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OPTIMIZATION OF THERMOSTABLE ALPHA-AMYLASE PRODUCTION FROM *GEOBACILLUS* SP. D413

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ABSTRACT

The qualitative and quantitative α-amylase production capacities of six thermophilic bacilli were screened. *Geobacillus* sp. D413 was selected for enzyme optimization, as it displayed higher α-amylase activity. The maximum enzyme activities of D413 and *G. stearothermophilus* ATCC 12980^T were observed at the time of 72 h. While the optimal pH of medium for bacterial growth and enzyme production of D413 (pH 7.0) differed from ATCC 12980^T (pH 8.0), the optimal temperature for enzyme production was 55°C for both. The effects of various carbon and nitrogen sources were determined by changing their concentrations. The highest bacterial growth and enzyme production were sustained by the starch and maltose containing medium. Both bacterial growth and enzyme production were inhibited by NH_4Cl. D413 and ATCC 12980^T amylases showed optimal activity at 65ºC, pH 9.0 and at 65ºC, pH 7.5, respectively. They remained active over temperature and pH ranges of 45-75ºC and 4.0-10.5. Their activities retained 65% and 54% when incubated at 75ºC for 10 min and 98-86.5% and 95-84.5% at pH 4.0-10.5 for 15 h at 37ºC. In conclusion, the α-amylase production conditions of D413 have been optimized which can be useful in biotechnological processes such as hydrolysis of starch to glucose.

Keywords: Thermostable, α-Amylase, Production, Optimization, *Geobacillus* sp.

INTRODUCTION

α-Amylases (EC 3.2.1.1, 1,4-α-D-glucan glucanohydrolases and endoamylases) degrade starch hydrolyzing enzymes by breaking internal α-1,4-glucosidic linkages and produce reducing sugars. Starch is an immanent polysaccharide composed of two high-molecular weight components such as amylose and amylopectin. Amylose is a linear chain consisting of units from glucose molecules linked by α-1,4 glycosidic bonds. Amylopectin is a branched molecule containing α-1,6 linking branch points in addition to α-1,4 glycosidic bonds. α-amylases are produced abundantly by plants and microorganisms and produce a wide class of industrial enzymes constituting approximately 30% of the world enzyme market **(Van der Maarel *et al.*, 2002).**

Of those amylases, microbial α-amylases have wide applications in industrial processes such as starch degradation, production of glucose and fructose syrup as well as fruit juices and alcoholic beverages, and also in applications such as detergent, paper and textile industries. Recent studies especially focus on the role of thermostable enzymes in biotechnology and industry, since many industrial enzymatic reactions are performed at elevated temperatures. Therefore, thermostable α-amylases take advantage of usage potential in these processes. The microbial α-amylases have been described and characterized in some species including *Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus licheniformis, Geobacillus thermodenitrificans* HRO10 **(Ezeji and Bahl, 2006)**, *Geobacillus stearothermophilus* ATCC 12016 and *Geobacillus stearothermophilus* ATCC 12980^T **(Underkofler, 1976; Suzuki *et al.*, 1984; Ferner-Ortner-Bleckmann *et al*,. 2009)**. Some amylase producing strains and their pH and thermal stabilities are also listed in Table 1. Here we report the characteristic properties of *Geobacillus* sp D413 α-amylase by presenting the thermal and pH activity and stability of its enzyme as well as the optimal culture conditions for enzyme production.

Table 1 Some source microorganisms and properties of their thermostable or pH stable α-amylases

Organism	Optimum Temperature (°C)	Residual Activity (%)	Optimum pH	Residual Activity (%)	Reference
Bacillus sp. I-3	70	50 (80°C-2.5 h)	7.0	80	**Goyal *et al.*, 2005**
Bacillus sp ANT-6	80	87.7 (100°C-60 min)	10.5	55 (pH:10 15 h)	**Arikan *et al.*, 2003**
Bacillus sp PS-7	60	78 (50°C -6h)	6.5	96 (pH:5.0 90 min)	**Sodhi *et al.*, 2005**
L. manihotivorans	55	70 (50°C-1 h)	5.5	80 (pH:5.5 60 min)	**Aguilar *et al.*, *2000***
Bacillus KSM-K38	55-60	20 (50°C - 30 min)	8.0-9.5	80 (pH:11 30 min)	**Hagihara *et al.*, 2001**
Bacillus sp. Strain PM1	50	67 (50°C-60min)	8.0	78 (pH:8.0 4h)	**Sharma *et al.*, 2014**
B. amyloliquefaciens P-001	60	73 (50°C -30 min)	6.5	-	**Deb *et al.*, 2013**
G.thermodenitrificans HRO10	75-80	0 (70°C-30 min)	5.5	-	**Ezeji and Bahl, 2006**

