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THERMAL STABILITY OF CRUDE ALPHA-AMYLASE PRODUCED BY *BREVIBACILLUS BORSTELENSIS* R1 ISOLATED FROM COASTAL AREA OF BAY OF BENGAL, VISAKHAPATNAM

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ABSTRACT

Thermal stability is a desired characteristic of most of the industrial enzymes. The highest amylase activity was found at 37^0C (383±3 U/ml for 3hrs) and the lowest at 60^0C (37±1 U/ml for 10hrs). The highest stability of amylase was found at 4^0C (307±7 U/ml for 9 hours) and the lowest was observed at 60^0C (37±1 U/ml for 10hours). Alpha amylase has many applications in preparation of Bakery products, Chapatti preparation, Ethyl alcohol dual fermentation, Treatment of Sago and Rice effluent, Sewage water treatment, fodder production, Desizing in Textile industry, Glucose Industry, Chocolate Syrup industry, Building product industry, Unmalted cereal liquefaction industry, Manufacture of maltose, Manufacture of high fructose containing syrups, Manufacture of high molecular weight branched dextrin etc.

KEYWORDS: α-amylase, Thermal stability, *Brevibacillus borstelensis* R1.

INTRODUCTION

Several enzymes are used industrially, half of them obtained from fungi and yeast, and one-third from bacteria and the remainder divided between plant (4%) and animal (8%) sources. Microorganisms are preferred to animals and plants as source of enzymes because of many reasons. Microorganisms have faster growth rate, short generation time and require less space for cultivation. Further, they require relatively cheap and inexpensive media to grow. High temperatures damage bacteria by denaturing enzymes, transport carriers and other proteins.

Bacterial membranes are also disrupted by temperature extremes. The lipid bilayer simply melts, disintegrates and leaks. At very low temperatures, membranes solidify and enzymes do not work rapidly. The broad range of temperatures and the enzyme's high activity at both moderate and lower temperatures make this enzyme highly attractive for both basic research studies and industrial processes. A modern trend among consumers is to use colder temperatures for laundry or dishwashing. The removal of starch from cloth and porcelain becomes more problematic at low temperatures. To overcome this problem, detergents with α-amylases at low and moderate temperatures can be used.[1]

Thermostable α-amylases were produced by mesophilic species of *Bacillus*.[2&3] Therefore, a high value is placed on extreme thermostability and thermoactivity of the enzymes. Several reports are available on α-amylase production by thermophilic bacteria.[4-14]

Extreme thermophiles like species of *Pyrococcus*[15], *Thermococcus spp.*[16], *Thermobifida fusca* NTU22[17] and *Geobacillus thermodenitrificans* HRO10[18] also produced α-amylase. Extreme optimum temperature for amylase activity range 75^0C-100^0C was reported in *Bacillus spp.*[19], *Pyrococcus spp.*[20] and *Thermococcus spp.*[21-23].

Effect of temperature on stability of α-amylase activity

All the enzymes have a narrow temperature range for their efficient functioning. The enzyme activity declines at temperature beyond optimum temperature. It is important to understand the thermostability of the enzyme. It was reported that the amylase was optimally active with a half-life of 3hrs at 100^0C in *Bacillus thrmooleovorans* NP5.[24] The enzyme was stable retaining more than 90% of its original activity after 60min exposure at 90^0C and 20min exposure at 95^0C in *Bacillus licheniformis* mutant 7902.[25] The enzyme was reported to be stable at 90^0C for 10min in *Bacillus sp.*[26] The optimum temperature for α-amylase activity was 40^0C and 71% of the activity was still maintained until 30 min after heating at 80^0C in *Bacillus sp.*[27] The thermal stability of amylase in bacteria was studied by Salva & Moraes.[28] and Schokker & Van Boekel.[29]

MATERIALS AND METHODS

Collection of the marine water samples

Marine water samples were collected from coastal areas of Visakhapatnam ranging 30kms across the Bay of Bengal: Rushikonda, Appughur, Fishing harbor and Gangavaram in Visakhapatnam, Andhra Pradesh, India. The water samples were collected from the above

four sites in sterile BOD bottles (Borosil) and brought to the lab, stored in the refrigerator until it was used.

