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Characterization and Optimization of α-amylase Activity of *Streptomyces clavifer*

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Abstract: Amongst seventeen previously identified *Streptomyces* species, *S. crystallinus*, *S. noboritoensis*, *S. anulatus* and *S. clavifer* were selected for α-amylase activity. All these species were grown in fermentation broth for α-amylase production in orbital shaker under optimum conditions. The fermentation broth cultures were then centrifuged at refrigeration temperature at 1000 rpm and supernatants were collected. The α-amylase from each supernatant was extracted by ammonium sulfate precipitation at 90% saturation and was dialyzed. The enzyme was assayed under standard assay conditions. *Streptomyces clavifer* was found to have the highest α-amylase activity amongst others. The effect of pH and temperature on α-amylase activity of *Streptomyces clavifer* was determined. The optimum pH and temperature for enzyme activity was found be 7.0 and 45°C, respectively. The enzyme was found stable at pH 6.0 to 8.0 and temperature upto 55°C. The kinetics of the enzyme was determined under standard conditions. The K_M and the V_{max} of the enzyme were determined as 4 mg and 0.03 mg sec^{-1}, respectively. α-amylase together with other amylolytic enzymes have tremendous applications in textile industries, confectioneries, pharmaceutical industries and other food industries as hydrolyzing bioagents.

Key words: *Streptomyces*, optimization, characterization, α-amylase, activity, enzyme kinetics

INTRODUCTION

Streptomyces is the most important genera of Actinomycetes, which looks like a fungus, but is finally accepted as bacteria as it is procaryotic organism (Gottlieh, 1973). *Streptomyces* belongs to the family Streptomycetaceae (Waksman and Henrichi 1948) that includes more than 500 species (Williams *et al*., 1989). *Streptomyces* is a Gram-positive, non-acid fast, aerobic bacterium of complex form. They form a threadlike net called mycelium that bears chains of spores at maturity. *Streptomyces* species are mostly chemoorganotrophs and widely distributed in soil and water. *Streptomyces* species exist in soil for long time as resting arthrospores that germinate giving rise to new individuals. Almost all species can produce life saving drugs that are used to control plant, animal and human ailments.

There are a very few *Streptomyces* species that have been reported to have amylolytic activity such as maltase α-amylases, pullulanase and glucosyltransferase activities (Hoque *et al*., 2003a, b; 2001; 1999). Amylases are hydrolases category of enzymes, which catalyze hydrolysis of α-glucosyl or β-glucosyl compounds of starch or glycogen. They catalyze the hydrolysis of large starchy material into smaller glucose sub-unit, which in turn is acted upon by other amylases to produce glucose (Teresita *et al.,* 1996). These enzymes are successfully used to produce glucose syrup, maltose syrup and some rare sugars such panose (Hoque *et al*., 1997).

α-amylases are distributed widely in microorganisms. Industrial α-amylases are produced by bacteria and fungi, e.g., *Bacillus subtilis*, *Basillus licheniformis*, *Aspergillus oryzae, Aspergillus niger, Micrococcus halobius*, etc.

α-amylases are generally stable at pH ranged from 5.5 to 8.0. Optimal activity of α-amylases occurs between pH 4.8 and 6.5. Most purified α-amylases lose activity rapidly above 50°C. In the presence of calcium they are quite resistant to extreme temperature, pH.

α-amylase has numerous biotechnological applications in the production of syrups containing oligosaccharides, maltose and glucose. Another product from starch hydrolysis by amylases is dextrin, which are important in food processing as viscosity improver, filter or ingredient. In the textile industry, amylase is used in the resizing process to degrade starch from clothing materials.

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The present interest of this research was to study with α-amylase from the isolate S-23 presumptively identified as *Streptomyces clavifer*.

MATERIALS AND METHODS

This research study has been carried in 2004 in the Department of Microbiology, University of Dhaka, Bangladesh.

Chemicals: Required chemicals were reagent grade and available in the Laboratory of Microbiology, University of Dhaka and used without further purification.

Microorganisms for the study: *Streptomyces crystallitus* (S-2), *S. noboritoensis* (S-10), *S. anulatus* (S-20), *Streptomyces clavifer* (S-23).