MATERIALS AND METHODS

Bacterial isolates

In this study, formerly isolated six thermophilic bacilli and reference strain *G. stearothermophilus* DSMZ 22^T were screened for their qualitative and quantitative amylolytic activities. These thermophilic bacilli were formerly isolated from a polyphasic study and known to be amylase-producing strains **(Coleri *et. al.*, 2009).** Their 16S rRNA gene sequence analyses were also determined from our previous studies and their gene sequences were found in GenBank databases **(Cihan *et al*., 2011 and 2013).** The thermophilic isolates used in this study were *Geobacillus stearothermophilus* A113 (FJ429596), *Geobacillus* sp. C304 (FJ429574) and *Geobacillus* sp. D413 (FJ430040), *Anoxybacillus caldiproteolyticus* D504 (FJ430047), and *Anoxybacillus caldiproteolyticus* D621 (FJ430050). The reference strain *Geobacillus stearothermophilus* ATCC 12980^T was also used for comparison purposes.

Qualitative and quantitative amylase screening assays

In qualitative screening assay, a modified method of **Dheeran *et al.* (2010)** medium was used for bacterial growth. Amylolytic activity was tested on this modified Dheeran medium containing 0.5% peptone, 0.5% yeast extract, 0.5% NaCl, 1.0% soluble starch and 3.0% agar (pH 7.0) after incubation for 24 h at 55°C. Then the plates were treated with 0.2% I_2 in 2% KI solution and isolates having starch digestion zones around their colonies were determined as amylolytic **(Coleri *et al.*, 2009).** The halo zone diameters were measured (cm) in order to compare the amylase producing strains, which were produced due to hydrolysis of starch.

When determining the quantitative amylolytic activity for the screening assay, a modified **Santos and Martins (2003)** medium (1.0% tryptone, 0.5% yeast extract, 1.0% soluble starch) was used for enzyme production. The pH of the medium was adjusted according to the bacteria. The growing cells were suspended in 0.85% sterile NaCl and adjusted to 0.2-0.4 absorbance at 660 nm, and then 500 µL from this suspension was inoculated into 5 mL of enzyme production medium and incubated at 55 °C by shaking at 150 rpm for 72 h. The cells were collected by centrifugation at 5000 rpm for 15 min at 4 °C, and the wet weights of the cells were measured. α-Amylase production capacities of the isolates were designated based on their total enzyme amount per cell-yield (U/g). All the experiments were at least triplicate.

Determination of optimal culturing conditions for enzyme production

In order to optimize the growth pH, temperature and time for achieving the maximum enzyme production, the modified Santos and Martins broth (1.0% tryptone, 0.5% yeast extract, 1.0% soluble starch,) was used when cultivating the bacteria before measuring the amylase activities. The supernatant was obtained as explained before in the quantitative amylolytic activity assay and was used as an enzyme source for further experiments. In order to compare the α-amylase production capacities of the bacteria, total enzyme activity values were divided into biomass of bacterial pellet wet weight (U/g) throughout all the experiments in this study.

When determining the optimal cultivation conditions for amylase production, either the incubation temperature or the pH of the modified Santos and Martins medium was changed. For the determination of thermal conditions on enzyme production, bacterial isolates were incubated in this medium by changing the incubation temperature from 50 to 65°C. On the other hand, the pH of the medium was adjusted to various pH values between 3.0 and 10.0 in order to determine the effect of pH on amylase production. In addition, a time-course was carried out during 96 h in this broth medium by taking samples at 24 h intervals. The pH changes in the medium and also the cell weights were also measured during 96 h cultivation. All the triplicate experiments were further taken into spectrophotometric amylase assay to measure their enzyme production.

Enzyme assay

The α-amylase activity was determined by spectrophotometric method with measuring the hydrolysis of soluble starch as substrate. The standard reaction mixture was prepared by adding appropriately diluted 0.5 ml enzyme solution to 0.5 ml 0.2 M sodium phosphate buffer with 0.5 ml 2% soluble starch. Reaction was carried out at 65°C for 10 min and it was stopped by boiling 5 min after addition of 1 ml DNS. When the reaction tube was cooled, the amylase activity was measured spectrophotometrically at 540 nm. One unit of α amylase activity was defined as the amount of enzyme that catalyzed the liberation of reducing sugar equivalent to 1 µmol of maltose per min under the assay conditions. The millimolar extinction coefficient of maltose at 540 nm, pH 7.0 and at 65°C were measured as 1.454 $M^{-1}.cm^{-1}$ and used to calculate the amount of product yielded. The enzyme assays were performed at least three times.

