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Review

Halophiles as a source of polyextremophilic α-amylase for industrial applications

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Abstract: Halophiles are perceived as an excellent source of novel enzymes possessing inherent ability to function under saline and hypersaline environment conditions. The article covers and puts in perspective the structural and biocatalytic features of α-amylases from halophilic sources. The choice of α-amylase as the target enzyme is based on the fact that this is among the largest selling enzymes. Oligosaccharide synthesis is favored in presence of organic solvents and at high temperature. For this reason, the demand for α-amylases that are functional at high temperature and salt as well as stable towards organic solvents, is on the rise in recent years. Halophilic α-amylases are deemed to possess all the above characteristics. They are generally salt stable. In terms of water activity, saline environments are similar to non-aqueous systems. Therefore halophilic α-amylases also exhibit stability in organic solvents. In this context, the review encompasses α-amylase producing predominant halophilic microorganisms from saline habitats; strategies adopted for purification of halophilic α-amylase; their salient structural features and unique functional characteristics. Halophilic α-amylase applications and future aspects in research are also analyzed.

Keywords: halophilic α-amylase; halophiles; α-amylase characteristics; polyextremophilic enzymes; starch hydrolysis

1. Introduction

The search for new enzymes endowed with novel activities and enhanced stability continues to be a desirable pursuit in enzyme research. This is fuelled by industrial requirements and necessity of enzymatic interventions in new and challenging bioprocesses. Industrial applications of enzymes necessitate them to be stable under harsh operational conditions. In this context, enzymes from extremophiles are often useful because they withstand and carry out catalysis under extreme physiological conditions. Extremophiles are therefore perceived as an excellent source of novel enzymes possessing inherent ability to function under extreme conditions [1–5].

Halophiles, a class among extremophiles, have the unique ability to thrive in environments rich in salt. The word halophile is derived from the Greek, meaning "salt loving". They are salt-loving organisms found in all three domains of life: Archaea, Bacteria and Eukarya. Halophiles have been mostly isolated and characterized from the habitat like saline water, saline soil, salt lakes, soda lakes, salted foods and salterns [6,7]. Their metabolic and physiological activities are therefore adapted to function under high salt conditions. Research on halophiles has gained considerable attention because of their potential usefulness in food industries, biodegradation of toxic pollutants, as a source of organic osmotic stabilizers, bioplastics, enzymes and bacteriorhodopsin [8–12].

Their enzymes possess unique features to exhibit high activity and stability in saline environment [13–15]. In general, halophilic enzymes are active at salt concentrations ranging from 0.2 M to 5.2 M and have been documented to be better catalysts for peptide synthesis, enhanced oil recovery and hypersaline waste treatment, wherein normal enzymes may not function optimally or may even get denatured [10,16].

Hydrolases from halophiles have been of particular interest in recent years [14,17]. Many of them have been investigated in details and characterized as novel biocatalyst. The present review summarizes the halophilic hydrolases in general and encompasses the α-amylases in particular. Halophilic α-amylases have been less studied so far but hold tremendous potential for the production of oligosaccharides, which are high in demand as prebiotics. They have the ability to catalyze reaction in high salt/ organic solvents, which favors oligosaccharide synthesis much better as compared to mesophilic amylases [18,19].

2. Enzymes from Halophiles

Enzymes from halophiles are stable in presence of high concentrations of salt. They perform the same function as their non-halophilic counterparts but in presence of high salt concentrations. The potential of halophilic enzymes has been extensively reviewed [10,20–23]. Some of their interesting attributes include: (i) optimum activity and stability at high salt concentrations, (ii) protective role of salt in maintaining the structure, (iii) higher resistance towards denaturation, and (iv) ability to catalyze in low water or non-aqueous medium [20,24,25]. One of the distinct points of halophilic enzymes is reports of highly reversible refolding from denaturations in some cases, such as beta-lactamase, nucleoside diphosphate kinase and *Kocuria varians* α-amylase [25–27]. High reversibility was caused by high solubility of halophilic proteins due to the "highly acidic properties/ non-aggregative characteristics" of them, even under denatured conditions.

Table 1. Industrially important enzymes from halophiles.

Enzymes	Microorganism(s)	Characteristics/ prospective applications	Reference(s)
Alcohol dehydrogenase	*Haloferax volcanii*	Solvent stable	[28]
Alkaline phosphatases	*Halomonas* sp. 593	Phosphomonoesters hydrolysis over broad salt range	[29]
α-Amylases	*Amphibacillus* sp. NM-Ra2	Salt, alkali, temperature solvent and surfactant stable	[30]
	Exiguobacterium sp.	Solvent stable and bakery industry	[31]
	Marinobacter sp. EMB8	Solvent stable and maltooligosaccharide synthesis	[19]
	Saccharopolyspora sp. A9	Detergents formulation	[32]
	Nesterenkonia sp. strain F	Starch hydrolysis	[33]
Cellulases	*Aspergillus terreus* UniMAP AA-6	Ionic liquid tolerant and saccharification of lignocelluloses	[34]
	Thalassobacillus sp. LY18	Salt and solvent stable	[35]
	Marinobacter sp. MSI032	Stable at alkaline pH	[36]
Chitinases	*Planococcus rifitoensis* strain M2-26	Salt and heat stable	[37]
Esterases	*Haloarcula marismortui*	Alkaline and salt stable	[38]
β-Galactosidase	*Halorubrum lacusprofundi*	Salt and solvent stable	[39]
	Haloferax alicantei	Salt stable	[40]
Lipases	*Haloarcula* sp. G41	Solvent tolerant and biodiesel production	[41]
	Marinobacter lipolyticus SM19	Eicosapentaenoic acid (EPA) production and solvent stable	[42]
	Salicola strain IC10	Alkaline and salt stable	[43]
Nucleases	*Bacillus* sp.	Alkaline, salt and thermal stable	[44]
	Micrococcus varians	Alkaline and salt stable	[45]
Proteases	*Bacillus* sp. EMB9	Solvent stable and detergent formulations	[46]
	Geomicrobium sp. EMB2	Solvent stable and detergent formulations	[47]
	Halobacterium sp.	Alkaline, salt stable and used in fish sauce preparation	[48]
	Halobacterium halobium	Solvent stable and used for peptide synthesis	[16]
Xylanases	*Gracilibacillus* sp. TSCPVG	Halo-acid-alkali-thermo-stable	[49]
	Thermoanaerobacterium saccharolyticum NTOU1	Salt stable	[50]
	Halophilic bacterium CL8	pH, salt and heat stable	[51]

Halophilic enzymes appear quite promising for industrial applications involving high salt or hypersaline conditions. Some interesting properties have been reported in amylases, proteases, nucleases, cellulases, chitinases, xylanases, esterases and lipases from halophiles. These have been mainly studied from the genera Haloarcula, Haloferax, Halobacterium, Micrococcus, Bacillus, Halobacillus and Halothermothrix. Their industrial properties are summarized in Table 1.

