African Journal of Biotechnology Vol. 9 (11), pp. 1555-1560, 15 March, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Review

Diversity and industrial potential of hydrolase-producing halophilic/halotolerant eubacteria

Mathabatha Evodia Setati

Discipline of Microbiology, School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, P/Bag X54001, Durban 4000, South Africa. E-mail: setatim@ukzn.ac.za. Fax: +27(31) 260 7809. Tel: +27(31) 260 7405.

Accepted 21 December, 2009

Halophilic and haloterant eubacteria have been isolated from different marine and hypersaline environments. Halophilic eubacteria also occur in environments typified by more than one soda lakes which are both hypersaline and extremely alkaline. These organisms have been shown to produce a wide array of hydrolytic enzymes including proteases, amylases, xylanases, cellulases as well as lipases and DNases. These enzymes are commonly applied in the production of fermented food and food supplements, in animal feed, laundry detergents and textile industries. Several studies have shown that enzymes derived from halophilic and halotolerant eubacteria are not only halostable but may also be thermostable and alkalistable. This extremophilicity make the enzymes suitable candidates in various fields of biotechnology and may even open up new application opportunities.

Key words: Halophilic/halotolerant, Hydrolases, biodiversity, alkaliphilic, proteases, amylases, xylanases, cellulases.

INTRODUCTION

Hypersaline environments are widely distributed on the earth's continent where they exist either as natural water bodies such as permanent saline lakes, ephemeral salt pans and salt marshes, or as artificial solar salterns which comprise a series of interconnecting evaporation and crystallizer ponds constructed for the production of salt, potash and soda. Hypersaline environments can be divided into two broad categories. These are the thalassohaline and athalassohaline. Thalassohaline water bodies have similar salt composition to seawater with sodium and chloride being the dominant ions; common examples include the Great Salt Lake in Utah, playas, brine springs from underground salt deposits and solar salterns (Litchfield and Gillevet, 2002). In contrast, athalassohaline water bodies such as the Dead sea, Lake Magadi in Kenya, Wadi Natrun in Egypt, the soda lakes of Antarctica and Big Soda Lake and Mono Lake in California are dominated by potassium, magnesium, or sodium (Oren, 2002; Litchfield and Gillevet, 2002). Hypersaline water bodies are commonly 9-10 times more concentrated than sea water, which is generally defined as having 3.5% (w/v) dissolved salts. Both natural and artificial hypersaline environments harbour remarkably high and diverse microbial cell densities. Microorganisms that thrive in these environments have been broadly classified into halophilic microorganism (that is, require salt for their viability) and halotolerant microorganisms which are able to grow in the absence as well as in the presence of NaCl. Halophiles can be further divided into slight halophiles that grow optimally in 3% (w/v) total salt, moderate halophiles [optimal growth at 3 - 15% (w/v) salt] and extreme halophiles that grow optimally at 25% (w/v) salt (Ventosa et al., 1998). Eubacteria are mainly represented within the halotolerant, slight halophiles and moderate halophiles, with only a few genera been shown to be extremely halophilic. To adapt to saline conditions, these bacteria have developed various strategies to maintain cell structure and function. These include the accumulation of osmolytes such as ectoine and hydroxyectoine, glycine and betaine (Vargas et al., 2008). While most research performed on hypersaline environments has focused on the microbial diversity and ecology of these environments, there is growing interest in the extracellular hydrolytic enzymes from moderately halophilic bacteria. Most halophilic hydrolase producers have been assigned to the family Halomonadaceae and were shown to produce industrially relevant enzymes such as cellulases, amylases, xylanases, proteases and lipases (Sánchez-Porro et al., 2003a; Govender et al., 2009; Rohban et al., 2009). It is generally believed that

while these halophilic enzymes perform the same enzyme function as their non-halophilic counterparts, they can catalyse such reactions under different conditions, such as high salt environments. In addition, some of the enzymes derived from bacterial strains that were isolated from soda lakes and solar salterns originating from athalassohaline environments could display polyextremophilicity due to their adaptation to high salt and alkaline pH typical of soda lakes. Consequently, these bacteria would be an excellent source of enzymes that exhibit optimal activities at various ranges of salt concentrations and pH.

