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α-AMYLASE FROM SUGARCANE WOOLLY APHID (*CERATOVACUNA LANIGERA* ZEHNTNER)

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ABSTRACT

Sugarcane woolly aphid, *Ceratovacuna lanigera* is one of the important pests of the sugarcane which is responsible for the low production of the sugarcane in India. The incidences of pest itself suggest importance of study on this pest. Carbohydrate metabolizing digestive enzyme, α-amylase was studied from this insect. Biochemical characterization of this enzyme and screening of natural inhibitors will prove vital strategies against infestation. As initial step to this goal here we report the detection of α-amylase of *Ceratovacuna lanigera* by starch agar well diffusion assay. Biochemical parameters like pH, temperature and effect of substrate concentration were studied and compared with α-amylases from other sources. The optimum pH of α-amylase of *Ceratovacuna lanigera* was found to be pH 8 in contrast to human salivary α-amylases and fungal α-amylases those shows optimum activity at pH 7 and pH 6 respectively. Maximum activity of α-amylase was found to be at 40°C. Protein profile by Native - PAGE showed banding pattern with varied intensities. The α-amylase of *Ceratovacuna lanigera* detected in the present study needs further exploration for its use in better management of sugarcane woolly aphid.

KEY WORDS: α-amylase, Native-PAGE, Sugarcane, woolly aphid.

INTRODUCTION

Sugar is a universal sweetening agent and sugarcane (*Saccharum officinarum* L.) is the primary age old source of it. Sugarcane is damaged by about 288 species of insects and non-insets (David and Nandagopal, 1986) and tissue borers, white grubs, white flies, rodents, mealy bugs, pyrilla, scale insects etc. in which Raychaudhuri (1984) listed 17 species of aphids associated with sugarcane. Sugarcane woolly aphid (*Ceratovacuna lanigera* Zehnter) is well known but comparatively less studied pest. The sugarcane woolly aphid was first reported from west Bengal in 1958 and later from other parts of India. Recently, in Maharashtra state during July-2002, an epidemic of sugarcane woolly aphid was noticed in Sangli, Kolhapur and Satara districts and later on spread in parts of Solapur, Pune and Ahmednagar districts. The aphid undergoes an anholocyclic life cycle on Poaceae (Joshi and Viraktamath, 2004). This aphid which constitute serious pest of sugarcane is dependent on their α-amylases for metabolism of carbohydrate. A detailed understanding of digestive α-amylases is essential when developing methods of insect pest control. Hence current study deals with the detection and biochemical characterization of α-amylase of the *C. lanigera*. This information may be exploited for planning the strategies for the better management of sugarcane woolly aphid.

MATERIALS AND METHODS

Collection of the *Ceratovacuna lanigera*

Ceratovacuna lanigera Zehnter was collected from the affected sugarcane field located at Rahuri, district Ahmednagar. Collection was made in the dry petri plates and preserved in freeze condition until use.

Extraction of enzyme

Two gram *Ceratovacuna lanigera* insects were crushed in 10 ml physiological saline, after complete homogenization the homogenate was centrifuged at 15000 rpm for 30 min at 4°C. Clear supernatant was used as source of α-amylase. Concentration of protein was determined by Lowry method (Lowry *et al.*, 1951).

Detection of enzyme activity

Clear supernatant obtained was screened for α-amylase activity by starch agar plate method. In this method agar plate containing 2 % starch was used. The various combinations of enzyme and buffer poured in wells of the agar plate, incubated at 37°C for 30 min. Plate was stained with iodine solution and zone of clearance (α-amylase activity) was observed visually.

Estimation of α-amylase activity

The α-amylase activity was determined by measuring the formation of reducing sugars when the crude supernatant was incubated with starch. The standard reaction mixture contained 0.2M sodium phosphate buffer (pH 7.0), 1 % starch and

0.2 ml enzyme. After incubation at 37°C for 30 min, the liberated reducing sugars were estimated using DNSA (1% 3, 5-Dinitrosalisylic acid, 30% Sodium potassium tartarate, 0.2M NaoH) reagent. One unit of enzyme activity was defined as the quantity of enzyme producing 1µM reducing sugar (maltose) per min at defined assay condition.

