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ARTICLE

Thermodynamic and kinetic characteristics of an α-amylase from *Bacillus licheniformis* SKB4

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ABSTRACT An amylolytic bacterial strain, *Bacillus licheniformis* SKB4 produced maximum amylase at pH 6.5 at 42 °C, and at late stationary phase (24 h) of growth. Starch and peptone were found the best supporting carbon and nitrogen source with C:N ratio of 1:2 for amylase production. The purified enzyme was non-responsive to most of the metal ions except K^+ and Mg^{++} (1.0 mM). The enzyme was stable and active at pH 6.5. The enzyme showed optimum temperature at 90 °C with 10 min of half life ($t_{½}$) at 100 °C. The Q_{10} of the enzyme was 1.0. The thermodynamic principles like activation energy, free energy for substrate binding and transition state of the enzyme were found 31.53, 5.53 and -17.4 KJ/Mol of starch, respectively. The kinetic constant like V_{max}, K_m, K_{cat} and catalytic efficiency (K_{cat}/K_m) for starch were found to be 1.04 µmol mg^{-1} min^{-1}, 6.2 mg ml^{-1}, 2 × 10^3 S^{-1} and 3.22 × 10^2 ml mg^{-1} S^{-1}, respectively. All these findings suggested that this amylase has unique characteristics for starch hydrolysis in respect to thermostability and kinetic properties. **Acta Biol Szeged 58(2):147-156 (2014)**

KEY WORDS

α-amylase
Bacillus licheniformis
thermodynamic characteristics
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Alpha-amylase (EC 3.2.1.1, 1,4-α-D-glucan glucano hydrolase, endoamylase) hydrolyzes starch, glycogen and related polysaccharides randomly by cleaving the internal α-1,4-glucosidic linkages and have great success by replacing the chemical hydrolysis of starch. α-Amylases account for ~30% of the enzyme market (Shivramakrishnan et al. 2006) and it is widely used in various industries like alcohol, brewing, sugar for liquefaction of starch; in textile industry for desizing of fabrics, etc (Gupta et al. 2003). The exploitation of amylase in world market is estimated to increase by 4% annually, significantly uses in detergents (37%), textiles (12%), starch (11%), baking (8%) and animal feed (6%) industries (Deb et al. 2013). Bacterial α-amylases, particularly from the *Bacillus* species are of special interest for their large-scale production under simple cultivation system and of remarkable thermostability (Prakash and Jaiswal 2010). Heat-resistant enzymes offer commercial opportunities because higher temperatures can overcome the viscosity problems of substrate (e.g. starch) and accelerated endothermic reactions (Kikani et al. 2012).

Microbial enzyme production is generally subjected to the influence of different parameters of culture condition; therefore it is necessary to standardize the cultural and nutritional conditions of the organism. In industry, submerged fermentation process is generally employed for the production of microbial enzymes due to the easy control of different physico-chemical parameters, less chance of contamination and enzyme remain in the culture fluid somehow as purified form (Coronado et al. 2000; Gangadharan et al. 2006).

In view of the versatile appliances of thermophilic α-amylase in different sectors, attempt has been made to optimize the submerged culture condition and characterize some relevant properties of the purified α-amylase from *Bacillus licheniformis* SKB4.

Materials and Methods

Microorganism

Bacillus licheniformis SKB4, a soil isolate (Samanta et al. 2009) was used for the present study.

Culture condition for amylase production

Enzyme production was carried out in 250-ml Erlenmeyer flask containing 50 ml basal medium (pH 6.5) containing (g l^{-1}): starch, 5; peptone, 10; beef extract, 5; KH_2PO_4, 3; $MgSO_4$ 0.5; $CaCl_2$; 0.02. It was inoculated with 1% (v/v) freshly prepared inoculum and incubated on a rotary shaker (120 rpm) at 42 °C for 28 h. The growth of the bacteria was measured at 620 nm. The pH of the culture medium was also measured in different time intervals. The culture supernatant obtained by centrifugation (5000 rpm for 10 min) was considered as crude source of enzyme and assayed for amylase activity.

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Optimization of culture conditions

To determine the optimal cultural condition for enzyme production, the most effective level of individual parameters, *viz*., initial medium pH (3.0-10.0), growth temperature (30 °C - 50 °C), soluble sugars (glucose, mannose, galactose, fructose, inositol, arabinose, sucrose, lactose, maltose, rhamnose, xylose, ribose, raffinose, starch in 0.1-0.5 g % w/v concentrations) and nitrogen sources (peptone, beef extract, tryptone, urea, yeast extract, thiourea, NH_4NO_3, $(NH_4)_2HPO_4$, $(NH_4)H_2PO_4$, $NaNO_3$ in 0.1-0.5 g % w/v concentrations) were studied following one variable at a time (OVAT) optimization protocol.

Assay of α-amylase

Amylase activity was determined by studying its saccharolytic properties according to Bernfield (1955). Briefly, the reaction mixture consisted 0.5 ml of 1% (w/v) starch, 0.4 ml of phosphate buffer (10 mM, pH 6.5) and 0.1 ml of enzyme solution and incubated for 5 min at 90 °C. The reaction was stopped by addition of 1 ml of 3,5 dinitrosalicylate (DNS) reagent. The quantity of reducing sugar was measured colorimetrically at 530 nm using glucose as standard sugar. The unit of amylase was defined as the amount of enzyme which produced 1μmol of reducing sugar as glucose in 1 min under specified condition.

