Purification, characterization, and statistical optimization of a thermostable α -amylase from desert actinobacterium *Streptomyces fragilis* DA7-7

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Abstract In this study, preliminary screening revealed that of 134 desert soil actinobacterial isolates, only 43 isolates produced amylase. Among these, an isolate DA7-7, which was identified as *Streptomyces fragilis* DA7-7, showed a prominent zone of clearance and significant amount of α -amylase production. The pre-optimization studies showed varying physicochemical and nutrients properties of the medium influenced the enzyme production significantly. Consequently, central composite design was employed with the selected variables (pH, temperature, dextrose, and peptone) for α -amylase production. The optimum fermentation conditions were 3.07% dextrose, 1.085% peptone, pH 6.0, and incubation temperature 27.27 °C. The predicted optimum α -amylase activity was 991.82 U/mL/min, which was similar to the experimental amylase activity of 973.5 U/mL/min. The crude α -amylase produced by *S. fragilis* DA7-7 was purified with ammonium sulfate precipitation, followed by gel filtration chromatography, and the estimated molecular mass was 51 kDa. The purified α -amylase was stable under the following conditions: pH (4–9), temperature (40–80 °C), NaCl (1–4 M), and

detergents (1–10 mM). The K_m and V_{max} values of enzyme were found to be 0.624 mU/mg and 0.836 mg/mL, respectively.

Keywords Desert actinobacterium · *Streptomyces fragilis* · α -Amylase · Central composite design · Gel filtration chromatography

Introduction

Streptomyces is the most well-known genus of actinobacteria, and it is widely distributed in soil. These bacteria produce a variety of industrially important extracellular and intracellular enzymes (Kim and Lee 1995). Although researchers have long been fascinated to discover the new actinobacteria for the production of industrially important enzymes from diverse environments, desert environment has only been recently explored. Desert soils are less explored, and also one of the important reservoirs of extremophilic actinobacteria. The genus of *Actinomadura*, *Micromonospora*, *Streptomyces*, and *Streptosporangium* were commonly reported in desert soils (Kurapova et al. 2012; Ding et al. 2013). In Saudi Arabia, more than 90% of the land area are deserts, but the actinobacterial diversity and the biotechnological potentials from them have been poorly explored (Atta et al. 2010; Ara et al. 2012; Al-Habib and Magda 2013; Nithya et al. 2015). Previously, there are many reports on α -amylase production by *Streptomyces* species from different environments including: from a brick kiln soil (Kar and Ray 2008), mushroom compost (Singh et al. 2011), marine soil (Krishnan and Sampath Kumar 2015), and marine sponge of *Ircinia* sp. (Krishnakumar et al. 2015). The reports on α -amylase from desert soil actinobacterium are very limited; Ruchika (2016)

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reported three amylase producing actinobacterium *Streptomyces* sp. TDI-10, *Streptomyces* sp. TDI-12, and *Streptomyces* sp. TDI-13 from soil of Thar desert.

Amylases are important enzymes that are used in many industries including brewery, bakery, detergent, saccharification, starch liquefaction, paper, textile, food, and pharmaceuticals (Gupta et al. 2003). The amylase enzyme yield is dependent on the strain, medium composition, cultivation methods, cell growth, nutrient requirements, pH, temperature, and time of incubation. Response surface methodology (RSM) is a mathematical experimental strategy for predicting the optimum conditions in a multi-variable system (He et al. 2004), and it is used for optimization of culture conditions (Rao et al. 1993). Statistical optimization not only allows quick screening of large experimental domains, but also reflects the role of each of the components. RSM has already been successfully applied for optimization of media and culture conditions in many cultivation process for the production of primary and secondary metabolites (Boyaci 2005), amino acids (Xiong et al. 2005), ethanol (Carvalho et al. 2003), and enzymes (Sunitha et al. 1999; Rao and Satyanarayana 2003; Anisha et al. 2007; Kar and Ray 2008; Abdel-Fattah et al. 2013; Gajdhane et al. 2016).

Previously, many researchers studied extracellular α -amylase production from different actinobacterial isolates. However, until now, there was no report on production, statistical optimization of α -amylase from desert soil actinobacterium *Streptomyces fragilis* DA7-7. Therefore, the aims of the present study are: (1) to isolate and screening of α -amylase production from the desert soil actinobacteria, (2) characterization of the potential isolate DA7-7, (3) pre-optimization and statistical optimization of α -amylase production, and (4) extraction, purification, and characterization of α -amylase produced by *S. fragilis* DA7-7.