Primary screening of α-amylase producing Bacteria

The collected marine water samples were diluted by serial dilution technique. The diluted samples of 10^{-4} to 10^{-6} (0.1ml) were spread with L-shaped glass rod by spread plate technique on the starch agar plates. After incubation at 37^0C for 24hours, the plates were flooded with Lugol solution (1% iodine in 2% potassium iodide w/v).[30] The average cfu/ml, number of colonies forming clear halo zone of hydrolysis and zone of starch hydrolysis measured in mm.

Estimation of amylase by DNS method

The starch substrate [0.5ml of 0.5% in 0.1M phosphate buffer (pH 6.8)] was mixed with 1% (0.2ml) NaCl in a test tube and pre incubated at 37^0C for 10 minutes. The supernatant collected from the centrifugation of the production media was used as enzyme source, 0.5ml of this was added to the reaction mixture. The reaction was terminated by the addition of 1.0 ml of 3, 5-dinitrosalicylic acid reagent [1.0 gm DNS in 0.8% NaOH, 60% Na K tartrate]. After incubation at 37^0C for 15 minutes, the contents were mixed well and kept in boiling water bath for 10 minutes. Then they were cooled and diluted with 10 ml of distilled H_2O. The color developed was read at 520nm. One unit of enzyme activity is defined as the amount of enzyme that releases 1.0 mmol of reducing sugar (maltose) per minute under the assay conditions.[31]

Effect of temperature on α-amylase activity

The effect of temperature on α-amylase activity was assayed by adding 0.5 ml of starch (0.5% in phosphate buffer at pH 6.8) to 0.2ml of NaCl (1%) and pre-incubated for 10minutes at 37^0C. For crude (0.5ml supernatant) was added and incubated at different temperatures 4^0C, 20^0C, 25^0C, 37^0C, 50^0C, 60^0C and 70^0C for 15 minutes. One milliliter of 3, 5-dinitrosalicylic acid reagent (1.0%) was added to stop the reaction.

Thermal stability of α-amylase activity at different temperatures

The effect of temperature stability on α-amylase activity was checked by adding 0.5 ml of starch (0.5% in phosphate buffer at pH 6.8) to 0.2ml of NaCl (1%) and pre-incubated for 10minutes at 37^0C. For crude (0.5ml supernatant) was added and incubated at different temperatures 4^0C, 20^0C, 25^0C, 37^0C, 50^0C, 60^0C and 70^0C for different hours (1, 2, 3, 4, 5, 6,

7, 8, 9 and 10). For control the crude enzyme was incubated at 37^{0}C for 15 minutes. One milliliter of DNS reagent (1%) was added to stop the reaction. The amylase activity was determined based on the standard assay method.

RESULTS

Effect of temperature on activity of crude α-amylase

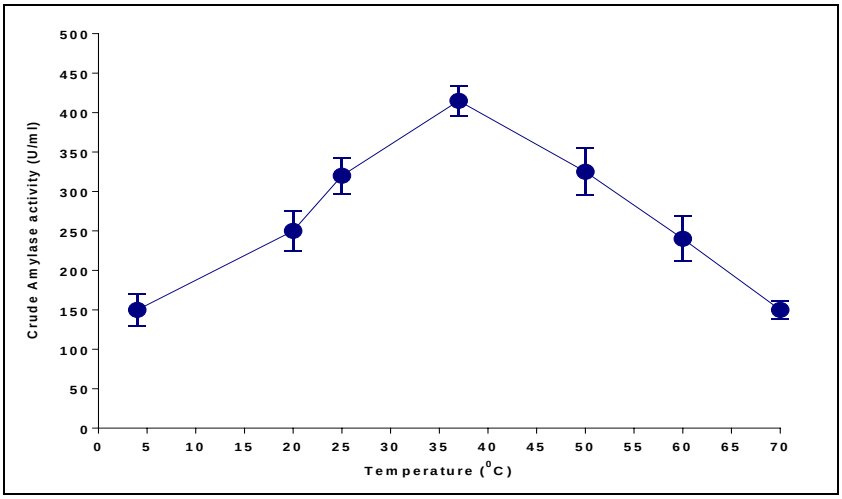

Fig: 1 Effect of temperature on activity of crude α-amylase.