ECOLOGICAL DISTRIBUTION OF HYDROLASE-PRODUCING BACTERIA

Hypersaline environments maintain remarkably high microbial cell densities and are biologically very productive ecosystems. Various culture-dependent and nutriational analyses carried in tandem with molecular culture-independent techniques have been used to characterize the microbial communities in hypersaline environments. Halophilic and extremely halotolerant microorganisms are present in each of the three domains of life: archaea, bacteria and eukarya (Oren, 2002). The domain bacteria typically contains many types of halophilic and halotolerant microorganisms that spread over a large number of phylogenetic subgroups. Most of these fall in the family Halomonadaceae (class Gammaproteobacteria, order Oceanospirillales) and they are moderate rather than extreme halophiles (Oren, 2002).

Research on hydrolytic enzymes from halophilic organisms was pioneered by Nordberg and Hofsten at the end of the 1960s (Nordberg and Hofsten, 1969). Since then, a considerable amount of effort has been dedicated towards the evaluation of extracellular salt-tolerant enzymes of the moderately halophilic bacteria and the use of such enzymes in biotechnological processes (Ventosa et al., 1998). Several researchers have screened halophilic bacteria from different hypersaline environments through direct plating on agar media amended with substrates specific for enzymes of interest. A wide variety of bacteria that secrete extracellular hydrolytic enzymes such as amylases, proteases, lipases, DNases, pullulanases and xylanases have been isolated and characterized (Sánchez-Porro et al., 2003b; Rohban et al., 2009; Govender et al., 2009). Greater hydrolytic activity is commonly observed amongst Gram-positive moderately halophilic bacteria than Gram-negative bacteria. Most of the Gram-positive bacteria belong to the *Bacillus* group including *Salibacillus*, *Halobacillus*, *Oceanobacillus*, *Gracilibacillus*, *Virgibacillus*, *Thalassobacillus* and *Piscibacillus* (Sánchez-Porro et al., 2003b; Rohban et al., 2009). Hydrolase-producing Gram-negative bacteria commonly comprise species of *Salinivibrio*, *Chromohalobacter* and *Halomonas* (Sánchez-Porro et al., 2003b; Rohban et al., 2009). Amylases, lipases, proteases, xylanases and cellulases are widely distributed amongst halophilic bacteria. While this could be unexpected, it is understandable since most natural and artificial hypersaline environments are open system with an influx and presence of plant and animal matter at any given time. Consequently, the microbial population in these environments can be expected to harbour the machinery to exploit the nutrient resources present in their surroundings.

OSMOADAPTATION IN HALOPHILIC BACTERIA

Hypersaline environments are characterized by high but variable osmotic strength and microorganisms present in these environments must be able to adapt to the changes in osmolarity. Most halophilic and halotolerant bacteria maintain viability in these environments by accumulating low-molecular weight water-soluble organic compounds commonly referred to as compatible solutes to counteract the deleterious effect of high salinity on cell physiology and loss of cell water (Louis and Galinski, 1997; Cánovas et al., 1998; Bursy et al., 2008). Both Gram-positive (e.g. *Marinococcus halophilus*, *Streptomyces coelicolor*, *Nesterenkonia halobia*) and Gram-negative bacteria (e.g. *Halomonas elongata*, *Chromohalobacter salexigenes*) are known to accumulate ectoines as the predominant class of osmolytes while other compounds such as glycine and betaine are only accumulated in small amounts (Louis and Galinski, 1997). These compounds are synthesized *de novo* or may be taken up from the external environment and they can be amassed by the cell in very high concentrations to provide osmotic balance without affecting essential cellular functions (Vargas et al., 2008; Bursy et al., 2008). Several studies have shown that osmolytes such as ectoines may serve as general stress protectants as they are produced both in response to salt and heat stresses (Bursy et al., 2008; Vargas et al., 2008). The ectoine synthesis pathway has been extensively studied and the ectoine synthesis gene cluster (*ectABC*) was found to be highly conserved among the ectoine-producing bacteria (Calderón et al., 2004; Vargas et al., 2008). The genes ectA, ectB and ectC encode the enzymes diaminobutrytic acid acetyl transferase, diaminobutrytic acid transaminase and ectoine synthase, respectively, which, altogether constitute the ectoine biosynthetic pathway (Calderón et al., 2004; Kuhlmann and Bermer, 2002; Vargas et al., 2008). While ectoine is generally thought to serve as an osmoprotectant, it has also been reported to play a critical role in stabilizing proteins and supporting correct folding of polypeptides under denaturing conditions (Bursy et al., 2008).