Materials and methods

Isolation of actinobacteria

The desert soil samples were collected from ten different places in Riyadh province of Saudi Arabia. One hundred grams of soil samples were taken in sterile petri dishes individually and placed in hot air oven at 70 °C for 10 min. The treated samples were serially diluted and plated on starch casein agar (SCA) medium [supplement with Nalidixic acid (10 µg/mL) and Amphotericin B (20 µg/mL)] using the spread plate technique, and the plates were incubated at 28 °C for 8–10 days. Morphologically distinct actinobacterial isolates were purified, and stock cultures were maintained in SCA medium.

Screening for amylase activity

For screening the amylase enzyme production, actinobacterial isolates were streaked in a single line on nutrient starch agar plates and incubated for 4 days at 28 °C. A positive test indicated the formation of zone of clearance of the medium around the colonies, visualized by flooding with Lugol's iodine solution (5 g iodine and 10 g potassium iodide in 100 mL of distilled water), and followed by measuring of the zone of clearance in millimeter (mm).

Enzyme production and amylase assay

The maximum zones of clearance produced by the isolates were further screened for enzyme production. The potential isolates were inoculated individually in 250-mL Erlenmeyer flasks containing 100 mL of International Streptomyces Project-2 broth (ISP-2 yeast extract–malt extract broth), and incubated at 28 °C for 4 days in a rotary shaker at 120 rpm. The fermented broth was centrifuged at 5000 rpm for 20 min at 4 °C, and the supernatant was recovered. Amylase assay was determined using a spectrophotometric method described by Bernfeld (1955). The culture filtrate (1 mL) was transferred into a test tube, and 1 mL of 1% soluble starch in 0.1 M sodium phosphate buffer (pH 7) was added. The tubes were covered and incubated at 35 °C for 10 min. Then, 2 mL DNS reagent was added in each tube to stop the reaction, followed by incubation in boiling water bath for 10 min. After cooling at room temperature, the final volume was adjusted to 10 mL by adding distilled water, and the absorbance was read at 540 nm using a spectrophotometer. The enzyme activity was described by international unit (IU). Based on maximum amylase production, the isolate DA7-7 was selected for further studies.

Morphological and biochemical characterization

For the isolate DA7-7, growth pattern, color of aerial and substrate mycelia, as well as diffusible pigment production were observed in ten different media: International Streptomyces project (ISP) media ISP1 (Tryptone-Yeast extract agar), ISP2 (Yeast extract-Malt extract agar), ISP3 (Oat meal agar), ISP4 (Inorganic salts agar), ISP5 (Glycerol asparagine agar), ISP6 (Peptone yeast extract agar), ISP7 (Tyrosine agar), Starch casein agar, Nutrient agar, and Glucose yeast extract agar. Similarly, spores, sporangia, aerial mycelium, and substrate mycelium were observed under high power magnification of phase-contrast microscope (Nikon, Japan). The isolate DA7-7 was subjected to the following tests: Gram staining, indole, methyl red, Voges Proskauer, citrate utilization, triple sugar iron agar,

nitrate reduction, urease, catalase, oxidase, starch, gelatin, lipid, and casein hydrolysis (Shirling and Gottlieb 1966).

Molecular characterization

The isolate DA7-7 was cultured in ISP 2 broth and genomic DNA was extracted using MN DNA extraction kit (Macherey–Nagel, Germany), and 16S rRNA gene was amplified using thermal cycler with 16S rRNA primers 27f (5'AGTTTGATCCTGGCTCAG3') and 1492r (5'ACGGCTACCTTGTTACGACTT3'). The amplified product was subjected to agarose gel electrophoresis (1.2%). The PCR product was purified using MN DNA purification kit, and sequenced by Roche GSFLX 454 technology (Macrogen, Republic of Korea). The similarity index was determined using BLASTn, and the sequence was submitted to GenBank under the accession number KT365285. Phylogenetic tree was constructed for the isolate DA7-7 and other closely related gene sequences by neighbor-joining method using the software MEGA v6.0 (Tamura et al. 2013).

Selection of medium for α -amylase production

The isolate DA7-7 was grown in seven fermentation media: tryptone yeast extract (ISP1), yeast extract–malt extract (ISP2), starch casein (SC), glucose yeast extract (GYE), nutrient broth (NB), Sabouraud dextrose (SD), and modified nutrient glucose (MNG) at 28 °C under 120 rpm for 4 days. Enzyme production in the fermented broth was estimated from the amylase assay, the detailed procedures of which are described in the previous section.

Pre-optimization of α -amylase production

Pre-optimization studies were carried out in Sabouraud dextrose medium (20 g/L dextrose, 10 g/L peptone, and the conditions: pH 8, temperature 28 °C, inoculum size 1%, 120 rpm, and incubation period 96 h), for α -amylase production using the one variant at time (OVAT) approach. The OVAT method was used to evaluate the effects of the following different conditions on α -amylase production by the isolate DA7-7: temperature, pH, inoculum concentration, incubation period, and nitrogen and carbon sources.

