Nep J Environ Sci (2025), 13(2), 23-30
https://doi.org/10.3126/njes.v13i2.74503
ISSN 2350-8647 (Print) 2542-2901 (Online)

Review Article

# Applications of halophilic enzymes in the pharmaceutical industry and medicine

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*(Received: 23 January 2025; Revised: 23 August 2025; Accepted: 26 August 2025)*

## Abstract
Halophiles are an important group of microorganisms that can grow under high salt conditions. The paper here describes the enzymes of halophiles and their mode of action. The application of halophiles in industries is also covered. The halophilic microorganisms have immense significance; hence, this paper describes the halophilic microorganisms and their enzymes. The paper highlights mainly the enzymes, i.e., cellulase, esterase, lipase, protease, laccase, L-asparaginase, and L-glutaminase from halophiles, which have industrial applications, and the study of these enzymes is very important.

***Keywords***: Eco-friendly, economical, intracellular, microorganisms, purification

## Introduction
Halophiles are micro-organisms that thrive in saline environment, and they have emerged as useful agents for synthesis of enzymes that have major applications in biotechnology, industry and health. The name "halophile" comes from the Greek words "halos," which means salt, and "philos," which means love (Oren, 2006). Halophiles have evolved unique adaptation strategies to survive in saline environments, capturing the curiosity of researchers globally.

By definition, halophiles can be classified into slight halophiles (optimum growth in 2-5 % NaCl), moderate halophiles (optimum growth in 5-20 % NaCl), and extreme halophiles (optimum growth in 20- 30% NaCl), owing to their growth in specific range of salt concentration (Oren, 2002, Thombre et al., 2016). Their presence challenges conventional views of environmental limits to life, since they have evolved to develop novel techniques to combat the osmotic stress caused by high salt concentrations. Halophiles are found in a variety of environments, including salt flats, salt marshes, saline soils, and salt mines (Ghosh et al., 2019). Their capacity to endure harsh settings makes them essential members of microbial communities in these environments, where they contribute to ecological processes including nutrient cycling.

Moderate halophiles prefer environments with salinities ranging from 3% to 15% (Haque et al., 2020) and bacteria from the genera *Halomonas* and *Chromohalobacter* are notable examples of moderate halophiles (Biswas & Paul, 2017; Ventosa et al., 2015). Extreme halophiles thrive in hypersaline salt concentrations archaea like *Haloferax* and *Halobacterium* are common examples of extreme halophiles (DasSarma & Arora, 2001; DasSarma & DasSarma, 2015). The extreme halophilic archaeon *Haloferax volcanii* is used as a model organism to study the molecular biology of extremophiles, and *Halomonas species* is a moderate halophile valued for its biotechnological potential and flexibility (Kanekar et al., 2012; Madern et al., 2000; Oren, 2006). The present chapter summarises the various applications of halophilic enzymes in industry.

### Halophilic enzymes: Types, Classification, and Mode of Action
Enzymes are the catalysts obtained from biological sources which increase the speed of biochemical reactions that occur in bacteria. All the living organisms synthesize specific enzymes to carry out basic metabolic processes. These enzymes can be isolated from bacteria and used as biocatalyst *in vitro,* for many industrially important processes (Robinson, 2015). Microbial enzymes have a wide application in many industries as they are known to be more stable and active than enzymes isolated from plants and animals. The culturing of microorganisms in high quantities is possible in short period using fermentation and in general, microorganisms are biochemically diverse. Bacteria can also be genetically modified with much ease as compared to any other eukaryotic cells (Anbu et al*.,* 2013).

Extremophilic microorganisms like halophiles are also known to produces enzymes. Halophiles are one such extremophiles which are known to exist in extreme saline environments, where most of the life forms fail to thrive (Ruginescu et al., 2020). These organisms synthesize special types of proteins, the salt tolerant enzymes, which can have promising role in the biological processes where salt concentrations are high. The halophilic enzymes are reported to have an excess of acidic amino acids imparting negative charge and less hydrophobicity (Siglioccolo et al*.,* 2011; Tadeo et al*.,* 2009). It is because of these properties of the enzymes, that the halophiles are known to adapt well in extreme saline conditions where other mesophilic proteins usually aggregate, denature and precipitate due to less water activity and limited solvation (DasSarma &

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DasSarma, 2015). Some reports suggest that the halophilic enzymes are polyextremophilic, i.e., they not only tolerate high salt but are also active and work stably in high temperature and pH conditions (Moreno et al., 2009).

The detailed structural study of halophilic enzymes and genome of halophiles in 1990's revealed the molecular aspects of these organisms to adapt to the extreme saline conditions, and it roused intense interest among the researchers (Dym et al., 1995) (Table 1). In last few years, many halophilic enzymes like proteases, lipases, DNases, pullulanase and glycosidases have been isolated, purified and characterized which can provide reliable applications in food processing, biofuel production industries and biodegradation of organic pollutants (Amoozegar et al., 2019; Cai et al., 2018; Le Borgne et al., 2014; Fathepure et al., 2014; Rohban et al., 2009).