It is quite apparent that they are stable under a variety of extreme conditions. These are therefore recommended as catalysts of choice for applications in (i) hypersaline waste treatment (ii) peptide synthesis (iii) detergents (iv) textile industry (v) pharmaceuticals and (vi) food [10,52,53].

3. Halophilic α-amylase

α-Amylase (E.C. 3.2.1.1, 1, 4-α-D-glucan glucanohydrolase) catalyzes hydrolysis of α-1, 4-glycosidic linkages in starch and related polysaccharides. They are endolytic enzymes, products of which are of varying glucose length with retention of α-anomeric configuration. α-Amylases are an important class of industrial enzymes, finding widescale applications in food, textile, paper, detergent, analytical chemistry, beverage and pharmaceutical industry. Demand for α-amylase is projected to increase further in the coming years due to its use in diverse industrial sectors [54–58].

α-Amylase is part of family 13 (GH-13) of the glycoside hydrolase. They share certain common characteristics such as (i) catalytic domain is formed by $(\beta/\alpha)_8$ or TIM barrel, (ii) catalytic triad consists of one glutamatic acid and two aspartatic acid residues and (iii) presence of four conserved sequences involved in catalysis and substrate binding [56,59].

α-Amylase from wide range of sources with distinct characteristics are available. Yet search continues for novel α-amylase to increase the realm of processes where it can be used. In this context, isolation and screening of extremophilic organisms for α-amylase of desired trait is a contemporary research area. The use of halophilic α-amylase in bioprocesses presents the advantage to obtain optimal activities at high salt concentrations. Halophilic α-amylases also might be particularly resistant to organic solvents because they function under conditions where water activity is low.

Amylases are normally constitutive enzymes. Surprisingly, very few α-amylases have been studied from halophilic sources. The prominent among these are *Natronococcus amylolyticus* [60], *Halomonas meridiana* [61], *Halothermothrix orenii* [62], *Haloferax mediterranei* [63], *Nesterenkonia* sp. strain F [33] and *Marinobacter* sp. EMB8 [64].

3.1. Isolation of amylase producer halophiles

Good and Hartman, screened about ten halophiles as early as 1970, for amylase activity. They reported *Halobacterium salinarum* (formerly *Halobacterium halobium*), to be the best producer [65]. Later Onishi [66] isolated amylase producing moderate halophilic *Micrococcus* from unrefined solar salt. An amylase producing *Acinetobacter* sp. strain was isolated by the same group from sea-sands [67].

Amylase producing halophiles have been isolated during screening and isolation of hydrolase producers from different ecosystems. In one such study, amylase producers represented by the genus *Salinivibrio*, *Bacillus* and *Halomonas* were isolated from hypersaline environments in south Spain [68]. Hundred seventy seven amylase producing halophilic strains were isolated from Howz Soltan Lake, Iran. Amylase producers were mainly Gram-positive rods followed by Gram-negative rods and Gram-positive cocci. They belonged to *Halobacillus*, *Gracilibacillus*, *Thalassobacillus*, *Oceanobacillus* and *Halomonas* genus [69]. Starch hydrolyzing bacteria, majority of which belonged to genus *Bacillus* and could tolerate upto 10% (w/v) sodium chloride (NaCl) concentration have been isolated from Ethiopian soda lakes [70]. Similar culture dependent diversity studies on halophiles for hydrolase producers have been done from Tuzkoy salt mine, Turkey [71], deep-sea sediments of the Southern Okinawa Trough [72], Tunisian Solar Saltern [73], Atacama Desert, Chile [74] and Argentinean salterns [75].

There are relatively fewer studies on halophilic amylase producing actinomycetes and fungi. In a biodiversity study on actinomycetes from salt lakes in Hami, China, out of total sixty three isolates, forty seven were halophilic actinomycetes. Amylase was the predominant hydrolase produced by forty six strains [76]. During another screening, sixty eight amylase producing marine actinomycetes were isolated from the Bay of Bengal, India [77]. Table 2 summarizes the major amylase producing halophiles reported so far, along with the place of their isolation.

Table 2. α-Amylase producing halophiles.

Isolate	Location	Reference(s)
Micrococcus	Unrefined solar salt, Japan	[66]
Natronococcus amylolyticus	Lake Magadi, Kenya	[60]
Haloferax mediterranei	Saline habitat of Spain	[63]
Haloarcula sp. strain S-1	Commercially available French solar salt	[78]
Haloarcula hispanica	Solar saltern in Spain	[79]
Halorubrum xinjiangense	Hypersaline Lake Urmia, Iran	[80]
Halomonas meridiana	Antarctic saline lakes	[61]
Bacillus dipsosauri	Nasal cavity of a desert iguana	[81]
Halobacillus sp. strain MA-2	Saline soil, Iran	[82]
Bacillus sp. strain TSCVKK	Soil samples, India	[83]
Chromohalobacter sp. TVSP 101	Solar evaporated saltern pond, India	[84]
Nesterenkonia sp. strain F	Aran-Bidgol Lake, Iran	[33]
Thalassobacillus sp. LY18	Saline soil of Yuncheng Salt Lake, China	[85]
Pseudoalteromonas spp.	Persian Gulf, Iran	[86]
Marinobacter sp. EMB8	Kozhikode, India	[87]
Amphibacillus sp. NM-Ra2	Wadi An Natrun, Egypt.	[30]

It is apparent that Spain, Iran and China have been prominent locations for isolation of amylase producing halophiles. In the Indian context, *Bacillus* sp. strain TSCVKK from soil samples of salt-manufacturing industry, Chennai [83]; *Chromohalobacter* sp. TVSP 101 from the solar evaporated saltern pond, Tuticorin [84]; *Streptomyces* sp. D1 [88] and *Saccharopolyspora* sp. A9 [32] from coastal regions of Goa and Mumbai; *Marinobacter* sp. EMB8 from Kozhikode and *Halobacillus* sp. EMB14 from Gujarat [87] and fungal amylase from *Mucor* sp. associated with marine sponge from Havelock Island, Andaman Sea [89] have been the major reports.

3.2. Amylase production by halophiles

Table 3. Amylase production in halophiles.