HALOPHILIC HYDROLASES

There has been growing interest in scientific research on salt tolerant enzymes derived from halophilic bacteria due

to the potential industrial application of these enzymes. It is generally believed and has been proven that many halophilic enzymes are polyextremophilic. These enzymes not only remain active and stable in high salt environments but are often also thermotolerant and alkaliphilic (Moreno et al., 2009). These properties made halophilic enzymes attractive for various biotechnological applications as they would be able to catalyze reactions under harsh conditions typical of many industrial processes.

α-Amylases

Amylases are a class of hydrolases which catalyse the degradation of starch polymers to produce dextrins and different gluco-oligosaccharides of variable lengths. Amylases are widely employed in different biotechnological applications including the food industry where they are used extensively in bread and baking industry to improve the volume of dough, colour and crumb softness. Amylases are also applied in detergents to promote stain removal and are utilise in the paper and pulp industry for the modification of starches for coated paper (Gupta et al., 2003). Halophilic amylases, commonly cyclomaltodextrinases (EC: 3.2.1.54), have been produced from bacteria such as *Micrococcus halobius* (Onishi and Sonoda, 1979), *Halomonas meridiana* (Coronado et al., 2000a), *Halobacillus* sp. (Amoozegar et al., 2003), *Halothermothrix orenii* (Mitjs and Patel, 2002; Tan et al., 2008), *Streptomyces* sp. (Chakraborty et al., 2009) as well as *Chromohalobacter* sp. (Prakash et al., 2009a). These enzymes generally display broad pH optima and stability and they remain active at temperatures above 50°C. For instance, the amylase from *Halobacillus* and *Chromohalobacter* species were found to be stable at pH 7 – 10. Some of the enzymes such as the *Chromohalobacter* amylase maintain their stability in the presence and absence of NaCl. Halophilic amylases display molecular weights ranging between 50-75 kDa. The stability of these enzymes at extremes of pH and NaCl, as well as their ability to function optimally at elevated temperatures make them attractive candidates for hydrolysis of starch in industrial processes which are commonly performed at low water activity such as the production of syrups and also in the treatment of saline water or waste water solutions containing starch residues in the presence of high salt (Margesin and Schinner, 2001). In addition, some of the halophilic enzymes such as the amylase form a marine *Streptomyces* sp. remain stable in the presence of commercial detergents and would therefore, be attractive additives in laundry detergents (Chakraborty et al., 2009). Currently, only a few halophilic amylase encoding genes have been sequenced. Phylogenetic analysis shows that the amylase from the moderate halophile *H. meridiana* clusters together with amylases from marine bacteria in a distinct clade away from other extremophilic amylases (Figure 1). The enzyme was reported to display 55 and 53% identity to the amylases from *Pseudoalteromonas haloplanktis* and *Aeromonas hydrophila*, respectively (Coronado et al., 2000b). In contrast, the amylase from the thermophilic, moderately halophilic anaerobic bacterium *H. orenii* display high homology with thermophilic amylases from *Dictyoglomus thermophilum* and *Bacillus* species, although it has narrow substrate specificity as it does not hydrolyse substrates such as pullulan and cyclodextrins (Mitjs and Patel, 2002). The *H. orenii* amylase also lacks transferase activity which means that it can perform the same catalytic reactions as the thermophilic amylases but will most probably generate a different range of products (Tan et al., 2008).