**Table 1.** Enzymes produced by representative halophiles

| Halophilic enzyme | Source | Reference |
|---|---|---|
| α-amylase | *Halothermothrix orenii* | Tan et al. (2008) |
| Hydrolases | *Halothermothrix orenii* | Tan et al. (2008) |
| Carbonic anhydrase | *Dunaliella salina* | Jeon et al. (2016) |
| Alkaline Phosphatase | *Halobacterium cutirubrum* | Fitt and Baddoo (1979) |
| Dihydrofolate reductase | *Haloferax volcanii* | Ortenberg et al. (2000) |
| Oxidoreductases | *Salinibacter ruber* | Madern and Zaccai (2004) |
| Glucose dehydrogenase | *Haloferax mediterranei* | José_Bonete et al. (1996) |
| Lipase | *Oceanobacillus* sp. | Rathakrishnan and Gopalan (2022) |
| Hydrolyase | *Gracilibacillus* sp. | Rohban et al. (2009) |
| Cellulase | *Virgibacillus* sp. | Rohban et al. (2009) |
| Inulinase | *Thalassobacillus* sp. | Rohban et al. (2009) |
| Protease | *Halobacterium* sp. | Chuprom et al. (2016) |
| L-asparaginase | *Bacillus* sp. | Shirazian et al. (2016) |
| L-glutaminase | *Salicola* sp. | Shirazian et al. (2016) |
| α-amylase | *Zunongwangia profunda* | Qin et al. 2014 |
| Lipase, Protease | *Salicolamarasensis* sp. | Maturrano et al. (2006); Ovreas et al. (2003) |
| β-1,3-xylanase | *Flammeovirga pacifica* | Cai et al. (2018) |
| α-amylase | *Haloarcula* sp. | Fukushima et al. (2005) |
| α-amylase | *Halorubrum xinjiangense* | Moshfegh et al. (2013) |
| Lipase | *Haloarula marismortui* | Camacho et al. (2009) |
| DNase | *Halorubrum* sp. | Makhdoumi et al. (2011). |
| Pectinase, Xylanase | *Halorubrum chaoviator* | Karray et al. (2018) |
| Cellulase, Lipase | *Natrinema altunense* | Karray et al. (2018) |
| Protease | *Halobacillus karajensis* | Karbalaei-Heidari et al. (2000) |
| Esterase | *Psychrobacter pacificensis* | Wu et al. (2015) |
| Arylsulfatase | *Flammeovirga pacifica* | Gao et al. (2015) |
| Prolidase | *Pyrococcus furiosus* | Ghosh et al. (1998) |

## Types of Halophilic Enzymes

The halophilic enzymes are divided mainly into three categories:

**Intracellular enzymes:** These enzymes are not in direct contact with the ionic concentrations of the surrounding areas.

**Membrane-bound enzymes**: These enzymes are in direct contact with the outside areas as well as the cytoplasm of the organism, they are also called as carrier proteins.

**Extracellular enzymes:** They are in direct contact with the outside medium (Patel & Saraf, 2015; Ventosa et al., 1998).

## Classification of halophilic enzymes

The enzymes produced by halophilic and halotolerant organisms are broadly classified into six major classes based upon the reaction they catalyse. Some enzymes break down the compounds while some join them, some are known to rearrange the atoms within the molecules and other transfers the electrons from one molecule to other (Martínez Cuesta et al., 2015).

## Mode of Action of Halophilic Enzymes

Since enzymes are catalysts, they are required in less concentrations. They stimulate the reaction without themselves getting consumed in the reaction (Robinson, 2015). German Physiologist, Wilhelm Kühne used the word 'enzyme' for the first time in 1878. It is derived from Greek word, *en* which means within and *zume* which means yeast. He was studying the ability of yeast to form alcohol from sugars (Robinson, 2015). Here, the enzymes isolated from different halophiles and extensively studied are discussed in brief.

**Amylases:** Amylases belong to hydrolases group of enzymes which has a molecular weight ranging from 50 to 75 kDa. Halophilic amylase is usually stable in extreme saline conditions, they are active in a broad pH range, and they can work efficiently at higher temperatures as compared to mesophilic enzymes. These properties make amylases an ideal candidate in industrial processes operating in less water activity (Margesin & Schinner, 2001). The major role of amylases is to break the glycosidic bonds present in the starch molecules and converting complex carbohydrates into simpler sugars (Tiwari et al., 2015). Many organisms have been studied to check for presence of amylases in last many years. One of the studies reported isolation, purification and characterization of α- amylase by *Halomonas meridian*. The molecular mass of the enzyme was 58 kDa, which required 3 M NaCl for optimum activity (Perez-Pomares et al., 2003). Prakash et al. (2009) reported that amylase isolated from *Chromohalobacter* sp. was stable even in the absence of NaCl which describes its versatile nature.

**Lipase:** Lipase belongs to hydrolase family of enzymes. They convert triglycerids into glycerol and fatty acids (Patel et al. 2019). Halophilic lipases isolated from *Salicolamarasensis* usually enhance their activity in presence of organic solvents like acetone, 1-butanol, 2-butanol, EDTA and metal ions like $Ca^{2+}$ and $Ni^{2+}$. They act on different substrates like p-nitrophenyldecanoate, p-nitrophenylvalerate, p- nitrophenyl butyrate, and p-nitrophenylcaprilate and are active even at 4M NaCl (Moreno et al., 2013).