Halophilic isolates	Production level	Comments	Reference(s)
Micrococcus sp.	90.0 U/mL	Starch acts as inducer; glucose represses production; no production in absence of salt (NaCl)	[66]
Natronococcus sp. strain Ah-36	0.12 U/mL	No production in absence of starch; glucose at 0.1% concentration inhibited production	[90]
Halobacterium halobium	1.84 U/mL	Soluble starch (1%, w/v) was best carbon source; 1% (w/v) peptone was best among nitrogen sources; 0.1 mM $ZnSO_4$ stimulated production	[91]
Micrococcus sp. 4	1.2 U/mL	Production in presence of starch, dextrin and wheat bran; no production in absence of salt	[92]
Halomonas meridiana	Not specified	No production in absence of starch; glucose acts as repressor; production starts in stationary phase reaching maximum in exponential phase	[61]
Halobacillus sp. strain MA-2	3.2 U/mL	Enzyme constitutively expressed; dextrin was best carbon source; no production in absence of salt; maximum production in presence of 15% (w/v) Na_2SO_4	[82]
Haloferax mediterranei	0.50 U/mL	Optimum production in presence of ammonium acetate and soluble starch	[63]
Haloarcula sp. strain S-1	4.56 U/mL	Production in medium containing 1.0% soluble starch and 4.3 M NaCl	[78]
Bacillus sp. strain TSCVKK	0.59 U/mL	Production best induced by dextrin followed by soluble starch; yeast extract in combination with tryptone resulted better production; 0.2% $CaCl_2$ stimulated production	[83]
Chromohalobacter sp. TVSP101	4.7 U/mL	Maximum production in presence of rice flour; tryptone was best nitrogen source; 50 mM $CaCl_2$ increased production by 29%	[84]
Halorubrum xinjiangense	0.7 U/mL	Maximum production with wheat starch; production in presence of glucose also; peptone best nitrogen source; Production was growth independent reaching maximum in mid exponential phase	[80]
Marinobacter sp. EMB8	48.0 U/mL	Production was inducible; maximum was obtained with starch as carbon and casein enzyme hydrolysate as nitrogen source	[64]

Production study and further characterization of very few halophilic amylases have been carried out. In some cases production optimization was carried out while in many others skipping this further

purification and other studies were done. Comparing production level in these studies is difficult as different assay methods and unit definitions have been used. Table 3 summarizes the production of amylase from halophiles in broth medium.

α-Amylase production levels in halophiles are generally low and very few studies have been done where many factors have been optimized to increase it. Carbon sources, nitrogen sources, salts and metal ions effects have been commonly investigated for amylase production. In halophiles, amylase production is usually growth dependent starting in exponential phase and reaching maximum in stationary phase [64]. An exception to this was observed in case of *Halorubrum xinjiangense* amylase production, which was growth independent starting in early exponential phase and reaching maximum in mid of this phase [80]. Amylase production was also growth independent for marine actinomycetes *Streptomyces* sp. D1 [88].

3.2.1. Carbon sources

Amylases from halophiles are mainly inducible. They get expressed in presence of suitable carbon sources such as starch, dextrin and maltose. Contrary to this, the amylase of *Halobacillus* sp. strain MA-2 was constitutively expressed [82]. Glucose acted as a repressor for production in several studies such as *Micrococcus* sp. [66], *Natronococcus* sp. strain Ah-36 [90], *Halomonas meridiana* [61] and *Marinobacter* sp. EMB8 [64]. Among inducers starch is best and supported better amylase production in case of *Halobacterium salinarum* (formerly *Halobacterium halobium*) [91], *Halomonas meridiana* [61] and *Marinobacter* sp. EMB8 [64], while for *Halobacillus* sp. strain MA-2 [82] and *Bacillus* sp. strain TSCVKK [83] dextrin gave the best result.

3.2.2. Nitrogen sources

Effect of nitrogen sources on amylase production among halophiles has not been much investigated. Peptone has been suggested to support better production, in case of *Halobacterium salinarum* (formerly *Halobacterium halobium*) [91] and *Halorubrum xinjiangense* [80]. Yeast extract at 8.3 g/L was optimum for production by *Rhodothermus marinus* ITI 990 [93]. Combination of yeast extract and tryptone worked best for production in case of *Bacillus* sp. strain TSCVKK. In this study tryptone, peptone and urea failed to induce production and there was no growth in absence of organic nitrogen source [83]. Amylase production by *Chromohalobacter* sp. TVSP101 was best in presence of tryptone while ammonium choride and urea did not back up production [84]. Casein enzyme hydrolysate at 1% (w/v) concentration was found to be best for amylase production by *Marinobacter* sp. EMB8 [64]. Thus no single nitrogen source may be termed as universally good.

3.2.3. Salts and metal ions

Salt is vital for the growth of halophiles, so in most of the cases growth as well as amylase production diminishes in absence of salt. Sodium chloride is the preferred salt for growth and amylase production. Optimum concentration of salt varies from 5–25% (w/v) for maximum production. Optimized salt concentrations in some of production studies were 5% (w/v) NaCl for

Halomonas meridiana [61]; 10% (w/v) NaCl for *Bacillus* sp. strain TSCVKK [83]; 20% (w/v) NaCl or 15% (w/v) KCl for *Chromohalobacter* sp.TVSP101 [84]; 25% (w/v) NaCl for *Halobacterium halobium* [91]. Sodium sulphate and potassium chloride are suggested to be an alternate replacement and better for amylase production by *Halobacillus* sp. strain MA-2 [82] and *Bacillus dipsosauri* [81] respectively. In *Micrococcus* sp. 4 though, amylase production was induced by other salts such as sodium nitrate, sodium sulphate and potassium nitrate but maximum production was attained with 1 M sodium chloride containing medium [92].

Divalent metal ions have shown stimulatory effect on amylase production in halophiles. Production by *Bacillus* sp. strain TSCVKK was enhanced by 0.2% (w/v) $CaCl_2$ [83]. Presence of 50 mM $CaCl_2$ increased the production by 29% in case of *Chromohalobacter* sp. TVSP101 [84]. Addition of zinc sulphate (0.1 mM) not only stimulated amylase production but also decreased the time for maximum production from 10 to 5 days for *Halobacterium halobium* [91]. In case of *Halobacillus* sp. strain MA-2 sodium arsenate served as best metal ion source, while copper sulfate decreased and lead nitrate failed to induce any change [82].

3.2.4. Effect of pH, temperature and aeration

The effect of above factors has not been investigated much for amylase production in halophiles. *Halobacillus* sp. produced maximum amylase at pH 7.8, temperature 30 °C and aeration rate of 200 rpm [82]. In *Bacillus* sp. strain TSCVKK amylase production was maximum at 30 °C and pH 8.0 [83]. For *Chromohalobacter* sp. TVSP101 optimum conditions were pH 9.0 and 37 °C [84]. Maximum α-amylase production was observed at pH 7.0–7.5, 35 °C and shaking speed of 200 rpm in case of *Marinobacter* sp. EMB8 [64]. In general, a slightly alkaline pH and a temperature range of about 30–37 °C supports amylase production.