Determination of the effect of different carbon and nitrogen sources on amylase production

Various carbon sources were used for the determination of their effects on amylase production. Therefore, thermophilic isolates were grown in different modified Santos and Martins medium containing 1 % carbon sources such as sucrose, lactose, dextrose, maltose, and soluble starch (**Kıran *et al.*, 2005, Pavithra *et al.*, 2014**). Of those sources, the concentration of starch was also screened in wider ranges from 0.2 to1 %. Moreover, different nitrogen sources including tryptone, yeast and ammonium chloride, in concentrations from 0.2 to 1%, were studied for their effect of enzyme production. In these assays, the pH and temperature were adjusted to the determined optimal value according to the bacteria or their enzymes used for both growth conditions and for enzyme assays.

Determination of the effect of temperature and pH on α-amylase activity and stability

The effect of temperature on enzyme activities was determined on the crude enzymes between 45°C to 75°C with 5°C intervals. For stability tests, enzymes were heated for 10 min at different temperatures, then quickly chilled on ice and assayed for the remaining activity at its optimal pH and temperature. The effect of pH on the enzyme activity was determined using 0.02 M sodium-citrate (pH 4.0, 4.5, 5, 5.5, 6.0), 0.1 M sodium phosphate (pH 6.5, 7, 7.5, 8.0) and 50mM glycine-NaOH (pH 8.5, 9.0, 9.5, 10.0, 10.5) buffers at 65°C, all instead of 0,2 M potassium phosphate buffer in the standard assay mixture. The effect of pH on α-amylase stability was examined by incubating the enzymes in the same buffers (pH 4.0-10.5) at 37°C for 15 h as reported in the activity test. The activity of the not-heated and not pH-treated enzyme was expressed as 100 %.

RESULTS

Screening results and optimization of culture conditions

In this study, six thermophilic bacilli and an amylase producing reference strain were screened for their α-amylase activity both qualitatively and quantitatively. The zone diameters and the specific α-amylase activity results obtained from screening assays were listed in Table 2. According to these results, the highest α-amylase producing strain was found as *Geobacillus* sp. D413 with a zone diameter of 0.5 cm and with a specific enzyme activity of 15.84 U/g, whereas these values were determined as 15.79 U/g and 0.3 cm for the reference strain *G. stearothermophilus* ATCC 12980^T. Consequently, as the the largest zone diameter after 24 h and the maximum specific activity after 72 h were observed in *Geobacillus* sp. D413, D413 isolate and the reference strain were selected for further optimization analyses.

Table 2 The zone diameters and specific enzyme activities of bacteria obtained from screening assays

Bacteria	Zone diameters (cm)	Enzyme activity/biomass (U/g)
Geobacillus sp. D413	0.5	15.84
Geobacillus stearothermophilus ATCC 12980^T	0.3	15.79
Geobacillus sp. C304	0.4	13.33
Geobacillus stearothermophilus A113	0.2	9.32
Anoxybacillus caldiproteolyticus D504	0.1	8.46
Anoxybacillus caldiproteolyticus D621	0.2	7.76
Anoxybacillus caldiproteolyticus D623	0.2	6.18

When the culture conditions were adjusted between the temperatures from 55-65°C (Fig. 1a) and pH values of 3.0-10.0 (Fig. 1b), the optimal incubation temperature for the maximum amylase production was determined as 55 ^{0}C for both of the bacteria, but they differed in optimal bacterial growth pH for the highest amylase production. pH 7.0 (12,9 U/g) and pH 8.0 (11,73 U/g) were found as optimal values for the isolate D413 and strain ATCC 12980^T, respectively. If the culture medium was adjusted to a pH value of 4.0, it seems that *Geobacillus* sp. D413 could not grow in the medium. This case was shown at previous studies. **(Dheeran *et al.*, 2010; Suman and Ramesh, 2010)**. Furthermore, enzyme production was diminished, when the temperature was below and above 55°C.

