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Short communication

Studies on the extraction and characterization of thermostable α-amylase from pericarp of *Borassus indica*

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Thermostable α-amylase was extracted and characterized from the fruits (pericarp) of *Borassus indica*. Analysis on the influence of various physico-chemical parameters on the extracted enzyme revealed a Vmax of 0.793 and a Km of 0.022. The optimum temperature was found to be 37^{0}C at pH 4.5. The stability studies on enzyme activity envisaged that the enzyme is stable up to 80^{0}C and retained its activity over a wide range of pH (4.0 – 8.5). Significant enhancement in the enzyme activity was observed in the presence of metal ions like Manganese and Strontium and an insignificant decrement in the presence of Sodium ions.

Key words: α-Amylase, *Borassus indica,* enzyme activity.

INTRODUCTION

Industrial application of enzymes have been receiving attention throughout the world. Most of these enzymes were obtained through fermentation process using microbial, plant and animal cells as source material. It is well documented that plants are one of the abundant sources of these enzymes (Bajpai and Bajpai, 1989). The enzyme, α-amylase is important in the metabolism of maltose and maltodextrins. The potential for commercial applications of alpha amylases is enormous and they are very extensively used in beverages, baby foods, medicinal and pharmaceutical manufacturing industries. Commercially α-amylases are produced mostly from fungal sources, but they are also being extracted from different plant sources like barely, millets, wheat sorghum, and maize.

In view of its importance in industrial application, an attempt has been made to isolate α-amylase from pericarp (outer covering of endosperm) of *Borassus indica* fruit and to study the possibility of its commercial production. *B. indica* was chosen for the present study because of its wide distribution in south Asia as a dry and summer crop and the fruit is a source of α-amylase.

MATERIALS AND METHODS

Source and preliminary studies

The fruit of *B. indica* plants were collected from forest land of Nambur, Guntur district, A.P, India and brought to the laboratory for experimentation. The pericarp of fruits was removed and preliminary experiments were conducted for presence of α-amylase activity.

Extraction of enzyme

The fresh pericarp were collected from fruits and thoroughly washed with distilled water. Then 5 g of pericarp was taken and ground to fine paste with 20 ml of cold 0.025 M sodium acetate

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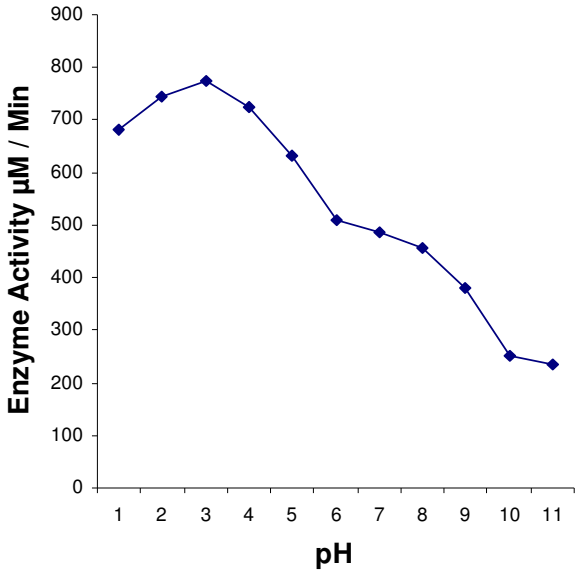

Figure 1. Effect of pH on the extracted enzyme activity.

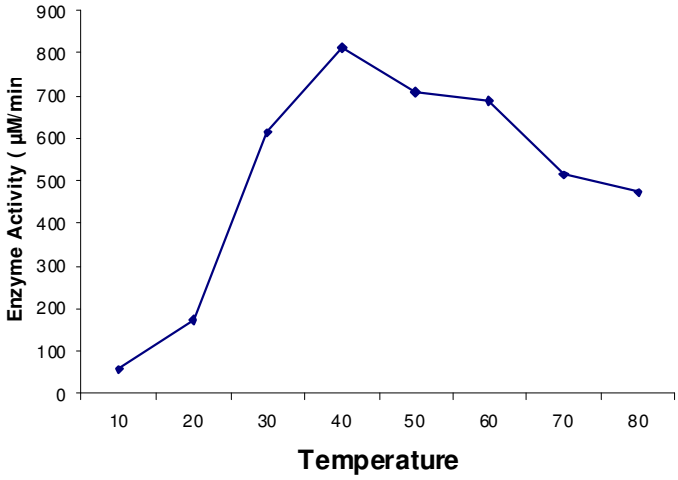

Figure 2. Effect of Temperature on stability of extracted enzyme.

buffer of pH 5.5, containing 0.02 M $CaCl_2$ as stabilizing agent. The contents were centrifuged at 6000 rpm for 15 min at 40°C. The supernatant was diluted (1:10) with sodium acetate buffer and used as enzyme source.

Chromatographic analysis

Starch was digested with extracted enzyme at 37°C for 10, 20, 30 and 60 min, respectively, in different sets of tubes. Sugar products at each time point were identified by ascending paper chromatography with Whatman no.1 filter in a solvent system of n-butanol, pyridine and water in 6:4:3 ratios (V/V) at room temperature. Chromatogram was developed by dipping the paper in silver nitrate/sodium hydroxide reagent. Further the RF values of sugars were calculated and compared with that of the standards.

