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Effect of Activators on Crude α-amylase produced by Brevibacillus borstelensis R1 isolated from coastal area of Bay of Bengal, Visakhapatnam

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ABSTRACT

Alpha amylase have many applications in Ethyl alcohol production, Treatment of Sago and Rice effluent, Sewage water treatment and fodder production, Textile industry, Glucose Industry, Chocolate Syrup industry, Building product industry, Feed Industry, Unmalted cereal liquefaction industry, Manufacture of high fructose containing syrups, Manufacture of maltotetraose syrup, Hydrolysis of starch to maltodextrins etc. All the activators studied showed more activity when compared with the control. The average enzyme activity of the crude enzyme found in $CaCO_3$(427±13-2223±9 U/ml), $CaCl_2$ (663±9-1813±13 U/ml), $CaSO_4$ (663±9-1430±6 U/ml), $MgCl_2$ (570±15-733±18 U/ml), $MgS0_4$ (577±15-707±7 U/ml) and NaCl (440±20-670±6 U/ml).The highest amylase activity was observed in $CaCO_3$ (2223±9 U/ml) at 0.5M and lowest (427±13 U/ml) in $CaCO_3$ at 0.2M.

Key words: α-amylase, $CaCl_2$, $MgCl_2$, $MgS0_4$, NaCl, $CaCO_3$, $CaSO_4$, *Brevibacillus borstelensis* R1.

1. INTRODUCTION

The total bacterial members on an average are high in coastal waters than in the open ocean [1]. The capability of amylase production widely occurs in various bacteria, fungi, plants and animals that have a major role in the utilization of polysaccharides [2-9].

The stimulatory effect of $CaCl_2$ on amylase activity was reported by several investigators in *Bacillus spp.* [10-19]. It was reported that the calcium ions stabilize the α-amylase activity in *Bacillus spp.* [20].

Magnesium sulphate reported to stimulate α-amylase in *Bacillus spp*. [21&22]. In contrary to this result $MgSO_4$ reported to inhibit the enzyme activity. Probably this metal block binding sites of enzyme or enzyme contain number of metals and displacement of these ions by another metal ions, either with some change result in inhibition of enzyme activity [23].

Stimulation of NaCl on activity of α-amylase was reported in *Bacillus spp.* [12,24 & 25], *Heliodiaptomus viduus* [26] and *Pyrococcus woesei* [27]. Inhibitory effect of NaCl on the enzyme activity was also reported in *Bacillus spp.* [28].

2. EXPERIMENTAL PROCEDURES, RESULTS AND DISCUSION

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

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) [29]. The average cfu/ml, number of colonies forming clear halo zone of hydrolysis and zone of starch hydrolysis measured in mm.

Maltose produced by the hydrolytic activity of α-amylase on α-1, 4 linkages present in polysaccharides, reduce 3, 5 dinitro salicylate to an orange red colored 5-nitro 3-amino salicylate which can be measured at 520nm. 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 was defined as the amount of enzyme that releases 1.0 mmol of reducing sugar (maltose) per minute under the assay conditions [30].

The bacterial isolates were characterized by their cultural, morphological and biochemical characters by adopting standard techniques [31].

The effect of various activators (calcium chloride, magnesium chloride, magnesium sulphate, sodium chloride, calcium carbonate and calcium sulphate) at 0.1, 0.2, 0.3, 0.4 and 0.5M concentrations on crude α-amylase was studied. The influence of various inhibitors (silver nitrate, mercury (II) chloride, Ethylene dinitrilo tetra acetic acid disodium salt, cupric sulphate, L-glutamic acid and zinc chloride) at 0.1, 0.2, 0.3, 0.4 and 0.5M concentrations on α-amylase activity was studied.

The effect of activators and inhibitors on α-amylase activity was determined by DNS method (0.5% phosphate buffered starch as substrate) using crude supernatant enzyme from *Brevibacillus borostelensis* R1 culture grown in Pikovskaya's medium under standardized conditions. The control in all tests was assayed without adding any influencing agent.

