ISSN 0975-6299

Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

BIO TECHENOLOGY

EFFECT OF pH, TEMPERATURE AND METAL IONS ON AMYLASE ACTIVITY FROM *BACILLUS SUBTILIS* KCX 006

K. AMUTHA[1] AND K. JAYA PRIYA[*2]

[1]Department of Biotechnology, VELS University, Old Pallavaram, Chennai 600117, Tamil Nadu, India.
[2]Department of Biotechnology, Prince Shri Venkateshwara Arts and Science College, Gowrivakkam, Chennai 601302, Tamil Nadu, India

K. JAYA PRIYA
Department of Biotechnology, Prince Shri Venkateshwara Arts and Science College, Gowrivakkam, Chennai 601302, Tamil Nadu, India

*Corresponding author

ABSTRACT

Under different growth conditions such as pH, temperature and metal ions, *Bacillus subtilis* KCX006 was tested for amylase production and kinetic properties of amylase was determined. The enzyme activity was more in the stationary phase and the value was 21.78 U/mL (at 30 h) and protein content was 0.303 mg/mL. The maximum amylase activity (38 U/mL) was also found at stationary phase when the culture was grown at 37°C. The enzyme was purified to homogeneity with an overall recovery of 24.2% and specific activity of 4133 U/mg. Either acidic or alkaline condition significantly affected the growth and enzyme production. Enzyme activity was more in reaction mixture containing $NiCl_2$ and its residual activity was found to be 117.5%. These results suggest possible application in food and detergent industries where moderate pH and temperature are employed.

ISSN 0975-6299 Vol 2/Issue 2/Apr-Jun 2011

KEYWORDS

Bacillus subtilis KCX006, Amylase, pH and Temperature.

INTRODUCTION

Enzymes have immense catalytic power and accelerate reaction by reducing energy for at least million times for its activation. Enzymes easily become denatured and lose catalytic activity. In general temperature higher than 40°C and extreme pH should be avoided at all times. Enzyme preparation should also be stored at low temperature, but not necessarily frozen, since freezing and thawing may causes loses of activity. Hydrolytic activity of alpha-amylase from *Aspergillus terricola* was found to vary symbiotically with the specific optic rotation in the above pH range[1].

Amylases are most important biocatalyst due to their ability to utilize a wide spectrum of substrates, high stability towards extreme temperature, pH etc. Among microbial, plant and animal enzymes, microbial amylases have immense applications in various fields[2]. On the whole, bacterial amylase preparations are preferred to those of fungal origin for this work because of their greater heat stability[3]. Number of amylases with different optimum pH and temperature has also been reported[4].

Enzymes are vulnerable to various environmental factors. Their activity may be significantly diminished or destroyed by a variety of physical or chemical agents resulting in a loss of the functions performed by the enzymes. The present study was aimed to optimize pH, temperature, substrate and salt concentration for maximum production of protease from *Bacillus subtilis* BS1 and its stability. Here in this work we report purification and characterization of an amylase from, *Bacillus subtilis* KCX006. Reports were there regarding alpha-amylase production from *Bacillus subtilis* KCC103[5].

MATERIALS AND METHODS

i. Culture condition

Bacillus subtilis KCX006 was brought from Prince Shri Venkateshwara Arts and Science College culture collection and was grown overnight in a 5 mL nutrient medium at 37°C. A 1 mL of this culture was transferred to 50 mL of nutrient medium containing 0.5% starch and grown for 48 h at 37°C in a shaker (200 rpm). At every 6 h, 1 mL of culture samples was collected and growth was measured at absorbance 600 nm. Amylase activity in the culture supernatant was estimated.

ii. Enzyme assay

Amylase activity was checked by measuring the reducing sugar formed by the enzymatic hydrolysis of soluble starch. The reaction mixture containing 0.5 mL of 1% (w/v) soluble starch in 0.45 mL of 50 mM acetate buffer (pH 5.5) and 50 µL of the enzyme was incubated at 60°C for 10 min. The reaction was stopped by addition of 1 mL of dinitrosalicylic acid (DNS) reagent[6]. The reaction mixture was heated for 10–15 min in boiling water bath and absorbance was read at 540 nm to estimate reducing sugars released[7].

