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Characterization and stability studies on surfactant, detergent and oxidant stable α-amylase from marine haloalkaliphilic *Saccharopolyspora* sp. A9

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ABSTRACT

A halopalkaliphilic marine *Saccharopolyspora* sp. stain A9 with an ability to produce surfactants, oxidant and detergent stable α-amylase was isolated from marine sediments collected from west coast of India. The α-amylase from strain A9 was purified to homogeneity with the aid of ammonium sulfate precipitation and gel filtration chromatography by using Sephadex G-75, insoluble corn starch and sephacryl S-100 column, with a 39.01-fold increase in specific activity. SDS-PAGE and zymogram activity staining showed a single band equal to molecular mass of 66 kDa. Enzyme was found to be stable in presence of wide range of NaCl concentration with maximum activity found at 11% (w/v) of NaCl. Enzyme showed remarkable stability towards laboratory surfactants, detergents and oxidants. Glucose, maltose and maltotriose were the main end product of starch hydrolysis, indicating it is α-amylase.

1. Introduction

Amylases (endo-1,4-α-D-glucanohydrolases EC 3.2.1.1) are extracellular endoenzymes that randomly hydrolyse starch molecules to give diverse products including dextrins and progressively smaller polymers composed of glucose units [1]. This class of industrial enzyme constitutes 25% of the sales in the enzyme market [2]. Amylase has a great significance with extensive biotechnological applications in many industrial processes such as sugar, textile, paper, brewing, baking and in distilling industries. It is also used in preparation of digestive juices, cakes, fruit juices, starch syrups and in pharmaceutical industry as a digestive aid. The demand for amylase is increasing day by day because of its extensive applications in all above mentioned industrial sectors [3].

α-Amylases are universally distributed throughout the animal, plant and microbial kingdoms. Over the past few decades, considerable researches have been undertaken with the extracellular α-amylase being produced by a wide variety of microorganisms. The major advantages of using microorganisms for the production of amylases are the economical bulk production capacity of microbes and their easier manipulation to obtain enzymes of desired characteristics. Since many of the commercially available amylases do not withstand industrial reaction conditions and also they do not meet a large industrial demand of this enzyme, therefore, isolation and characterization of novel amylases with desirable properties such as thermostability, alkaline stability and halophilicity are very important to meet the industrial demands [4]. These may be the reasons why researchers all over the globe are now trying to exploit extremophiles which are the valuable source of novel enzymes [4–6]. Among the extremophiles, halophiles are microorganisms that live, grow, and multiply in highly saline environments. Moderately halophilic bacteria are able to grow over a wide range of salt concentrations from 0.4 to 3.5 M with optimum growth at 0.5–2.0 M [7]. Exoenzymes from these organisms with polymer-degrading ability at low water activity are of interest in many harsh industrial processes where concentrated salt solutions would inhibit enzymatic conversions [3].

Marine microorganisms are capable of catalyzing various biochemical reactions with novel enzymes such as amylase, lipase, deoxyribonuclease and protease [8]. There are many reports on thermostable α-amylase production from bacteria belonging to genus *Bacillus* such as *Bacillus coagulans*, *Bacillus stearothermophilus* and *Bacillus candolyticus* [9,10]. Amylase production also has been

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reported in eubacterial moderate halophiles e.g. *Acinetobacter* [11], *Micrococcus halobius* [12], yeast such as *Cryptococcus flavus* [13] and *Halomonas meridiana* [14]. But very limited research has been directed towards α-amylase production from marine actinomycetes [15]. The present study was carried out to select a haloalkaliphilic *Saccharopolyspora* strain that produces detergent, oxidant and thermostable amylase and to investigate its properties and stabilities.

2. Materials and methods

2.1. Source of marine actinomycetes strain

Sediment samples were collected from Goa, Alibagh and Mumbai coastal region of India at the time of low tide. Heat pretreatment at 40 °C for 30–60 days was employed for isolation of marine actinomycetes [16]. Marine soil samples were suspended in sterile water and thoroughly mixed on rotary shaker at 150 rpm for 20 min. Different marine actinomycetes were isolated by using different selective media such as glycerol yeast extract agar, starch casein agar, maltose yeast extract agar and glucose aspargine agar. The isolated strains were screened for amylase production by using starch agar medium prepared in artificial sea water. The artificial sea water (ASW) contains (g/l) NaCl, 23.37; Na_2SO_4, 3.91; $NaHCO_3$, 0.19; KCl, 0.66; KBr, 0.09; $MgCl_2$, 4.98; $CaCl_2$, 1.10; $SrCl_2$, 0.02 and H_3BO_3 0.02. Maximum amylase producing marine *Saccharoplospora* sp. A9 was maintained on glycerol yeast extract agar medium.

2.2. Identification of strain A9

Identification of strain was done by scanning electron microscopy (SEM), 16S r-DNA sequencing, biochemical and cultural characterization. The method adopted for preparation of slide culture for SEM analysis was as described by Williams and Davies [17].