Maintenance and preservation of the organisms: The *Streptomyces* species were maintained throughout the work on oatmeal agar slants (Shirling and Gottiieb, 1966). The isolates in 20% glycerol broth were kept at -20°C for long time preservation.

Media: Two types of media were used for this study such as the seed culture medium (Glucose 3%, peptone 0.3%, C.S.L 2%, $CaCO_3$ 0.4% and pH 7.3) and the fermentation medium (Glucose 2%, Corn starch 4%, C.S.L. 2%, NH_4Cl 0.5%, $CaCO_3$ 1.5% and pH 7.0 (Iwasa *et al*., 1970).

Enzyme production in fermentation medium: The crude α-amylase enzyme was obtained from *Streptomyces clavifer* growing first in seed culture broth medium and then in fermentation broth medium (Iwasa *et al*., 1970) following the method described by Hoque *et al*. (2003b, 2005). Solid ammonium sulfate was added to the crude α-amylase enzyme solution upto 90% saturation and allowed to precipitate protein at 4°C for 24 h. The precipitate was harvested by refrigerated centrifugation at 10000 rpm for 20 min and dissolved in 20 mM Tris-HCl buffer (pH 7.0). The enzyme solution was dialyzed in 20 mM Tris-HCl buffer (pH 7.0) at 4°C for 48 h. The dialyzate was collected for enzyme assay. The same experiment was carried out for other *Streptomyces* spp. employed.

Enzyme assay: α-amylase activity of each isolate was determined by DNS method (Miller, 1969) using 1% soluble starch (w/v) as the substrate under standard assay conditions (Hoque *et al*., 2003b). The enzyme activity was expressed as unit/mL, which corresponded to μmole of glucose equivalent released per min under the assay conditions.

Purification of α-amylase by ammonium sulfate fractionation: Successive fractionation of the crude enzyme solution was made by precipitating with ammonium sulfate at the saturation level of 25 to 90%. The precipitated fraction was collected every time after refrigerated centrifugation at 1000 rpm for 15 min and dissolved in 20 mM Tris-HCl buffer (pH 7.0) and was then dialyzed at 4°C against same buffer for 48 h. Enzyme activity of each fraction was done under the standard enzyme assay conditions.

Effect of pH and temperature on α-amylase activity of *S. clavifer*: The effect of pH (optimum) and temperature (optimum) on partially purified α-amylase was determined by varying the pH of the buffer system between 3.0 and 10.0 and at temperature between 30 and 70°C, respectively under standard assay conditions (Hoque *et al*., 2003b).

For pH stability the enzyme preparations in different buffer solutions (between 3.0 and 10.0) were kept at 4°C for 24 h and for thermal stability, the enzyme preparations were exposed at various temperatures (4, 10, 25, 37, 45, 60, 70 and 80°C) for 15 min. Residual enzyme activities were determined under standard assay conditions (Hoque *et al*., 2003b).

RESULTS AND DISCUSSION

Screening of *Streptomyces* sp. for better extra-cellular α-amylase production: As mentioned earlier that four *Streptomyces* spp. were tested for their ability to produce extra-cellular α-amylase of industrial interest in shake-flask culture. The highest enzyme activity was observed in *Streptomyces clavifer*, which exhibited the enzyme activity of 3.78 U mL^{-1} (Fig. 1). So this species was selected for further study.