**Proteases:** One of the most industrially important group of enzymes is protease. This enzyme is widely used in detergent making, baking, brewing, cheese making and tanning industry (Chand et al., 2003; Li et al., 2009). Proteases can degrade proteins into their small counterparts, amino acids and peptides (Sharma et al., 2017). The proteases produced by different halophilic organisms exhibited variation as far as their optimum conditions for activity were studied. Haloprotease isolated from *Pseudoalteromonas ruthenica* was active at a high temperature and alkali pH while protease produced by *Halobacillus karajensis* strain MA-2 had optimum activity at pH 10 and inhibited its activity in the presence of EDTA, PMSF etc (Karbalaei-Heidari et al., 2009; Sanchez-Porro et al., 2003). This strongly suggests that the nature of the enzyme depends upon the source from which the enzyme is isolated.

**Cellulases:** These enzymes degraded cellulose to their basic reducing sugars (Lee and Fan 2005). Halophile, *Halocella cellulolytica* was the first cellulase producing organism that was discovered by Bolobova and his colleagues in 1992 (Bolobova et al., 1992). *Marinobacter* sp. MSI032 produced maximum cellulase at 27 °C in the presence of 2 % NaCl and alkaline pH (Wang et al., 2009).

It can be said that the halophilic enzymes isolated from halophilic organisms are unique as they are highly stable in extreme conditions of salt, pH, temperature and presence of organic solvents. There are reports which have shown that these enzymes maintain solvation and solubility by binding to water tightly in hypersaline environments. This is also the reason why halophilic producers of these enzymes can survive in such harsh saline condition. Due to the virtue of different enzymes, the halophilic organism can have extensive use in bioremediation, agriculture or biofuel production as the hypersaline conditions can allow transformations to be carried out in the relaxed state of sterile conditions due to growth inhibition of unwanted, non-halophilic organisms. Due to these unique properties of halophiles, they and their enzymes both can play a very pivotal role in the basic and applied research in next coming years.

## Application of Halophilic Enzymes in Pharmaceutical Industry

Several mesophilic enzymes have been investigated and are used as a biocatalyst in the production of active pharmaceutical ingredients (APIs) and for enzyme therapy purposes. Robust characteristics like stability under unique set of conditions such as at different salt concentrations, alkaline pH, high temperature and low water activity conditions makes halophilic enzymes, an attractive but unfortunately less explored candidates for pharmaceutical applications. Studies conducted depict the ability of several halophiles in production and release of amylase, protease, lipase, esterase etc. which must be further explored for their pharmaceutical applications (Das et al., 2019).

**Protease:** Various linear and cyclic peptides play crucial role *in-vivo* and they are even more important *in-vitro* with wide applications in pharmaceutical, healthcare, and cosmetic industry. These peptides can be used either as an active biological substance or as a precursor in the treatment of many metabolic disorders and tumors. Peptides are also used in skin care products such as anti-wrinkle and anti-aging formulations. Some peptides can even be useful as food stabilizers, antimicrobials or as a supplement for better health benefits. Chemical synthesis of peptides is labor intensive, complicated, and outdated. Use of biocatalysts in peptide synthesis is a more practical and commercially feasible option. Proteases carry out trans-esterification, ammonolysis, thiolysis reactions helpful for stereo-selective, region-selective and chemo-selective changes in a substrate in industrial processing (Mokashe et al., 2018). Halophilic proteases with altered amino acid composition compared to mesophilic proteases provide high stability and bioactivity under required optimum conditions, further increasing the possibilities of the process optimization.

**L-asparaginase and L-glutaminase:** L-asparaginase and L- glutaminase are widely used as a therapeutic enzyme in the treatment of cancers like lymphoblastic leukemia, breast cancer for being target specific, safe and efficient in their actions compared to radiotherapy and chemotherapy. But prolonged use of mesophilic enzymes causes problem of immunological responses, which could be potentially resolved by halophilic L-asparaginase and L- glutaminase with novel immunological properties (Benítez-Mateos et al., 2023; Shirazian et al., 2016; Zolfaghar et al., 2019). L-asparaginase from *Halomonas elongate* was expressed in *E. coli* and put for anti tumor tests turned out to be potential alternative without any signs of cytotoxicity (Ghasemi et al., 2017). 15 halophilic strains isolated from the western coast of the Red Sea, Egypt showed extracellular production of L-glutaminase and successfully tested for their antitumor potential on MCF-7, HepG-2 and HCT-116 carcinoma cell lines revealing its therapeutic potential (Gomaa, 2022). L-glutaminase is also reported to be helpful in the treatment of HIV (Rahamat et al., 2014). Genetically engineered L-glutaminase stopped the replication in HIV infected cells and was patented (Roberts et al., 2001). L-asparginase from halophiles is more stable in comparison to L-asparginase from mesophilic microorganisms. Future studies on halophilic L-glutaminase may help in relishing its possible use in HIV treatment.