Biochemical parameters

The following parameters were investigated for their effects on the activity of enzyme: temperature, pH and substrate concentration.

Native-PAGE

Polyacrylamide Gel Electrophoresis of the native protein under non-denaturating conditions (Davis, 1964) was conducted by loading crude supernatant. The gel was later stained in 0.2% Coomassie Brilliant Blue R-250 in methanol, acetic acid and water (30:10:60 v/v), destained in the same solution without dye and the protein bands were visualized.

RESULTS

The clear supernatant obtained after homogenization of sugarcane woolly aphid was screened for detection α-amylase. It was found that starch agar plate showed strong α-amylase activity (figure 1). Digestive α-amylase activity of *Ceratovacuna lanigera* was assayed using the dinitrosalicylic acid (DNS) method and activity was compared with human salivary and fungal α-amylase activities. pH optima of these three amylases was determined using a broad pH range starting from pH 4 to pH 10. It was found that all these three amylases exhibited different pH optima i.e. pH 6 for fungal α-amylase, pH 7 for human salivary α-amylase and pH 8 for woolly aphid α-amylase (fig. 2). Usually α-amylases are most active at neutral or acidic pH but interestingly sugarcane woolly aphid α-amylase showed maximum activity at alkaline pH. All the three amylases studied exhibited maximum activity at 40°C (fig. 3). The optimum substrate concentration was determined by using different substrate concentrations (fig. 4) from 0.2 % to 2.0% with the increment of 0.2%. The Km for this enzyme was found to be 0.6. The native protein profile of the crude supernatant is presented in fig. 5. Protein bands with varied intensities were observed on polyacrylamide gel. The specific activity of the α-amylase was found to be 120 units/mg of protein.

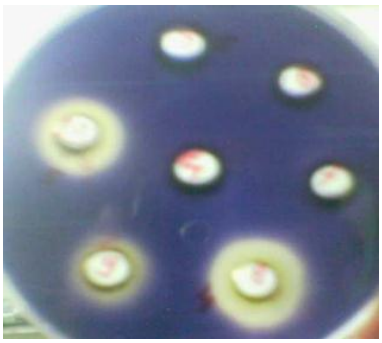

Figure 1. Detection of α-amylase activity by starch agar plate method

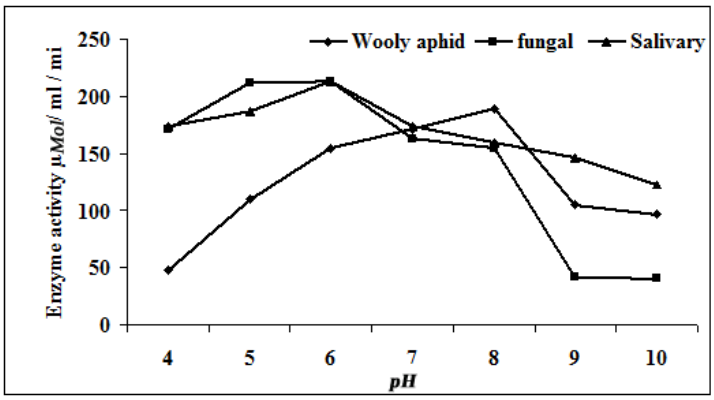

Figure 2. Effect of pH on the activity of woolly aphid, fungal and salivary α-amylase.

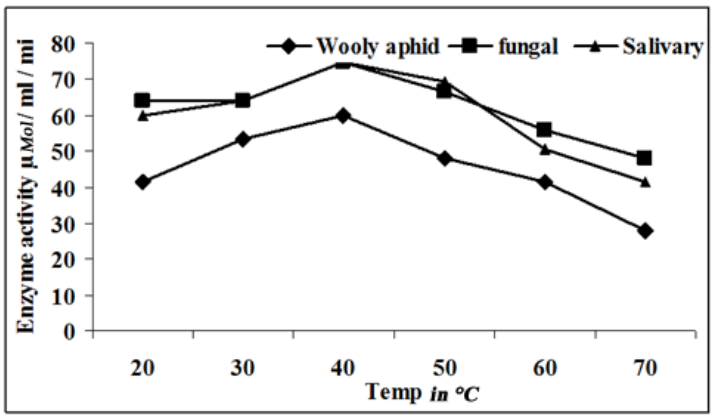

Figure 3. Effect of temperature on activity of woolly aphid, fungal and salivary α-amylases.