Cell extract (5 μl), standard amino acids [DL-DAP (5 μl)] and glycine mixture (1 μg/ml)] were added to the baseline of the Whatmann filter paper No. 1 to analyse the amino acids present in the cell wall. An ascending chromatography was performed using methanol:distilled water:6N HCl:pyridine (80:26:4:10 v/v) respectively for 4 h. Chromatographic paper was air dried and developed with 0.2% (w/v) of ninhydrin. Finally, paper was kept at 100 °C for 3 min.

Cell hydrolysate and standard sugar solution were applied to the baseline of the Whatman filter paper No. 1 to determine the sugar content of cell wall. Finally ascending chromatography was performed in solvent system [*n*-butanol:distilled water:pyridine:toluene (10:6:6:1 v/v)] and sugar spots were developed by spraying aniline phthalate and by keeping at 100 °C for 4 min [18–20].

2.3. Inoculum preparation and culture condition

The glycerol yeast extract medium containing starch (Hi-Media) 1% (w/v) prepared in artificial sea water (ASW) was used for development of inoculum. The seed culture was prepared in 100 ml of conical flask containing 50 ml of medium by inoculating 2.0 ml of spore suspension containing 2.5–3.0 × 10^6 CFU ml^{-1} and cultivated under agitation at 200 rpm at 55 °C for 4 days. Then 50 ml of seed culture was inoculated in the 500 ml of fermentation medium containing 2% starch, 0.2% beef extract, 0.2% tryptone and 11% (w/v) NaCl prepared in artificial sea water. The pH of the medium was adjusted to 11 and fermentation was carried out 14 days under agitation at 200 rpm at 55 °C. After removal of cells by centrifugation at 3000 × *g* for 20 min, cell free supernatant was subjected to purification and characterization.

2.4. Purification of α-amylase enzyme

The enzyme was precipitated by bringing the culture filtrate to 90% saturation with solid ammonium sulfate and kept at 4 °C for overnight. The precipitate was centrifuged at 12,000 × *g* for 30 min. The precipitate was dissolved in 50 mM glycine–NaOH buffer of pH 11 and dialyzed for 48 h against the same buffer.

Enzyme obtained (2 ml) from the above step was loaded onto a Sephadex G-50 column (1.2 × 135 cm), pre-equilibrated with 50 mM glycine–NaOH buffer, pH 11, at flow rate of 10 ml/h. Fractions (3.5 ml) were collected and those having specific activities more than 200 U/mg in the void volume were pooled and used for the next step. The enzyme obtained after Sephadex G-50 was loaded onto an equilibrated DEAE-Cellulose column (2 cm × 10 cm). The fractions with specific activities more than 300 U/mg were pooled and loaded onto insoluble corn starch column [21].

The enzyme obtained from the above step was loaded onto an insoluble corn starch column (2.5 cm × 10 cm), pre-equilibrated with 50 mM glycine–NaOH buffer, pH 11. The column was washed with cold water and the bound enzyme was eluted by incubation in 50 mM of glycine–NaOH buffer of pH 11 at 55 °C. Fractions with high amylase activities (above 1200 U/mg) were pooled and dialyzed against 50 mM of glycine–NaOH buffer of pH 11. Enzyme (2 ml) from starch column was loaded onto a Sephacryl S-400 column (1.2 cm × 130 cm). The fractions (3.5 ml) were collected and checked for the enzyme activities and protein concentrations [22].

2.5. Assay of amylase

The activity of α-amylase was estimated by determining the amount of reducing sugar released from starch. 1 ml of enzyme solution was added to 1 ml of starch solution (1%, w/v) in 50 mM of glycine–NaOH buffer (pH 11) and in presence of 10 mM of Ca^{2+}. The mixture was incubated at 55 °C for 60 min. The reaction was stopped by the addition 2 ml of 3,5-dinitrosalicylic acid (Himedia) and $A_{540\,nm}$ was measured in Jasco V-530 spectrophotometer. One unit (U) of enzyme activity is defined as the amount of enzyme required for the liberation of 1 μmol of reducing sugar as glucose per minute under assay condition [23].

2.6. Effect of metal ions, EDTA and various chemicals on enzyme activity

The effect of metal ions and EDTA on amylolytic activity was determined by the addition of different concentrations of each ion (5 mM and 10 mM) to the standard assay. All metal ions were used in salt form such as $CaCl_2$, $MgCl_2$, $HgCl_2$, $FeCl_3$, $CoCl_2$ $CuSO_4$, KCl, urea (8 mM), glycerin (1%, v/v) olive oil (1%, v/v) and EDTA (5 mM and 10 mM).

The effects of metal ions, various chemicals and chelating agent on amylolytic activity were determined by pre-incubating the enzyme in the presence of additives for 30 min at 55 °C and then performing the assay in the presence of the same concentrations of additives at optimum temperature for 60 min. Enzyme activity was determined as percentage relative activity as compared to control (without additives), considered to have 100% of relative activity.