Effect of activators on crude α-amylase activity

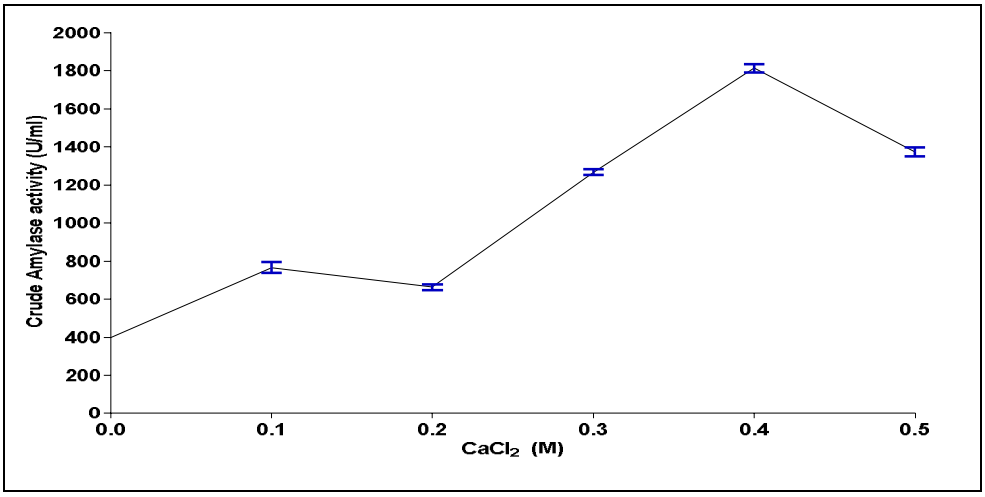

Figure 1 Effect of $CaCl_2$ on crude α-amylase activity
Y bars indicate the standard deviation of mean value

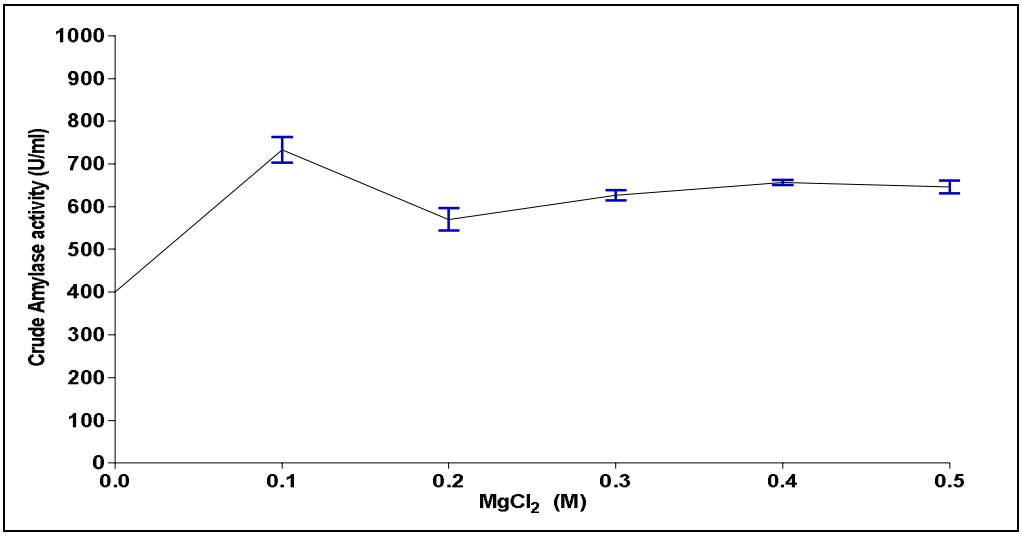

Figure 2 Effect of $MgCl_2$ on crude α-amylase activity
Y bars indicate the standard deviation of mean value

