slopes of the same medium. The MH medium contained ($g.L^{-1}$): Yeast extract 10; Protease peptone, 5; Glucose, 1; NaCl, 100; $MgCl_2$, $6H_2O$, 7; $MgSO_4$, $7H_2O$, 9.6; $CaCl_2$, $2H_2O$, 0.36; KCl, 2; $NaHCO_3$, 0.06 and NaBr 0.026, pH-7.2.

Characterization of isolates

Morphological, physiological and biochemical characteristics of the selected isolates were determined following different standard methods. Gram nature, motility, color of colony, IMViC tests were performed as recommended by Smibert and Krieg (Smibert and Krieg, 1994). Optimum temperature and pH for growth and tolerance to NaCl% (w/v) were also routinely determined. Fermentation of different carbohydrates was tested by using phenol red medium supplemented with 1% (w/v) carbohydrate. The inhibitory effect of different antibiotics was determined by disc-diffusion method and expressed in terms of antibiotic resistance index which denotes the ratio of number of resistant antibiotics to the total number of antibiotics tested.

Screening for extracellular enzyme

These sixteen halophilic bacteria were screened for production of different extracellular enzymes like lipase, caseinase, cellulase, glutaminase, asparaginase, inulinase, xylanase, pectinase and urease. The basal MH medium supplemented with respective substrates was used for production of these enzymes. Amylase and lipase production were performed according to Gonzalez et al. (1978). For urease production, bacterial strains were inoculated in Urea agar medium and incubated at 37^0C for 3-5 days. Production of pink coloration around the growth indicates its urease activity. Detection of asparaginase and glutaminase activities was made as described by Gulati et al. (1997). Gelatinase activity was tested in basal MH broth supplemented with gelatin (120 $g.L^{-1}$). Tubes inoculated with test organisms were incubated for 7 days at 37^0C and tested for liquefaction of gelatin in an ice bath. Production of xylanase was performed as described by Wejse and Ingvorsen (2003). Inulinase activity was detected on basal MH agar medium supplemented with 0.5% inulin. Bacterial isolates were grown on these plates for 3-5 days at 37^0C were flooded with Burk's iodine solution and formation of clear hallows zones around the bacterial growth indicated inulinase activity. Cellulase activity was screened according to Farkas et al. (1985) using 0.5% carboxy methyl cellulose as substrate. Casein hydrolysis was tested in basal MH agar medium supplemented with 1% casein and isolates were grown on these plates for 3-5 days at 37^0C. Presence of clear hallow around the growth indicated hydrolysis of casein. Pectinase activity was detected according to Soares et al. (1999) using 0.5% pectin as substrate.

RESULTS

Isolation of halophilic bacteria

Halophilic bacterial strains were isolated from the soil and water samples collected from multipond solar salterns distributed along the coasts of Orissa and West Bengal, India. The samples were serially diluted and plated on MH agar medium supplemented with 10% (w/v) NaCl. Pure cultures of bacteria were maintained on slopes of MH agar medium at 4^0C and subculture at an interval of 30 days.

Characterization and identification of halophilic isolates

A total of sixteen isolates were obtained in pure form and analyzed for standard morphological, physiological and biochemical characters and are represented in Table1 Results indicate that most of the isolates were Gram-negative, motile rods and stay singly. None of them produce endospores and diffusible pigments. Colony color of most isolates was either cream or white with the exception of isolate BKC01 which was orange red. They did not grow in McConky agar medium. According to their salt tolerance, most of them were categorized as moderate halophiles or halotolerant. They were able to tolerate wide range of pH but mostly neutrophilic. None of them gave positive response for MR-VP test, indole production, arginine decarboxylase and H_2S production. Only four isolates utilized citrate and showed presence of ornithine and lysine decarboxylase activities. Except two isolates (BKS304 and BKS313), all produced nitrate reductase. Two (SUR106 and SUR306) of them showed the presence of phenylalanine deaminase while one (SUR306) was positive tryptophan deaminase.