2.7. Stability of amylase

The pH stability of amylase was determined by incubating the enzyme in presence of different buffer systems in presence of 11% NaCl and 10 mM Ca^{2+} at 55 °C. Residual amylase activity was expressed as % relative activity as compared to control which was considered as 100% of relative activity. Enzyme activity after pre-incubation at 55 °C for 60 min in glycine–NaOH buffer (pH 11)

containing 11% (w/v) of NaCl and10 mM Ca^{2+} was considered as control.

The enzyme was incubated at different temperatures (55, 65, 75, 85, 95 and 100 °C) for 6–48 h in glycine–NaOH buffer (pH 11) containing 11% (w/v) NaCl in absence and presence of Ca^{2+} (10 mM) for determination of temperature stability of amylase. Residual activity of enzyme was determined as mentioned above.

Salt tolerability of amylase was determined by incubating the enzyme in presence of different percentage of NaCl (3.5%, 7% 11% 14% and 17%, w/v) in glycine–NaOH buffer (pH 11) containing10 mM Ca^{2+} for 6–48 h. Residual activity was determined as described above.

Surfactant stability of amylase was determined by incubating the enzyme in presence of anionic and nonionic surfactants in glycine–NaOH buffer (pH 11) containing 11% (w/v) of NaCl and10 mM Ca^{2+} at 55 °C. The residual activity was measured as described above.

Amylase enzyme was incubated in presence of commercial detergents [0.1%, w/v] such as rin (Hindustan Unilever Ltd., India), surf (Hindustan Unilever Ltd., India), ariel (Procter & Gamble Company, Mumbai, India) and tide (Hindustan Unilever Ltd., India) in glycine–NaOH buffer (pH 11) containing 11% (w/v) of NaCl and 10 mM Ca^{2+} for 7–90 days to determine the detergent stability of amylase. The residual activity was measured as described above.

For the determination of stability of amylase towards oxidants, amylase was incubated for 2 h in presence of H_2O_2 and $NaClO_3$ in glycine–NaOH buffer (pH 11) containing 11% (w/v) of NaCl and 10 mM Ca^{2+}. The residual activity of enzyme was determined as described above.

2.8. Electrophoretic method

SDS-PAGE was used to determine protein purity and the molecular mass of the purified enzyme using a 10% acrylamide gel [24]. After electrophoresis, gel was stained with Coomassie R-250. Phosphorylase b (97 kDa), bovine serum albumin (66 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa) and soyabean trypsin inhibitor (20.1 kDa) were used as molecular mass markers. For activity staining, gel was suspended in 20% (v/v) isopropanol and incubated for 30 min, then transferred to 100 mM glycine–NaOH buffer (pH 11) and incubated for 30 min. Amylolytic activity [25] was determined by placing the gel onto agarose gel containing 1% (w/v) starch and incubating at 55 °C for 3 h. The transparent band on the amylase containing agarose gel was observed after flooding with I_2–KI solution.

2.9. HPTLC analysis of enzyme

Amylase (test sample) was incubated with 1% (w/v) starch in glycine–NaOH buffer at pH 11 containing 10 mM Ca^{2+} and 11% (w/v) at 55 °C for 2 h. Standards (glucose, maltose and maltotriose) and test sample were spotted as bands (width, 6 mm) with a CAMAG microlitre syringe on precoated silica gel aluminium plate 60F-2 54 (20 cm × 10 cm with 250 μm thickness, E. Merck, Germany) using a CAMAG Linomat IV spotter (Switzerland). The plates were developed using *n*-butanol–absolute ethanol–water (5:3:2) mixture as mobile phase. Plates were dried and derivatized by spraying aniline–diphenylamine reagent [aniline 1% (v/v):diphenylamine 1% (w/v):phosphoric acid 1% (v/v)] in acetone followed by baking at 120 °C for 20 min. Densitometric scanning was carried out at 370 nm for detection of hydrolysed products.

2.10. Statistical analysis of data

All the data were expressed as mean ± standard error mean (SEM). The experimental data were analysed using two-way analysis of variance (ANOVA) followed by Bonferroni post test. Statistical differences yielding $P < 0.001$ were considered significant. The analysis was performed using Graph Pad Prism software (version 5.01).

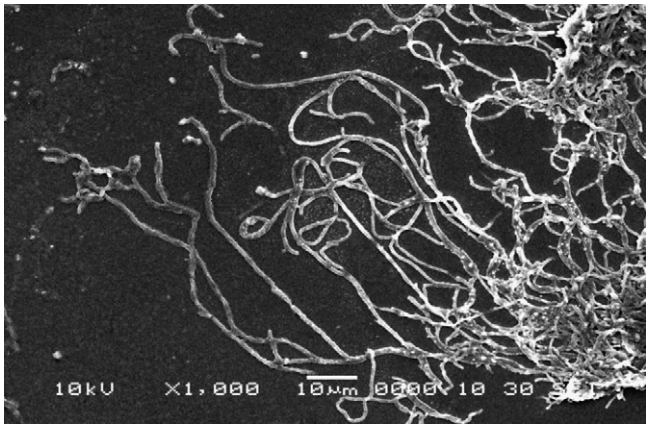

Fig. 1. Scanning electron microscopy of strain A9 at ×1000 resolution grown on glycerol yeast extract agar plate at 55 °C for 15 days.