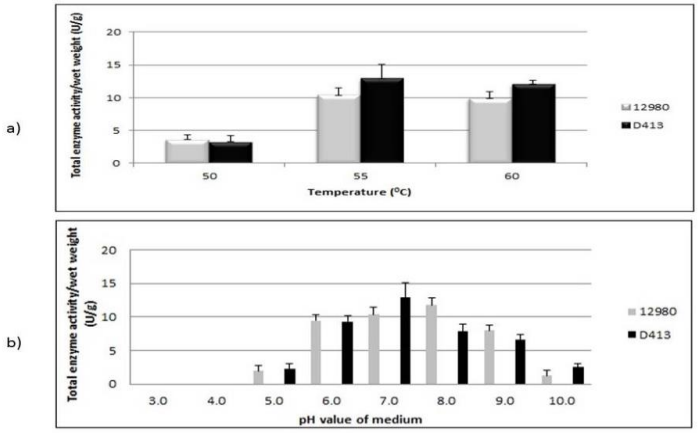

Figure 1 Enzyme production of *G.stearothermophilus* ATCC 12980^T and *Geobacillus* sp. D413 in medium containing 1 % starch, 0.5 % yeast extract and 1 % tryptone at different a) temperatures, and b) pH.

In addition, *Geobacillus* sp. D413 isolate and standard strain were incubated at 55°C and at 150 rpm (pH 7.0 or 8.0) for 96 h in broth medium containing 1% starch (Fig. 2). The experiments on growth patterns and time-course of α-amylase production by D413 and ATCC 12980^T revealed that the maximum extracellular enzyme activity was observed at the time of 72 h, for both D413(15.84 U/g) and ATCC 12980^T (15.79 U/g). Moreover, time-course revealed that the highest α-amylase production was achieved when the pH of the medium were diminished up to 5.0 on 72 h from their optimal pH values (7.0 or 8.0) for both of the strains. Therefore, in order to reach to the highest amylase production, both D413 and ATCC 12980^T were incubated at 55°C and pH 7.0 or 8.0 by shaking at 150 rpm, in rest of the studies, respectively.

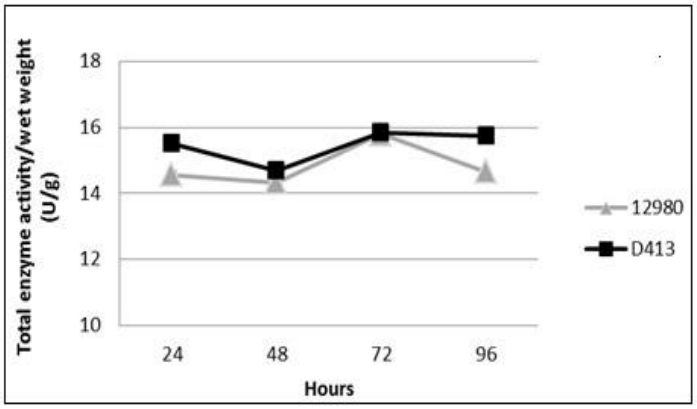

Figure 2 Time course of *G.stearothermophilus* ATCC 12980^T (Δ) and *Geobacillus* sp. D413 (□) during 96 h in medium containing 1 % starch, 0.5 % yeast extract and 1% tryptone at 55°C.

Effects of different carbon sources on enzyme activities

Among the different carbon sources such as starch, maltose, sucrose, dextrose and lactose, used in a concentration of 1%, the maximum enzyme production was observed with maltose for both of the bacteria. The specific activity values of 17.85 and 24.85 U/g were found with the addition of maltose for ATCC 12980 and D413 amylases, respectively. These results are presented in Fig. 3.
As starch is an important substrate for fermentation experiments for amylases, and as it was found to be the second carbon choice for amylase production by isolate D413 (15,38 U/g) and strain ATCC 12980^T (13,6 U/g), the effects of different starch concentrations were also experimented as presented in Fig. 4. The optimum concentration for soluble starch was determined as 10 g/L and below this value a speed decline was observed especially in the case of ATCC 12980 amylase production.