**Esterase***:* Esterase isolated from the *Halomonas elongata* were successfully tested for the hydrolysis of non-steroidal anti-inflammatory drugs (NSAIDS) in the presence of 10% organic co-solvent. This study highlighted the importance of halophilic enzymes in the synthesis of anti-inflammatory drugs (Roura Padrosa et al., 2019; Benítez-Mateos AI et al., 2023).

**Transferases***:* Ectoine is a natural bioactive agent used in many pharmaceuticals and cosmetic preparations. DABA transaminase, DABA acetyltransferase and ectoine synthase are involved in the synthesis of ectoine. Halophile *Halomonaselongata* exhibited natural accumulation of ectoine at high salt concentration as an osmolyte. Heterologous expression of these halophilic enzymes in other microorganisms have assisted in avoiding the use sophisticated fermentation technologies, reducing the cost. Another enzyme of *Halomonas elongata*, ω-transaminase (HewT) is heavily used in the synthesis of high value pharmaceuticals like L-pipecolic acid which combine with natural anticancer and antiviral products and is also a precursor of amide anaesthetic drugs (Cerioli et al., 2015; Roura Padrosa et al., 2020).

Nucleoside phosphorylases are exploited for synthesis of antiviral and anticancer drugs. Purine nucleoside phosphorylase (HePNP) and a thymidine phosphorylase (HeTP) from *Halomonaselongata*, in the presence of 50% DMSO and 10%ethanol have given the highest yield of valuable pharmaceuticals (Benítez-Mateos AI et al., 2023). Transaminases are involved in the synthesis of chiral amines for API synthesis such as antidiabetic drug sitagliptin. The study of halophilic enzymes in API synthesis showed that transaminase Ad2-TAm, derived from the halophilic bacterium, *Halomonas* sp. CSM-2 exhibited halotolerance (1.5M NaCl) and tolerance to organic solvent at pH 9. Another transaminase BC61-TAm reported from the haloarchaeon *Halorubrum* sp. CSM-61 displayed even higher salt tolerance (4M NaCl and KCl), higher tolerance for organic solvent (30 % DMF) and rare (R)-selective enantio-preference (Kelly et al., 2017; Kelly et al., 2018).

**PHA synthase***:* Polyhydroxyalkanoates (PHA), are microbial polyester with wide applications in therapeutics. Halophiles are considered as potential candidates for the cheaper production of PHA because, halophiles sustain high salinity environment lowering the risk of microbial contamination, easy recovery of PHA and have ability to thrive on low-cost substrates. Precise metabolic engineering of halophiles is needed to further enhance the PHA production (Mitra et al., 2020). PHA is highly appreciated as an efficient and controlled drug delivery agent and is also explored for production of multivalent low-cost vaccines like PHA bead vaccine displaying mycobacterial antigens for skin test of bovine TB (Parlane et al., 2012; Xu Zhang et al., 2022). PHA nanoparticles have shown significant advantage not only in oncotherapy but also as a base for healthcare biosensors (Xu Zhang et al., 2022). PHA synthase is major enzyme which polymerises the hydroxyalkanoate monomers to form PHA chain. *Halomonas sp. SF2003* is found to be a PHA-producing strain and especially of poly-3-hydroxybutyrate (P-3HB) and poly-3-hydroxybutyrate-co-3-hydroxyvalerate (P-3HB-co-3HV). PhaC1 and PhaC2 synthases of *Halomonas sp. SF2003* were expressed in the non-PHA-producing strain, *Cupriavidusnecator* H16 PHB¯4 (DSM 541) shows promising results (Thomas et al., 2020).

**Ferredoxin (coenzyme):** D-phenylglycine is non proteinogenic α amino acid. It serves as a precursor in the formation of semi-synthetic antibiotics including cephalosporin and penicillin (Müller et al., 2006). When D-phenylglycine was combined with L-dopa to form D-phenylglycine-L-dopa as a dipeptide prodrug of L-dopa facilitated the better absorption of L-dopa in the treatment of Parkinson's disease (Wang et al., 2010). In a study carried out by Javid and his colleagues in 2014, short peptides (A1 αhelix, A2 α-helix, and ALAL, which was a hybrid of A1 and A2) of ferredoxin coenzyme present in *Halobacterium salinarum* were fused with the N-terminus of D-Phenylglycine aminotransferase (D-PhgAT) from *Pseudomonas stutzeri* ST-201 to improve its solubility, tolerance and catalytic performance at high substrate concentration enhancing synthesis of enantiomerically pure D-phenylglycine (Javid et al., 2014).

**Laccase*:*** Laccases play role in imparting the antimicrobial and antioxidant properties on different surfaces such as lignocellulose fibres, chitosan and catheters (Mohit et al., 2020). Laccase is also important is prostaglandin production, sedatives etc. (Chaurasia et al., 2016). Although there are reports on halophilic laccase production, there are no reports on its use in biomedicine or pharmaceutical industry but could be explored for the same in future.