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Table 1 Morphological, physiological and biochemical characteristics of some selected halophilic bacteria isolated from solar salterns

	Bacterial isolate															
	BKC01	BKS201	BKS210	BKS304	BKS313	DPS01	DPS102	DPS407	DPS501	DPS502	DPW204	DPW304	DPW508	SUR101	SUR106	SUR306
						Morphological characters										
Colony shape and color	Red, tiny, round	White, round	White, round	Cream, round	Cream, round	Cream, round	Cream, round	White, round	White, round	Cream, round	Cream, round	White, round	White, round	Cream, round	Cream, round	Cream, round
Gram nature	-	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-
Cell size, µm	0.8-0.9	1.8-2.0 ×0.5-0.6	1.4-1.6 × 0.7- 0.8	1.5-1.6× 0.5- 0.6	0.70- 0.8× 0.50-0.6	1.2-1.4× 0.45-0.5	1.2- 1.4× 0.45-0.5	1.2-1.5 × 0.7-0.8	1.5-1.5 × 0.6-0.7	1.8-2.0 × 0.5 -0.6	1.4-1.5× 0.45- 0.50	2.0-2.5 × 0.6-0.8	0.80- 0.90 × 0.60- 0.65	1.5-1.6 × 0.6-0.6	1.5-1.5× 0.6-0.7	1.5-1.5 × 0.6-0.5
Cell arrangement	Single	Single	Single	Single	Single	Single	Single	Single	Single	Single	Single	Single	Single	Single	Single	Single
Endospore formation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
						Physiological characters										
pH range for growth	5-10	5-10	5-10	5-10	5-11	5-11	5-11	5-10	5-10	5-11	5-11	5-10	5-10	5-10	5-10	5-10
pH optimum for growth	7	7	6	7	7	9	6	7	9	5	6	7	6	7	7	7
Temp. range for growth (°C)	28-45	28-45	28-45	28-40	28-45	28-45	28-45	28-45	28-45	28-45	28-45	28-45	28-37	28-37	28-37	28-37
Opt. temp. for growth (°C)	37	28	28	32	28	28	28	37	40	37	28	37	28	32	32	32
NaCl tolerance (%)	5-35	5-20	0-20	5-20	0-20	5-35	0-15	5-20	0-20	0-20	0-20	5-25	0-20	0-20	0-20	0-20
NaCl optimum for growth (%)	15	5	5	5	5	5	5	5	2.5	2.5	2.5	15	5	5	5	5
Growth on McConcy	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth on King's B	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Biochemical characters																
MR test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VP test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate utilization	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+
Production of Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H_2S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	
Lysine decarboxylase	-	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-
Ornithine decarboxylase	-	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-
Arginine decarboxylase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenylalanine deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Tryptophan deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Nitrate reductase	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+

(+) indicates positive response; (-) indicates negative response

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Table 2 shows the pattern of sugar fermentation as tested on Phenol red medium. Four of them (DPS407, DPS501, BKS201 and DPW304) showed strong carbohydrate fermenting ability by fermenting 14 – 15 of the total carbohydrate used. On the other end, isolate DPS01 showed least carbohydrates fermentation and was followed by isolates BKS313, SUR106, SUR101 and SUR306. Based on these characteristic features, thirteen out of sixteen isolates were identified tentatively as members of the genus *Halomonas.* Two isolates (BKS313 and DPW508) with coccobacilli morphology were categorized as *Cobetia* while one (BKC01) was identified as *Halococcus*. Susceptibility to antibiotics of these isolates was investigated following disc-diffusion method and the antibiotic resistance index of the isolates was calculated (Figure 1). It was evident that bacterial isolate BKC01 (ARI = 0.916) was resistant to almost all the antibiotics tested and was followed by BKS210 and DPS502 (ARI = 0.625) and BKS313 (ARI = 0.583), while isolates DPS01 and DPW508 showed least resistance (ARI = 0.25) to the tested antibiotics.

Table 2 Carbohydrate fermentation pattern of some selected halophilic bacteria isolated from solar salterns