3.3. Purification, characterization and specific properties of halophilic α-amylase

There have been very limited studies on the purification and characterization of halophilic amylases. The reason for this could be that halophilic strains produce rather very low amounts of α-amylase, as evident from the production data in the previous section. The purification strategies and characteristics of amylase from various halophiles are compiled in Table 4. A combination of chromatographic matrices has been used in multistep for halophilic amylase purification. The purification methods used suggest that there is no generic protocol for obtaining purified amylase. They are monomeric proteins with molecular weight mostly in the range of 50-100 kDa. Compared to other halophilic amylases *Thalassobacillus* sp. LY18 has a lower molecular mass of 31 kDa [85].

Table 4. α-Amylase purification and characteristics.

Halophiles	Purification procedure	Fold purification and recovery	Characteristics	Additional properties	Reference
Acinetobacter sp.	Glycogen-complex formation, DEAE-Sephadex A-50 and Sephadex G-200 gel filtration chromatography	-	M_w amylase I 55 kDa and amylase II 65 kDa; pH_{opt} 7.0 in 0.2-0.6 M NaCl or KCl; T_{opt} 50-55 °C	Activity lost by dialysis against water	[67]
Micrococcus halobius	Glycogen-complex formation, diethylaminoethyl-cellulose and Bio-Gel P-200 gel filtration chromatography	474; 47%	M_w 89 kDa; pH_{opt} 6.5-7.5; T_{opt} 50-55 °C; $Salt_{opt}$ 0.25 M NaCl or 0.75 M KCl	Dialysis against distilled water and EDTA leads to complete loss of activity; Calcium ions provided stability	[94]
Natronococcus sp. strain Ah-36	Ethanol precipitation, hydroxylapatite, butyl Sepharose 4B and Sephacryl S-200 gel filtration chromatography	2,000; 10%	M_w 74 kDa; pH_{opt} 8.7; T_{opt} 55 °C; $Salt_{opt}$ 2.5 M NaCl	Starch stabilized amylase; Inhibition by N-bromosuccinimide	[90]
Haloferax mediterranei	Hydroxylapatite, Sepharose-4B, DEAE-cellulose and Sephadex-G50 chromatography	48; 1.8%	M_w 58 kDa; pH_{opt} 7.0-8.0; T_{opt} 50-60 °C; $Salt_{opt}$ 3 M NaCl; Salt stability 2-4 M NaCl	EDTA resulted in irreversible loss of activity; Activation by calcium chloride	[63]
Haloarcula sp. strain S-1	Centriprep, Phenyl C-650 toyopearl and Sephadex G-100 chromatography	34; 17%	M_w 70 kDa; pH_{opt} 7.0; T_{opt} 50 °C; $Salt_{opt}$ 4.3 M NaCl	Organic solvent tolerant; Activity not observed at low salt concentration	[78]
Haloarcula hispanica	Ultrafiltration, β-cyclodextrin-sepharose chromatography	-	M_w 50 kDa; pH_{opt} 6.5; T_{opt} 50 °C; $Salt_{opt}$ 4-5 M NaCl	Activity loss in absence of salt is reversible; calcium ions support catalysis	[79]
Rhodothermus marinus	Ammonium sulfate precipitation, Q-Sepharose ion-exchange, Superdex-200 gel filtration chromatography and preparative native page	-	M_w 66 kDa; pH_{opt} 6.0; T_{opt} 80 °C; $Salt_{opt}$ 0.5 M NaCl; Active in 0-4.0 M NaCl	Amylolytic and transferase activity; Magnesium ions increased activity by 15%	[95]
Chromohalobacter sp. TVSP 101	Ultrafiltration, ethanol precipitation, hydrophobic interaction chromatography on Butyl Sepharose 4B and Sephacryl S-200 chromatography	-	M_w amylase I 72 kDa and amylase II 62 kDa; pH_{opt} 9.0; T_{opt} 65 °C; active in 0-20% (w/v) NaCl; K_m 125 and 166 mM; V_{max} 5.88 and 5.0 U/mg, respectively	Active over broad salt concentration	[84]

Nesterenkonia sp. strain F	Ethanol precipitation, Q-Sepharose anion exchange and Sephacryl S-200 gel filtration chromatography	10.8; 6.4%	M_w 100 kDa; pH_{opt} 7.5; T_{opt} 45 °C; $Salt_{opt}$ 0.5 M NaCl; Active in 0-4.0 M NaCl; K_m 4.5 mg/mL and V_{max} 1.18 mg/mL/min	Detergent and surfactant stable; Inhibited by EDTA	[33]
Saccharopolyspora sp. A9	Ammonium sulphate precipitation, Sephadex G-75, DEAE-Sephadex, insoluble corn starch and sephacryl S-400 chromatography	39.01; 25.27%	M_w 66 kDa; pH_{opt} 11.0; pH stability 8.0-12.0; T_{opt} 55 °C; $Salt_{opt}$ 11% (w/v) NaCl; Salt stability 7-17% (w/v) NaCl	Stable in various surfactants, commercial detergents and oxidising agents; Activated by calcium ions	[32]
Thalassobacillus sp. LY18	Ammonium sulfate precipitation, Q-Sepharose ion exchange and Sephacryl S-100 chromatography	6.4; 14.9%	M_w 31 kDa; pH_{opt} 9.0; pH stability 6.0-12.0; T_{opt} 70 °C; Temperature stability 30-90 °C; $Salt_{opt}$ 10% (w/v) NaCl; Salt stability 0-20% (w/v) NaCl	Active and stable in hydrophobic solvents; Calcium ions enhanced activity	[85]
Marinobacter sp. EMB8	Ultrafiltration, DEAE cellulose and Sephadex G-75 chromatography	76; 52%	M_w 72 kDa; pH_{opt} 7.0; pH stability 6.0-11.0; T_{opt} 45 °C; $T_{1/2}$ 80 minutes at 80 °C; $Salt_{opt}$ 1% (w/v) NaCl; Salt stability 3-20% (w/v) NaCl; K_m 4.6 mg/mL and V_{max} 1.3 mg/mL/min	Stable in organic solvents and surfactants; Activity unaffected by calcium ions	[19]
Halorubrum xinjiangense	Ethanol precipitation and starch-affinity chromatography	119; 56%	M_w 60 kDa; pH_{opt} 8.5; T_{opt} 70 °C; $Salt_{opt}$ 4 M NaCl or 4.5 M KCl; K_m 3.8 mg/mL and V_{max} 12.4 U/mg	Stable in SDS, detergents and a range of organic solvents	[80]
Aspergillus gracilis	Ammonium sulfate precipitation and Sephadex G-100 gel filtration chromatography	6; 47%	M_w 35 kDa; pH_{opt} 5.0; T_{opt} 60 °C; $Salt_{opt}$ 30% (w/v) NaCl; K_m 6.33 mg/mL and V_{max} 8.36 U/mg	Active in presence of inhibitors	[96]
Amphibacillus sp. NM-Ra2	Ethanol precipitation, anion exchange on Q-sepharose FF and Superdex^{TM} 75 gel filtration chromatography	4.5; 15.4%	M_w 50 kDa; pH_{opt} 8.0; T_{opt} 54 °C; $Salt_{opt}$ 1.9 M NaCl	Stable in organic solvents, surfactants and oxidising agents	[30]