**Application of Halophilic Enzymes and Compounds in Medicine and Cancer**
The ability of halophilic organisms to produce different enzymes has been reported (Rohban et al*.,* 2009). The enzymes have anti-tumor activity as they target the cancer cells. The enzymes disrupt the metabolism of cancer cells. The application of various enzymes and other compounds produced by halophiles is represented in Table 2.

**Table 2.** Application of enzymes and other compounds produced by halophilic organisms

| Halophiles | Enzymes | Applications | Reference |
|---|---|---|---|
| *Haloferax volcanii* | azoreductase | Decolorization of dye | Vigneshwari et al. (2021) |
| *Halomonas* sp. | L-asparaginase | Used to treat leukemia | Vigneshwari et al. (2021) |
| *Marinobacter* sp. | L-glutaminase | Used as antiviral agent | Vigneshwari et al. (2021) |
| *Halomonas* sp. | L-araginase | Important in urea cycle | Vigneshwari et al. (2021) |
| *Halococcus dombrowskii* | lipase | Wastewater treatment, in improvement of food aroma | Salameh and Wiegel (2007) |
| *Halobacterium salinarum* | protease | In peptide synthesis | Enache and Kamekura (2010) |
| *)Pseudoalteromonas ruthenica* | | In food industries | Raza and Ameen (2017) |
| *Nesterenkonia* sp. | amylase | starch saccharification | Raza and Ameen (2017) |
| *Halogeometricum limi* strain RO1-6 and *Haloplanus vescus* | carotenoids | Antioxidant activity | Hou and Cui (2018) |

**Future Prospects**
The study of halophilic enzymes and their prospective uses in the pharmaceutical industry opens the door to various opportunities. Future research will focus on enzyme engineering strategies to improve the functionality of halophilic enzymes (Munawar & Engel, 2013). The promise of tailoring these enzymes for specific pharmaceutical applications, such as drug production or bioconversion processes, is that it will improve their efficiency and adaptability.

**Drug discovery and synthesis:** Due to their unique properties, halophilic enzymes are predicted to play an important role in drug discovery and synthesis (Danis et al., 2015; Quillaguamán et al., 2010). Enzymatic catalysis in high-salt conditions may open up new avenues for pharmaceutical drug synthesis, offering environmentally acceptable and cost-effective alternatives to established chemical procedures (Dutta & Bandopadhyay, 2022).

**Bioprocessing technologies:** One promising path is the incorporation of halophilic enzymes into bioprocessing technologies. Pharmaceutical applications may include the creation of high-value bioactive chemicals, therapeutic enzymes, and the optimisation of pharmaceutical manufacturing processes (Biswas & Paul, 2017). Halophilic enzymes have the potential to revolutionise the manufacture of biopharmaceuticals and medicines (DasSarma & DasSarma, 2015). Their exceptional resilience in high-salt environments may give advantages in pharmaceutical product composition and delivery, assuring greater stability.

**Environmental bioremediation:** Beyond pharmaceutical applications, halophilic enzymes' future may include environmental bioremediation. Harnessing their ability to degrade contaminants in saline conditions could lead to long-term solutions to environmental problems (Edbeib et al., 2016; Oyewusi et al., 2020). The research is needed on protein engineering aspects of the enzymes produced by halophilic microorganisms.

## Conclusions
Halophilic organisms are known to produce salt tolerant enzymes that are stable at high salinity conditions. The study of halophilic enzymes reveals an array of possibilities with far-reaching ramifications for the pharmaceutical industry. Their adaptable nature, refined through evolution in high-salt conditions, presents them as valuable instruments for solving current issues in pharmaceutical processes. The adaptability of halophilic enzymes, which range from mild halophiles such as *Halomonas* to extreme halophiles such as *Haloferax*, highlights their potential for a wide range of applications. As we traverse the future of

pharmaceutical research, the incorporation of these enzymes provides not only fresh drug development pathways, but also sustainable and efficient bioprocessing solutions. However, problems persist, and more research is needed to fully understand the potential of halophilic enzymes. This includes gaining a better knowledge of their biological mechanisms, optimising production techniques, and integrating them into industrial-scale applications. Collaboration across disciplines will be critical in realising the practical application of halophilic enzymes in pharmacological and medical environments. In essence, the voyage into the realm of halophilic enzymes is a story of scientific curiosity, invention, and the potential of defining a future in which these unique enzymes become indispensable tools in furthering pharmaceutical research and medical applications. As we engage on this scientific journey, the convergence of halophiles and medicine has the potential to reshape the landscape of biotechnological and pharmacological developments.

**Author Contributions:** All authors have equal contributions.

**Conflicts of Interest:** The author declares no conflicts of interest.

**Data Availability Statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

## References

Amoozegar, M.A., Safarpour, A., Noghabi, K.A., Bakhtiary, T., & Ventosa, A. (2019). Halophiles and their vast potential in biofuel production. *Frontiers in Microbiology*, 10, 1895.

Anbu, P., Gopinath, S.B., Cihan, A.C., & Chaulagain, B.P. (2013). Microbial enzymes and their applications in industries and medicine. Biomed Research International, 2013, 204014.

Biswas, J., & Paul, A. (2017). Diversity and production of extracellular polysaccharide by halophilic microorganisms. *Biodiversity International Journal*, 1, 32-39.