Y bars indicate the standard deviation of mean value

The Highest amylase activity was observed at 37^{0}C (415±10 U/ml), lowest at 70^{0}C (150±6U/ml) and the range of amylase activity in crude enzyme (150-420U/ml) were shown in fig.1.

Thermal stability of crude α-amylase

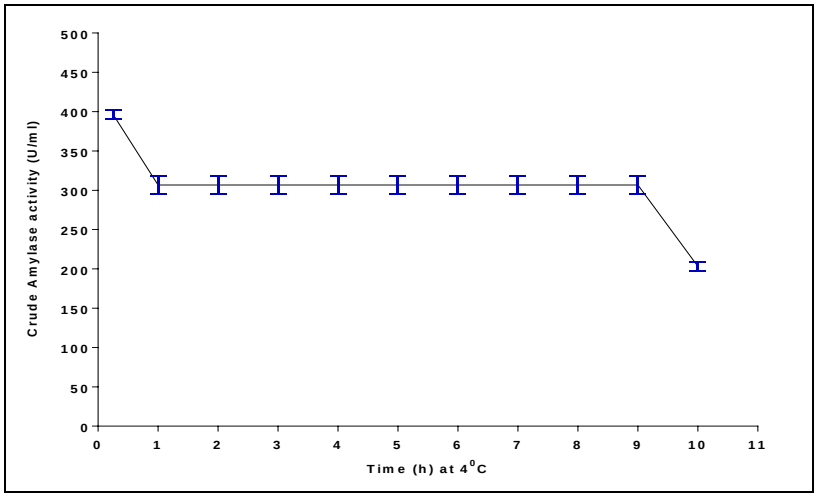

Fig: 2 Thermal stability of crude α-amylase at 4^{0}C.

Y bars indicate the standard deviation of mean value

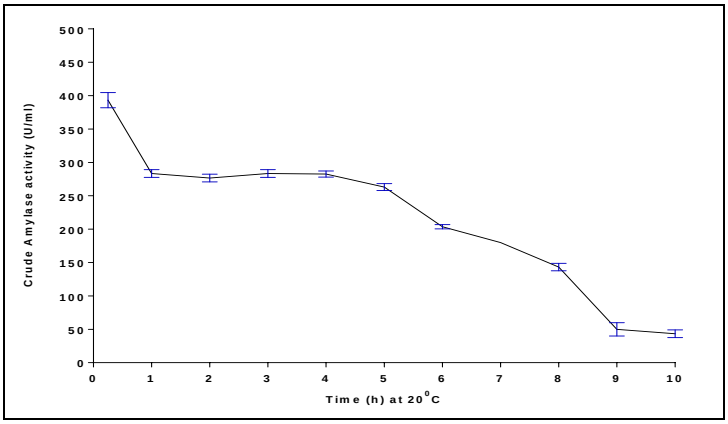

Fig: 3 Thermal stability of crude α-amylase at 20^{0}C.

Y bars indicate the standard deviation of mean value

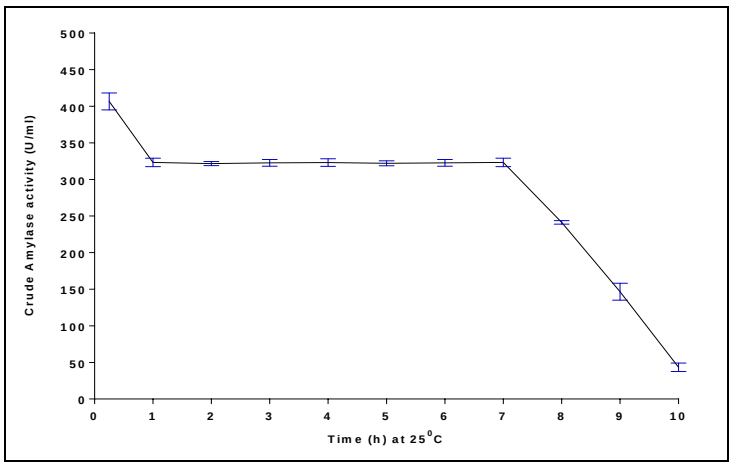

Fig: 4 Thermal stability of crude α-amylase at 25^{0}C.