One unit of enzyme activity was defined as the amount of enzyme that liberated 1 µM of reducing sugar as glucose equivalents in 1 min under the assay condition. The enzyme was purified by ammonium sulphate precipitation method and amylase enzyme was separated by DEAE-sephadex anionic exchange column chromatography method[8]. Temperature effect of pure enzyme was obtained by incubating 1 mL of

pure enzyme at different temperature ranging from 40 to 80°C. At every 10 min of incubation 160 µL of sample was taken and kept in ice. Enzyme activities of these samples were checked. Metal ions such as $NiCl_2$, $AgSO_4$, $BaCl_2$, $CaCl_2$, $NiCl_2$ and $MgSO_4$ were also tested for the enhanced production of amylase.

iii. Kinetic properties of pure enzyme

Maximum velocity (V_{max}) Michaelis Menten constant (K_m) were determined from Line Weaver Burk Plot[9] various concentrations (0.05–0.5 M) of substrate the activity of the enzyme was determined. Influence of pH, temperature, and metal ion effect on amylase production were calculated.

RESULTS

i. Amylase activity

The amylase enzyme activity was assayed (Table 1). Enzyme activity was more in the stationary phase and the values increased upto 21.78 U/mL (Table 2). Production of amylase increases till the stationary phase and reduce gradually with decline phase[10].

Table 1
Activity of Amylase from Bacillus subtilis KCX006

Steps	Total activity (U)	Total protein (mg/mL)	Enzyme activity (U/mL)	Specific activity (U/mg)	Activity yield %	Purification fold
Culture supernatant	2556.89	0.032	12.78	393.97	100	1
70% Ammonium sulphate	2350.73	0.024	11.75	474.60	91.93	1.20
Pure enzyme	566.93	0.002	11.33	4969.01	22.17	12.61

Table 2
Enzyme Activity at Stationary Phase

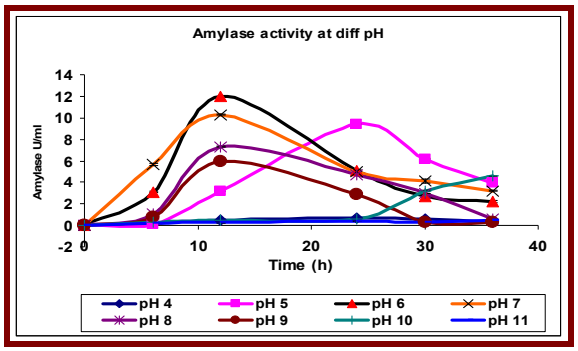
S. No	Time (h)	Enzyme activity (U/mL)	Total protein (mg/mL)
1	0	0	0
2	6	1.894	0.165
3	12	3.761	0.205
4	24	20.870	0.298
5	30	21.780	0.303
6	36	18.192	0.288
7	48	11.325	0.337

Amount of reducing sugar = Absorbance at 540 / Slope of glucose standard
Amylase activity = Amount of reducing sugar / Molecular weight of glucose × Amount of enzyme × time

ii. Effect of pH on growth and amylase production

At pH 5, growth was similar to that at pH 6 but with slow rate of growth. When the pH was ≥8 and 4 there was no good growth. The enzyme production was high at pH 6 after 12 h (11.94 U/mL) (Fig. 1).

Figure 1
Amylase Activity at Different pH



No amylase production was seen at pH 4, 10 and 11. These results indicate that the strain is not able to tolerate acidic and alkaline pH (Table 3).