Proteases

Microbial proteases are one of the most extensively studied enzymes and they are widely applied in industrial processes. They are commonly used as additives in laundry detergents, food processing, pharmaceuticals, leather and diagnostic reagents, waste management as well as silver recovery (Amoozegar et al., 2007; Karbalaei-Heidari et al., 2009). Halophilic proteases have been isolated and characterized from several bacterial species including *Bacillus* sp. (Kamekura and Onishi, 1974; Kumar et al., 2004; Setyorini et al., 2006; Shivanand and Jayaraman, 2009), *Pseudoaltermonas* sp. (Sanchez-Porro et al., 2003b), *Salinivobrio* sp. (Amoozegar et al., 2007), *Salicola* sp. (Moreno et al., 2009), *Halobacillus* spp. (Namwong et al., 2006; Karbalaei-Heidari et al., 2009), *Filobacillus* sp. (Hiraga et al., 2005), *Chromohalobacter* sp. (Vidyasagar et al., 2009), *Nesterenkonia* sp. (Bakhtiar et al., 2005) and *Virgibacillus* sp. (Sinsuwan et al., 2009). These enzymes display optimal activity in the presence of NaCl and maintain stability over a wide pH range (pH 5-10). In addition, the enzymes were active at temperatures of 40 – 75°C. While some of the enzymes display an absolute requirement of NaCl for activation, the protease from *Chromohalobacter* was reported to retain 100% stability in the absence of NaCl (Vidyasagar et al., 2009). In addition, some of the enzymes may display polyextremophilicity. For instance, the enzymes may be haloalkaliphilic (Gupta et al., 2005) or halothermophilic (Vidyasagar et al., 2009). Consequently, halophilic and halotolerant bacteria harbour a pool of proteases that will be more suitable for application in food production processes that are performed under saline conditions but can also be applied in saline free systems. For instance, saline fermentation processes involved in the production of various protein rich foods including processing of fish and meat-based products and the production of soy sauce (Setyorini et al., 2006). Moreover, the enzymes derived from halophiles make excellent additives for