Effects of pH and temperature

SD broth was prepared, and initial pH values of 5, 6, 7, 8, 9, 10, or 11 were adjusted using 1 N HCl and 1 N NaOH. The isolate DA7-7 was inoculated at different pH values and incubated in a rotary shaker at 28 °C with 120 rpm for 4 days. The isolate DA7-7 was also inoculated in SD broth and incubated at various temperatures, including 25, 28, 31, 34, 37, and 41 °C, in a rotary shaker (120 rpm) for

4 days. The enzyme production in the fermented broth was determined from the amylase assay.

Effects of inoculum and incubation period

The SD broth was prepared, and the DA7-7 isolates were inoculated in varying concentrations (v/v) such as 0.5, 1, 1.5, 2, and 4%, in a rotary shaker (120 rpm) at 28 °C for 4 days. The isolate DA7-7 was also inoculated in SD broth and incubated in a rotary shaker at 28 °C with 120 rpm. The broth was recovered at regular intervals of 24, 48, 72, 96, 120, 144, and 168 h of incubation. The enzyme production in the fermented broth was determined from the amylase assay.

Effects of different carbon and nitrogen sources

To investigate the effects of different carbon sources (20 g/L), the SD medium was supplemented with maltose, lactose, sucrose, mannose, dextrose, galactose, glycerol, starch, arabinose, and mannitol. Meanwhile, the effect of different nitrogen sources (10 g/L) was determined by supplementing the SD medium with peptone, beef extract, yeast extract, malt extract, tryptone, alanine, cysteine, glycine, histidine, and urea. The DA7-7 isolates were inoculated in the flasks and incubated in a rotary shaker at 28 °C with 120 rpm for 4 days. The enzyme production in the fermented broth was determined from the α -amylase assay.

Effects of dextrose and peptone concentrations

To determine the effects of different concentrations of dextrose on the enzyme production, SD medium was prepared with different concentrations of dextrose: 10, 20, 30, 40, and 50 g/L. The effects of different concentrations of peptone were also studied. SD medium was prepared with peptone 5, 7.5, 10, 12.5, and 15 g/L. The isolate DA7-7 was inoculated and incubated in a rotary shaker at 28 °C with 120 rpm for 4 days. The enzyme production in the fermented broth was determined from the amylase assay.

Statistical optimization of α -amylase production by RSM

To determine the optimum levels of the significant variables, statistical optimization was adopted for improving α -amylase production for the isolate DA7-7 using RSM. The statistical model was developed using central composite design (CCD) with four independent variables such as pH (A), temperature (B), dextrose (C), and peptone (D). Each factor in this model was calculated at five different levels (Supplementary Table S1). In total, 31 sets of experiments

were carried out, and the central coded value was considered as zero for all the variables. The details of all the experimental variables with respect to their values are presented in Supplementary Table S2. The prediction of optimal point, a second-order polynomial function, was fitted to correlate relationship between independent variable and production of α -amylase. The equation for the four factors was as follows:

$$Y = b_0 + b_1A + b_2B + b_3C + b_4D - b_{11}A^2 - b_{22}B^2 - b_{33}C^2 - b_{44}D^2 - b_{12}AB + b_{13}AC + b_{14}AD + b_{23}BC - b_{24}BD + b_{34}CD,$$

where Y is response enzyme activity; A , B , C , and D are the coded independent variables; β_1 , β_2 , β_3 , and β_4 are linear co-efficients; β_{11} , β_{22} , β_{33} , and β_{44} are the quadratic co-efficients; and β_{12} , β_{13} , β_{24} , and β_{34} are the interactive co-efficients.

The response surface regression analysis was performed to obtain a second-order polynomial equation. The data obtained from RSM on α -amylase production were subjected to analysis of variance (ANOVA) test. The surface plots were also generated for all the variables using MINITAB v17.1.0.

Purification of amylase

Mass-scale fermentation was carried out by inoculating the isolate DA7-7 in optimized SD medium. After fermentation, 2 L of the fermented broth was centrifuged at 10,000 rpm using a refrigerated centrifuge (Eppendorf, Germany), and the obtained supernatant was precipitated with 85% of ammonium sulfate. Then, the precipitate was disbanded in 0.05 M glycine–NaOH buffer (pH 10). The mixture was dialyzed overnight by glycine–NaOH buffer (Nithya et al. 2016).