In some cases two amylases were obtained after purification. For instance, two halotolerant extracellular amylases have been purified from *Chromohalobacter* sp. TVSP 101 by ultrafiltration, ethanol precipitation, hydrophobic interaction chromatography on Butyl Sepharose 4B and Sephacryl S-200 size exclusion chromatography. They were designated as amylase I and amylase II having

molecular mass of 72 and 62 kDa respectively. Both had maximum activity at pH 9.0 and temperature 65 °C. They showed activity in 0–20% (w/v) NaCl and even at 30% NaCl concentration 50% activity was retained. In absence of NaCl, amylase I was more stable compared to amylase II [84]. *Acinetobacter* sp. was the first halophilic amylase to be purified and characterized. Purification process employing Glycogen-complex formation, DEAE-Sephadex A-50 chromatography and Sephadex G-200 gel filtration led to two pure amylases namely, amylase I and amylase II of molecular masses 55 kDa and 65 kDa respectively. Both enzymes had pH optima at 7.0 in 0.2–0.6 M NaCl or KCl. The maximum activity was observed at 50–55 °C [67].

Salt is an important additive, modulating activity and stability of halophilic α-amylase. Salt requirement is more in cases of archaea as compared to bacteria. Archaeal amylase from *Natronococcus* sp. strain Ah-36 loses its activity irreversibly at low salt concentration, therefore 2.5 M NaCl concentration was maintained throughout the purification process. Optimum 2.5 M NaCl is required for its activity and stability. No activity is detectable below 1.0 M NaCl and less than 20% activity is recordable at 5.0 M and above. KCl, RbCl and CsCl at higher concentration could replace NaCl for optimum activity. Complete loss of activity was observed below 1.3 M NaCl whereas; at higher salt concentration it was quite stable with retention of 70% activity at 4.4 M NaCl. In presence of 0.5% soluble starch, amylase was completely stable from 2.1 to 3.6 M NaCl concentration [90]. The amylase from *Haloferax mediterranei* was stable in the salt range of 2–4 M NaCl with maximum activity at 3 M NaCl [63]. Similar to other haloarchaeal amylases, *Haloarcula hispanica* amylase also worked well at high salt concentrations with optimum activity at 4–5 M NaCl. Interestingly, it was active even at lower salt concentrations and showed 30% activity even in absence of NaCl. Activity loss in absence of salt is reversible as it was recovered as soon as optimum salt concentration was restored [79].

Among bacteria, *Rhodothermus marinus* was active in 0–4.0 M NaCl, optimum being 0.5 M NaCl [95]. Extracellular amylase from *Nesterenkonia* sp. strain F was active in 0–4.0 M NaCl with optimum at 0.5 M. It was stable in 1 to 4 M NaCl [33].

Their pH optima were mostly in the neutral range and some of them showed better activity at alkaline pH. A few of them showed better activity and stability at higher temperature. *Micrococcus halobius* amylase showed optimum activity at 50–55 °C and at pH 6.5. The enzyme was stable in the pH range of 6.5 to 7.5 [94]. Amylase from *Natronococcus* sp. strain Ah-36 has pH optimum of 8.7 with good stability in the pH range of 6.0–8.6. The activity declined sharply in acidic range. Maximum amylase activity is attained at 55 °C, although 50% of activity is lost at this temperature [90]. Extracellular amylolytic enzyme from *Rhodothermus marinus* showed maximum activity at pH 6.0 and was highly thermostable with temperature optima at 80 °C. At 80 °C and 85 °C, it had a half-life of 73.7 and 16.7 minutes respectively [95]. Amylase from *Saccharopolyspora* sp. A9 haloalkaliphilic marine actinomycetes was alkaline in nature with optimum pH at 11.0 and stability in buffers of pH 8.0 to 12.0. It was quite heat stable and maximum activity was shown at 55 °C [32]. A haloalkaline and thermostable amylase with stability in the temperature range (30–90 °C), pH range (6.0–12.0), and NaCl concentrations (0–20%) has been reported from *Thalassobacillus* sp. LY18, while optimum activity was at pH 9.0, 70 °C and 10% (w/v) NaCl [85].

α-Amylases are metalloenzymes, well-known to contain calcium ion. Similar behavior has also been observed in case of halophiles. Calcium ions acted as activator for α-amylase in some studies

and dialysis against ethylenediaminetetraacetic acid (EDTA) has resulted in loss of activity. Mercury ions are potent inhibitor for this class of amylase. *Micrococcus halobius* amylase activity was completely lost against dialysis with distilled water and 0.01 M EDTA in 24 and 8 h respectively. It could not be restored by dialysis against $CaCl_2$. The enzyme was dependent on calcium ions for stability [94]. Addition of EDTA resulted in irreversible loss of amylase activity of *Haloferax mediterranei*. Among bivalents, magnesium chloride acted as an inhibitor, while calcium chloride activated the enzyme [63]. Calcium seems to support the catalysis of *Haloarcula hispanica* amylase. Presence of calcium ions in assay mixture enhanced activity, optimum being 2 mM $CaCl_2$. In absence of calcium ions, the activity is considerably reduced. EDTA treatment caused complete loss of activity, which was restored by the addition of calcium ions thus confirming its role in the enzyme activity [79]. Calcium ions activated the amylase from *Saccharopolyspora* sp. A9 to greater extent. Other divalent metal ions such as Mg^{2+}, Mn^{2+}, Co^{2+} and Cu^{2+} also led to increase in activity but to a lesser extent as compared to calcium. Activity was inhibited by Hg^{2+}, Zn^{2+} and Fe^{3+} ions. Incubation with EDTA caused decrease in amylase activity confirming the role of calcium ions in catalysis [32]. Calcium ions enhanced activity while mercury inhibited it in case of *Thalassobacillus* sp. LY18 amylase. Other metal ions did not have any significant effect on activity. EDTA inhibited activity significantly confirming amylase to be a metalloenzyme [85].