Determination of α-amylase activity

The activity levels of α-amylase was determined by measuring the maltose liberated in µ M / minute when treated 1 mg of protein using 3-5 dinitrosalicylic acid (Williams and Wilson, 1974). The protein content was determined by Lowry method (Lowry et al., 1951).

Determination of pH optima

To determine the optimum pH, the enzyme was incubated in a set of different concentrations of acetate buffers of pH ranging from 3.5 to 8.5 and the reaction was performed at 37°C for 30 min.

Optimum temperature

The optimum temperature for maximum enzyme activity was determined by varying incubation temperature of the reaction mixture from 10 to 80°C.

Incubation time

To find out the appropriate incubation time, the reaction mixture was incubated at different time intervals from 5 to 60 min at 37°C.

Substrate concentration

To determine the optimum substrate concentration, various amounts of starch ranging from 100 µg to 1000 µg were used and reactions were carried out at 37°C for 30 min.

Effect of metal ions

Different metal ions at a concentration of 20 µg/ml of assay buffer were incubated with the enzyme and the activity assayed.

RESULTS AND DISCUSSION

Preliminary experiments showed that α-amylase is very abundant in pericarp of *B. indica* fruit. The amylolytic activity has also been reported in bananas (Bassinello et al., 2002). Using paper chromatography we confirmed that the extracted α-amylase can hydrolyse starch to glucose, maltose and dextrins. Similar analysis with paper chromatography was performed for the confirmation of α-amylase activity from *Cryptococcus* species and thin layer chromatography was used in *Bacillus* species (Lefuji et al., 1996). Furthermore, it was observed that the enzyme is very stable and effective at pH 4.5 and it is also stable over a broad range of pH (4.5 to 8.5). The broad peak of the curve in Figure 1 signifies that pH has very little influence on the activity and stability of enzyme. The results with pH stability were

comparable with that of the thermophilic *Bacillus* α-amylase, which was reported to have stability from a pH of 5 to 9.5 (Mamo and Gessesse, 1999). However the stability of yeast α- amylases were reported to be very limited pH at 6.0 (Lefuji et al., 1996).

Table-1. Effect of incubation time on α-amylase activity.

Time of Incubation (in min)	Enzyme Activity (µM / min)
5	131
10	257
20	613
30	801
40	823
50	837
60	839

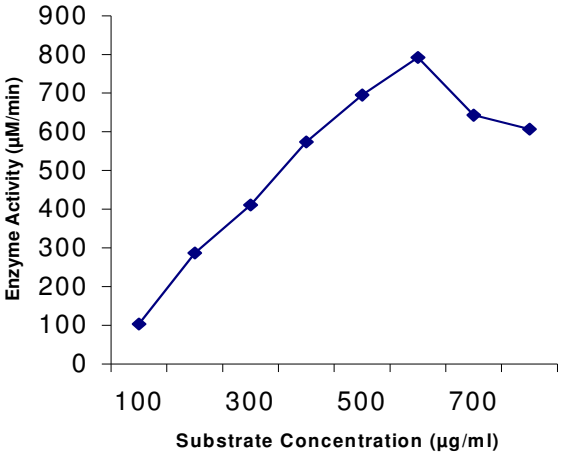

Figure 3. Effect of Temperature on stability of extracted enzyme.

Table 2. Effect of Metal Ions on α-amylase activity.

Metal Ions (20 mg/L)	Enzyme Activity (µM / min)
Control	760
Na^+	787
K^+	793
Mg^{2+}	777
Mn^+	857
Sn^+	823

The studies on the effect of different temperatures on the activity of α-amylase revealed that with the rise in temperature, the activity of the enzyme increases up to 37^0C and thereafter decrease slightly in activity probably because of enzyme denaturation (Figure 2). In similar studies α-amylases of *Saccharomyces* origin were reported to have optimum activity at 30^0C (Kocher, 2002). Generally α-amylases are thermostable up to 70^0C. The α-amylases obtained from pericarp of *Borassus indica* fruit has also shown activity up to 80^0C, with the loss of 40% activity. Thermostability of the α-amylases from *Bacillus* species was reported be up to 90^0C with 50% loss of activity (Lefuji et al., 1996; Mamo and Gessesse, 1999).

The enzyme activity increased exponentially with the time of incubation up to 30 min, afterwards there was a slight increase in activity (Table 1). It was also observed that with the increase in substrate concentration, the enzyme activity also increased up to 550 µg/ml, following first order kinetics, and thereafter the enzyme activity was reduced. This might be due to maximum saturation (Figure 3). Vmax and (maximum velocity of reaction) and Km (Michaeli's constant) values of enzyme activity were determined by using Lineweaver - Burk plot and the values were found to be 0.793 and 0.022, respectively.

The influence of metal ions on the activity of α-amylase was also studied (Table 2). It was found that Na^+, K^+, and Mg^{2+} have very little influence on the enzyme activity. However, Mn^{++} and Sn^+ have pronounced effect in increasing the enzyme activity.

The present study revealed that the pericarp of *B. indica* is a rich source of α-amylase activity having high pH and thermostability and it can be used for commercial scale operations.

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