Purification of amylase

The concentrated culture broth was brought to 35% saturation with analytical grade $(NH_4)_2SO_4$ and kept overnight at 4 °C. The supernatant was separated by centrifugation (10 000 × g for 10 min) and the precipitate was discarded after examining its amylolytic activity. Then 80% saturation was brought by adding additional $(NH_4)_2SO_4$ and was allowed to stand overnight at 4 °C. The precipitate was collected by centrifugation (12 000 × g for 30 min at 4 °C) and dissolved in 10 mM phosphate buffer (pH 6.5). The precipitate was then dialyzed against the same buffer for 24 h with a periodical change of buffer solution. The dialyzed enzyme was then applied to a DEAE cellulose column (Merck, Mumbai, India) and the enzyme was eluted with a linear gradient of KCl (0.01-0.1M). The active fraction in the flow through were collected and concentrated in lyophilizer. At the next stage, the amylase solution put on a Sephadex G-100 column (1.5 x 92 cm) that pre-equilibrated with 10 mM phosphate buffer (pH 6.5) and then eluted with the same buffer. The active enzyme fraction were collected and concentrated and stored at 4 °C for further use.

Effect of metal ions on amylase activity

Different metal ions (at a concentration of 1-100 mM) in assay buffer (10 mM phosphate buffer pH 6.5) were incubated with the enzyme at 37 °C for 30 min. The residual activities were examined under standard assay conditions.

Influence of pH on amylase activity and stability

The optimum pH of enzyme activity was measured at different pH levels from 3.0-11.0 [10 mM citrate buffer (pH 3.0-5.8), 10 mM phosphate buffer (pH 6.0-8.0) and 10 mM bi-carbonate buffer (pH 9.0 to 11.0)]. To determine the pH stability, the enzyme solution was mixed with suitable buffer at different pH values (10 mM, pH 3.0-11.0) and incubated at 4 °C for 24 h and then residual activity was measured at optimum temperature.

Determination of optimum temperature and apparent half life ($t_{1/2}$) of amylase

The optimum temperature of enzyme activity was determined by incubating the reaction mixture separately at different tem-

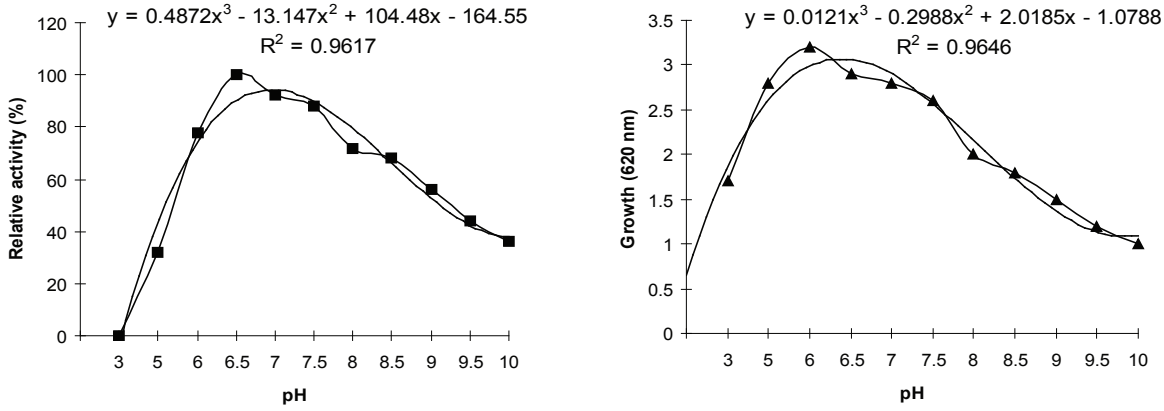

Figure 1. Effect of initial medium pH on amylase production (■) and growth (▲) of *B. licheniformis* SKB4. The pH of medium adjusted after sterilization. Bacteria were grown on a rotary shaker (120 rpm) at 42 °C for 28 h.