3. Results and discussion

Actinomycetes are mostly exploited for antibiotics production but they are less extensively studied for enzyme production especially those from marine origin [15,26]. In our laboratory, we have isolated forty actinomycetes strains from marine sediments collected from west coast of India by pre-heat treatment at 40 °C. Isolate A9 was selected for further studies because it appeared to be the best producer of extracellular amylase in both liquid and solid media.

3.1. Identification of marine strain A9

The species showed good growth at 37–55 °C at alkaline pH in 7 days on glycerol yeast extract medium but optimum temperature for growth was found to be 55 °C. The aerial and substrate mycelium were observed by light microscopy and both the mycelium fragmented into short rods (Fig. 1). Short spiral spore chain was also observed on aerial mycelium. Meso-diaminopimelic acid, galactose and arabinose were found to be major constituents of the cell wall. 16S r-DNA sequencing (Fig. 2) and SEM confirmed that the isolated strain was found to be a member of *Saccharopolyspora* genus. The strain A9 was deposited in Gene Bank with an accession number of HM440345.

3.2. Growth characteristics and amylase production

Actinomycetes have slow growth rate. Enzyme production had started in early log phase but there was drastic increase in production of enzyme at late growth phase and early stationary phase; and amylase production was continued up to late stationary phase, after that it declined (Fig. 3). It indicates that the kinetics of enzyme production was more of growth associated than non-growth associated type. Effective induction may not occur until the stationary phase has been reached and the readily available carbon source was depleted [27,28].

3.3. Purification of enzyme

The enzyme was precipitated by ammonium sulfate for partial purification and followed by gel filtration (Sephadex G-75) to remove low molecular weight proteins and desalting. Ion exchange chromatography and starch column were used to remove

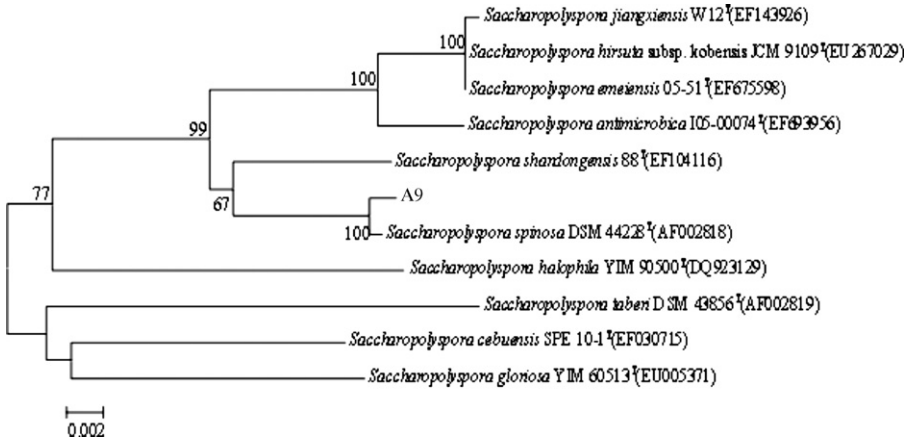

Fig. 2. Neighbour-joining phylogenetic tree of strain A9. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; only values >50% are given. NCBI accession numbers are given in parentheses. Bar, 0.002 nucleotide substitutions per site.

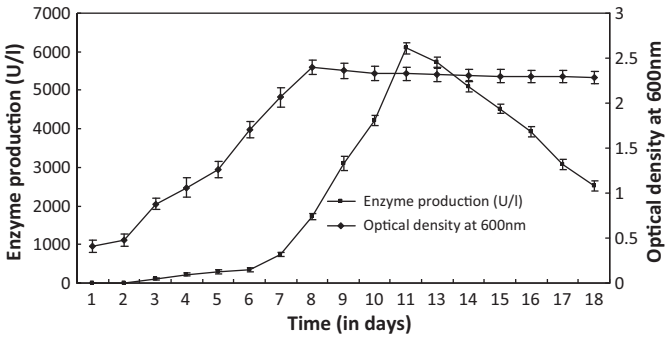

Fig. 3. Growth kinetics of strain A9 with reference to amylase production. Cell growth (OD_{600}) (♦) and amylase production (■).