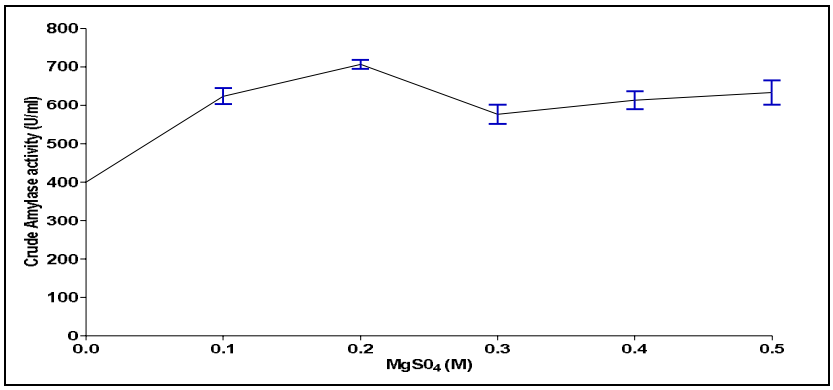

Figure 3 Effect of $MgSO_4$ on crude α-amylase activity
Y bars indicate the standard deviation of mean value

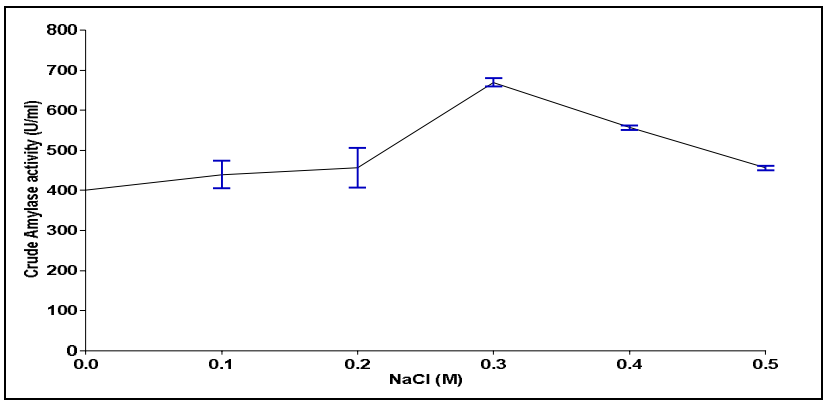

Figure 4 Effect of NaCl on crude α-amylase activity
Y bars indicate the standard deviation of mean value

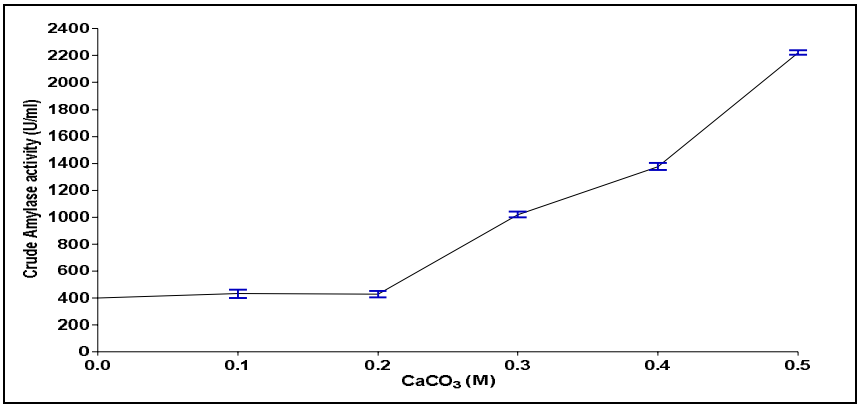

Figure 5 Effect of $CaCO_3$ on crude α-amylase activity
Y bars indicate the standard deviation of mean value