Bolobova, A.V., Simankova, M.V., & Markovitch, N.A. (1992). Cellulase complex of a new halophilic bacterium, *Halocella cellulolytica*. *Microbiology*, 61, 557-562.

Bonete, M.J., Pire, C., Llorca, F.I., & Camacho, M.L. (1996). Glucose dehydrogenase from the halophilic Archaeon *Haloferax Mediterranei*: Enzyme purification, characterisation and N-terminal sequence. *FEBS Letters*, 383, 227-229.

Cai, Z.W., Ge, H.H., Yi, Z.W., Zeng, R.Y., & Zhang, G.Y. (2018). Characterization of a novel psychrophilic and halophilic β-1,3-xylanase from deep-sea bacterium, *Flammeovirgapacifica* strain WPAGA1. *International Journal of Biological Macromolecules*, 118, 2176-2184.

Camacho, R.M., Mateos, J.C., González-Reynoso, O., Prado, L.A., & Cordova, J. (2009). Production and characterization of esterase and lipase from *Haloarcula marismortui*. *Journal of Industrial Microbiology and Biotechnology*, 36, 901-909.

Chand, S., & Mishra, P. (2003). Research and application of microbial enzymes. India's. Contribution. *Advances in Biochemical Engineering/ Biotechnology*, 85, 95-124.

Chuprom, J., Bovornreungroj, P., Ahmad, M., Kantachote, D., & Dueramae, S. (2016). Approach toward enhancement of halophilic protease production by *Halobacterium* sp. strain LBU50301 using statistical design response surface methodology. *Biotechnology Reports*, 10, 17-28

Marden, D., & Zaccai, G. (2004). Molecular adaptation: the malate dehydrogenase from the extreme halophilic bacterium *Salinibacterruber* behaves like a non-halophilic protein. *Biochimie*, 86, 295-303.

Danis, O., Ogan, A., Tatlican, P., Attar, A., Cakmakci, E., Mertoglu, B., & Birbir, M. (2015). Preparation of poly (3-hydroxybutyrate-co-hydroxyvalerate) films from halophilic archaea and their potential use in drug delivery. *Extremophiles*, 19, 515-524.

DasSarma, S., & DasSarma, P. (2015). Halophiles and their enzymes: negativity put to good use. *Current Opinion in Microbiology*, 25, 120-126.

Dutta, B., & Bandopadhyay, R. (2022). Biotechnological potentials of halophilic microorganisms and their impact on mankind. *Beni-Suef University Journal of Basic and Applied Sciences*, 11, 75.

Dym, A., Mavaerech, M., & Sussman, J.L. (1995). Structural features that stabilize halophilic malate dehydrogenase from an archaebacterium. *Science*, 267, 1344-1346.

Edbeib, M. F., Wahab, R.A., & Huyop, F. (2016). Halophiles: biology, adaptation, and their role in decontamination of hypersaline environments. *World Journal of Microbiology and Biotechnology*, 32, 1-23.

Enache, M., & Kamekura, M. (2010). Hydrolytic enzymes of halophilic microorganisms and their economic values. *Romanian Journal of Biochemistry*, 47, 47-59.

Fathepure, B.Z. (2014). Recent studies in microbial degradation of petroleum hydrocarbons in hypersaline environments. *Frontiers in Microbiology*, 5, 173.

Fitt, P.S., & Baddoo, P. (1979). Separation and purification of the alkaline phosphatase and a phosphodiesterase from *Halobacterium cutirubrum*. *Biochemical Journal*, 181, 347-53.

Fukushima, T., Mizuki, T., Echigo, A., Inoue, A., & Usami, R. (2005). Organic solvent tolerance of halophilic α-amylase from a Haloarchaeon, *Haloarcula* sp. strain S-1. *Extremophiles*, 9, 85-89.

Gao, C., Jin, M., Yi, Z., & Zeng, R.J. (2015). Characterization of a recombinant thermostable arylsulfatase from deep-sea bacterium

*Flammeovirgapacifica*. *Journal of Microbiology and Biotechnology*, 25, 1894-1901.

Ghosh, M., Grunden, A.M., Dunn, D.M., Weiss, R., & Adams, M.W. (1998). Characterization of native and recombinant forms of an unusual cobalt-dependent proline dipeptidase (prolidase) from the hyperthermophilic archaeon *Pyrococcusfuriosus*. *Journal of Bacteriology*, 180, 4781-4789.

Ghosh, S., Kumar, S., & Khare, S. K. (2019). Microbial diversity of saline habitats: an overview of biotechnological applications. In Giri, B., & Varma, A. (Eds.), *Microorganisms in Saline Environments: Strategies and Functions*. Springer, Cham, pp. 65-92.

Haque, R., Paradisi, F., & Allers, T. (2020). *Haloferax volcanii* for biotechnology applications: challenges, current state and perspectives. *Applied Microbiology and Biotechnology*, 104, 1371-1382.

Hou, J., & Cui, H.L. (2018). In-vitro antioxidant, antihemolytic, and anticancer activity of the carotenoids from halophilic archaea. *Current Microbiology*, 75, 266-271.