The partially purified enzyme was subjected to gel filtration chromatography on Sephadex G-100 column (Mamo and Gessesse 1999). Five grams of Sephadex G-100 were suspended in 100 mL of 50 mM Tris–HCl buffer (pH 9) and incubated for swelling overnight. The column was pre-equilibrated with 50 mM Tris–HCl buffer (pH 9). The dialyzed and concentrated fraction was loaded onto the Sephadex G-100 column and eluted using the same buffer. The eluted fractions were collected in test tubes and were maintained at 4 °C. The protein contents (Lowry et al. 1951) and α -amylase activities of the collected fractions were determined.

Kinetic properties of α -amylase

Kinetic properties of purified α -amylase were determined according to the method of Lineweaver–Burk plot

(Lineweaver and Burk 1934). Starch was used as substrate for this study in concentration ranged from 0.5 to 1.5%, and the reaction conditions: pH at 8 and temperature at 55 °C. The kinetic constant K_m (mg/mL) and values of maximum rate V_{max} (mU/mg/min) were determined, and the enzyme kinetic data were plotted using Microsoft Excel 2010.

Characterization of α -amylase

The pH stability of purified amylase was studied by pre-incubating the enzyme (80 μ g/mL) in different pH value buffers (4–12), at 37 °C for 1 h. Thermal stability of the enzyme was determined from 40 to 100 °C for 1 h. Similarly, halostability of the enzyme was tested different concentrations of NaCl (0–4 M) at 37 °C for 1 h. The effect of various detergents (10 mM each) was tested at 37 °C for 30 min: cetyltrimethylammonium bromide, sodium dodecyl sulfate, Triton X-100, Tween 20, and Tween 80 (Nithya et al. 2016). The enzyme activities were determined and compared to the control.

Statistical analyses

The experimental conditions were analyzed in triplicate ($n = 3$), and data were expressed as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) and regression co-efficient analysis were carried out.

Results

Isolation and screening

In the present study, 134 actinobacterial isolates were obtained from Saudi Arabian desert soil samples, and qualitative testing of α -amylase was performed. Among them, only 43 isolates produced α -amylase. From these, nine isolates produced α -amylase ranging from 320.62 to 864.25 U/mL/min, and one isolate DA7-7 produced the maximum zone of clearance (16 mm) with an enzyme activity of 939.91 U/mL/min (Table 1).

Characterization and identification of isolate DA7-7

The isolate DA7-7 grew well in all tested media, and produced ash grey colored aerial spores on ISP-2 medium, which was more suitable than the other tested media. Diffusible pigments were not observed in any tested media (Table 2; Fig. 1). Isolate DA7-7 was Gram-positive, urease, lipase, amylase, and catalase positive. The morphological, physiological, and biochemical characterization revealed that the isolate was closely related to the genus *Streptomyces*. The 16S rRNA gene was amplified

Table 1 Screening of α -amylase production

Name of the isolates	Zone of clearance (mm)	Amylase production (U/mL/min)
DA3-2	10	520.26
DA3-7	12	440.75
DA3-9	13	513.42
DA3-12	13	864.25
DA5-5	10	296.45
DA5-7	11	340.38
DA6-1	11	320.62
DA7-1	12	480.96
DA7-4	12	420.68
DA7-7	16	939.91

and sequenced for the isolate DA7-7. The BLASTn search for the isolate DA7-7 showed 99% similarity with *S. fragilis*. The 16S rRNA gene sequence of the isolate was submitted to the GenBank (NCBI) under the accession KT365285. The phylogenetic relationship between 16S rRNA gene sequence of isolate DA3-7 and other similar sequences available in the GenBank database revealed that the isolate formed a separate cluster with *S. fragilis* strain YJ-RT6 (Fig. 2).

Media selection and pre-optimization

The selection of the fermentation medium is an important prerequisite in enzyme production. In the present study, seven different fermentation media were tested, of which Sabouraud dextrose (SD) was found to be the most suitable medium for the production of α -amylase (959.91 U/mL/min after 72 h) by *S. fragilis* DA7-7 (Table 3).

Pre-optimization studies included testing the effects of pH, temperature, inoculum size, incubation period, carbon, nitrogen sources, dextrose, and peptone concentrations on

α -amylase production by *S. fragilis* DA7-7, and the results are illustrated in Supplementary Fig. 1a–h. The highest yield (940.36 U/mL/min) was obtained at pH 6, and the lowest yield was obtained at pH 4 and 11 as 724.16 and 736.20 U/mL/min, respectively. Maximum α -amylase production was obtained on incubation at 28 °C (923.12 U/mL/min), followed by 31 °C (902.24 U/mL/min), while further increase in the incubation temperature decreased the α -amylase production. *S. fragilis* DA7-7 was found to produce maximum α -amylase with 2% inoculum size (976.20 U/mL/min) and 72 h of incubation period (970 U/mL/min).