α-Amylases are endolytic enzymes and show preferential activity on starch. Among halophiles, favored substrate is starch though they are active on amylose, amylopectin and glycogen. They do not use cyclodextrin and pullulan as substrate. *Haloarcula hispanica* [79], *Marinobacter* sp. EMB8 [19] and *Halorubrum xinjiangense* [80] amylase was active on starch, amylose, amylopectin and glycogen but no activity was detected towards pullulan and β-cyclodextrin.

In some cases the properties of crude amylases have been studied without any attempt to purify it e.g. *Halomonas meridiana*, *Halobacillus* sp. strain MA-2, *Bacillus* sp. strain TSCVKK. Crude *H. meridiana* amylase showed maximum activity at pH 7.0, temperature 37 °C and 10% (w/v) NaCl concentration. It was more active in alkaline pH range. Maltose and maltotriose were formed as major end products after starch hydrolysis by this enzyme [61]. Optimum assay conditions for *Halobacillus* sp. amylase were pH 7.5 to 8.5, 50 °C and 5% NaCl [82]. *Bacillus* sp. strain TSCVKK amylase was partially purified by acetone precipitation and was optimally active at pH 7.5, 55 ºC and 10% NaCl concentration. This amylase did not show activity in the absence of salt and was stable in various surfactants and detergents [83].

3.3.1. Organic solvent stability

Stability in presence of organic solvents has been observed as a generic feature for enzymes from halophiles [87]. This is because of the fact that presence of salts decreases the water activity of medium; the same effect is caused by organic solvents. So, it is hypothesized that if the enzyme is stable under presence of salts, it will also be stable in organic solvents. Organic solvent stability of α-amylase from halophiles is reported in some studies. *Haloarcula* sp. strain S-1, a halophilic archaea isolated from a commercial French solar salt has been described to produce an organic solvent tolerant extracellular amylase. The activity and stability of enzyme was maintained in various organic solvents such as n-decane, n-nonane, n-octane, xylene, styrene, toluene, benzene and

chloroform. However, the activity is lost in presence of hydrophilic solvents [78]. As a useful feature, *Thalassobacillus* sp. LY18 amylase showed activity and stability in hydrophobic solvents of log $P_{ow} \geq 2.13$ [85]. *Marinobacter* sp. EMB8 α-amylase exhibited stability in 25% (v/v) concentration of DCM (dichloromethane), benzene, toluene, hexane, cyclohexane and decane up to 24 h. The enzyme was effectively utilized for maltooligosaccharide synthesis in presence of solvents [19]. *Halorubrum xinjiangense* amylase was stable in a range of organic solvents and as a unique feature was able to hydrolyze raw starches in aqueous/ hexadecane two phase system [80]. Organic solvent tolerant amylases from halophiles have also been reported from *Exiguobacterium* sp. DAU5 [31] and *Amphibacillus* sp. NM-Ra2 [30].

3.3.2. Polyextremophilicity

Halophiles inhabit environments of various extreme conditions in addition to salts. Such circumstances can be extremes of pH, temperature and pressure. It can be said that they live in polyextreme situations. Their metabolic machinery and enzymes are stable and functional under these conditions. Similar property of activity and stability under polyextreme conditions *viz.* increased salt concentration, high pH, elevated temperature, presence of organic solvents, detergents and surfactants has been exhibited in some reports of α-amylase from halophiles. α-Amylase from *Marinobacter* sp. EMB8 was stable up to 20% NaCl (w/v) as well as in broad pH range of 6.0-11.0. It showed considerable thermal stability with half-life of 80 minutes at 80 °C. In addition to the above, it was stable in various organic solvents, detergents and surfactants [19]. A haloalkaline thermally stable extracellular amylase from haloarchaea *H. xinjiangense* was stable in SDS, detergents and a range of organic solvents [80]. Analogous polyextremophilic performance was given away by *Amphibacillus* sp. NM-Ra2 amylase having additional pullulanase activity. Enzyme showed stability in salts, high pH, elevated temperature, organic solvents, surfactants and oxidizing agents [30].

3.4. Cloning and overexpression of halophilic α-amylase

Evidently, the halophilic enzymes have typical enzymatic properties with additional polyextremities *viz.* salt requirement for activity and stability, alkaline inclination and resistance to unfolding even with high concentration of chaotropic reagents. This raises interest in their structure and adaptive features at molecular level. The nucleotide sequence, cloning and overexpression of halophilic enzymes have been studied only scantly. *E. coli* does not serve well as suitable host for halophilic protein expression, although attempted in few cases. The lack of NaCl/ KCl/ osmolytes in the cytoplasm of *E. coli* (being mesophilic), may affect the correct folding of translated nascent protein. Albeit, the *Haloferax volcanii* has been a successful host in quite many cases of halophiles. The progress on the molecular characterization of halophilic amylase, available so far is compiled in Table 5.

Table 5. Cloning and overexpression of halophilic α-amylase.

Donor	Vector	Host	Characteristics	Reference
Natronococcus sp. strain Ah-36	pANAM121 pWL102	*Haloferax volcanii*	α-Amylase gene was of 1512 bp with signal peptide of 43 amino acids; The activity of recombinant amylase was over 100 times higher than that of native *Natronococcus* sp. strain Ah-36	[97]
Halomonas meridiana	pML122/123 pVK102 pMJC21-28	*Halomonas elongata*; *E. coli*	Amylase protein (AmyH) contained a high content of acidic amino acids as well as the four highly conserved regions in amylases; First 20 codons of the amylase precursor protein constituted signal peptide	[98]
Halothermothrix orenii	pSK5A6 pTrcHisB pTH5A6	*E. coli* strain TOP10	α-Amylase gene was of 1545 bp encoding signal peptide of 25 amino acid and a 490 amino acid mature protein; Over 90% activity was observed at high salt concentration	[62]
Kocuria varians	pTAF	*E. coli* BL21 (DE3)	The kva gene of 2211 bp codes 736 amino acids residue protein; presence of starch binding domain (SBD) enables the enzyme to hydrolyse raw starches	[27]
Exiguobacterium sp. DAU5	pET-AmyH-sp. pET-32a	*E. coli* BL21 (trxB)	The 1545 bp ORF encodes 514 amino acid protein; Amylase was highly stable in presence of organic solvents	[31]
Haloarcula japonica	pET-21b pWL102	*Haloarcula japonica*	The ORF of 1989 nucleotides encodes an intracellular α-amylase of 663 amino acids; It shows high activity on soluble starch, amylose and amylopectin	[99]
Escherichia coli JM109	pSE380	*E. coli* XL10-Gold	A halophilic α-amylase was obtained from a non-halophilic microorganism and retains activity in high salt concentrations	[100]
Zunongwangia profunda	pGEX-6P-1	*E. coli* DH5α; *E. coli* BL21 (DE3)	Gene of 1785 bp encodes an α-amylase of 594 amino acids; A cold active and salt stable amylase	[101]