Carbohydrate	Bacterial isolate															
	BKC01	BKS201	BKS210	BKS313	BKS304	DPS01	DPS102	DPS407	DPS501	DPS502	DPW204	DPW304	DPW508	SUR101	SUR106	SUR306
Arabinose	-	+	+	-	+	-	+	+	+	-	+	+	-	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	-	-	-	-	+	-	-	+	+		+	+	-	-	-	-
Glycine	-	+	+	-	-	-	+	+	+	-	-	+	-	+	+	+
Galactose	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+
m-Inositol	-	+	+	+	+	-	+	+	+	-	+	+	-	-	-	-
Maltose	-	+	+	-	+	+	+	+	+	+	-	+	+	-	-	-
Mannose	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+
Mannitol	-	+	+	-	+	-	+	+	+	-	-	+	-	-	-	-
Maltotriose	-	+	+	-	-	-	+	+	+	+	-	+	+	-	-	-
Na-Acetate	-	+	-	-	-	-	-	+	+	-	-	+	-	-	-	-
Na- Benzoate	-	-	+	-	+	-	-	+	-	+	-	+	-	-	-	-
Na-Fumarate	-	+	-	-	-	-	+	+	+	+	-	+	+	-	-	-
Na-Fofmate	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-
Na-Succinate	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Lactose	-	+	+	-	-	-	+	+	+	-	-	+	-	-	-	-
Rhamnose	+	+	+	-	+	-	+	+	+	-	-	+	-	-	-	-
Sucrose	+	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-
Salicin	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-
Xylose	+	+	+	-	+	-	+	+	+	-	-	+	-	-	-	-

(+) indicates positive response; (-) indicates negative response

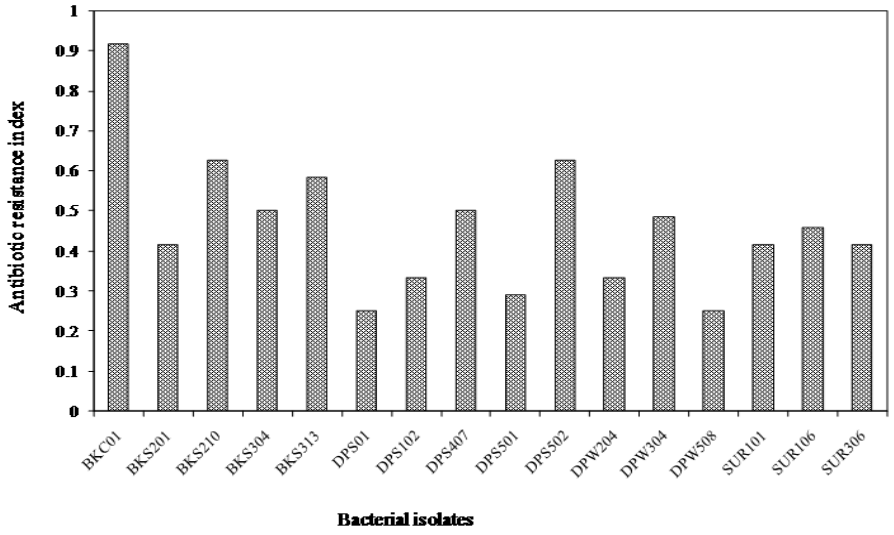

Figure-1: Antibiotic resistance index (ARI) of the selected halophilic bacteria isolated from solar salterns.

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Screening for extracellular enzymes

All sixteen bacterial isolates were tested for their ability to hydrolyze a number of different substrates such as starch, gelatin, casein, Tween 80, CM-cellulose, xylan, inulin, pectin, glutamine, asparagine and thereby production of respective hydrolytic enzymes. Twelve out of 16 isolates produced asparaginase and glutaminase and eleven of them produced caseinase and lipase. Amylase and gelatinase were produced by six and three isolates respectively. When the extracellular enzyme production profile of the isolates was considered (Table 3) individually, the isolate DPS01 was found to produce six different enzymes; this was followed by five different isolates (BKS210, BKS313, DPS502, DPW204 and DPW304) each of them producing five different enzymes. Isolate DPS407 was exceptional being unable to produce anyone of the targeted extracellular enzymes.

Table 3 Production of extracellular enzymes by some selected halophilic bacteria isolated from solar salterns