Among the various carbon sources tested, mannitol and glycerol showed poor enzyme yields (< 500 U/mL/min), whereas arabinose, dextrose, galactose, lactose, maltose, mannose, starch, and sucrose showed high α -amylase enzyme yields (> 900 U/mL/min). Different nitrogen sources such as peptone, beef extract, yeast extract, malt extract, tryptone, alanine, cysteine, glycine, histidine, and urea were found to increase the α -amylase production by *S. fragilis* DA7-7. The studies on effects of carbon and nitrogen sources revealed that dextrose (30 g/L) and peptone (10 g/L) were suitable for maximum α -amylase production.

Statistical optimization of α -amylase production using RSM

To determine the optimum level of α -amylase production, effects of four independent fermentation variables, that is, pH, temperature, dextrose, and peptone concentrations, were studied. The results of predicted and observed responses for α -amylase production by CCD experiments are presented in Supplementary Table S2. Based on the results of CCD, the final response equation for a suitable model for α -amylase production, obtained after ANOVA, is given below:

Table 2 Morphological features of the isolate DA7-7 on different media

Media	Aerial mycelium	Substrate mycelium	Diffusile pigment	Growth
ISP 1	Pale yellow	Pale yellow	—	++
ISP 2	Ash grey	Yellowish brown	—	+++
ISP 3	Whitish grey	Ash	—	+
ISP 4	Whitish grey	Pale yellow	—	++
ISP 5	Ash grey	Yellowish white	—	++
ISP 6	Whitish grey	Pale yellow	—	++
ISP 7	Golden yellow	Yellowish white	—	+++
ISP 8	Whitish ash	Pale yellow	—	++
SCA	Pale yellow	Pale yellow	—	+++
NA	Greyish white	Pale yellow	—	++
GYEA	Greyish white	Yellowish white	—	+++

Fig. 1 Colony morphology and microscopic view of *Streptomyces fragilis* DA7-7. **a** Isolate DA7-7 cultured on ISP-2 medium. **b** Phase-contrast microscopic view of isolate DA7-7

$$\begin{aligned}
 Y = & 22,102 + 6393A + 249.06B + 14.88C + 50.4D \\
 & - 496.9A^2 - 2.0313B^2 - 1.2500C^2 \\
 & - 4.140D^2 - 24.38AB + 7.000AC + 2.25AD \\
 & + 0.3250BC - 0.1625BD + 0.9900CD,
 \end{aligned}$$

where Y = response enzyme activity, A = pH, B = temperature ($^{\circ}\text{C}$), C = dextrose (w/v), and D = peptone (w/v).

To determine the effects of medium components and physical factors on α -amylase production, statistical analyses were performed using Student's t and p tests, and the results are illustrated in Table 4. The linear effect factors, namely A and B , showed negative values, while C and D showed positive t values. The significant p values for A , B , C , and D were 0.000. The square effect factors, namely, AA , BB , CC , and DD showed negative t values, and the p values were 0.000. The interaction effect factors, namely, AB and BD , showed negative t values, and the p values were 0.000 and 0.097, respectively, while AC , AD , BC , and CD showed positive t values, and the p values were 0.000 for AC , BC , and CD , and 0.240 for AD . The co-efficient of determination (R^2) was calculated as 99.75% of variability in the response for α -amylase production (Table 4). The predicted R^2 of 98.58% is in reasonable agreement with adjusted R^2 of 99.54%, indicating good agreement between the experimental and predicted values for α -amylase production by *S. fragilis* DA7-7.

ANOVA was also performed to test the significance and adequacy of the second-order polynomial model, and the results are presented in Table 5. The significance of regression was assessed by f and p values. High f and low p values of 461.42 and 0.000, respectively, were found in

the regression model, indicating that the model was highly significant. The linear, square, and intersection factors were also highly significant, as denoted by the high f values of 177.94, 1252.31, and 123.16, respectively, and low p value of 0.000.

Response surface and contour plots were generated by plotting the response (α -amylase production) on the z -axis against two independent variables, while keeping the other independent variable at zero. In total, six response surfaces were obtained by considering all the possible combinations. Three-dimensional response surface plots and contour plots were assessed, and they are presented in subfigures of Fig. 3. Interactions between pH and temperature, which were highly elliptical in nature, are displayed in Fig. 3a1, a2. Hence, interaction between pH and temperature is highly significant. Similarly, in Fig. 3f1, f2, contour plot was also highly elliptical in nature, which suggests highly significant interaction between dextrose and peptone concentration. In Fig. 3b1, b2, d1, d2, contour plots were slightly elliptical in nature. Hence, the interactions between pH vs. dextrose and temperature vs. dextrose are less significant. Contour plots in Fig. 3c1, c2, e1, e2 were circular in nature, indicating that the interactions between pH vs. peptone and temperature vs. peptone do not have significant effects on α -amylase production.