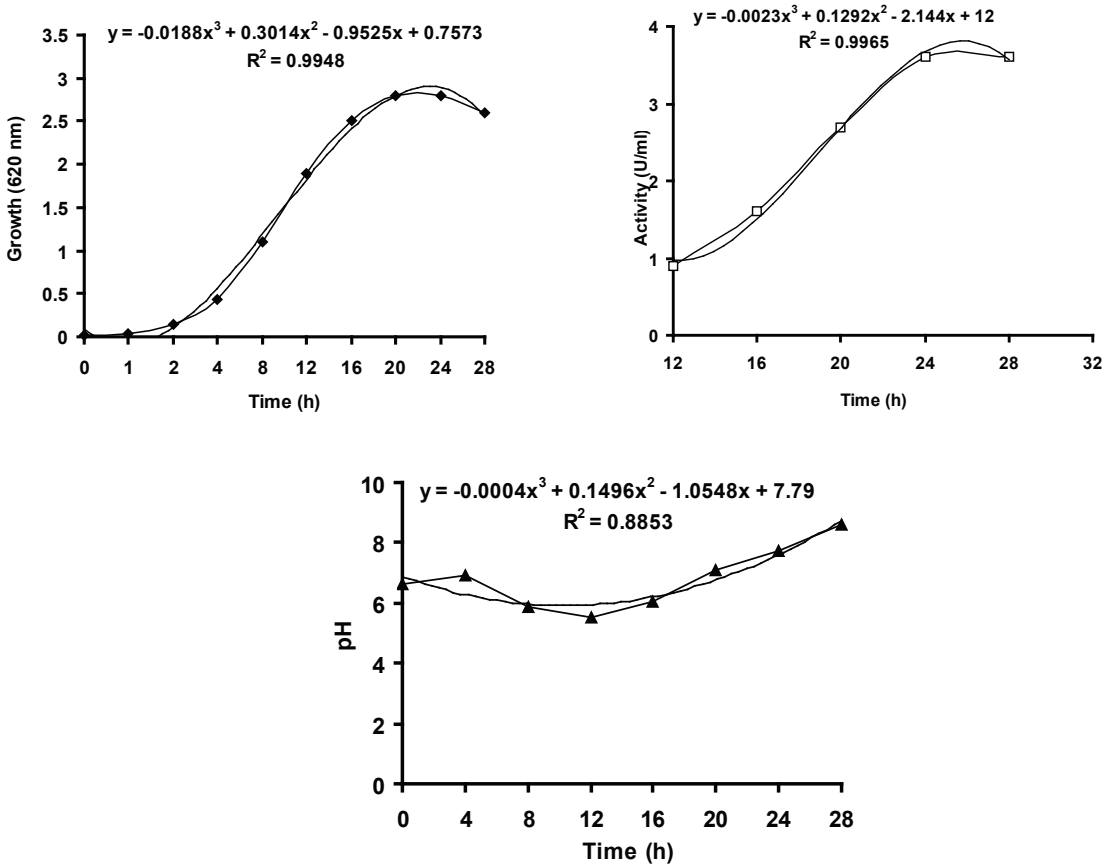

Figure 2. Cell growth (♦) and enzyme production (□) of *B. licheniformis* SKB4. Incubation period was 28 h at 42 °C on a rotary shaker (120 rpm). Initial medium pH adjusted at 6.5. The pH (▲) of the growing medium increased during growth of the bacteria.

perature from 30-100 °C. The apparent half life ($t_{1/2}$) of amylase at 100 °C was estimated on the basis of thermostability of enzyme in the absence of substrate. The enzyme solution was taken in a stoppered test tube and placed in a water bath at 100 °C. The enzyme solution was taken out after regular interval and assayed immediately after addition of substrate. $t_{1/2}$ was estimated at when 50% activity was restored.

Thermodynamic characteristics of amylase activity

The activation energy (Ea) for substrate hydrolysis under the given experimental conditions was determined by plotting the data in an Arrhenius plot (Das et al. 2012). The free energy for substrate binding and transition state formation was calculated using the following derivations (Saqib et al. 2010):

$\Delta G^*_{E\text{-}S}$ (free energy of substrate binding) = -RT *ln* Ka

Where, Ka = $1/K_m$, R is the universal gas constant (8.314 JK^{-1} mol^{-1}) and T is the absolute temperature (K).

$\Delta G^*_{E\text{-}T}$ (free energy of transition state formation) = -RT *ln* (K_{cat}/K_m)

The temperature coefficient (Q_{10}) is the rate of an enzymatic catalytic reaction changes for every 10 °C rise in temperature, was calculated by the Dixon and Webb equation (Dixon and Webb 1979).

$ln\ Q_{10} = E_a \times 10/RT^2$

Where, Ea is the activation energy of the enzyme (J mol^{-1}).

Determination of kinetic constants for substrate

The Michaelis constant (K_m), maximum reaction velocity (V_{max}), turn over number (K_{cat}) and catalytic efficiency (K_{cat}/K_m) of purified amylase for starch were determined by using Lineweaver and Burk plot (Lineweaver and Burk 1934).

Results and Discussion

Effect of initial medium pH and incubation time on amylase production

pH is one of the important determinant for growth and overall activities of microbes. The catalytic activity and three dimensional structure of enzyme are of particularly very sensitive to hydrogen ion concentration. The experimental organism, *B.*

Table 1. Effect of soluble sugar on growth and amylase formation of *B. licheniformis* SKB4. Fermentation condition: temperature 42 °C, initial medium pH 6.5, incubation time 28 h. Data represented as the average of three separate experiments.

Carbon source	Concentration (g% w/v)	Growth (620 nm)	Enzyme activity (U/ml)
Control (without C source)		1.10	0.10
Glucose	0.1	2.40	0.23
	0.3	2.40	0.15
	0.5	3.6	0.10
Mannose	0.1	2.25	0.03
	0.3	3.60	0.05
	0.5	3.20	0.26
Galactose	0.1	3.00	0.15
	0.3	3.15	0.20
	0.5	3.30	0.27
Fructose	0.1	2.85	0.12
	0.3	3.60	0.09
	0.5	3.60	0.07
Inositol	0.1	2.10	0.06
	0.3	3.30	0.10
	0.5	3.90	0.10
Arabinose	0.1	2.70	0.06
	0.3	2.40	0.09
	0.5	2.25	0.09
Sucrose	0.1	2.25	0.21
	0.3	2.55	0.17
	0.5	3.00	0.05
Lactose	0.1	2.40	0.11
	0.3	2.40	0.27
	0.5	1.80	0.50
Maltose	0.1	2.55	0.11
	0.3	2.85	0.21
	0.5	3.00	0.30
Rhamnose	0.1	1.86	0.02
	0.3	3.15	0.08
	0.5	3.45	0.05
Xylose	0.1	2.40	0.08
	0.3	3.30	0.08
	0.5	3.90	0.10
Ribose	0.1	2.40	0.18
	0.3	2.10	0.15
	0.5	3.30	0.04
Raffinose	0.1	2.40	0.06
	0.3	3.30	0.06
	0.5	3.60	0.03
Starch	0.1	2.6	0.45
	0.3	2.7	0.65
	0.5	3.0	0.90

Table 2. Effect of different nitrogen sources on growth and amylase production of *B. licheniformis* SKB4 (incubation time 28 h, initial medium pH 6.5, temperature 42 °C). Data represented as the average of three separate experiments.