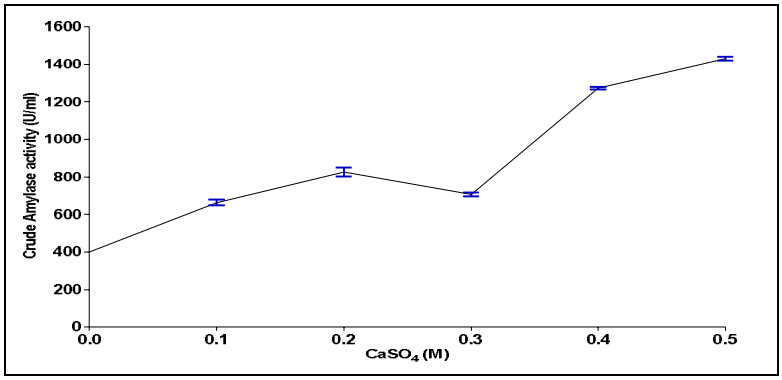

Figure 6 Effect of $CaSO_4$ on crude α-amylase activity
Y bars indicate the standard deviation of mean value

The effect of six activators on crude enzyme activity was studied were shown in figure 1 -6. The control was the activity of the crude enzyme without activator under standard conditions. All the activators studied showed more activity when compared with the control. The highest activity of the crude enzyme was found in $CaCO_3$ (2223±9 U/ml) at 0.5M, $CaCl_2$ (1813±13 U/ml) at 0.4 M, $CaSO_4$ (1430±6 U/ml) at 0.5 M, $MgCl_2$ (733±18 U/ml) at 0.1 M, $MgS0_4$ (707±7 U/ml) at 0.2 M and NaCl (670±6 U/ml) at 0.3 M.

The lowest activity of the crude enzyme was found in $CaSO_4$ (663±9 U/ml) at 0.1M, $CaCl_2$ (663±9 U/ml) at 0.2M, $MgS0_4$ (577±15 U/ml) at 0.3M, $MgCl_2$ (570±15 U/ml) at 0.2M, NaCl (440±20 U/ml) at 0.1M and $CaCO_3$ (427±13 U/ml) at 0.2M.

The average enzyme activity of the crude enzyme found in $CaCl_2$ (663±9-1813±13 U/ml), $CaSO_4$ (663±9-1430±6 U/ml), $MgS0_4$ (577±15-707±7 U/ml), $MgCl_2$ (570±15-733±18 U/ml), NaCl (440±20-670±6 U/ml) and $CaCO_3$(427±13-2223±9 U/ml).The highest amylase activity was observed in $CaCO_3$ (2223±9 U/ml) at 0.5M and lowest (427±13 U/ml) in $CaCO_3$ at 0.2M.

The effect of six activators ($CaCl_2$, $MgCl_2$, $MgS0_4$, NaCl, $CaCO_3$ and $CaSO_4$) on crude enzyme activity was studied. All the activators studied showed more activity when compared with the control. The highest activity of the crude enzyme was found in $CaCO_3$ (2223±9 U/ml) at 0.5M. The stimulating effect of Ca^{2+} on the affinity of alpha amylase was much stronger than any other ions and the calcium ions stabilize the enzyme activity was reported in *Bacillus sp*s. [11 & 20], *Thermus sps.* [32], *Pyrococcus furiosus* [33] and *Bacillus pumilus* [34].

The effect of six activators ($CaCl_2$, $MgCl_2$, $MgS0_4$, NaCl, $CaCO_3$ and $CaSO_4$) on crude enzyme activity was studied. All the activators studied showed more activity when compared with the control. The highest activity of the crude enzyme was found in $CaCO_3$ (2223±9 U/ml) at 0.5M, $CaCl_2$ (1813±13 U/ml) at 0.4 M, $CaSO_4$ (1430±6 U/ml) at 0.5 M, $MgCl_2$ (733±18 U/ml) at 0.1 M, $MgS0_4$ (707±7 U/ml) at 0.2 M and NaCl (670±6 U/ml) at 0.3 M.

3. CONCLUSION

The control was the activity of the crude enzyme without activator under standard conditions. All the activators studied showed more activity when compared with the control. The highest activity of the crude enzyme was found in $CaCO_3$ (2223±9 U/ml) at 0.5M and the lowest activity of the was found in $CaCO_3$ (427±13 U/ml) at 0.2M.

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