Experimental model validation

The experimental model for α -amylase production was validated under basal and predicted optimal conditions. The enzyme activity in the basal medium was 937.5 U/mL/

Fig. 2 Phylogenetic tree of the 16S rRNA gene sequence of *Streptomyces fragilis* DA7-7 and other closely related gene sequences. Numbers in the parentheses indicate GenBank accession numbers

Table 3 Selection of the optimum medium for α -amylase enzyme production

Incubation time (h)	Medium for amylase enzyme production (U/mL/min)						
	ISP II	SC	GYE	NB	ISP I	SD	MNG
48	899.80	420.68	917.32	140.22	245.40	934.85	900.38
72	911.48	409.00	923.17	151.91	222.03	959.91	934.85
96	905.64	403.16	899.80	137.31	198.66	947.38	901.55
120	888.11	297.98	891.03	122.70	204.50	934.85	876.42
144	876.42	198.66	889.57	116.86	146.07	929.01	835.52
168	818.00	140.22	853.05	111.01	116.86	891.80	794.62

min, while in the optimized medium, the maximum α -amylase activity was 979.3 U/mL/min after 72 h. The predicted activity value from the polynomial model was 991.82 U/mL/min, based on optimum conditions of variables: 3.07% dextrose, 1.085% peptone, pH 6.0, temperature 27.27 °C, and incubation time 72 h.

Purification of α -amylase

The α -amylase from *S. fragilis* DA7-7 was purified to homogeneity with 17.13-fold and 24.62% yield (Table 6). The purity was confirmed from SDS-PAGE, which showed a single band with a molecular weight of 51 kDa (Fig. 4).

Table 4 Estimated regression co-efficient analysis for optimization of α -amylase production

Term	Co-efficient	SE co-efficient	<i>t</i> value	<i>p</i> value
Constant	988.00	1.39	708.58	0.000
<i>A</i>	− 3.833	0.753	− 5.09	0.000
<i>B</i>	− 11.250	0.753	− 14.94	0.000
<i>C</i>	4.417	0.753	5.87	0.000
<i>D</i>	15.583	0.753	20.69	0.000
<i>AA</i>	− 19.875	0.690	− 28.81	0.000
<i>BB</i>	− 32.500	0.690	− 47.11	0.000
<i>CC</i>	− 31.250	0.690	− 45.30	0.000
<i>DD</i>	− 25.875	0.690	− 37.51	0.000
<i>AB</i>	− 19.500	0.922	− 21.14	0.000
<i>AC</i>	7.000	0.922	7.59	0.000
<i>AD</i>	1.125	0.922	1.22	0.240
<i>BC</i>	6.500	0.922	7.05	0.000
<i>BD</i>	− 1.625	0.922	− 1.76	0.097
<i>CD</i>	12.375	0.922	13.42	0.000
$R^2 = 99.75\%$	R^2 (adj) = 99.54%	R^2 (pred) = 98.58%		

A pH, *B* temperature (°C), *C* dextrose (w/v), *D* peptone (w/v), *SE* standard error, *t* student test, *p* probability

Table 5 Analyses of variance

Source	<i>df</i>	Adj SS	Adj MS	<i>f</i> value	<i>p</i> value
Regression	14	87,915.7	6279.7	461.42	0.000
Linear	4	9686.5	2421.6	177.94	0.000
Square	4	68,172.4	17,043.1	1252.31	0.000
Interaction	6	10,056.8	1676.1	123.16	0.000
Error	16	217.7	13.6		
Lack-of-fit	10	217.7	21.8		
Pure error	6	0.0	0.0		
Total	30	88,133.4			

df degrees of freedom

Kinetic properties of α -amylase

After purification of α -amylase, the kinetic properties were determined. The Michaelis–Menten constant (K_m) was found to be 0.624 mU/mg, and the Lineweaver–Burk plot showed that the maximum velocity (V_{max}) was found 0.836 mg/mL (Supplementary Fig. 2).

Characterization of α -amylase

Stability of purified α -amylase was assessed at varying conditions of pH, temperature, NaCl, and detergents, and the results are illustrated in Fig. 5. The enzyme was stable in a wide range of pH values from 5.0 to 9.0, and the relative activities at pH 5 and pH 9 were 82 and 72%, respectively. Purified α -amylase retained 100% stability when pre-incubated at pH 6 and 7 for 24 h. In addition, the enzyme was highly stable in a temperature range from 40

to 80 °C. The relative activities at 40 and 80 °C were 83 and 72%, respectively, while 100% relative activity was observed at 50 °C after 180 min of incubation. The enzyme was also stable in different detergents, including SDS (88%), CTAB (73%), Triton X-100 (82%), Tween 20 (74%), and Tween 80 (78%).