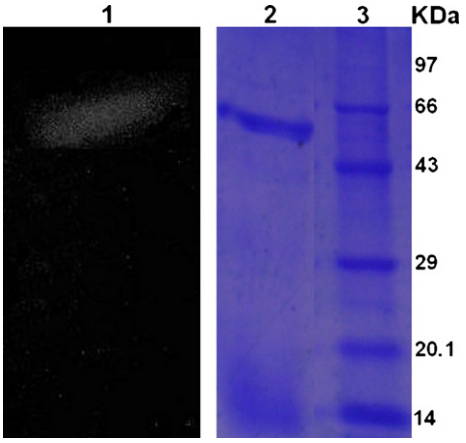

Fig. 4. SDS-PAGE and activity staining analysis of amylase enzyme. Lane 1, activity staining of enzyme, lane 2, amylase enzyme, lane 3, molecular mass standard.

contaminated proteins and induction of specific activity up to 1348.28 U/mg. Finally, the enzyme was subjected to Sephacryl S-400 column to obtain a homogeneous amylase with specific activity of 1640.80 U/mg. It corresponds to 39.01 fold purification with 25.27% of yield (Table 1). The purified enzyme showed a single band about 66 kDa on SDS-PAGE and activity staining of gel confirmed high enzyme purity (Fig. 4).

3.4. Role of metal ions, EDTA, various chemicals and surfactants on enzyme activity

Calcium ion appeared to be most critical factors for amylase production and activity. Amylase activity was increased to 125% (5 mM) and 142% (10 mM) of Ca^{2+}. Other divalent cations such as Mg^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+} were also found to potentiate the enzyme activity but not to the extent of calcium ion. But amylase activity was inhibited by Hg^{2+}, Zn^{2+} and Fe^{3+} ions. Metal ions such as K^{+} have no effect on amylase activity (Table 2). In previous reports, most of the amylases were inhibited in the presence of Ni^{2+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Pb^{2+}, Fe^{2+}, and Zn^{2+}. α-Amylases from *Bacillus* sp. KSM-1378 [29] and *Bacillus firmus* [25] were strongly inhibited by Ni^{2+}, Cd^{2+}, Zn^{2+} and Hg^{2+}. Earlier, there was report on inhibition of α-amylase from *Thermus* sp. by Cu^{2+} and Fe^{2+}. In previous investigations α-amylases from *Bacillus subtilis*, *B. amyloliquefaciens* I and *B. amyloliquefaciens* II were strongly inhibited by Zn^{2+}, Ag^{+}, Cu^{2+} and Fe^{2+} [25,30]. EDTA was found to decrease the activity of amylase, indicating calcium ion dependent nature of amylase. This is due to the fact that the EDTA forms complex with the Ca^{2+} which is required for activity and stability of enzyme, therefore causing decrease in enzyme activity [2,3]. Urea has strong inhibitory effect on amylase activity as it is a strong denaturant, therefore unfolds the proteins.

3.5. Stability studies

The adaptation and survival abilities of halophilic microorganisms in a wide range of salinities (0.5–25%) offer potential

Table 1
Summary of purification of amylase from *Saccharoployspora* sp. A9.

Step	Total activity	Total protein (mg)	Specific activity	Yield (%)	Purification fold
Cell free extract	37,070.5	881.25	42.06	100	1
Ammonium sulfate fractionation	34,291.6	273.27	125.48	92.5	2.98
Sephadex G-75	30,783	68.76	447.68	83.04	10.64
DEAE-Sephadex	25,137	51.27	490.29	67.80	11.65
Starch column	18,458	13.69	1348.28	49.79	32.05
Sephacryl S-400	9369	5.71	1640.80	25.27	39.01

Table 2
Effect of metal ions and different chemicals on enzyme activity.

Metal ions and chemicals	Concentration	% Relative activity
Ca^{2+}	5 mM	125
Ca^{2+}	10 mM	142
Mn^{2+}	5 mM	110
Mn^{2+}	10 mM	126
Co^{2+}	5 mM	104.5
Co^{2+}	10 mM	110.21
Cu^{2+}	5 mM	108.79
Cu^{2+}	10 mM	117.47
K^{+}	5 mM	100
K^{+}	10 mM	99.96
Mg^{2+}	5 mM	109.87
Mg^{2+}	10 mM	117.61
Hg^{2+}	5 mM	64.23
Hg^{2+}	10 mM	46.78
Fe^{3+}	5 mM	56.67
Fe^{3+}	10 mM	42.12
EDTA	5 mM	2.13
EDTA	10 mM	Nil
Urea	8 mM	9.45
Glycerin	1%	78.90
Olive oil	1%	84.23
Control	–	100

applications in various fields of biotechnology [31]. Amylase isolated from strain A9 was found to be stable in presence of higher concentration of NaCl as it retained only 4% of its activity in presence of 3.5% (w/v) of NaCl after 48 h incubation; whereas it retained 72% and 33% of its activity in presence of 11% (w/v) and 17% (w/v) of NaCl respectively after 48 h incubation (Fig. 5). Similar behavior has been reported for bacteria belonging to genus *Bacillus* [3]. But this is probably the first report on salt tolerant amylase from marine *Saccharopolyspora* sp.