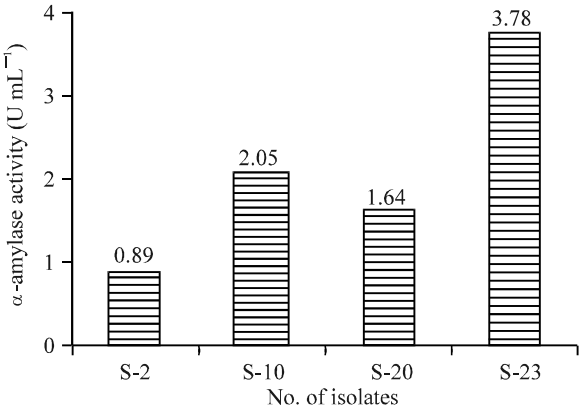

Fig. 1: Screening of *Streptomyces* sp. for better α-amylase production

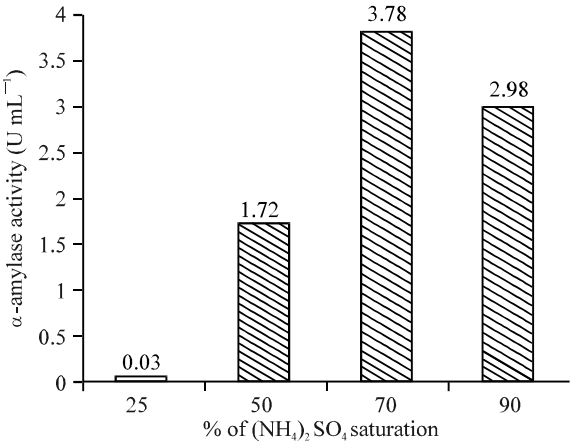

Fig. 2: $(NH_4)_2SO_4$ fractionation of α-amylase

Partial purification of α-amylase by ammonium sulfate precipitation: α-amylase activity was determined by precipitating with ammonium sulfate at the saturation level of 25-90% and highest α-amylase activity was found at 70% saturation (Fig. 2).

Effect of pH and temperature on α-amylase activity of *S. clavifer*: The optimum pH of partial purified α-amylase from *S. clavifer* is shown in Fig. 3a. The highest activity was found at pH 7.0. α-amylase activity was determined at different temperatures. Maximum activity was observed at 45°C, so optimum temperature for α-amylase activity was found at 45°C (Fig. 3b). The α-amylase was stable at pH from 6.0 to 8.0 and was then decreased above pH 8.0 and below pH 6.0 (Fig. 3c). The enzyme retained almost its full activity upto 55°C. At 70°C the enzyme retained almost 70%of the original activity. Then enzyme activity diminished gradually below 60°C (Fig. 3d).

Kinetic properties of α-amylase from *S. clavifer*: The α-amylase activity with different substrate concentrations was estimated. The K_M and V_{max} values of the α-amylase were determined by using Lineweaver-Burk double-reciprocal plot. The reciprocal of reaction velocity, 1/V, is plotted against the reciprocal of the substrate concentration, 1/[S].

Plotting the results gives a straight line; the best fit to the experimental points is $1/V = 2.4(1/[S]) + 0.6$. The y-intercept is $1/V_{max}$ and the slope of the line is K_M/V_{max}. The x-intercept is $-1/K_M$. Hence the V_{max} and K_M values of the α-amylase from *S. clavifer* were calculated from Lineweaver-Burk double-reciprocal plot as 0.03 mg sec^{-1} and 4 mg, respectively (Fig. 4).

Streptomyces species have not been yet studied extensively for the production of industrially important enzymes. There are relatively few reports of industrially important enzymes from *Streptomyces* spp. α-amylase from *Streptomyces* spp. has not yet been well reported from Bangladesh.