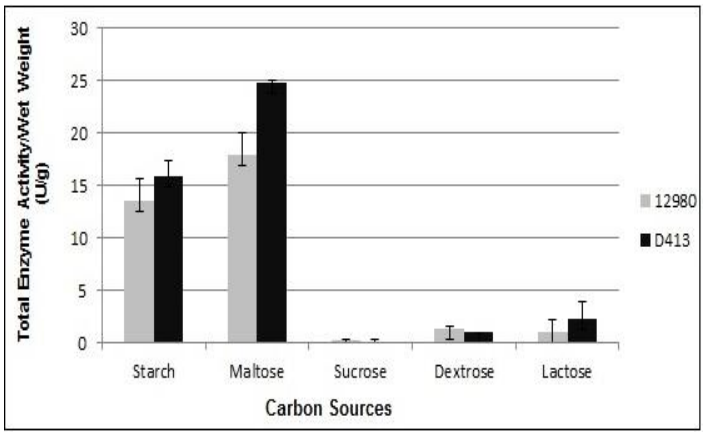

Figure 3 Effects of different carbon sources on amylase production by *G.stearothermophilus* ATCC 12980^T and *Geobacillus* sp. D413 at 55°C.

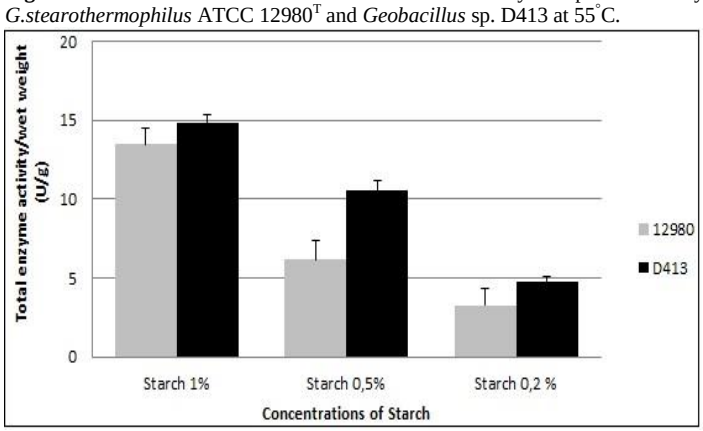

Figure 4 Effects of different concentrations of starch on amylase production by *G.stearothermophilus* ATCC 12980^T and *Geobacillus* sp. D413 at 55°C.

Effects of different nitrogen sources on amylase activities

When determining the effects of different nitrogen sources on amylase production, various concentrations of inorganic and organic compounds were added to the amylase production medium (Fig. 5). If only the inorganic nitrogen source ammonium chloride was used, the bacterial growth and enzyme production was inhibited. While the amylase production of strain D413 was diminished to 1.56 U/g, this value was found to be 0.59 U/g for strain ATCC 12980^T. In the other case, if the ammonium chloride in addition to tryptone or yeast extract were used with conjunction as double nitrogen sources, bacterial growth continued, but the enzyme production diminished for both of the bacteria ≤1 U/g). Therefore, it can be concluded that ammonium chloride had inhibitory effects for these α-amylase.

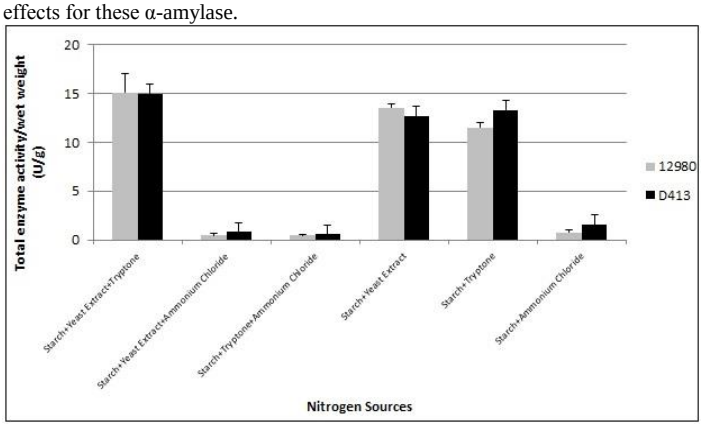

Figure 5 Effects of different nitrogen sources on amylase production by *G.stearothermophilus* ATCC 12980^T and *Geobacillus* sp. D413 at 55°C.