The amylase enzyme was found to be stable in buffer systems such as phosphate buffer, Tris–Cl buffer, glycine–NaOH buffer and disodium hydrogen phosphate buffer of pH from 7 to 12 after 48 h incubation (Fig. 6). This clearly indicates haloalkaliphilic nature of isolate A9 and haloalkaline nature of isolated amylase. There was report on isolation alkaline amylase from bacteria belonging to genus *Bacillus*. This is probably the first report on haloalkaline amylase from marine *Saccharopolysora* sp. A9. Several researchers all over the world are now trying to exploit microbes for the isolation of alkaline enzymes because their tremendous potentiality in detergent industry [7]. Therefore, it can be concluded that amylase from strain A9 may have wide spread applications in detergent, food and other industrial processes containing high salt concentration [4].

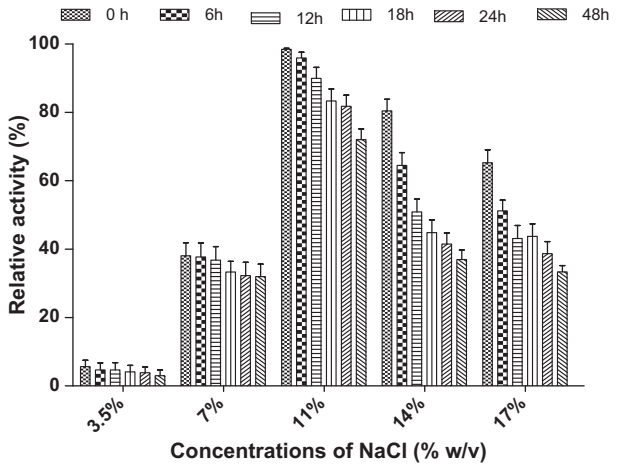

Fig. 5. Salt tolerability of amylase.

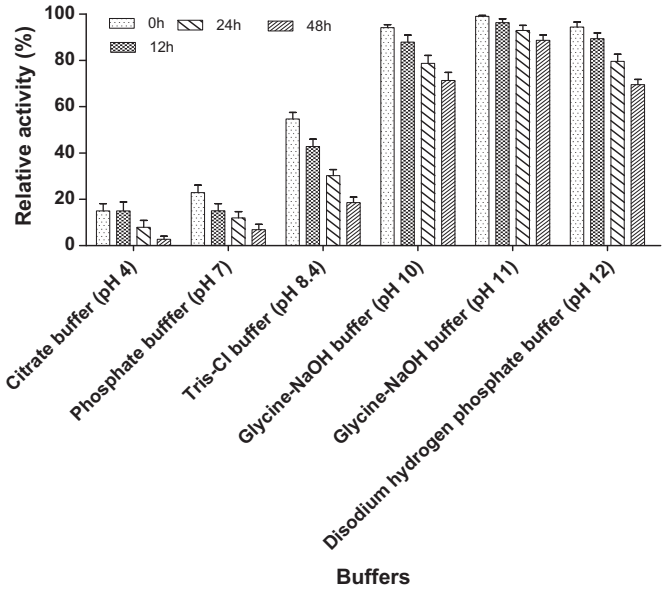

Fig. 6. pH stability of amylase.

Amylase was found to be stable at higher temperature, indicating thermostable nature of enzyme. Enzyme retained 54% of its original activity after 6 h pre-incubation at 100 °C but there was sharp decrease in activity after 48 h of pre-incubation (Fig. 7a). Calcium ion found to play an essential role in the stability of enzyme at higher temperature as there was drastic increase in the stability especially at higher temperature (Fig. 7b). This clearly indicates calcium-dependent nature of enzyme where calcium ion was needed for stability, activity and structural integrity of enzyme [32]. Similar effect of calcium on the thermostability of amylase has

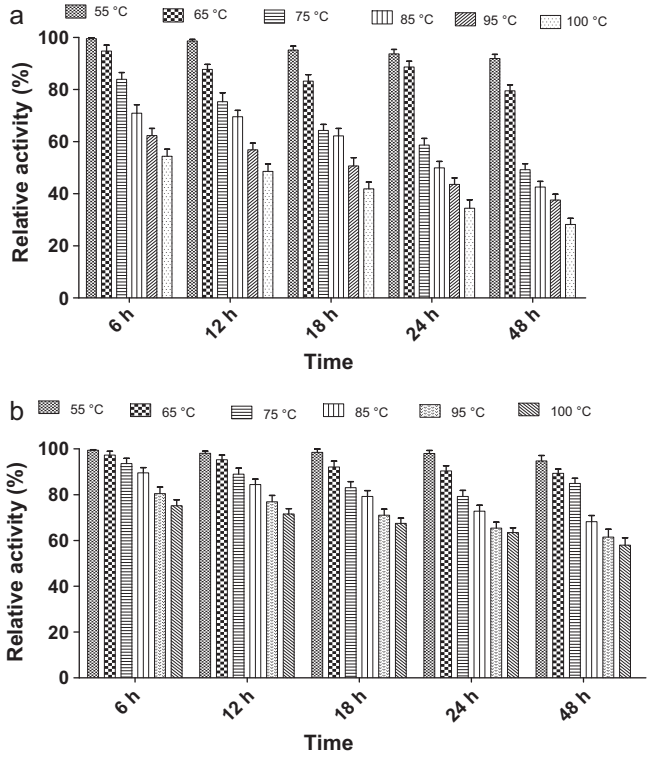

Fig. 7. Temperature stability of amylase (a) in absence and (b) presence of calcium ion.