Discussion

Diversity of actinobacteria in the desert habitats and their biotechnological potentials has not been well documented. Recently, studies on actinobacteria from unexplored environments, especially from desert, have explored novel actinobacteria and production of natural products, including antimicrobial compounds and enzymes (Zitouni et al. 2004; Badji et al. 2006; Hozzein et al. 2007; Rateb et al. 2011; Nithya et al. 2015). The extremophilic/alkalophilic

Fig. 3 Graphical representation of surface and contour plots. **a1**, **a2** pH and temperature. **b1**, **b2** pH and dextrose. **c1**, **c2** pH and peptone. **d1**, **d2** Temperature and dextrose. **e1**, **e2** Temperature and peptone. **f1**, **f2** Dextrose and peptone

Table 6 Purification of α -amylase

Enzyme fraction	Volume (mL)	Enzyme activity (units)	Protein conc. (mg)	Specific activity (U/mg)	Purification (fold)	Yield (%)
Culture filtrate	100	9180.28	792.34	11.58	1.00	100
Ammonium sulfate precipitation	12	6420.37	78.45	81.84	7.06	69.94
Sephadex G-100 column chromatography	7	2260.61	11.26	200.76	17.34	24.62

Fig. 4 Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) showing the purified α -amylase enzyme produced by *Streptomyces fragilis* DA7-7. Lane 1 Molecular marker protein. Lane 2 Crude α -amylase enzyme in the culture filtrate. Lane 3 Purified α -amylase enzyme

actinobacteria produced a variety of enzymes that are functional under extreme conditions, which could have applications in industrial processes. In this study, 134 actinobacterial isolates were obtained from desert soil collected from Riyadh province of Saudi Arabia, and among these, 43 isolates produced α -amylase. The isolate DA7-7 produced higher amount of α -amylase, as compared to the other isolates, and was identified as *S. fragilis* DA7-7. Therefore, it is clear that actinobacteria can produce a variety of enzymes, and show high potential for exploring novel industrial enzymes from desert actinobacteria.

The production of α -amylase is mostly dependent on the strain, medium composition, cultivation methods, cell growth, nutrient requirements, pH, temperature, and time of incubation (Stanbury et al. 1995; Fogarty et al. 1999). Among the physicochemical parameters, pH of the fermentation medium, in particular, plays an important role in the production of α -amylase (Pandey et al. 2000). In the

present study, maximum α -amylase production was found at pH 6, and increase or decrease of the pH from the optimum level led to a gradual decline in α -amylase production, probably due to poor microbial growth under acidic and alkaline conditions (Heese et al. 1991). The previous report has also supported that pH 6 was optimum for α -amylase production in *Streptomyces erumpens* (Kar and Ray 2008). Similarly, the pH range 6–7 was reported to be optimum for amylase production by several *Streptomyces* species including *Streptomyces megasporus* SD 12 (Dey and Agarwal 1999), *S. tendae* TK-VL 333 (Kavitha and Vijayalakshmi 2010), *Streptomyces* sp. MSC702 (Singh et al. 2011), *S. clavifer* (Yassien and Asfour 2012), and *S. cheonanensis* VUK-A (Naragani et al. 2015). Deviation from the optimum temperature range resulted in decreased enzyme production. In this study, temperature range of 28–31 °C was revealed as optimum temperature for α -amylase production by *S. fragilis* DA7-7. Similar findings have also obtained for *S. tendae* TK-VL 333 (Kavitha and Vijayalakshmi 2010). Narayana and Vijayalakshmi (2008) reported that the optimum temperature for α -amylase production in *Streptomyces albidoflavus* at 30 °C. Another recent study also confirmed the optimum α -amylase production by *S. cheonanensis* VUK-A at 30 °C (Naragani et al. 2015). The incubation period is one of the major factors that influence α -amylase production (Dhanasekaran et al. 2006; Nithya et al. 2016). In this study, the optimum incubation period for α -amylase production by *S. fragilis* DA7-7 was found to be 72 h. In the previous report, Naragani et al. (2015) also supported that the average incubation period employed was 72 h for α -amylase production by *S. cheonanensis* VUK-A.

The nature and quantity of carbon and nitrogen sources and supplements with specific carbon and nitrogen sources also influenced the enzyme production, and the results vary in different types of bacteria (Hillier et al. 1996). In the present study, various carbon and nitrogen sources were tested, and dextrose (20 g/L) and peptone (10 g/L) were found to produce maximum quantity of α -amylase. However, Narayana and Vijayalakshmi (2008) reported starch and yeast extracts as carbon and nitrogen sources, respectively, resulted in maximum α -amylase production in *S. albidoflavus*. The recent reports of Krishnakumar et al.