Among the organic nitrogen sources, neither_solely the yeast extract nor the tryptone addition increased the amylase activity for both of the enzymes. However, maximum enzyme production was observed at medium supplemented with both 0.5% yeast extract and 1% tryptone together as shown in Fig. 6. The amylase activities in the presence of yeast extract (0.5%) and tryptone (1%) were found to be 15.38 U/g and 13.6 U/g for D413 and ATCC 12980, respectively. If the concentrations of tryptone and yeast extract fell down in the medium, enzyme production of the reference strain showed a sharper decrease than the isolate D413. In a previous study of **Hamilton *et al.* (1999)** yeast extract turned out to be

the best nitrogen source for maximum amylase activity. However, **Saxena *et al.* (2007)** found the maximum amylase activity at medium supplemented with 0.5% peptone and 0.3% yeast extract for *Bacillus* sp. PN5. Therefore, it seems that the effects of various nitrogen sources also vary with the source organisms as in the case of carbon sources. As a consequence, the inorganic nitrogen source of 0.5 % ammonium chloride inhibit both the bacterial growth and amylase production; however, the organic sources like 0.5 % tryptone and 1 % yeast extract supported the growth and enzyme secretion for both strain ATCC 12980 and isolate D413. The effects of different carbon and nitrogen sources on the amylase production of these bacteria were also listed totally in Table 3.

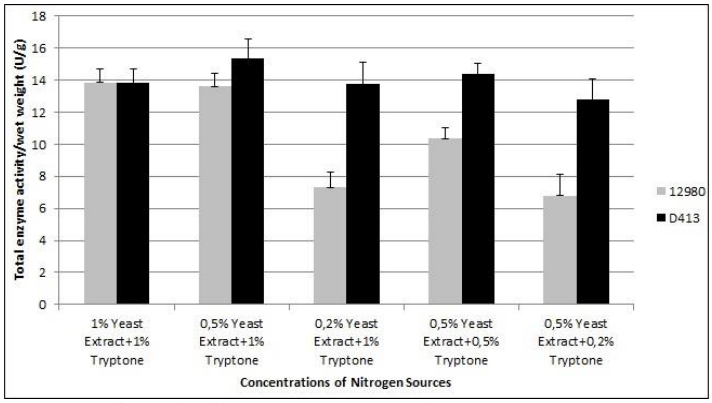

Figure 6 Effects of different concentrations of yeast extract and tryptone on amylase production by *G.stearothermophilus* ATCC 12980^T and *Geobacillus* sp. D413 at 55°C.

Table 3 Effects of different carbon and nitrogen sources on amylase production

Media (Content %)	*G. stearothermophilus* ATCC 12980		*Geobacillus sp*. D413	
	Enzyme activity (U/ml)	Enzyme activity/biomass (U/g)	Enzyme activity (U/ml)	Enzyme activity/biomass (U/g)
Starch + Yeast Extract + Tryptone (1; 0.5;1)	**0.95±0.16**	**13.6±1.48**	**1.23±0.05**	**15.38±1.59**
Starch + Yeast Extract + Tryptone (0.5; 0.5; 1.0)	0.55±0.10	6.19±1.21	0.73±0.04	10.53±0.66
Starch + Yeast Extract + Tryptone (0,2; 0.5;1.0)	0.16±0.04	3.29±1.03	0.38±0.02	4.79±0.29
Starch + Yeast Extract + Tryptone(1.0; 1.0; 1.0)	0.96±0.05	13.84±0.84	1.06±0.05	13.85±0.81
Starch + Yeast Extract + Tryptone (1.0; 0.2;1.0)	0.58±0.07	7.31±0.92	1.37 ±0.13	13.73±1.36
Starch + Yeast Extract + Tryptone (1.0; 0.5; 0.5)	0.73±0.05	10.36±0.64	1.44±0.21	14.38±2.10
Starch + Yeast Extract + Tryptone (1.0; 0.5; 0.2)	0.54±0.10	6.79±1.30	1.28±0.09	12.77±0.90
Starch + Yeast Extract (1.0; 0.5)	0.79±0.02	13.51±0.47	1.01±0.10	12.63±1.67
Starch + Tryptone (1.0;1.0)	0.92±0.04	11.48±0.54	1.06±0.10	13.32±1.35
Starch + Yeast Extract + NH_4Cl (1.0; 0.5; 0.5)	0.03±0.01	0.46±0.18	0.08±0.02	0.80±0.23
Starch + Tryptone + NH_4Cl (1.0; 1.0; 0.5)	0.03±0.008	0.44±0.07	0.06±0.01	0.55±0.10
Starch + NH_4Cl (1.0; 0.5)	0.02±0.001	0.59±0.21	0.05±0.01	1.56±0.33
Sucrose + Yeast Extract + Tryptone (1.0; 0.5;1.0)	0.01±0.008	0.21±0.018	0.01±0.007	0.14±0.11
Dextrose + Yeast Extract + Tryptone (1.0; 0.5; 1.0)	0.08±0.009	1.35±0.19	0.09±0.005	0.96±0.05
Lactose + Yeast Extract + Tryptone (1.0; 0.5; 1.0)	0.06±0.08	1.05±0.16	0.21±0.10	2.30±1.56
Maltose + Yeast Extract + Tryptone (1.0; 0.5; 1.0)	0.89±0.06	17.85±1.25	1.49±0.20	24.85±3.29