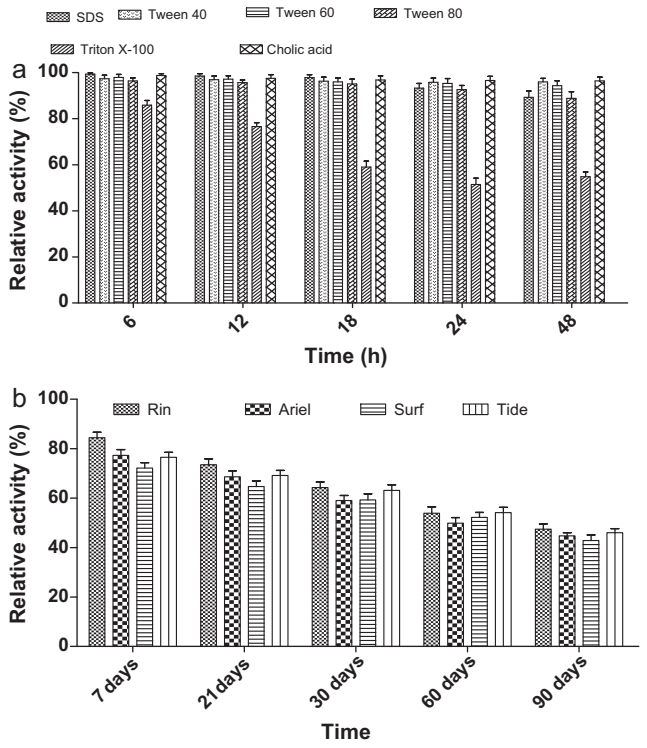

Fig. 8. Stability of amylase towards (a) laboratory surfactants and (b) commercial detergents.

reported for *B. licheniformis, Pyrococcus juriosus* and *Thermococcus litoralis* [33]; and protease from alkaliphilic *Bacillus* sp. It is desirable that α-amylase should be active at high temperature for gelanization (100–110 °C) and liquefaction (80–90 °C) for the use in the starch industry. Until today, α-amylases from bacteria belonging to genus *Bacillus* such as *B. subtilis* var. *amyloliquefaciens* [33–35], *B. stearothermophilus* and *Bacillus licheniformis* are heavily used in the starch-processing industry [36,37]. There is also report on novel thermostable acidophilic α-amylases from new thermophilic *Bacillus sp. Ferdowsicous* [38]. To the best of our knowledge, this is probably the first report of thermostable α-amylase from *Saccharopolyspora* sp. Since, thermostability is an important feature for use of amylolytic enzymes in starch-processing, amylases from thermophilic and hyperthermophilic bacteria are of special interest as a source of novel thermostable enzymes [39]. The amylase from strain A9 reveals that it can be industrially exploited for starch liquefaction [2,33,34].

Amylase enzyme was found to be very stable towards laboratory surfactants such as Tween 40, Tween 60, Tween 80 and cholic acid as enzyme retained above 90–95% of its activity; when incubated in presence of these surfactants 0.5% (w/v) for 6–48 h. Though enzyme production decreased in presence of Triton X-100 but reported amylase retained almost 50% of its activity in presence of Triton X-100 after 48 h incubation (Fig. 8a). There is a report on SDS resistant amylase from genus *Bacillus* [40], but to the best of our knowledge this is probably the first report on amylase from marine *Saccharopolyspora* sp. possessing stability towards an array of laboratory surfactants.

Enzyme possessed a good stability in presence of commercial detergents such as rin, surf, aerial and tide as it retained 84–42% of it activity after incubation of 7–90 days respectively (Fig. 8b).

Amylase was found to be stable in presence of oxidizing agents such as sodium hypochlorite and H_2O_2 as enzyme retained almost 100–75% of its activity in the concentration range of 0.2–1.2% (w/v) respectively (Fig. 9).

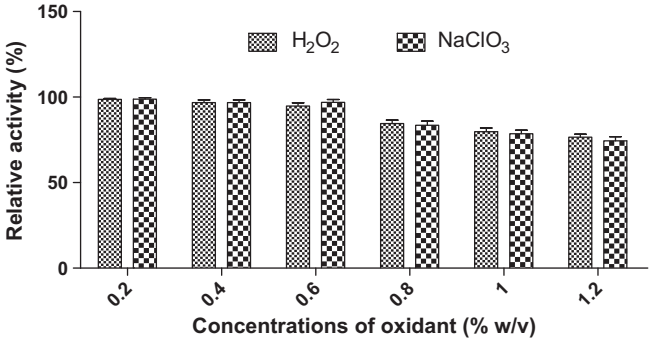

Fig. 9. Stability of amylase towards oxidizing agents.

