EFFECT OF INHIBITORS ON CRUDE ALPHA-AMYLASE PRODUCED BY BREVIBACILLUS BORSTELENSIS R1 ISOLATED FROM COASTAL AREA OF BAY OF BENGAL, VISAKHAPATNAM

K. Suribabu1* and K. P. J Hemalatha2

1PG Department of Microbiology and Research Centre, Dr.Lankapalli Bullayya Post-Graduate College, Visakhapatnam-530 013, A.P, India.
2Department Microbiology, Andhra University, Visakhapatnam-530 003, A.P, India.

ABSTRACT

Alpha amylase have many applications in Bakery industry, Detaining in Laundry industry, Automation Dishwashing (Restaurants), Alcohol dual fermentation, Textile industry, Glucose Industry, Chocolate Syrup industry, Building product industry, Poultry feed Industry, Unmalted cereal liquefaction industry, Manufacture of maltose, Manufacture of high fructose containing syrups, Manufacture of high molecular weight branched dextrins etc. The control was the activity of the crude α-amylase without inhibitor under standard conditions. All the inhibitors decreased the enzyme activity when compared with the control. The following were the activities of the crude enzyme in the presence of 0.3M AgNO3 (410±6 U/ml), 0.1M HgCl2 (383±3 U/ml), 0.1M ZnCl2 (370±6 U/ml), 0.1M CuSO4 (347±7 U/ml), 0.1M EDTA (327±7 U/ml) and 0.1M L-Glutamic acid (243±3 U/ml).

KEY WORDS: Brevibacillus borstelensis R1, α-amylase, AgNO3, HgCl2, EDTA, CuSO4, L-glutamic acid, ZnCl2.

INTRODUCTION

Microorganisms account for more than 90% of marine biomass and a majority of them remain unknown because it is difficult to isolate them on synthetic media. Amylases derived from bacterial sources have economically dominated applications in industrial sectors.[1] The
literature survey revealed that bacterial amylases are widely used than fungal amylases. Species of *Bacillus* are ubiquitous in terrestrial, fresh water and marine habitats.\[^{[2]}\] Higher metal ion concentrations often inhibit microbial growth and enzyme production. Adequate concentrations of specific metal ions are very essential for microbial growth of *Bacillus subtilis*.\[^{[3]}\] Previous literature indicated that most amylase activities were inhibited in the presence of Ni\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\), Ag\(^{+}\), Pb\(^{2+}\), Fe\(^{2+}\) and Zn\(^{2+}\). The α-amylases from *Bacillus* were strongly inhibited by Ni\(^{2+}\), Cd\(^{2+}\), Zn\(^{2+}\) and Hg\(^{2+}\).\[^{[4]}\] Zinc chloride has inhibitory effect on the activity of enzyme in *Aspergillus flavus*.\[^{[5]}\] The α-amylase from *Thermus* sp. was strongly inhibited by Cu\(^{2+}\) and Fe\(^{2+}\)\[^{[6]}\] and the α-amylase from *Bacillus subtilis* was strongly inhibited by Zn\(^{2+}\), Ag\(^{+}\), Cu\(^{2+}\) and Fe\(^{2+}\).\[^{[7]}\] However, the activity of *Nocardiopsis* sp.7326 amylase was not affected by Zn\(^{2+}\), Ni\(^{2+}\) and Fe\(^{2+}\) but activated by Cd\(^{2+}\) and Cu\(^{2+}\). Alpha-amylase was strongly inhibited by Ag\(^{2+}\) in *Bacillus* spp.\[^{[8-14]}\] and α-amylase was inhibited by Hg\(^{2+}\) in *Bacillus* spp.\[^{[15-20]}\] The activity was inhibited in the presence of zinc ions in *Bacillus* spp.\[^{[21-26]}\], *Bacillus* dipsosauri\[^{[27]}\], *Bacillus* stearothermophilus,\[^{[28]}\] *Bifidobacterium adolescentis*\[^{[29]}\] and *Bacillus* pumilus.\[^{[30]}\] Ethylenediamine tetraacetic acid exhibited no effect on amylase activity in *Bacillus* spp.\[^{[31-34 &25]}\] and *Bifidobacterium adolescentis*\[^{[29]}\] but stimulated the activity of amylase in *Bacillus* spp.\[^{[17 & 22]}\] Stimulatory effect of Cu\(^{2+}\) was reported by Krishnan & Chandra in *Bacillus licheniformis* CUNC305.\[^{[35]}\]

**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 of 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\(^{0}\)C for 24hours, the plates were flooded with Lugol solution (1% iodine in 2% potassium iodide w/v).\[^{[36]}\] The average cfu/ml, number of colonies forming clear halo zone of hydrolysis and zone of starch hydrolysis was measured in mm.