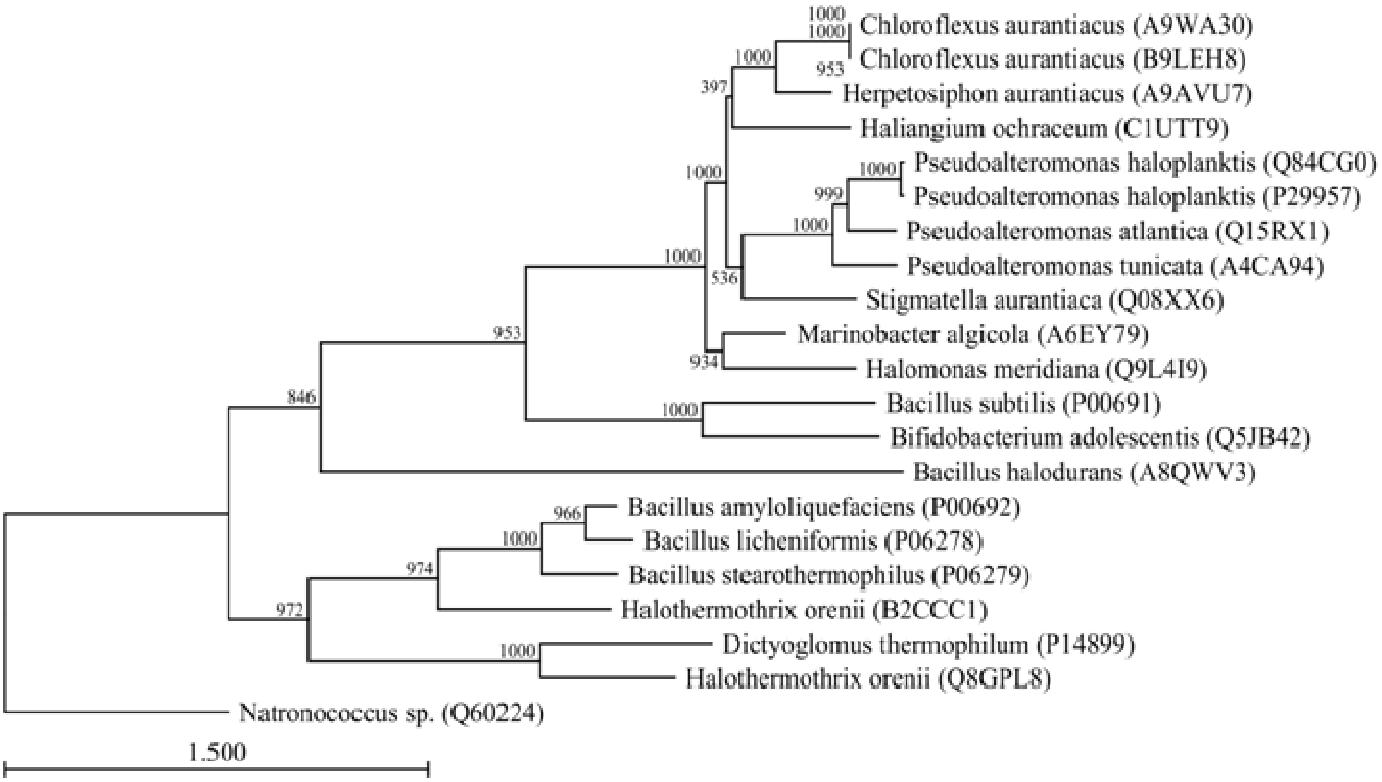

Figure 1. Neighbour-joining phylogenetic tree inferred from alignment of α-amylase protein sequences of selected extremophilic bacteria. The protein sequence of the haloalkaliphilic archaeon *Natronococcus amylolyticus* was used as the outgroup. Numbers at the nodes indicate the level of bootstrap support on 1000 resamplings. The numbers in parentheses are accession numbers.

laundry detergent as most of them are either alkalitolerant or alkaliphilic. Some proteases such as those from *Nesterenkonia* species have been reported to display unique substrate specificities which might open up new application opportunities (Bakhtiar et al., 2005).

Xylanases

Xylanases play a pivotal role in the degradation of xylan. They are widely used in the baking industry to improve the properties of dough, and also for the past two decades the potential use of xylanases in biobleaching of paper and pulp has been growing perpetually (Mamo et al., 2009). However, efficient application of xylanases in biobleaching requires them to be alkaliphilic and thermotolerant. Halophilic organisms are the most likely source of enzymes with such properties although research in this arena is currently limited. Only a few halophilic/ halotolerant xylanases have been described. They include enzymes derived from marine and hypersaline bacteria such as *Glaciecola mesophila* (Guo et al., 2009) and *Chromohalobacter* sp. (Prakash et al., 2009) and *Nesterenkonia* sp. (Govender et al., 2009). Some of these enzymes display stability at wide pH (6-11), remain active at temperatures above 60°C and may display an absolute requirement for NaCl (Wejse et al., 2003; Guo et al., 2009; Prakash et al., 2009).

Cellulases are mainly applied in textile industry for biopolishing of fabrics and production of stonewashed denims, as well as in laundry detergents for fabric softening and brightening (Aygan and Arikan, 2008). Interest in cellulases is also increasing in the production of bioethanol as the enzymes are used to hydrolyse pretreated cellulosic materials to fermentable sugars (Wang et al., 2009). Currently, halophilic and halotolerant cellulases derived from *Bacillus* sp. (Aygan et al., 2008), *Salinivibrio* sp. (Wang et al., 2009) and metagenome library (Voget et al., 2006) have been characterized. The enzymes were reported to be thermostable, halostable and alkalostable, making them ideal candidates for various industrial applications.