Y bars indicate the standard deviation of mean value

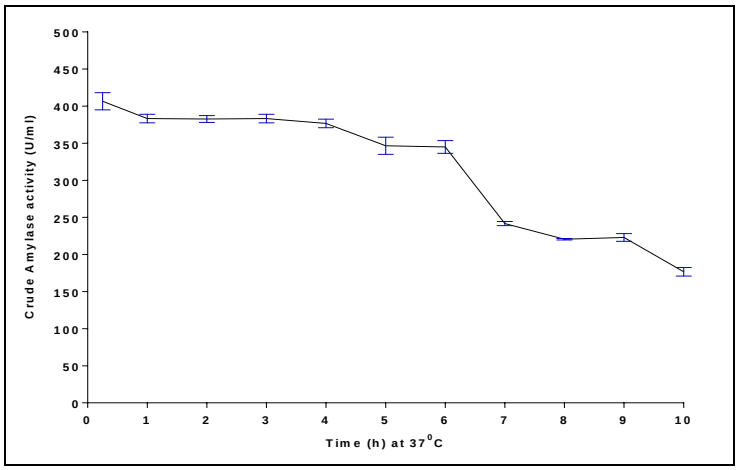

Fig: 5 Thermal stability of crude α-amylase at 37^{0}C.

Y bars indicate the standard deviation of mean value

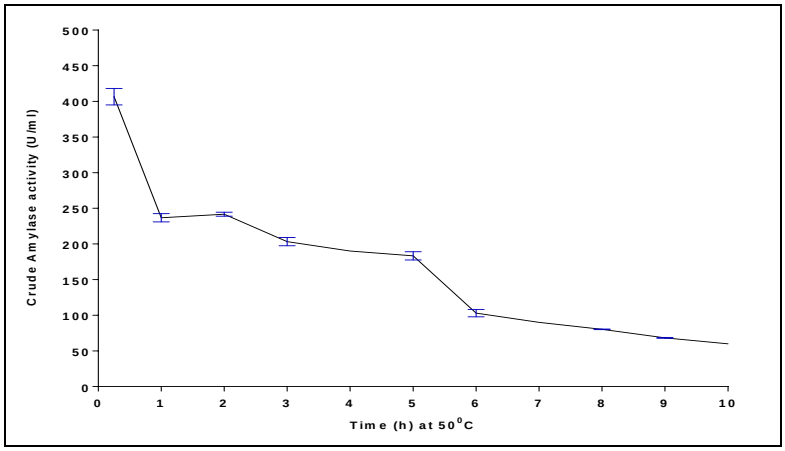

Fig: 6 Thermal stability of crude α-amylase at 50^{0}C.

Y bars indicate the standard deviation of mean value

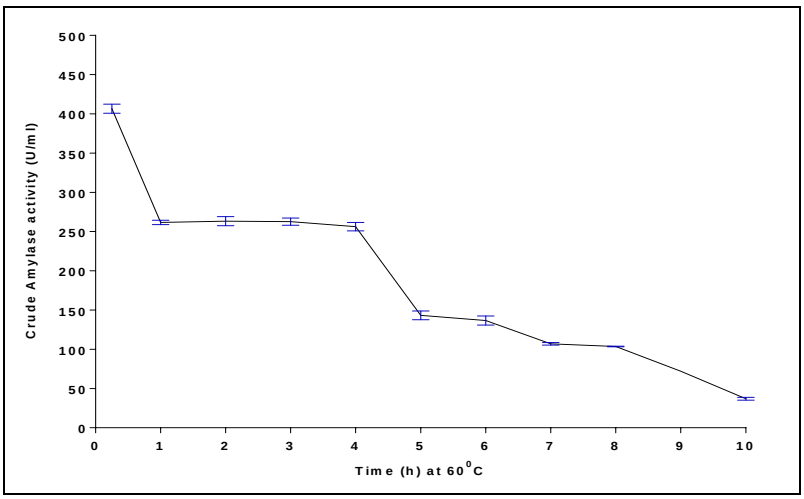

Fig: 7 Thermal stability of crude α-amylase at 60^{0}C.