Table 3
Amylase Activity at Different pH

S. No	Time (h)	Amylase activity (OD at 540 nm) (U/mL)							
		pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 11
1	0	0	0	0	0	0	0	0	0
2	6	0.167	0.153	3.112	5.662	1.077	0.803	0.274	0.180
3	12	0.495	3.179	11.961	10.301	7.329	5.930	0.502	0.294
4	24	0.669	9.417	5.113	5.026	4.738	2.918	0.629	0.401
5	30	0.595	6.191	2.663	4.149	2.965	0.321	3.192	0.301
6	36	0.267	3.935	2.215	3.219	0.595	0.267	4.571	0.455

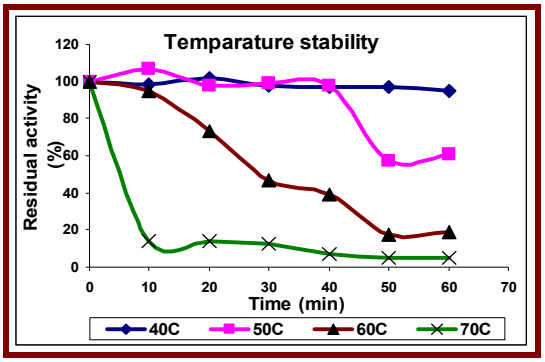
iii. Optimal temperature for amylase activity

Thermal stability was checked by incubating the enzyme at different temperature of 40–70°C in a water bath upto 60 min. Samples were withdrawn at regular 10 min interval and the activity was assayed. The result obtained was plotted with residual activity versus time shown in Fig. 2 and Table 4.

Table 4
Optimal Temperature for Amylase Activity

S. No	Temperature (°C)	Time (min)						
		0	10	20	30	40	50	60
1	40	100	98.4	101.5	98.0	97.2	97.2	94.8
2	50	100	106.8	97.8	98.7	97.4	56.9	60.7
3	60	100	94.8	73.1	47.0	39.2	17.0	19.0
4	70	100	13.8	13.8	12.5	7.0	5.0	4.5

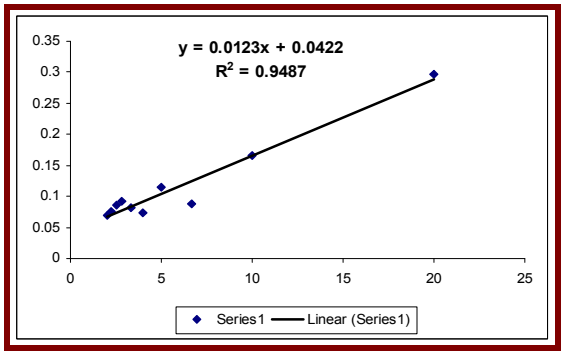
Figure 2
Temperature Stability of Pure Amylase



iv. Kinetic parameter of a pure enzyme

Substrate concentration from 0.05 to 0.5% was taken and enzyme activity at each substrate concentration was checked to study Michaelis–Menten kinetics. This was plotted in a graph as shown in Fig. 3.

Figure 3
Michalis–Menten Kinetic of Pure Enzyme

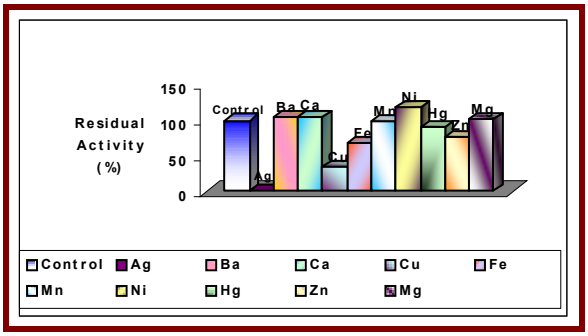


The K_m was found to be 0.291 mg/mL and V_{max} was calculated as 23.69 U/mL

v. Metal ion effect on pure enzyme

Enzyme activity was more in reaction mixture containing $NiCl_2$ and its residual activity was found to be 117.5%. Significant level of activity was seen in mixture containing $BaCl_2$, $CaCl_2$, $NiCl_2$ and $MgSO_4$. No activity was seen in mixture containing $AgSO_4$. The reaction mixture containing $CuSO_4$ shows less activity and it residual activity was found to be 34.54% (Fig. 4).