However, not all of currently used detergent enzymes are active in presence of bleaching agents. Hence, the latest trend in enzyme-based detergents is to introduce site directed mutagenesis and protein engineering techniques to produce enzyme with better oxidation and heat stability [41,42]. However, the reported amylase from strain A9 already possessed good oxidation stability.

3.6. HPTLC analysis of enzyme

The hydrolysis pattern presented by amylase from strain A9 showed similarity with that of *H. meridiana* [14] and *Bacillus* sp. strain TSCVKK [3]. It was observed that the test sample (soluble starch and enzyme) gave three peaks having similar R_f to that of standard glucose, maltose and maltotriose (Fig. 10). These results provide an insight on degradation mode of the enzyme suggesting that there are both α-1-4 and α-1-6 (debranching) and also α-glucosidase activity.

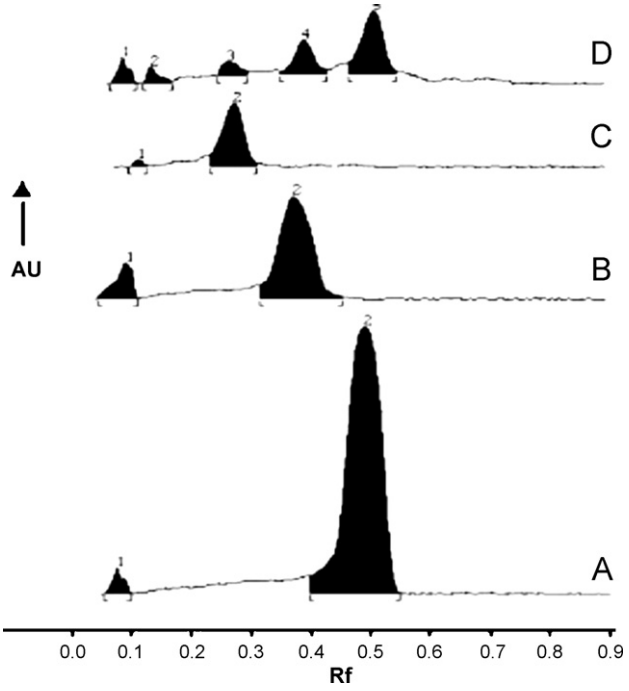

Fig. 10. Analysis of hydrolytic products of starch digested by isolated enzyme. (A) Peak of standard glucose (Himedia), (B) peak of standard maltose (Himedia), (C) peak of standard maltotriose (Sigma), (D) peak of hydrolysis product of starch consisting of peaks corresponding to glucose, maltose and maltotriose generated by isolated amylase enzyme.

Table 3
Comparison of amylase from isolate A9 with other microbial amylase.

Name of microorganisms	Optimum pH	pH stability	Optimum temperature (°C)	MW	Stability	Stability towards NaCl	References
Saccharoplyspora A9	11	8–12	55	66	Surfactant, detergent and oxidant stable	11–17%, (w/v)	This study
Bacillus subtilis	8.0	5.5–10	70				[27]
Bacillus sp. WN 11	5.5	5.5–9.0	75–80	76.0; 53.0			[28]
Bacillus sp. I-3	7.0		70				[35]
Bacillus sp. TS-23	9	7–10	60	66.3	SDS resistant		[40]
B. subtilis AX20	6.0	pH 5–9 for 24 h at 4 °C	55 °C	79			[21]
Bacillus sp. Ferdowsicous	4.5		70	53			[38]
Bacillus sp. YX-1	5.0	4.5–11.0	40–50	56.0			[28]
Bacillus isolate	6.5	5–8	60–70	55	Oxidant stable	0.5 M	[41]
Nesterenkonia sp. strain F	7.5		45				[4]
Bacillus sp. isolate A3–15	11	10–12	70	86 and 60.5	SDS and chelator resistant		[33]
Bacillus sp. TSCVKK	11				Surfactant & detergent stable	10%	[3]
B. amyloliquefaciens	6.0	5.0–8.0, 1 h	55	52			[37]
A. oryzae 245 (ATCC 9376)		5–6	30–40				[1]
*A. us*amii		3.0–5.5	60–70	54.			[1]
Cryptococcus S-2		6.0	50–60	66.0			[1]
Thermomonospora curva		6.0	65	60.9			[1]

4. Conclusion

The comparative study of purified α-amylase from strain A9 with other reported α-amylases in relevant literatures was presented in Table 3. The enzyme showed excellent stability towards high concentrations of NaCl, surfactant, commercial detergents, oxidant and was found to be stable over wide range of pH especially in alkaline pH. The reported amylase was also found to possess significant thermostability.

The enzyme exhibited specific activity of 1640.80 U/mg and molecular mass was found to be 66 kDa. Enzyme may have tremendous applications in detergent, starch liquefaction and pharmaceutical industries where higher salt concentration, surfactant and detergents inhibit enzymatic conversions.

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