Bacterial isolate	Enzymes produced										
	Amylase	Asparaginase	Cellulase	Caseinase	Gelatinase	Glutaminase	Inulinase	Lipase	Pectinase	Urease	Xylanase
BKC01	-	-	-	+	-	-	-	+	-	-	-
BKS201	-	-	-	+	-	-	-	+	-	-	-
BKS210	+	+	-	+	-	+	-	+	-	-	-
BKS313	+	+	-	+	-	+	-	-	-	+	-
BKS304	-	+	-	-	-	+	-	-	-	-	-
DPS01	+	+	-	+	+	+	-	+	-	-	-
DPS102	+	+	-	+	-	+	-	-	-	-	-
DPS407	-	-	-	-	-	-	-	-	-	-	-
DPS501	-	-	-	+	+	-	-	+	-	-	-
DPS502	-	+	-	+	+	+	-	+	-	-	-
DPW204	+	+	-	+	-	+	-	+	-	-	-
DPW304	+	+	-	+	-	+	-	+	-	-	-
DPW508	-	+	-	+	-	+	-	+	-	-	-
SUR101	-	+	-	-	-	+	-	+	-	-	-
SUR106	-	+	-	-	-	+	-	-	-	-	-
SUR306	-	+	-	-	-	+	-	+	-	-	-

(+) indicates positive response; (-) indicates negative response

DISCUSSION

The present study indicated that most of the Gram-negative rods isolated from solar salterns belonged to the genus *Halomonas* and two isolates (BKS313 and DPW508) were assigned to the genus *Cobetia*. In addition, we were able to isolate a strain of *Halococcus* (BKC01). While, Sanchez-Porro et al. (2003) have reported the isolation of strains belonging to the genus *Chromohalobacter*, but in the present study we were not able to identify any member of this genus. They have also reported the abundance of different hydrolytic extracellular enzymes like amylases, proteases, lipase, DNase, and pullulanase in halophilic bacteria isolated from solar salterns of Spain. It was also (Sanchez-Porro et al., 2003) indicated that Gram-positive species have more hydrolytic activities than the Gram-negative halophilic isolates. However, in our studies, Gram-negative halophiles were proved to be more efficient extracellular enzyme producers. Asparaginase and glutaminase activities were most prevalent in these isolates followed by production of caseinase and lipase. The phenomenon of urea hydrolysis was of rare occurrence in these halophilic strains. Combined hydrolytic activities appeared to be a common criterion in most of the *Halomonas* strains. Similarly, Zavaleta and Cardenas-Farnandez (2007) have reported the production of amylase, lipase and protease by halophilic bacteria isolated from Pilluana brines of Peru. Moreno et al. (2007) on the other hand have investigated the diversity of extreme halophiles as producers of lipase, protease, amylase and other extracellular enzymes.

CONCLUSION

Extracellular enzymes from halophilic bacteria have been identified with great economical potential in industrial, agricultural, chemical, pharmaceuticals and biotechnological applications. The present study demonstrated the production of enzymes like lipase, asparaginase, glutaminase which are very common in halophilic bacteria isolated from solar salterns deserve special attention due to their importance in biotechnology and industries.

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REFERENCES

Bhatnagar T., S. Boutaiba, H. Hacene, J. Cayol, M. Fardeau, B. Ollivier and J. Baratti.(2005). Lipolytic activity from halobacteria: screening and hydrolase production. FEMS Microbiol. Lett. 248:133-140.

Farkas V., M. Liskova and P. Biely. (1985). Novel media for detection of microbial producers of cellulase and xylanase. FEMS Microbiol. Lett. 28:137-140.

Gulati R., R. K. Saxena and R. Gupta. (1997). A rapid plate assay for screening L-asparaginase producing microorganisms. Lett. Appl. Microbiol. 24:23-26.

Gonzalez C., C. Gutierrez and C. Ramirez. (1978). *Halobacterium vallismortis* sp. Nov. an amylolytic and carbohydrate metabolizing, extremely halophilic bacterium. Can. J. Microbiol. 24:710-715.

Grant W. D., R. T. Gemmell and T. J. Mcgenity. (1998). halobacteria- the evidence for longevity. Extremophiles. 2:279-288.

Kamekura M., and H. Onishi. (1978). Properties of the halophilic nuclease of a moderate halophile, *Micrococcus varians* subsp. *Halophilus* to commercial production of flavoring agent 5′-GMP. Appl. Environ. Microbiol. 44:994-995.

Kulkarni N., A. Shendnye and M. Rao. (1999). Molecular and Biotechnological Aspects of Xylanases. FEMS Microbiol. Rev. 23:411-456.

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.

Kobayashi T., M. Kamekura, W. Kanlayakrit and H. Onishi. (1986). Production, purification and characterization of an amylase of the moderate halophile *Micrococcus varians* subsp.*Halophilus*. Microbios. 46:165-170.