Nitrogen source	Concentration (g% w/v)	Growth (620 nm)	Enzyme activity (U/ml)
Control		0.08	0.10
NH_4NO_3	0.1	0.10	0.08
	0.3	0.20	0.07
	0.5	0.32	0.02
$(NH_4)_2HPO_4$	0.1	0.45	0.10
	0.3	0.62	0.14
	0.5	0.80	0.08
$(NH_4)H_2PO_4$	0.1	0.30	0.07
	0.3	0.72	0.05
	0.5	0.65	0.03
$NaNO_3$	0.1	0.22	0.07
	0.3	0.46	0.04
	0.5	0.58	0.04
Urea	0.1	0.90	0.08
	0.3	0.85	0.05
	0.5	0.55	0.05
Beef extract	0.1	2.0	0.10
	0.3	3.0	0.16
	0.5	3.5	0.18
Peptone	0.1	2.2	0.17
	0.3	2.8	0.20
	0.5	3.2	0.32
Tryptone	0.1	0.95	0.10
	0.3	0.95	0.06
	0.5	1.30	0.07
Yeast extract	0.1	0.90	0.12
	0.3	2.6	0.12
	0.5	2.8	0.10
Thiourea	0.1	0.35	0.10
	0.3	0.10	0.03
	0.5	0.10	0.03

licheniformis SKB4 grew well over a wide range of pH, *i.e.*, 3.0 to 10.0, produced maximum amylase at pH 6.5 (Fig. 1), but it showed maximum growth at pH 6.0. Most of the starch degrading bacterial strains are reported to produce enzyme in a pH range between 6.0 and 7.0 (Haq et al. 2002; Gupta et al. 2003; Rasooli et al. 2008).

B. licheniformis SKB4 initiated to synthesize amylase from the 12th h of active log phase, and maximum amount was noted during its late stationary phase (24 h) (Fig. 2). Similar pattern of amylase synthesis was also observed by Boži et al. (2011) in *Bacillus subtilis* IP 5832. Wanderley et al. (2004) suggested that effective induction of amylase did not occur until the stationary phase of growth and it related to the depletion of readily available carbon source. Moreover, maximum α-amylase production by *B. licheniformis* Shahed-07 occurred when cell growth reached to the stationary phase (26 h), suggesting that enzyme secretion is not growth related rather it depends on the quantity of cell mass (Rasooli et al. 2008).

In relation to growth, pH of the media was tended to acidic up to 12 h of growth, but, thereafter changed towards alkalinity (Fig. 2). The initial decrease of pH is due to fast growth of the organisms which produced acids from carbohydrate fermentation, and the latter change of pH towards alkalinity may due to cell lysis (de Oliveira et al. 2010). Similar fashion of cell growth, enzyme production and changes in pH of the culture media were observed in case of *Bacillus* sp. IMD 434

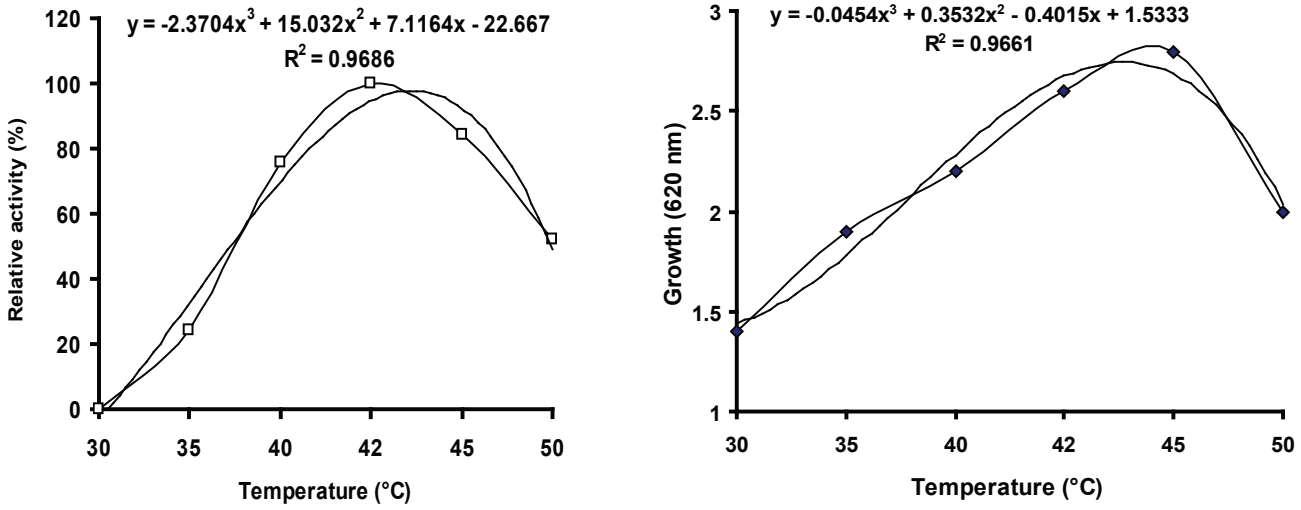

Figure 3. Effect of incubation temperature on amylase production (□) and growth (♦) of *B. licheniformis* SKB4. The pH of medium adjusted at 6.5. Bacteria were grown on a rotary shaker (120 rpm) for 28 h at different temperatures (30-50 °C).