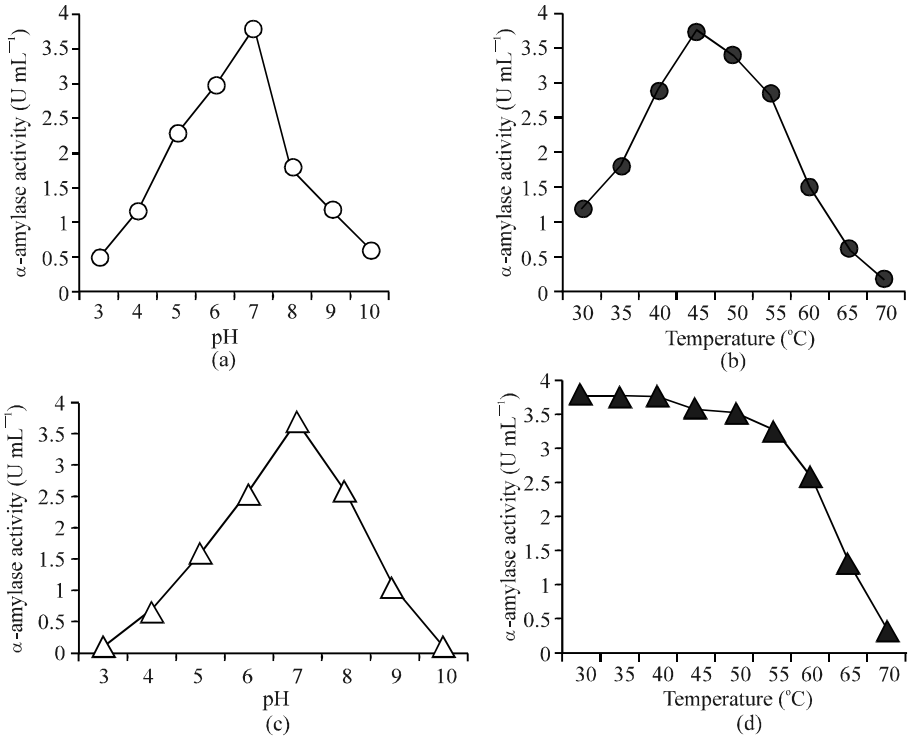

Fig. 3: Effect of pH and temperature on α-amylase activity of *S. clavifer* (a) Optimum pH, (b) Optimum temperature, (c) pH stability, (d) Thermal stability

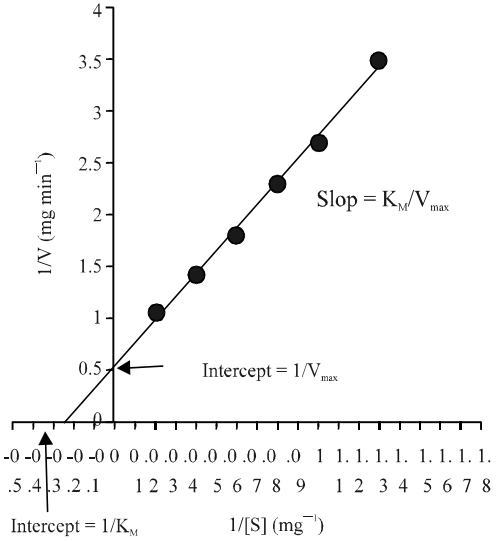

Fig. 4: A Lineweaver-Burk double reciprocal plot of Enzyme kinetics of α-amylase from *S. clavifer* (S-23).

Hoque *et al.* (2003b) started to study with *Streptomyces* species for the production of amylolytic enzymes and identified 17 species out of 24 isolates of indigenous *Streptomyces* spp.

As mentioned earlier *Streptomyces clavifer* was screened as the best α-amylase producer amongst four species studied (Fig. 1).

As described earlier that α-amylase from *S. clavifer* was found to give maximum activity at pH 7.0 (Fig. 3a). It lost ca. 60% of its original activity when kept under the same conditions at pH 8.0. The enzyme was highly unstable at high acidic or high alkaline conditions and stable at pH ranged from 6.0 to 8.0 (Fig. 3c).

A sharp peak of temperature optimum at 45°C was observed. Then the activity decreased suddenly to ca. 40% (Fig. 3b). The enzyme exhibited thermal stability upto 50°C (Fig-3d). Then the activity decreased gradually. At 60°C, the enzyme activity was about half of its activity obtained at 45°C. The enzyme was inactivated at 90° C.

From experimental findings of many investigators, it was evident that *Streptomyces* species showed highest activity at pH ranged from 4.6 to 7.0 and temperature ranged from 40 to 60°C (Fogarty, 1983). Hoque *et al.* (2003b) reported that α-amylase from *S. misakiensis* showed highest activity at pH 6.0 and 45°C. From the present investigation it was apparent that α-amylase from *S. clavifer* showed highest activity at pH 7.0 and 45°C. These results suggested that the α-amylase from *S. clavifer* showed more or less similar activity as activity of *S. aureofaciens*, *S.* praecox (Fogarty, 1983) and *S. misakiensis* (Hoque *et al.*, 2003b).

α-amylase is used in starch hydrolysis together with pullulanase or glucoamylase to produce high glucose or maltose syrups. Since these amylases have pH optima from 4.6 to 8.0 and temperature optima at 45°C for easier avoidance of contamination during use (Takashaki *et al.*, 1993).

α-amylase from *S. clavifer* exhibited pH and temperature optima within the range of industrial bioconversion process, can be used in many hydrolysis processes.

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