Y bars indicate the standard deviation of mean value

The control in all the temperature stability tests was treated for 15minutes at respective temperatures. The crude enzyme was tested for its amylase activity stability time (1-10 hours) at temperatures ranging from 4^{0}C-60^{0}C as shown in the fig. 2-7.

At 4^{0}C the activity of amylase in control (397±3 U/ml) slowly decreased after 1hrs (307±7 U/ml) and remained constant for 9hrs and later increase in activity at 10hrs (203±3 U/ml) (fig.2).

At 20^0C the activity of amylase in control (373±7 U/ml) remained stable up to 5hrs (263±3 U/ml) with little decrease in activity but later decreased each hour and reached the lowest after 10hrs (43±3 U/ml) (fig.3).

At 25^0C the activity of amylase in control (330±100 U/ml) remained stable up to 7hrs (323±3 U/ml) and slowly the decrease in activity at 10hrs (43±3 U/ml) (fig. 4).

At 37^0C the activity of amylase in control (407±7 U/ml). The activity remained stable from 1hour (383±3 U/ml) to 3hrs and later slowly decreased to lowest at 10hrs (177±3U/ml) was showed in fig. 5.

At 50^0C the activity of amylase in control was 343±3 U/ml. The activity of the amylase constantly decreased from 1hr (237±3 U/ml) to the lowest 10hrs (60 U/ml) (fig. 6).

At 60^0C the activity of amylase in control (363±3 U/ml). The activity of the amylase constantly decreased from 1hr (262±2 U/ml) and slowly decreased to the lowest at 10hrs (37±1 U/ml) (fig. 7).

The highest amylase activity was found at 37^0C (383±3 U/ml for 3hrs) and the lowest at 60^0C (37±1 U/ml for 10hrs). The highest stability of amylase was found at 4^0C (307±7 U/ml for 9 hours) and the lowest was observed at 60^0C (37±1 U/ml for 10hours).

DISCUSSION

The effect of temperature on activity of crude α-amylase was studied. The highest α-amylase activity was observed at 37^0C (383±3 U/ml). Several authors reported the optimum activity of amylase between the range of 30-50^0C in *Bacillus sps.*[32-39]

The thermal stability of crude α-amylase was tested for its stability time (1-10 hours) at temperatures ranging from 4^0C-60^0C. The highest amylase activity was found at 37^0C (383±3 U/ml for 3hrs) and the lowest at 60^0C (37±1 U/ml for 10hrs). Inactivation of enzymes at high temperature due to incorrect conformation as a result of hydrolysis of the peptide chain, destruction of amino acid or aggregation.[40&41] The enzyme retained 94% activity in 1h at 60°C in *Streptomyces hygroscopicus* SF-1084.[42] The optimum temperature for α-amylase activity was 40^0C and 71% of the activity was still maintained until 30 min after heating at 80^0C in *Alternaria alternate*.[43] The optimum incubation temperature was 35°C in amylase.

The purified enzyme had maximum activity after 30hrs of incubation. The enzyme was stable when it was pre-incubated 1h at 30^0C, while inactivation was observed at 60^0C.[44]

CONCLUSION

The highest amylase activity was found at 37^0C (383±3 U/ml for 3hrs) and the lowest at 60^0C (37±1 U/ml for 10hrs). The highest stability of amylase was found at 4^0C (307±7 U/ml for 9 hours) and the lowest was observed at 60^0C (37±1 U/ml for 10hours).

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