(Hamilton et al. 1999) and *Bacillus* sp. IMD 370 (Mc Tigue et al. 1995).

Effect of temperature on amylase production

The studied bacterium, *B. licheniformis* SKB4 grown well at 30 °C, but it produced notable quantity of amylase at 35 °C. Maximum level of enzyme production was found at 42 °C, but highest growth was achieved at 45 °C (Fig. 3). Previously, it was reported that maximum level of α-amylase production by *B. subtilis* 147 (Avdiiuk and Varbanets 2008) and *Bacillus* sp. GHA1 (Ahmadi et al. 2010) occurred at 42 °C. Aiba et al. (1983) reported that the high temperature may inactivate the expression of genes responsible for the starch degrading enzymes.

Effect of carbon and nitrogen sources on amylase production

A range of soluble sugars at various concentrations (0.1-0.5%, w/v) were tested for growth and amylase production of *B. licheniformis* SKB4 and the results are summarized in Table 1. Among the carbon sources, starch was found to be best inducer for amylase synthesis followed by lactose > maltose > galactose > mannose. Whereas, other sugars like fructose, inositol, arabinose, rhamnose, xylose, and raffinose could be considered as poor producer of amylase. Similar type of results was also noted in *B. licheniformis* (Chandra et al. 1980, Rasooli et al. 2008). Aiyer (2004) and Rasooli et al. (2008) also suggested that starch is a good inducer for amylase synthesis in *B. licheniformis*. Glucose, sucrose and ribose in 0.1% (w/v) concentration act as moderate carbon source for amylase production (Table 1), but enzyme synthesis was inhibited at their higher concentrations (>0.1%, w/v). Similar type of findings was also noted in case of *Bacillus polymyxa* and *B. subtilis* (Nickless et al. 2001) and their amylase producing gene was turned off (catabolite repression) in presence of glucose or fructose. The bacterial cells growing on easily utilizable carbon sources such as glucose or sucrose did not need to waste valuable energy for the biosynthesis of amylase. Therefore, the gene for amylase is turned off (Nickless et al. 2001). Haseltine et al. (1996) argued that a sensory system facilitated the detection of exogenous starch which acted as mediator for induction of α-amylase synthesis. The present findings suggest that the *B. licheniformis* SKB4 α-amylase induction by starch and repression by glucose was subjected to multiple forms of regulations.

It was found that enzyme production increased along with starch concentration upto 0.5% (w/v) and above it decreased (Fig. 4). But growth of the bacteria reached highest level at 1% (w/v) starch concentration. Rukhaiyar and Srivastava (1995) explained that culture broth viscosity has increased at high concentration of starch which interfere the oxygen transfer leading to limitation of dissolved oxygen for the growth of bacteria as well as its enzyme synthesis.

The effect of nitrogen sources over the concentration range of 0.1 to 0.5% (w/v) on growth and amylase production of *B. licheniformis* SKB4 was tested and represented in Table 2. Among the various nitrogen sources, peptone (0.5%, w/v) favoured maximum amylase production. The increasing order of amylase production by the stimulatory nitrogen sources can be arranged as: peptone (0.5%, w/v) > beef extract (0.5%, w/v) > $(NH_4)_2HPO_4$ (0.3%, w/v) > yeast extract (0.1%, w/v). Low concentration of nitrogen is inadequate for the enzyme production and excess nitrogen is equally detrimental causing inhibition of microbial growth as well