Jeon, H., Jeong, J., Baek, K., McKie-Krisberg, Z., Polle, J.W., & Jin, E. (2016). Identification of the carbonic anhydrases from the unicellular green alga *Dunaliella salina* strain CCAP 19/18. *Algal Research*, 19, 12-20.

Kanekar, P., Kanekar, S., Kelkar, A., & Dhakephalkar, P. (2012). Halophiles-taxonomy, diversity, physiology and applications. In Satyanarayana, T., & Johri, B. (Eds.), *Microorganisms in Environmental Management*. Springer, Dordrecht, pp. 1-34.

Karbalaei-Heidari, H.R., Amoozegar, M.A., Hajighasemi, M., Ziaee, A.A., & Ventosa, A. (2009). Production, optimization and purification of a novel extracellular protease from the moderately halophilic bacterium *Halobacillus karajensis*. *Journal of Industrial Microbiology and Biotechnology*, 36, 21-27.

Karray, F., Ben Abdallah, M., Kallel, N., Hamza, M., Fakhfakh, M., & Sayadi, S. (2018). Extracellular hydrolytic enzymes produced by halophilic bacteria and archaea isolated from hypersaline lake. *Molecular Biology Reports*, 45, 1297-1309.

Le Borgne, S., Paniagua, D., & Vazquez-Duhalt, R. (2014). Biodegradation of organic pollutants by halophilic bacteria and archaea. *Journal of Molecular Microbiology and Biotechnology*, 15, 74-92.

Lee, Y.H., & Fan, L.T. (1980). Properties and mode of action of cellulase. In *Advances in Biochemical Engineering*. Springer, Berlin, Heidelberg.

Li, A.N., & Li, D.C. (2009). Cloning, expression and characterization of the serine protease gene from *Chaetomium thermophilum*. *Journal of Applied Microbiology*, 106, 369-380.

Madern, D., Ebel, C., & Zaccai, G. (2000). Halophilic adaptation of enzymes. *Extremophiles*. 4, 91-98.

Makhdoumi, K.A., Amoozegar, M.A., & Mahmodi, K.E. (2011). Diversity of hydrolytic enzymes in haloarchaeal strains isolated from Salt Lake. *International Journal of Environmental Science and Technology*, 8, 705-714.

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

Martínez Cuesta, S., Rahman, S.A., Furnham, N., & Thornton, J.M. (2015). The classification and evolution of enzyme function. *Biophysical Journal*, 109, 1082-6.

Maturrano, L., Valens-Vadell, M., Roselló-Mora, R., & Antón, J. (2006). *Salicolamarasensis* gen. nov., sp. nov., an extremely halophilic bacterium isolated from the Maras solar salterns in Perú. *International Journal of Systematic and Evolutionary Microbiology*, 56, 1685-1691.

Moreno, M.L., Dolores, P., María, T.G., & Encarnación, M. (2013). Halophilic bacteria as a source of novel hydrolytic enzymes. *Life*, 3, 38-51.

Moreno, M.L., García, M.T., Ventosa, A., & Mellado, E. (2009). Characterization of *Salicola* sp. IC10, a lipase- and protease-producing extreme halophile. *FEMS Microbiology Ecology*, 68, 59-71.

Moshfegh, M., Shahverdi, A.R., Zarrini, G., & Faramarzi, M.A. (2013). Biochemical characterization of an extracellular polyextremophilic α-amylase from the halophilic archaeon *Halorubrum xinjiangense*. *Extremophiles*, 17, 677-687.

Munawar, N., & Engel, P. (2013). Halophilic enzymes: Characteristics, structural adaptation and potential applications for biocatalysis. *Current Biotechnology*, 2, 334-344.

Oren, A. (2002). Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. *Journal of Industrial Microbiology and Biotechnology*, 28, 56-63.

Oren, A. (2006). Life at high salt concentrations. *The Prokaryotes*. 3, 263-282.

Ortenberg, R., Rozenblatt-Rosen, O., & Mevarech, M. (2000). The extremely halophilic archaeon *Haloferax volcanii* has two very different dihydrofolate reductases. *Molecular Microbiology*, 35, 1493-1505.

Ovreas, L., Bourne, D., Sandaa, R.A., Casamayor, E.O., Benlloch, S., & Goddard, V. (2003). Response of bacterial and viral communities to nutrient manipulations in sea water mesocosms. *Aquatic Microbial Ecology*, 31, 109-121.

Oyewusi, H.A., Wahab, R.A., & Huyop, F. (2020). Dehalogenase-producing halophiles and their potential role in bioremediation. *Marine Pollution Bulletin*, 160, 111603.

Patel, N., Rai, D., Shivam, S., Shahane, & Mishra, U. (2019). Lipases: Sources, production, purification, and applications. *Recent Patents on Biotechnology*, 13, 45-56.

Patel, S., & Saraf, M. (2015). Perspectives and application of halophilic enzymes. In Maheshwari, D., & Saraf, M. (Eds.), *Halophiles: Sustainable Development and Biodiversity*. Springer, Cham.