The first report of α-amylase gene cloning and overexpression from archaea *Natronococcus* sp. strain Ah-36 came in the year 1994 by Kobayashi et al. [97]. Though amylase was purified and characterized previously [90]. Amylase was expressed in *Haloferax volcanii* with correct cleavage of signal peptide. The heterologous protein expression was growth associated and enhanced by presence of starch in medium. Purified expressed protein showed properties similar to native. The α-amylase gene of *Natronococcus* sp. strain Ah-36 was sequenced and showed an open reading frame of 1,512

base pairs [97]. Extracellular amylase from moderate halophile *H. meridiana* was characterized by Coronado et al. [61]. The *H. meridiana* was the first moderate halophile α-amylase gene to be cloned. The α-amylase gene amyH was sequenced and showed high degree of homology with amylase from *Alteromonas haloplanktis*. Further in this study thermostable α-amylase from *Bacillus licheniformis* were expressed in *H. meridiana* and *H. elongata*. This established that moderately halophilic bacteria can be used as cell factories for heterologous protein expression [98].

Haloarcula japonica is an extremely halophilic archaea whose genome sequence has been determined recently. A cytoplasmic α-amylase gene malA was identified from genome sequence and was subsequently cloned and expressed. The His-tagged expressed amylase was purified by Ni-affinity column. The amylase was of 663 amino acid residues and the catalytic domain showed homology to GH13 family. Amylase showed optimum activity at pH 6.5, 45 °C with 2.6 M NaCl. The enzyme activity spans over broad salt range, *viz.* about 83% and 95% activity in 0.6 and 4.2 M NaCl respectively. It was unaffected by calcium ions and EDTA. Examination of primary protein sequence revealed that calcium binding residues were replaced by other amino acids, thus the amylase activity was calcium-independent [99]. The study on *Escherichia coli* JM109 was unique in sense that for the first time a halophilic α-amylase was reported from a non-halophilic bacteria. This α-amylase (EAMY) gene was expressed in *E. coli* XL10-Gold cells and later purified and characterized. Amylase showed maximum activity at pH 7.0, temperature 55 °C in 2 M NaCl. It is activated in presence of NaCl. With specific activity of about 1,087 U/mg, it is quite superior over typical halophilic amylases. The K_m and K_{cat} are represented as 4.3 mg/mL and 825/s respectively [100]. Halophilic α-amylase with psychrophilic character has been reported from *Zunongwangia profunda.* The recombinant amylase exhibited maximum activity at 35 °C and retained 39% activity even at 0 °C [101]. Cold active α-amylase with salt tolerance property has investigated in detail from *Pseudoalteromonas haloplanktis* (formerly *Alteromonas haloplanctis*) [102,103].

α-Amylase from bacterial halophiles have shown properties and phylogenetic similarity with amylase of eukaryotic origin. Amylase from *Halomonas meridiana* exhibited substantial homology with amylase from insects and mammals and was a member of family 13 of glycosyl hydrolase. It contained four conserved regions found in this class of enzyme. Amino acids involved in catalysis, substrate, calcium ion and chloride ion binding were also conserved [98]. *Kocuria varians* amylase catalytic domain (468 amino acids) showed similarity with human salivary and the porcine pancreatic α-amylases with presence of four conserved regions of amylase family. It is inhibited by proteinaceous inhibitor from *S. nitrosporeus*. This amylase inhibitor acts on animal amylases, whilst ineffective on *B. subtilis* α-amylase [27]. Bioinformatic analysis of *Marinobacter* α-amylase revealed its phylogenetic closeness with mammals. Other members of halophilic Gammaproteobacteria such as *Halomonas meridiana, Kocuria varians* and *Nesterenkonia* sp. strain F also clustered with animal α-amylase. *Marinobacter* and other halophilic α-amylase having sequence similarity with mammals showed existence of N-terminal signature sequence [104]. Previous study has revealed that presence of eukaryotic α-amylase domain among bacteria results from horizontal gene transfer [105].

An additional property demonstrating similarity between halophilic and mammalian α-amylase is chloride activation. Chloride ions play a vital role in activating numerous α-amylases and similar property is encountered in mammalian amylase, such as human salivary amylase. Chloride ions are

reported to act as allosteric activator in case of human as well as halophilic α-amylases such as *Pseudoalteromonas haloplanktis*. Binding of chloride ions lead to interaction with catalytic residues and ultimately activation of α-amylase activity [106,107]. Chloride activation was encountered in halophilic amylase of *Marinobacter* sp. EMB8. It was also activated with other anions of similar size to chloride such as acetate, nitrate and azide [64]. Bromide and iodide ions which are of comparable size to chloride ions also acted as activators but to a lesser extent. Analogous performance was also demonstrated by *Natronococcus* sp. strain Ah-36 amylase. Among the various anions, activity was best in citrate followed by chloride and acetate, while no activity was detected in NaF, NaBr, and $NaClO_4$ [90].

3.5. Structure and function relationship

Normally high concentrations of salt result in precipitation of proteins due to salting out effect. On account of differential amino acid composition and their precise structural orientation halophilic proteins maintain their solubility, structural integrity and activity in high salt environment. They comprise of high proportion of acidic amino acids compared to their non-halophilic counterpart [20,108,109]. Presence of acidic aspartic and glutamic amino acids on surface helps in binding to water dipoles. This enables proteins of halophiles in maintaining essential water molecules and neutralizes their surface charge to make them soluble even under high salt conditions [110]. Apart from excess acidic amino acids, halophilic proteins have low lysine content, increased small hydrophobic amino acids instead of large hydrophobic residues and increased number of salt bridges [111]. Parallel pattern is also observed in α-amylase from halophiles. Amino acid composition of *Natronococcus* sp. strain Ah-36 amylase revealed high frequency of glutamic acid or glutamine and glycine. Lysine, serine and threonine content were low. The study thus indicated role of acidic residues in activity and stability [90]. Amylase from *Halomonas meridiana* too exhibited typical halophilic inclination *viz.* high content of acidic amino acids, lower number of basic amino acids and lower pI [98]. Likewise *Kocuria varians* amylase showed surplus of acidic amino acids over basic and had lower pI of 3.97 [27]. *Marinobacter* α-amylase characteristics were also similar with greater proportion of acidic amino acids, low lysine content and excess of small hydrophobic amino acids as compared to larger ones [104]. On the contrary *Halothermothrix orenii* amylase did not demonstrate surplus of acidic amino acids [62].