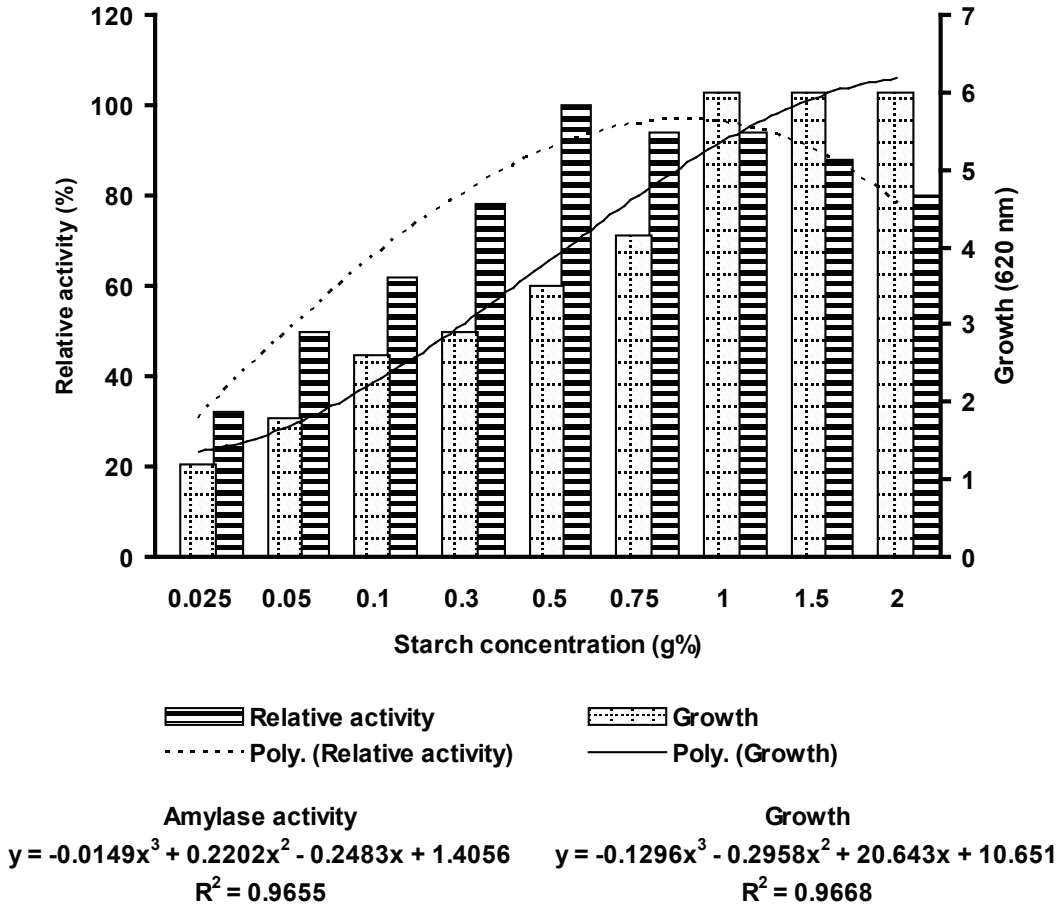

Figure 4. Effect of starch concentration on growth and amylase production of *B. licheniformis* SKB4. Basal medium contained starch at various concentration (0.025-2.0 % w/v), initial pH 6.5, temperature 42 °C, incubation time 28 h.

as enzyme synthesis (Aiyer 2004). Mc Tigue et al. (1995) also reported that peptone was a better nitrogen source for amylase production of .*B. licheniformis* SPT 278 than that of other organic and inorganic nitrogen sources. The maximum amylase yield was achieved when peptone concentration was at 1.0% with C/N ratio of reached 0.5 (Fig. 5, Table 3). This indicated that the nature and relative concentrations of carbon and nitrogen sources are important for amylase production from *B. licheniformis* SKB4. Earlier reports also revealed the C/N ratio of 1:1 to 1:2 was very effective for α-amylase production by different strains of *Bacillus* sp. (Aiyer 2004, Avdiiuk and Varbanets 2008).

Table 3. Effect of C/N ratio on amylase production.

C/N ratio	Activity of enzyme (%)
2.5	44
1.0	72
0.66	86
0.50	100
0.33	100

Effect of metal ions on enzyme activity

The effectiveness of a group of metal ions on the activity of purified amylase (M_w 60 kDa) was tested and represented in Table 4. Among them, Mg^{2+} and K^{+} only increased the enzyme activity at very low concentration (1 mM). It was observed that Zn^{2+}, Fe^{2+}, Cu^{2+} inactivated the enzyme activity at higher concentration (0.1 M), whereas, Hg^{2+} inhibited at lower concentration. This observation indicated that the activity of amylase from *B. licheniformis* SKB4 may not metal dependent. The results were comparable with the findings of other studies (Shaw et al. 1995; Rao et al. 2002).

Effect of pH on stability and activity of amylase

The optimum pH of the amylase activity was found at 6.5 (Fig. 6). The enzyme was stable at this condition (Fig. 6). The enzyme retained considerable activity in the pH range of 6.0–9.0. It also retained about 58% and 18% of its original