ENZYME HALOPHILISM

The enzymes described above are typically secreted into the extracellular environment throughout the growth cycle of halophilic bacteria in the presence of suitable substrates which would act as indirect inducers of respective genes. It can therefore, be expected and has been proven that these enzymes are adapted to

functioning under high NaCl concentrations. Halophilic enzymes remain highly stable under these conditions while most non-halophilic enzymes often aggregate and become non-functional. It is generally believed that this stability is governed by the high acidic amino acid content of halophilic enzymes (Ventosa et al., 1998). Acidic amino acid residues have a high water binding capacity when they are deprotonated and can thus form a solvation shell on the surface of the proteins and keep them hydrated under high salt conditions (Mamo et al., 2009). The common characteristics of halophilic enzymes include: (i) optimal activity at high NaCl concentrations, (ii) higher reversibility to denaturing stresses, (iii) higher stability in the presence of NaCl and (iv) slow mobility on SDS-PAGE due to lower SDS binding (Tokunaga et al., 2008).

CONCLUSION AND FUTURE PROSPECTS

Halophilic and halotolerant bacteria secrete a wide range of hydrolytic enzymes into their surrounding environment. Several of these enzymes which include amylases, proteases, xylanases and cellulases display polyextremophilic properties. They are generally haloalkaliphilic and thermotolerant which renders them amenable to an array of industrial processes, normally performed at extreme conditions of temperature and pH. However, only a limited number of these enzymes have been well characterized and only a few of them are exploited commer-cially, mainly because research has largely focused on microbial diversity in hypersaline environments rather than the industrial potential of halophiles. In order to fully reap the benefits of newly described bacterial species, it is necessary to understand their metabolic and physiological properties. This will allow the generation of valuable information and the definition of the repertoire of extreme enzymes that has the potential to open new biotechnological applications. Therefore, it is necessary to expedite research on the sequence analyses, expression and characterization of halophilic enzymes so that the potential of these enzymes for industrial applications can be explored.

REFERENCES

Amoozegar MA, Fatemi ZA, Karbalaei-Heidari HR, Razavi MR (2007). Production of an extracellular alkaline metalloprotease from a newly isolated, moderately halophile, *Salinivibrio* sp. strain AF-2004. Microbiol. Res. 162: 369-377.

Amoozegar MA, Malekzadeh F, Malik KA (2003). Production of amylase by newly isolated moderate halophile, *Halobacillus* sp. strain MA-2. Microbiol. Methods, 52: 353-359.

Aygan A, Arikan B (2008). A new halo-alkaliphilic, thermostable endoglucanase from moderately halophilic *Bacillus* sp. C14 isolated from Van soda lake. Int. J. Agric. Biol.10: 369-374.

Aygan A, Arikan B, Korkmaz H, Dinçer S, Çolak Ö (2008). Highly thermostable and alkaline α-amylase from a halotolerant alkaliphilic *Bacillus* sp. AB68. Braz. J. Microbiol. 39: 547-553.

Bakhtiar S, Estiveira RJ, Hatti-Kaul R (2005). Substrate specificity of alkaline protease from alkaliphilic feather-degrading *Nesterenkonia* sp. AL20. Enzyme Microb. Technol. 37: 534-540.

Bursy J, Kuhlmann AU, Pittelkow M, Hartmann H, Jebbar M, Pierik AJ, Bremer E, (2008). Synthesis and uptake of the compatible solute ectoine and 5-hydroxyectoine by *Streptomyces coelicolor* A3(2) in response to salt and heat stresses. Appl. Environ. Microbiol. 74: 7286-7296.

Calderón MI, Vargas C, Fernando R, Iglesias-Guerra F, Laszlo NC, Ventosa A, Joaquín JN (2004). Complex regulation of the synthesis of the compatible solute ectoine in the halophilic bacterium *Chromohalobacter salexigens* DSM 3043. Microbiology, 150: 3051-3063.