There is a lack of structural studies on halophilic amylase to ascertain basis of stability under multitude of harsh conditions. Report on *Marinobacter* sp. EMB8 α-amylase established the role of salt in proper folding and structure maintenance. Solvent stability of α-amylase was established by structural studies demonstrating preserved tertiary structure in presence of hydrophobic solvents [104]. Loss of structure with decrease in fluorescence intensity in absence of salt was also encountered for α-amylase of *Haloarcula hispanica* [79]. The non-availability of three dimensional structures restricts our understanding about structure-function relationship of halophilic α-amylases. Three dimensional structure of halophilic α-amylase has been deduced only in case of *Halothermothrix orenii* [112]. *M. algicola* α-amylase 3D structure was modelled using *Pseudoalteromonas haloplanktis* α-amylase as a template. Presence of a higher proportion of acidic residues on α-amylase surface imparts stability in saline environment [104]. The three dimensional

structure of α-amylase from halophilic archaea is not available. To understand the haloadaptation mechanism in archaea structural modeling of *Haloarcula marismortui*, *Haloarcula hispanica* and *Halalkalicoccus jeotgali* α-amylase were done. The haloarchaeal α-amylases have increased proportion of coil forming region as compared to helix formation. Further, excess acidic amino acids, low hydrophobicity, augmented salt bridges and diminished hydrophobic interaction on the surface provide them stability at increased salt concentrations [113].

3.6. Applications of halophilic α-amylase

α-Amylase is one among the top selling industrial enzymes. Whereas, mesophilic α-amylases have been widely used in diverse sectors like food, detergent, textile and pharmaceuticals [54,114]. Halophilic amylases have been least explored, even though they offer advantage of working better towards saline samples, due to their activity and stability in presence of varying concentrations of salt. Other features of industrial importance encountered in this class of amylases are stability in alkaline pH, high temperature and low water activity (presence of organic solvents) conditions.

Table 6. Starch hydrolysis products of various halophilic α-amylase.

Source of α-amylase	Hydrolysis products	Reference(s)
Halobacterium halobium	Maltose, maltotriose and glucose	[65]
Micrococcus halobius	Maltose, maltotriose, maltotetraose and small amount of glucose	[94]
Natronococcus sp. strain Ah-36	Maltotriose with lesser amount of maltose and glucose	[90]
Halomonas meridiana	Maltose and maltotriose	[61]
Haloferax mediterranei	Maltose and lesser amount of maltohexaose	[63]
Bacillus sp. strain TSCVKK	Glucose, maltose and higher molecular weight MOS	[83]
Chromohalobacter sp. TVSP 101	Maltotetraose, maltotriose, maltose and glucose	[84]
Nesterenkonia sp. strain F	Maltose, maltotriose and maltotetraose	[33]
Saccharopolyspora sp. A9	Maltose, glucose and maltotriose	[32]
Thalassobacillus sp. LY18	Maltose and maltotriose	[85]
Marinobacter sp. EMB8	Maltose, maltotriose and maltotetraose	[19]
Exiguobacterium sp. DAU5	Maltotriose and maltopentaose along with various MOS	[31]
Amphibacillus sp. NM-Ra2	Maltose and maltotriose	[30]

One of the major applications of α-amylases has been in starch saccharification to yield maltooligosaccharides (MOS) of varying glucose units. MOS are used in food and pharmaceuticals. In this context, halophilic amylases have a good potential for effective starch hydrolysis and

generation of various type of MOS. Table 6 summarizes starch hydrolysis products formed by various halophilic amylases.

In one report on immobilization, *Marinobacter* sp. EMB8 amylase immobilized on silica nanoparticle demonstrated better starch hydrolysis compared to free enzyme [64]. Other then hydrolysis of soluble starches, raw starch hydrolyzing capability has been shown in halophilic amylase from *Nesterenkonia* sp. strain F [33] and *Amphibacillus* sp. NM-Ra2 [30]. *Halorubrum xinjiangense* also showed this property as it was able to hydrolyse raw starches in aqueous/ hexadecane two phase system [80]. Raw starch hydrolysis saves energy as heating process employed for starch solubilisation is energy intensive.

Alkaline α-amylases are used in detergent formulations. Among halophilic sources *Streptomyces* sp. D1 [88], *Bacillus* sp. strain TSCVKK [83] and *Saccharopolyspora* sp. A9 [32] α-amylases have been marked for detergent application. These halophilic amylases showed compatibility with commercial detergents, surfactants and oxidising agents.

Stability and activity at high temperatures desired for starch liquefaction have been observed in many halophilic amylases. α-Amylase from hyperthermophilic bacterium *Thermotoga maritima* showed maximum activity at 90 °C on α -1, 4-linked carbohydrates [115]. *Rhodothermus marinus* amylase can be used for production of branched oligosaccharides at high temperature and salinity [95]. Cold active amylase from *Psychromonas antarcticus* and *Pseudoalteromonas haloplanktis* could prove beneficial for bioprocessing of starch at low temperature [103,116].

Since saline environment has low water activity, stability in non-aqueous medium has been observed among many halophilic enzymes. *Haloarcula* sp. strain S-1 α-amylase was the first report of halophilic α-amylase showing stability in presence of n-decane, n-octane, xylene, toluene, benzene and chloroform [78]. Later, organic solvent stability was seen in amylases from *Nesterenkonia* sp. strain F [117], *Thalassobacillus* sp. LY18 [85], *Exiguobacterium* sp. DAU5 [31] and *Halorubrum xinjiangense* [80]. Enzymes active in organic sovents/ low water activity are required in many biotransformation processes. In recent years, halophilic amylases have been envisaged to be uniquely suitable for bioremediation of hypersaline wastes containing starch and organic solvents. Polyextremophilic *α*-Amylase from *Aspergillus gracilis* showed potential for application in the bioremediation of saline and low water activity effluents. The performance of enzyme in synthetic waste water remediation was better as compared to the commercial one at higher salt concentrations [96].

4. Conclusions

α-Amylases among halophiles have mostly been reported from bacteria and archaea. They are highlighted as efficient catalysts under high salt, alkaline pH and in presence of organic solvents. The polyextremophilic characteristics make halophilic α-amylases as prospective entrant for starch hydrolysis, food and bioremediation applications. The new directions in their research are envisaged as (i) metagenomic approach to investigate the α-amylases from non-culturable halophiles will add to existing repository. (ii) Their adaptive structural features yet to be completely comprehended will enable better functional understanding of such enzymes.

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Conflict of Interest

All authors declare no known conflict of interest.

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