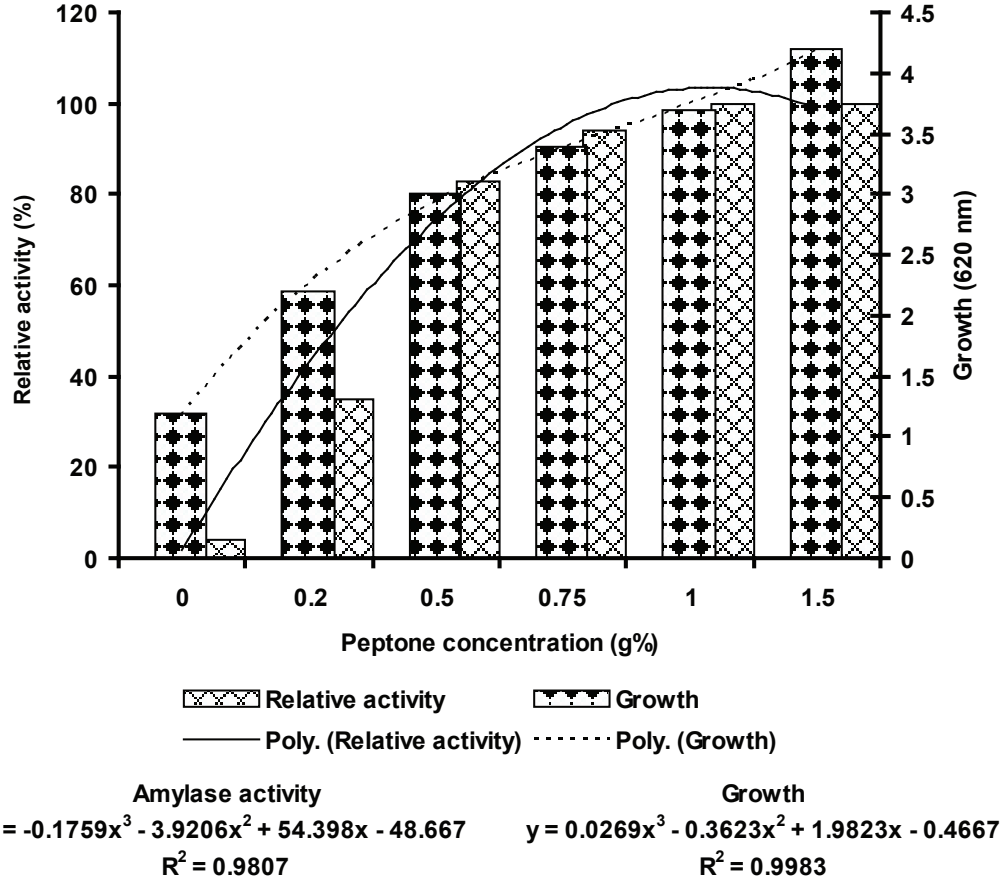

Figure 5. Effect of peptone concentration on growth and amylase production of *B. licheniformis* SKB4. Basal medium contained various concentration of peptone (0.2-1.5% w/v). Initial pH, temperature and incubation were 6.5, 42 °C and 28 h, respectively.

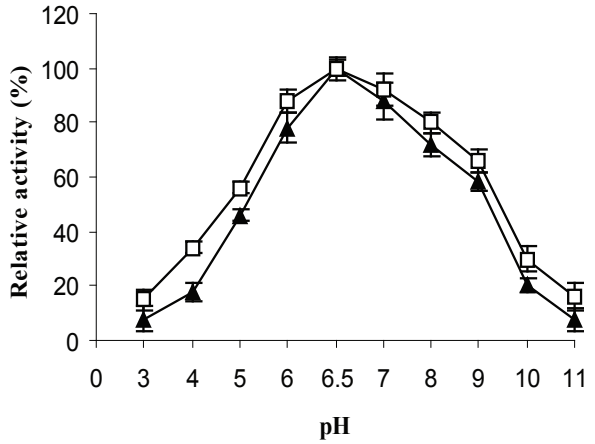

Figure 6. Effect of pH on enzyme activity (▲) and stability (□) of *B. licheniformis* SKB4 amylase. pH of the reaction buffer was varied (3-11). Amylase activity measured at 90 °C.

activity at pH 9.0 and 4.0, respectively. Our results are comparable with α-amylase from *B. subtilis* ITBCCB148 (Yandri et al. 2010) and *Bacillus cereus* GUF8 (Mahdavi et al. 2010).

Effect of temperature on amylase activity

Activity of α-amylase from *B. licheniformis* SKB4 was measured over a temperature range of 30 °C to 100 °C. The enzyme showed optimum activity at 90 °C (Fig. 7). It was also observed that 90% of its original activity was retained at 70 °C after 1 h incubation (Fig. 8). Other reports indicated that α-amylase from *B. licheniformis* (Adeyanju et al. 2007), *B. cereus* MK (Mrudula and Kokila 2010) were also active at 90 °C.

The half-life ($t_{1/2}$) of α-amylase of *B. licheniformis* SKB4 was 10 min at 100 °C and the temperature coefficient value (Q_{10}) was found to be 1.0 (Table 5). The Q_{10} for enzymes generally achieved between the range of 1 and 2 (Singh and Chhatpar 2011).

Determination of kinetic and thermodyanamic indices

The kinetic constants of the α-amylase like K_m (the Michaelis constant) and V_{max} (the maximum reaction rate) for starch

Table 4. Effect of metal ions on the activity of purified *B. licheniformis* SKB4 amylase. Enzyme activity was measured at pH 6.5 and 90 °C. Data represented as the average of three separate experiments.

Metal ions	Concentrations (M)	Relative activity (%)	% of change
Control		100	-
$MnCl_2$	0.1	20	80 (-)
	0.01	80	20 (-)
	0.001	86.6	13.4 (-)
$ZnCl_2$	0.1	0	100 (-)
	0.01	23	77 (-)
	0.001	76.6	23.4 (-)
$HgCl_2$	0.1	0	100 (-)
	0.01	8	92 (-)
	0.001	13	87 (-)
$CoCl_2$	0.1	20	80 (-)
	0.01	63	37 (-)
	0.001	76.6	23.4 (-)
$FeCl_3$	0.1	10	90 (-)
	0.01	86.6	13.4 (-)
	0.001	93	7 (-)
$CaCl_2$	0.1	24	76 (-)
	0.01	76	24 (-)
	0.001	93	7 (-)
KCl	0.1	86.6	13.4 (-)
	0.01	95	5 (-)
	0.001	103	3 (+)
$MgCl_2$	0.1	80	20 (-)
	0.01	86.6	13.4 (-)
	0.001	110	10 (+)
$CuCl_2$	0.1	0	100 (-)
	0.01	46.6	53.4 (-)
	0.001	60	40 (-)

(+) indicates stimulatory effect, (-) indicates inhibitory effect.

were found to be 6.2 mg ml^{-1} and 1.04 µmol mg^{-1} min^{-1} (Table 5), respectively, at 90 °C and pH 6.5. The K_m and V_{max} of α-amylase from *Bacillus stearothermophilus* GRE1 were also found to be 4.78 mg ml^{-1} and 6.67 mg ml^{-1} min^{-1}, respectively (Hossain et al. 2006). The turn over number (K_{cat}), which is the second-order rate constant for the conversion of the enzyme-substrate complex to the product, was calculated as 2×10^3 S^{-1} (Table 5). The apparent second-order rate constant, also called catalytic efficiency (K_{cat}/K_m), was 3.22×10^2 ml mg^{-1} S^{-1} (Table 5). All these characteristics are unique in the studied amylase, indicating its higher catalytic efficiency against starch like homopolysaccharides. α-Amylase from a mutant strain of *B. licheniformis* EMS-6 also showed similar K_{cat} and K_{cat}/K_m values (Haq et al. 2010).