Margesin R., and F. Schinner. (2001). Potential of halotolerant and halophilic microorganisms for biotechnology. Extremophiles. 5:73-83.

Moreno M. L., E. Mellado, M. T. Garcia and A. Ventosa. (2007). Diversity of extreme halophiles producing hydrolytic enzymes in hypersaline habitats. Halophiles- 2007 booklet.59-60.

Mellado E., C. Sanchez-Porro, S. Martin, and A. Ventosa. (2004). Extracellular hydrolytic enzymes produced by moderately halophilic bacteria. *In*: Ventosa, A., (ed) Halophilic Microorganisms, Springer-Verlag, Berlin Heidelberg, 2004, pp 285-295.

Niehaus F., C. Bertoldo, M. Kahler and G. Antranikian. (1999). Extremophiles as a source of novel enzymes for industrial application. Appl. Microbiol. Biotech. 51:711-729.

Onishi H., T. Mori, S. Takeuchi, K. Tani, T. Kobayashi and M. Kamekura. (1983). Halophilic nuclease of moderately halophilic *Bacillus* sp.: Production, purification and characterization. Appl. Environ. Microbiol. 45:24-30.

Pandey A., S. Benjamin, C. R. Soccol, P. Nigam, N. Krieger and V. T. Soccol. (1999). The realm of microbial lipases in biotechnology. Biotechnol. Appl. Biochem. 29:119-131.

Pandey A., P. Nigam, V. T. Soccol, D. Singh and R. Mohan. (2000). Advances in microbial amylases. Biotechnol. Appl. Biochem. 31:135-152.

Rao M. B., A. M. Tanksale, M. S. Ghatge and V. V. Deshpande. (1998). Molecular and biotechnological aspects of microbial proteases. Microbiol. Mol. Biol. Rev. 62:597-635.

Rohban R., M. A. Amoozegar and A. Ventosa. (2009). Screening and isolation of halophilic bacteria producing extracellular hydrolyses from Howz Soltan Lake, Iran. J. Ind. Microbiol. Biotechnol. 36:333-340.

Rodriguez F. -Valera. (1988). Characteristics and microbial ecology of hypersaline environments, p. 3–30. *In* F. Rodriguez-Valera (ed.), Halophilic bacteria, vol. 1. CRC Press, Inc., Boca Raton, Fla.

Smibert R.M. and N. R. Krieg. (1994). Phenotypic characterization. *In: Gerhardt P. (ed)* Methods for general and molecular bacteriology. American Society for Microbiology Washington, DC, pp 607-654.

Sanchez-Porro C., S. Martin, E. Mellado and A. Ventosa. (2003). Diversity of moderately halophilic bacteria producing extracellular hydrolytic enzymes. J. Appl. Microbiol. 94:295-300.

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Soares Marcia M. C. N., R. De Silva and E. Gomez. (1999). Screening of bacterial strains for pectinolytic activity: Characterization of the polygalacturonase produced by *Bacillus* sp. Rev. Microbiol. 30:299-303.

Ventosa A., J. J. Nieto and A. Oren. (1995). Biology of moderately halophilic aerobic bacteria. Microbiol. Mol. Biol. Rev. 62:504-544.

Ventosa A., M. T. Garcia, M. Kamekura, H. Onishi and F. Ruiz-Berraquero. (1989). *Bacillus halophilus* sp. Nov., a moderately halophilic *Bacillus* species. Syst. Appl. Microbiol. 12:162-166.

Ventosa, A. and J. J. Nieto. (1995). Biotechnological applications and potentialities of halophilic microorganisms. W. J. Microbiol. Biotechnol. 11:85-94.

Wejse P. L. and K. Ingvorsen. (2003). Purification and characterization of two extremely halotolerant xylanase from a novel halophilic bacterium. Extremophiles. 7:423-431.

Waino M. and K. Ingvorsen. (2003). Production of β-xylanase and β-xylosidase by the extremely halophilic archeon *Halorhabdus utahensis*. Extremophiles. 7:87-93.

Zavaleta A. I. and A. M. Cardenas-Fernandez. (2007). Diversity of moderately halophilic bacteria producing extracellular hydrolytic enzymes isolated from Pilluana salt brine in Peru. Halophiles-2007 Congress Booklet. pp 50-51.