Fig. 5 Effects of pH, temperature, NaCl, and detergents on stability of purified α -amylase enzyme. Relative activity (%) of purified α -amylase was determined by comparing to the control activity of 100%

(2015) and Naragani et al. (2015), and α -amylase from *Streptomyces* sp. SBU3 and *S. cheonanensis* VUK-A were also strongly influenced by sorghum flour and glucose as carbon sources, while peptone and casamino acid as nitrogen sources, respectively. Thus, it is clear that the specific carbon and nitrogen sources do not uniformly influence the enzyme production in *Streptomyces* species.

RSM used in this study suggested the importance of various fermentation parameters at different levels. It is one of the fastest, accurate, and less time consuming experimental designs that could be commonly used for the optimization of many fermentation processes (Kunamneni and Singh 2005). In the present study, to determine the optimum conditions for α -amylase production, effects of four independent fermentation variables (pH, temperature, dextrose, and peptone) on enzyme yields were studied. RSM revealed high similarity between the predicted and experimental results, which reflected accuracy and applicability of RSM to optimize enzyme production in submerged fermentation. In this study, we observed the following optimum conditions of variables that resulted in maximum α -amylase yields: dextrose (30.7 g/L), peptone (10.85 g/L), pH (6.0), and temperature (27.27 °C). Many previous studies have reported that RSM is a suitable method to optimize the enzyme production in bacteria, actinobacteria, and fungi (Sunitha et al. 1999; Rao and Satyanarayana 2003; Anisha et al. 2007; Kar and Ray 2008; Abdel-Fattah et al. 2013; Gajdhane et al. 2016).

The crude α -amylase produced by *S. fragilis* DA7-7 was purified using ammonium sulfate precipitation, followed by gel filtration chromatography, and purified α -amylase was characterized. The molecular weight of purified α -amylase was determined as 51 kDa. Earlier, Yassien and Asfour (2012) have reported similar type of molecular mass 50 kDa α -amylase from *S. clavifer*. Similarly, molecular

masses of α -amylase 44 kDa and 55 kDa have been reported from *Streptomyces* sp. PDS1 (Ragunathan and Padhmadas 2013) and *S. gulgargensis* (Syed et al. 2009), respectively. Thermal and pH stability are very important features for any industrial enzymes. In this study, pH and thermal stability of purified α -amylase were determined, and the enzyme was able to tolerate maximum values of pH and temperature of 9 and 80 °C, respectively. The optimal pH and temperature values were 6 and 50 °C, respectively, and the enzyme relative activity was 100%. The purified enzyme could also withstand salinity up to 4 M and detergent concentration of 10 mM. Many previous studies have reported that α -amylase from *Streptomyces clavifer* and *Streptomyces* spp. was stable at pH of 6–7 and at temperatures ranging from 40 to 50 °C (Ammar et al. 2002; Kaneko et al. 2005; Yassien and Asfour 2012).

The purified α -amylase was further characterized by the kinetic properties, which showed that Michaelis–Menten constant (K_m) and V_{max} were 0.624 mU/mg and 0.836 mg/mL, respectively. It is difficult to compare of kinetic values of α -amylase reported by other researchers, because they used different concentration of starch and different assay conditions. Shafiei et al. (2010) reported α -amylase from *Nesterenkonia* sp. and found K_m and V_{max} values were 4.5 mg/mL and 1.18 mg/mL/min, respectively. Similarly, Singh et al. (2014) found K_m and V_{max} α -amylase produced by *Streptomyces* sp. MSC702 as 2.407 mg/mL and 21,853 mol/min/mg, respectively. The enzyme α -amylase mostly belonging to the Glycoside Hydrolase family 13 (GH13) are listed in <http://www.CAZy.org> (Lombard et al. 2014), and the enzyme further classified into 42 subfamilies on the basis of sequence similarity (Stam et al. 2006; Lombard et al. 2014). In CAZy, most α -amylase produced by *Streptomyces* species are coming under family GH13 and subfamily 32 (GH13_32). The N-terminal amino acid

sequence of α -amylase produced by *S. fragilis* DA7-7 has not been studied, and in future, research will be carried out. Previously, N-terminal amino acid sequences of α -amylase have been reported from *S. griseus* (Vigal et al. 1991), *S. thermoviolaceus* (Bahri and Ward 1993), and *Streptomyces* sp. (Kaneko et al. 2005). Thus, the present study revealed that the enzyme α -amylase from *S. fragilis* DA7-7 shows pH, temperature, NaCl, and detergent stability, and has great potential in many industrial applications, including in additives in laundry detergents and in starch saccharification.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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