Thermodynamic parameters provide a detailed portrait of inside of many chemical and biological reactions (Tanaka and Hoshino 2003). The activation energy (E_a) of the amylase of *B. licheniformis* SKB4 for starch hydrolysis was found to be 31.53, KJ/mol at 90 °C (Table 5). Previously it was reported that α-amylase from *B. licheniformis* EMS-6 was 25.14 KJ/mol (Haq et al. 2010). The free energy for substrate binding

Table 5. Summary of different kinetics and thermodynamic properties of purified *B. licheniformis* SKB4 amylase. Thermodynamic parameters were calculated at 90 °C.

Parameters	Purified amylase
K_m (mg/ml)	6.2
V_{max} (µmol/mg/min)	1.04
K_{cat} (S^{-1})	2×10^3
K_{cat} / K_m (ml mg^{-1} S^{-1})	3.22×10^2
E_a (KJ mol^{-1})	31.53
Q_{10}	1.0
$\Delta G_{E\text{-}S}$ (KJ mol^{-1})	5.53
$\Delta G_{E\text{-}T}$ (KJ mol^{-1})	-17.4
$T_{1/2}$ (min) (100 °C)	10

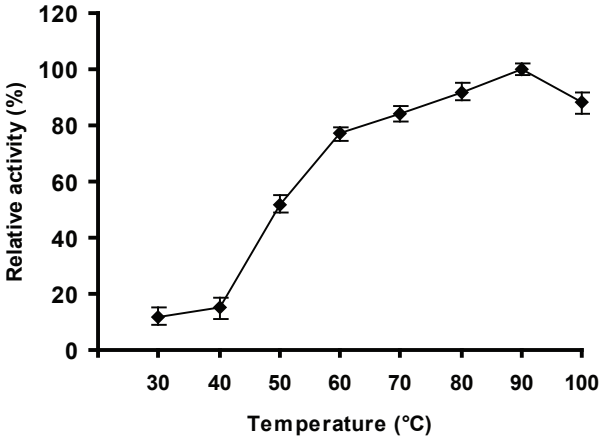

Figure 7. Effect of temperature on the activity of *B. licheniformis* SKB4 amylase. Temperature range of the reaction was 30-100 °C and amylase activity measured at pH 6.5.

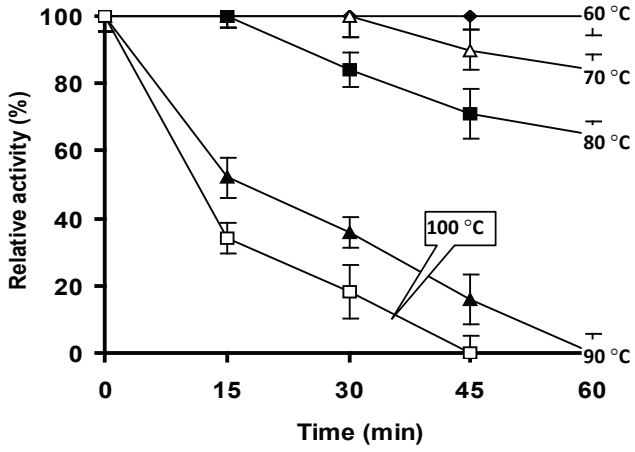

Figure 8. Study of thermostability of amylase from *B. licheniformis* SKB4 in absence of substrate after 1 h incubation. Amylase activity measured at pH 6.5 and 90 °C.

($\Delta G^*_{E\text{-}S}$) and transition state for formation of an activated enzyme substrate complex ($\Delta G^*_{E\text{-}T}$) of the enzyme at 90 °C were found to be 5.53 and –17.4 KJ/mol, respectively (Table 5). The free energy is required for the formation of activation complex and catalytic activity. The values of $\Delta G^*_{E\text{-}S}$ and $\Delta G^*_{E\text{-}T}$ indicated higher affinity of enzyme towards soluble starch for hydrolysis. The values of $\Delta G^*_{E\text{-}S}$ $\Delta G^*_{E\text{-}T}$ for glucoamylase from *Arachniotus citrinus* were found to be –2.95 and –17.7 kJ mol^{-1}, respectively (Perveen et al. 2006).

The present study revealed that *B. licheniformis* SKB4 is a potential strain for alpha amylase production in presence of soluble starch as substrate and complex nitrogen sources such as peptone. The high thermostability and metal independence of this amylase will be very effective for soluble starch hydrolysis. Various kinetic and thermodynamic parameters of the enzyme suggested that it has great affinity to catalyze soluble starch and suitable for many commercial applications, such as pharmaceutical, detergent and food industries.

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