Figure 4
Effect of Metal Ions on Pure Amylase



DISCUSSION

Production of amylase increases till the stationary phase and reduces gradually with decline phase. The enzyme activity was more in the stationary phase and the value was found to be 21.78 U/mL (at 30 h) and its protein content was 0.303 mg/mL. The maximum amylase activity (38 U/mL) was found at stationary phase when the culture was grown at 37°C[11]. The enzyme was purified to homogeneity with an overall recovery of 24.2% and specific activity of 4133 U/mg. The optimum pH was 6 and temperature of the amylase at 55°C. Amylase activity was found to be maximum at neutral pH 6–7. At strong acidic and basic pH the amylase activity starts to decrease.

At optimum temperature 65°C *Bacillus subtilis* KCX006 culture produce maximum amount of amylase. *Bacillus subtilis* strain DMO3 grown optimally at 52–55°C and secret significant amount of alpha-amylase at pH 8[12]. The optimum temperature stability was obtained at 40°C and lowest at 70°C. In my present study K_m was found to be 0.291 mg/mL and V_{max} was 23.6 U/mL. The obtained data in the study by using 10 metal ions had shown various residual activities (%) of the amylase, *Bacillus subtilis* KCX006. More than 100% of enzyme activity in reaction mixture contains $BaCl_2$, $NiCl_2$, $CaCl_2$ and $MgCl_2$.

REFERENCES

1. Varnavskaia OV, Illarionova NG, Selezneva AA, and Samsonov GV, Effect of pH on the conformation properties and enzyme activity of alpha-amylase from Aspergillus terricola. Prikl Biokhim Mikrobiol, 4:866–870, (1978).
2. Dutta KT, Jana M, Pahari PR and Bhattscharya T, The effect of temperature, pH, and salt on amylase in *Helidioptomous viduus* (Gurney) (Crustacea: Copepoda: Calanoida). Turk J Zool, 30:187–195, (2006).
3. Funglsang CC, Johanson C, Christagen S, and Alder NJ, Microbial enzymes: Applications and future potential in the food industry. Trends Food Sci Tech, 6:388–391, (1995).

4. Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D and Mohan R, Advances in microbial amylase (Review article). Biotechnol Appl Biochem, 31: 135–152, (2000).
5. Nagarajan DR, Rajagopalan G and Krishnan C. Purification and characterization of a maltooligosaccharide-forming alpha-amylase from a new *Bacillus subtilis* KCC103. Appl Microbiol Biotechnol, 73:591–597, (2006).
6. Shukun Y, Carl Erik O and Jan M. Methods for the assay of 1,5-anhydro-D-fructose and α-1,4-glucan lyase. Carbohydr Res, 305:75–82, (1998).
7. Miller GL, Use of Dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem, 31:426–428, (1959).
8. Dicko MH, Searle-van Leeuwen MJF, Hilhorst R and Traore AS. Extraction, partial purification and characterization of β-amylase from the bulbs of G. klattianus. Bioresour Technol, 73:183–185, (2000).
9. Line Weaver H, Burk D. The determination of enzyme dissociation constants. J Am Chem Soc, 56:658–666, (1934).
10. Amoozegar MA, Malekzadeh F and Khursheed AM. Production of amylase by newly isolated moderate halophile, *Halobacillus* sp. strain MA-2. J Microbiol Methods, 52:353–359, (2002).
11. Mohsen Fathi N, Dileep D and Deepti D. Purification and characterization of an extracellular α-amylase from *Bacillus subtilis* AX20. Protein Expr Purif, 41:349–354, (2005).
12. Kishore D and Ashis KM. Characterization of biochemical properties and biological activities of biosurfactants produced by *Pseudomonas aeruginosa* mucoid and non-mucoid strains isolated from hydrocarbon-contaminated soil samples. Appl Microbiol Biotechnol, 69:192–199, (2005).