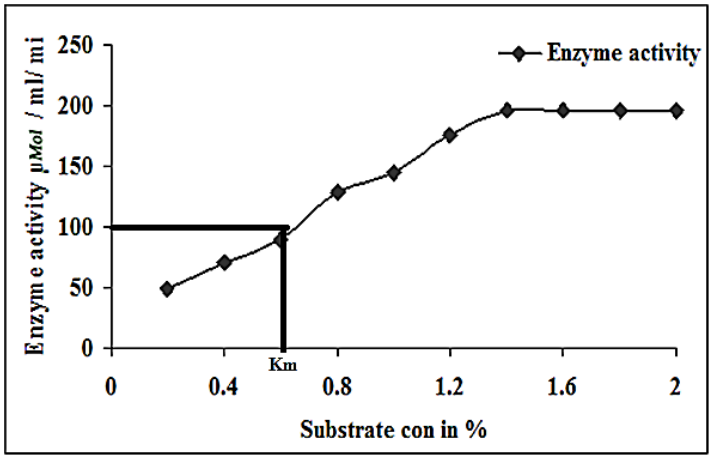

Figure 4. Effect of substrate concentration on enzyme (α-amylase) activity

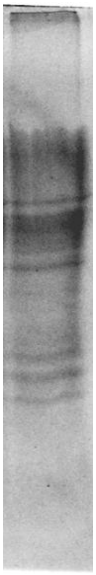

Figure 5. Electrophoretic protein profile of *Ceratovacuna lanigera* crude extract on 10 % native PAGE.

DISCUSSION

α-amylases, a starch digestive enzyme play vital role in carbohydrate digestion in insects. Sugarcane woolly aphids (*Ceratovacuna lanigera* Zehnter) are insect pest of sugarcane, induce biochemical changes in sugarcane(Padul et al

2008). Here we report the α-amylase activity from *C. lanigera* insect by simple and sensitive starch agar plate method and compare this activity with α-amylase activities from human saliva and fungal α-amylases which works well in neutral pH range. But our results show that α-amylase activity in *C. lanigera* is optimum at slight alkaline pH, which is consistent with the optimum pH reported for other insects (Alfonso et al., 1997, Valencia-Jimenez et al., 2008). All studied enzymes worked well at 40°C. Plant themselves have defense system for the pest and microbial attack. Some plants have α-amylases inhibitors, proteinase inhibitors, lectins and class of pathogenesis-related proteins. Recently proteinase inhibitors are detected in pigeonpea leaves and are found inducible (Padul et al. 2012). Till date people used different methods for the eradication of the *C. lanigera* like chemical pesticides, predators and fungal pathogens etc. but these methods not only have the limitations but also many of them have side effects which may lead another serious problems. Therefore there should be an eco-friendly approach to control this pest which is more effective and has no side effects. This could be possible by studying major digestive enzymes of the pest. In future it is possible to search the inhibitors of this enzyme. If these inhibitors exogenously added in plant cell and expressed, this would be better strategy for the pest control. α-amylase inhibitors in variety of plants are being studied for their possible use in strengthening plant defence against insect and microbial attacks (Garcia-Olmedo *et al.*, 1987). Further, inhibition of digestive enzymes of insect gut microbial flora is suggested for control of insect (Shinde *et al.*, 2012). α-amylase inhibitors from wheat (WAAI) and common bean (BAAI) has been characterized. Transgenic tobacco expressing WAAI gene has been reported to increase mortality of the lepidopteran larvae between 30 to 40 % (Carbonero et al. 1993). Also transgenic pea (*Pisum sativum*) expressing bean (*Phaseolus vulgaris*) α-amylase inhibitor in developing seeds has been found to be resistant to pea weevil, *Bruchus pisorum* (Schroder *et al.*, 1995) and storage pests *Callosobruchus maculatus* and *C. chinesis* (Shade *et al.*, 1994). In same way the transgenic variety of the sugarcane expressing the strong amylase inhibitory activity may be effectively used for the control of *C. lanigera*.

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