Effect of temperature and pH on enzyme activities and stabilities

Some distinctions were determined in both of these enzymes revealed by the temperature and pH activity and stabilities. The strain D413 extracellular α-amylase had temperature optima of 65°C and pH optima of 9.0. These values were found to be 65°C and pH 7.5 for strain ATCC 12980, respectively (Fig. 7a, b). After this temperature, especially for *Geobacillus* sp. D413, α-amylase activity was decreased. Both of these enzymes were very stable between temperature ranges from 45°C to 75°C and pH ranges from 4.0 to 10.5 (Fig. 8a, b). It is noteworthy that although the optimal growth pH (8.0) of ATCC 12980 was higher than D413 (pH 7.0) enzyme, the optimal amylase activity was observed in a value lower than D413 amylase. Notably, when the extracellular D413 α-amylase was incubated at 75°C for 10 min, it retained 65 % of its activity. This value was determined to be 54% for the reference strain. In addition, when D413 amylase was treated with buffers having pH:4.0 and 10.5 at 37°C for 15 h, it gained 98% and 86.5% of its activity, respectively. These were observed as pH 95% and 84.5% for ATCC 12980^T amylase.

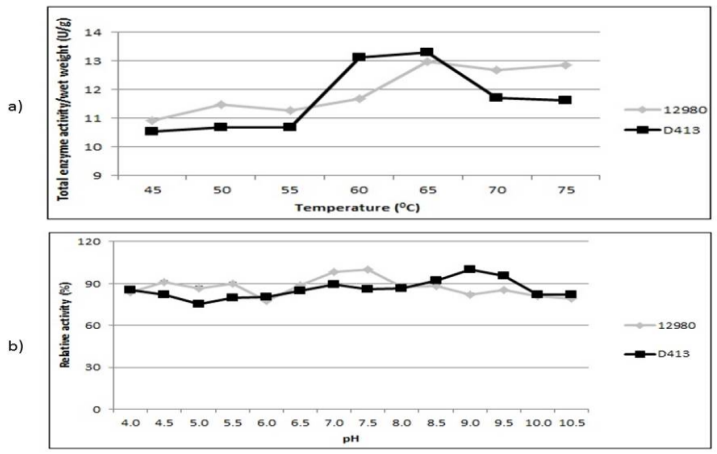

Figure 7 Effects of *G.stearothermophilus* ATCC 12980^T (◊) and *Geobacillus* sp. D413 (□) amylase activities under different a) temperatures at pH 7.0 and b) pH values at 65°C.

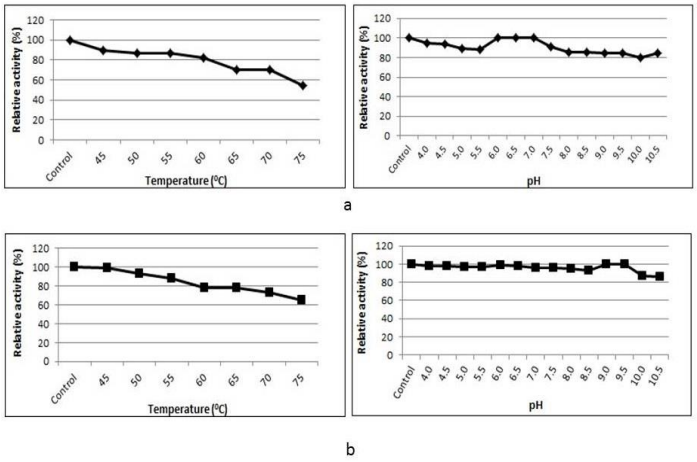

Figure 8 Temprerature and pH stabilities of amylases from a) *G. stearothermophilus* ATCC 12980^T (◊) and b) *Geobacillus* sp. D413 (□)

DISCUSSION

Amylases are among the most important enzymes and are of great significance in biotechnological processes. Particularly thermostable alpha amylases are used in many industrial processes at high temperature. In the present paper, we report the characterization of novel thermostable amylase produced by thermophilic strain *Geobacillus* sp. D413, which efficiently hydrolyzes the starch biomass. As a result of scanning performed amylase activity; *Geobacillus* sp. D413 has been selected as the best enzyme producing isolate. Enzyme production of *Geobacillus* sp. D413 has been optimized and used in *G. stearothermophilus* ATCC 12980.