Perez-Pomares F., Bautista, V., Ferrer, J., Pire, C., Marhuenda-Egea, F.C., & Bonete, M.J. 2003. α-amylase activity from the halophilic archaeon *Haloferax mediterranei*. *Extremophiles*, 7, 299-306.

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

Qin, Y., Huang, Z., & Liu, Z. (2013). A novel cold-active and salt-tolerant α-amylase from marine bacterium *Zunongwangia profunda*: Molecular cloning, heterologous expression and biochemical characterization. *Extremophiles*, 18, 271-281.

Quillaguamán, J., Guzmán, H., Van-Thuoc, D., & Hatti-Kaul, R. (2010). Synthesis and production of polyhydroxyalkanoates by halophiles: current potential and future prospects. *Applied Microbiology and Biotechnology*, 85, 1687-1696.

Rathakrishnan, D., & Gopalan, A.K. (2022). Isolation and characterization of halophilic isolates from Indian salterns and their screening for production of hydrolytic enzymes. *Environmental Challenges*, 6, 100426.

Raza, S., & Ameen, A. (2017). Halophiles and their important enzymes used for biotechnology application. *Lahore Garrison University Journal of Life Science*, 1, 1-5.

Robinson, P.K. (2015). Enzymes: principles and biotechnological applications. *Essays in Biochemistry*, 59, 1-41.

Rohban, R., Amoozegar, M.A., & Ventosa, A. (2009). Screening and isolation of halophilic bacteria producing extracellular hydrolyses from HowzSoltan Lake, Iran. *Journal of Industrial Microbiology and Biotechnology*, 36, 333-340.

Ruginescu, R., Gomoiu, I., Popescu, O., Cojoc, R., Neagu, S., Lucaci, I., Batrinescu-Moteau, C., & Enache, M. (2020). Bioprospecting for novel halophilic and halotolerant sources of hydrolytic enzymes in brackish, saline and hypersaline lakes of Romania. *Microorganisms*, 8, 1903.

Salameh, M.A., & Wiegel, J. (2007). Purification and characterization of two highly thermophilic alkaline lipases from *Thermosyntropha lipolytica*. *Applied and Environmental Microbiology*, 73, 7725-7731.

Sanchez-Porro, C., Martin, S., Mellado, E., & Ventosa, A. (2003). Diversity of moderately halophilic bacteria producing extracellular hydrolytic enzymes. *Journal of Applied Microbiology*, 94, 295-300.

Sharma, K.M., Kumar, R., Panwar, S., & Kumar, A. (2017). Microbial alkaline proteases: Optimization of production parameters and their properties. *Journal of Genetic Engineering and Biotechnology*, 15, 115-126.

Shirazian P., Asad, S., & Amoozegar, M.A. (2016). The potential of halophilic and halotolerant bacteria for the production of antineoplastic enzymes: L-asparaginase and L-glutaminase. *EXCLI Journal*, 15, 268-279.

Siglioccolo, A., Paiardini, A., Piscitelli, M., & Pascarella, S. (2011). Structural adaptation of extreme halophilic proteins through decrease of conserved hydrophobic contact surface. *BMC Structural Biology*, 11, 50.

Tadeo, X., López-Méndez, B., Trigueros, T., Laín, A., Castaño., D., & Millet, O. (2009). Structural basis for the amino acid composition of proteins from halophilic archea. *PLoS Biology*, 7, e1000257.

Tan, T.C., Mijts, B.N., Swaminathan, K., Patel, B.C., & 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. *Journal of Molecular Biology*, 378, 850-868.

Tiwari, S., Srivastava, R., Singh, C.S., Shukla, K., Singh, R.K., Singh, P., Singh, R., Singh, N.L., & Sharma. R. (2015). Amylases: an overview with special reference to alpha amylase. *Journal of Global Biosciences*, 4, 1886-1901.

Ventosa, A., Nieto, J.J., & Oren, A. (1998). Biology of moderately halophilic aerobic bacteria. *Microbiology and Molecular Biology Reviews*, 62, 504-544.

Ventosa, A., de la Haba, R. R., Sanchez-Porro, C., & Papke, R.T. (2015). Microbial diversity of hypersaline environments: a metagenomic approach. *Current Opinion in Microbiology*, 25, 80-87.

Vigneshwari, J., Gnanasekaran, A., Kumar, D.K., Manikandan, P., Senthilkumar, P.K., & Vijayakumar, N. (2021). Review on halophilic microbes and their applications. *Bulletin of Environment, Pharmacology and Life Sciences*, 10, 23-36.

Wang, C.Y., Hsieh, Y.R., Ng, C.C., Chan, H., Lin, H.T., Tzeng, W.S., & Shyu, Y.T. (2009). Purification and characterization of a novel halostable cellulase from *Salinivibrio* sp. strain NTU05. *Enzyme and Microbial Technology*, 44, 373-379.

Wu, G., Zhang, X., Wei, L., Wu, G., Kumar, A., Mao, T., & Liu, Z. (2015). A cold-adapted, solvent and salt tolerant esterase from marine bacterium *Psychrobacter pacificensis*. *International Journal of Biological Macromolecules*, 81, 180-187.