Cánovas D, Vargas C, Laszlo NC (1998). Synthesis of glycine betaine from exogenous choline in the moderately halophilic bacterium *Halomonas elongata*. Appl. Environ. Microbiol. 64: 4095-4097.

Chakraborty S, Khopade A, Kokare C, Mahadik K, Chopade B (2009). Isolation and characterization of novel α-amylase from marine *Streptomyces* sp. D1. J. Mol. Catalysis B. Enzymatic. 58: 17-23.

Coronado MJ, Vargas C, Hofemeister J, Ventosa A, Nieto JJ (2000a). Production and biochemical characterization of an α-amylase from the moderate halophile *Halomonas meridiana*. FEMS. Microbiol. Lett. 183: 67-71.

Coronado MJ, Vargas C, Mellado E, Tegos G, Drainas C, Nieto JJ, Ventosa A (2000b). The α-amylase gene *amy*H of the moderate halophile *Halomonas meridiana*: cloning and molecular characterization. Microbiology, 146: 861-868.

Govender L, Naidoo L, Setati ME (2009). Isolation of hydrolase producing bacteria from Sua pan solar salterns and the production of endo-1,4-β-xylanase from a newly isolated haloalkaliphilic *Nesterenkonia* sp. Afr. J. Biotechnol. 8: 5458-5466.

Guo B, Chen XL, Sun CY, Zhou BC, Zhang YZ (2009). Gene cloning, expression and characterization of a new cold-active and salt-tolerant endo-β-xylanase from marine *Glaciecola mesophila* KMM 241. DIO: 10.1007/s00253-009-2056-y. Appl. Microbiol. Biotechnol. 84: 1107–1115.

Gupta R, Gigras P, Mohapatran H, Goswami KV, Chauhan B (2003). Microbial α-amylase: a biotechnological perspective. Process Biochem. 38: 1599-1616.

Gupta A, Roy I, Patel RK, Singh SP, Khare SK, Gupta MN (2005). One-step purification and characterization of an alkaline protease from haloalkaliphilic *Bacillus* sp. J. Chromatogr. A. 1075: 103-108.

Hiraga K, Nishikata Y, Namwong S, Tanasupawat S, Takada K, Oda K (2005). Purification and characterization of serine proteinase from a halophilic bacterium, *Filobacillus* sp. RF2-5. Biosci. Biotechnol. Biochem. 69: 38-44.

Kamekura M, Onishi H (1974). Protease formation by a moderately halophilic *Bacillus* strain. Appl. Microbiol. 27: 809-810.

Karbalaei-Heidari HR, Ziaee AA, Amoozegar MA, Cheburkin Y, Budisa N (2008). Molecular cloning and sequence analysis of a novel zink-metalloprotease gene from the *Salinivibrio* sp. strain AF-2004 and its extracellular expression in *E. coli*. GENE. 408: 196-203.

Karbalaei-Heidari HR, Amoozegar MA, Ziaee AA (2009). Production, optimization and purification of a novel extracellular protease from the moderately halophilic bacterium. Ind. Microbiol. Biotechnol. 36: 21-27.

Litchfield CD, Gillevet MP (2002). Microbial diversity and complexity in hypersaline environments: A preliminary assessment. Ind. Microbiol. Biotechnol. 28:48-55.

Louis P, Galinski AE (1997). Characterization of the genes for the biosynthesis of the compatible solute ectoine from *Marinococcus halophilus* and osmoregulated expression in *Escherichia coli*. Microbiology, 143: 1141-1149.

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

Mamo G, Thunnissen M, Hatti-Kaul R, Mattiasson B (2009). An alkaline active xylanase: Insights into mechanisms of high pH catalytic adaptation. Biochimie. 91: 1187-1196.

Mijts BN, Patel BKC (2002). Cloning, sequencing and expression of an α-amylase gene, amyA, from the thermophilic halophile *Halothermothrix orenii* and purification and biochemical characterization of the recombinant enzyme. Microbiology, 148: 2343-2349.