**Estimation of amylase by DNS method:** 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°C 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°C 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 H2O. 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.\[37\]

**Effect of inhibitors on α-amylase activity:** The influence of various inhibitors (silver nitrate, mercury (II) chloride, Ethylenedinitrilotetraacetic 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 inhibitors on α-amylase activity was determined by DNS method (0.5% phosphate buffered starch as substrate) using crude supernatant enzyme from *Brevibacillus borstelensis* R1 culture grown in Pikovskaya’s medium under standardized conditions. The control in all tests was assayed without adding any influencing agent.

**Statistical analysis:** All the experiments were conducted in triplicate. The results were given as mean value ± standard deviation.

**RESULTS**

**The effect of inhibitors on crude enzyme**

![Fig: 1 Effect of AgNO₃ on crude α-amylase activity](image)
Y bars indicate the standard deviation of mean value

**Fig: 2 Effect of HgCl₂ on crude α-amylase activity**

Y bars indicate the standard deviation of mean value

**Fig: 3 Effect of EDTA on crude α-amylase activity**

Y bars indicate the standard deviation of mean value

**Fig: 4 Effect of CuSO₄ on crude α-amylase activity**
The effect of six inhibitors on crude enzyme activity was shown in fig. 1-6. The control was the activity of the crude enzyme without inhibitor under standard conditions. All the inhibitors studied showed less activity when compared with the control. The highest activity of the crude enzyme was found in AgNO₃ (410±6 U/ml) at 0.3 M, HgCl₂ (383±3 U/ml) at 0.1 M, ZnCl₂ (370±6 U/ml) at 0.1 M, CuSO₄ (347±7 U/ml) at 0.1 M, EDTA (327±7 U/ml) at 0.1 M and L-Glutamic acid (243±3 U/ml) at 0.1 M.

The lowest activity of the crude enzyme was found AgNO₃ (210±6 U/ml) at 0.5 M, ZnCl₂ (160 U/ml) at 0.5 M, L-Glutamic acid (83±3 U/ml) at 0.5 M, CuSO₄ (61±1 U/ml) at 0.5 M, EDTA (47±7 U/ml) at 0.5 M and HgCl₂ (38±4 U/ml) at 0.5 M. The average enzyme activity of the crude enzyme found in AgNO₃ (210±6-410±6 U/ml), ZnCl₂ (160-370±6 U/ml), L-
Glutamic acid (83±3-243±3 U/ml), CuSO₄ (61±1-347±7 U/ml), EDTA (47±7-327±7 U/ml), and HgCl₂ (38±4-383±3 U/ml). The highest amylase activity was observed in AgNO₃ (410±6 U/ml) at 0.3 M, and lowest in HgCl₂ (38±4 U/ml) at 0.5 M.

DISCUSSION
The effect of six inhibitors (AgNO₃, HgCl₂, EDTA, CuSO₄, L-Glutamic acid, and ZnCl₂) on crude enzyme activity was studied. The inhibition of α-amylase activity was observed in CuSO₄ (61±1-347±7 U/ml) at 0.1M when compared with control but stimulatory effect of Cu²⁺ was reported in Bacillus licheniformis CUMC-305. [35]

In the present study the highest inhibitory effect of α-amylase activity was exhibited by HgCl₂ (38±4 U/ml in 0.5M), in crude preparation. It was due to the binding of mercuric ions to the indole amino acid residues in the enzyme. The α-amylase was strongly inhibited by HgCl₂ was reported. [1, 14-16, 19-20 and 24]

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Effect of activators on crude α-amylase and Inhibitors on partially purified alpha-amylase produced by Brevibacillus borstelensis R1 was studied by Suribabu and Hemalatha. [38& 39]

CONCLUSION
The control was the activity of the crude enzyme without inhibitor under standard conditions. All the inhibitors studied showed less activity when compared with the control. The lowest activity of the crude enzyme was found AgNO₃ (210±6 U/ml) at 0.5 M, ZnCl₂ (160 U/ml) at 0.5 M, L-Glutamic acid (83±3 U/ml) at 0.5 M, CuSO₄ (61±1 U/ml) at 0.5 M, EDTA (47±7 U/ml) at 0.5 M and HgCl₂ (38±4 U/ml) at 0.5 M.

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REFERENCES