Culture conditions (medium pH, incubation temperature, incubation time, and carbon and nitrogen sources) were determined for maximum enzyme production. For the maximum enzyme production optimal temperature and pH was found to be 55°C and pH:7.0, respectively for our isolate. In general, most of the thermophilic members from *Bacillaceae* family were reported to produce maximum enzyme production at temperatures ranging from 35°C to 60°C **(Kiran *et al.*, 2005; Dheeran *et al.*, 2010; Suman and Ramesh, 2010).** On the other hand, the enzyme production was found to be inhibited (≤1.0 U/g) when sucrose, lactose and glucose were used as carbon sources. The previous studies have revealed that especially production of carbohydrate degrading enzymes in most species of the genus *Bacillus* is exposed to catabolic repression being readily metabolized in the presence of substrates such as glucose **(Lin *et al.*, 1998).** As starch is an important substrate for fermentation experiments for amylases and as it was found to be the second carbon choice for amylase production by isolate D413 (15.38 U/g) and strain ATCC 12980^T (13.6 U/g). Also, the enzyme activity was found to be associated with the starch concentration. Therefore, a direct proportion was observed between the starch concentration in the medium and the amylase production for both of the bacteria. Similar to this finding, **Suman and Ramesh (2010)** demonstrated the optimum concentration of soluble starch to be 20 g /L for *Bacillus* strain KCPSS-12ss amylase.

The optimal temperature and pH for α-amylase activities were within the ranges (30 to 105°C) of the reported α-amylases from endospore-forming bacilli **(Teodoro and Martins, 2000; Ezeji and Bahl, 2006; Asgher *et al.*, 2007; Saxena *et al.*, 2007; Arikan, 2008; Asoodeh *et al.*, 2010).** Among the previous studies, **Ezeji and Bahl (2006)** reported that *G. thermodenitrificans* HRO10 amylase retained 53.4% of its original activity after incubation without substrate at 70°C for 10 min. These results are in agreement with the reports of **Ezeji and Bahl (2006)** and **Teodoro and Martins (2000)**; and in those studies optimum temperature was diminished beyond of 55°C and was inactivated at 95°C for ten min. Also **Oziengbe and Onilude (2012)** have determined that deterioration took place in the stability of *B. licheniformis* amylase after temperature above 70°C.

Similar results were reported in the study of **Asgher *et al.*, 2007**. In that study, they found that the a-amylase of *B. subtilis* JS-2004 strain lost only its %6 of its original activity after incubation for 24 h at its optimum pH. But in the study of **Ezeji and Bahl (2006)**, *G. thermodenitrificans* HRO10 α-amylase showed maximum activity in an acidic pH such as 5.5. Therefore, when compared with the ATCC 1280 and other source microorganisms, the extracellular α-amylase of D413 differed from other bacilli enzymes by means of its temperature and pH optima values, by its high thermostability at its optimal temperature and also by its broad pH stabilities. Especially the α-amylase of D413 can be more active and stable on an alkaline pH such as 9.0, and differed from ATCC 12980 enzymes having optimum pH 7.0 at neutral conditions.

CONCLUSION

Microbial α-amylases are in use in industrial processes occupying approximately 30% of the world enzyme market. As in these processes the desired parameters are thermal and pH stability, the α-amylase production, activity and stability conditions were determined for optimal amylase production from *Geobacillus* sp. D413 by comparing the reference strain *G. stearothermophilus* ATCC 12980 in this study. As a result of the optimization studies, it was observed that when D413 enzyme was incubated at 55°C and pH 7.0 in media containing 1% soluble starch, 0.5% yeast extract and 1% tryptone, it showed maximum activity at pH 9.0 and at 65°C. D413 α-amylase enzyme was also found to be stable at 75°C and pH 10.5. *Geobacillus* sp. D413 α-amylase possesses high conformational stability at elevated temperatures, has an optimal pH at alkaline environments and shows activity in broad pH ranges; all these characteristic properties of the extracellular *Geobacillus* sp. D413 α-amylase are required parameters in enzymes desired for biotechnologically important industrial applications. Thus, when its pH optima and conformational stability are considered, the D413 α-amylase may prove to have a potential in biotechnological processes such as hydrolysis of starch to glucose.

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