Moreno MDL, Garcia MT, Ventosa A, Mellado E (2009). Characterization of *Salicola* sp. IC10, a lipase- and protease-producing extreme halophile. FEMS Microbiol. Ecol. 68:59-71.

Nordberg P, von Hofsten B (1969). Proteolytic enzymes from extremely halophilic bacteria. J. Gen. Microbiol. 55: 251-256.

Onishi H, Sonoda K (1979). Purification and some properties of an extracellular amylase from a moderate halophile, *Micrococcus halobius*. Appl. Environ. Microbiol. 38: 616-620.

Oren A (2002). Diversity of halophilic microorganisms: Environments, phylogeny, physiology, and applications. Ind. Microbiol Biotechnol. 28: 56-63.

Prakash S, Veeranagouga Y, Kyoung L, Sreeramulu K (2009a). Xylanase production using inexpensive agricultural wastes and its partial characterization from a halophilic *Chromohalobacter* sp. TPSV 101. Process Biochem. 25: 197-204.

Prakash B, Vidyasagar M, Madhukumar MS, Muralikrishna G, Sreeramulu K (2009). Production, purification, and characterization of two extremely halotolerant, thermostable, and alkali-stable α-amylase from *Chromohalobacter* sp. TVSP 101. Process Biochem. 44: 210-215.

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

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

Sánchez-Porro C, Mellado E, Bertoldo C, Antranikian G, Ventosa A (2003b). Screening and characterization of the protease CP1 produced by the moderately halophilic bacterium *Pseudoalteromonas* sp. strain CP76. Extremophiles, 7: 221-228.

Setyorini E, Takenaka S, Murakami S, Aoki K (2006). Purification and characterization of two novel halotolerant extracellular protease from *Bacillus subtilis* strain FP-133. Biosci. Biotechnol. Biochem. 70: 433-440.

Shivanand P, Jayaraman G (2009). Production of extracellular protease from halotolerant bacterium, *Bacillus aquimaris* strain VITP4 isolated from Kumta coast. Process Biochem. DOI:10.1016/ j.procbio.2009.05.010.

Tan TC, Mijts BN, Swaminathan K, Patel BKC, Divne C (2008). Crystal structure of the polyextremophilic α-amylase AmyB from *Halothermothrix orenii*: Details of a productive enzyme-substrate complex and an N domain with a role in binding raw starch. J. Mol. Biol. 378: 852-870.

Tokunaga H, Arakwa T, Tokunaga M (2008). Engineering of halophilic enzymes: Two acidic amino acid residues at the carboxy-terminal region confer halophilic characteristics to *Halomonas* and *Pseudomonas* nucleoside diphosphate kinases. Protein Sci. 17: 1603-1610.

Vargas C, Argandona M, Reina-Bueno M, Rodriguez-Moya J, Fernandez-Aunion C, Joaquin JN (2008). Unravelling the adaptation responses to osmotic and temperature stress in *Chromohalobacter salexigens*, a bacterium with broad salinity tolerance. Saline Syst. 4: p. 14

Ventosa A, Joaquin JN, Oren A (1998). Biology of moderately halophilic aerobic bacteria. Microbiol. Mol. Rev. 62: 504-544.

Vidyasagar M, Prakash S, Mahajan V, Shouche YS, Sreeramulu K (2009). Purification and characterization of an extreme halothermophilic protease from a halophilic bacterium *Chromohalobacter* sp. TVSP101. Braz. J. Microbiol. 40: 12-19.

Voget S, Steele HL, Streit WR (2006). Characterization of a metagenome-derived halotolerant cellulase. J. Biotechnol. 126: 26-36.

Wang CY, Hsieh YR, Ng CC, Chan H, Lin HT, Tzeng WS, Shyu YT (2009). Purification and characterization of a novel halostable cellulase from *Salinivibrio* sp. strain NTU-05. Enzyme Microb. Technol. 44: 373-379.

Wejse PL, Ingvorsen K, Mortensen KK (2003). Purification and characterization of two extremely halotolerant xylanases from a novel halophilic bacterium. Extremophiles, 7: 423-431.