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Study of Amylase and Invertase Activity in Adults of *Chironitis Arrowi* (Janssens) (Coleoptera: Scarabaeidae: Scarabaeinae)

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Abstract:

Some characters of Amylase and Invertase from mid gut (MG) hind gut (HG) of adult; male and female dung beetle; Chironitis arrowi were studied. The pH maxima, the optimal temperature and Km of these enzymes were determined. Both enzymes were showing the pH maxima at 7.2 in both sexes of MG and HG .Temperature optima for these enzymes were occurred at 45^0C in both sexes and guts. The Km values of amylase were 0.47040% (MG) and 0.7272% (HG) in male and 0.266 %(MG) and 0.888 % (HG) in female dung beetle. For invertase the Km values were 4.247 X 10^{-3}M (MG) and 4.58 X 10^{-3}M (HG) in male and 8.49 X 10^{-3}M (MG) and 3.895 X 10^{-3}M (HG) in female. The 50% inhibition of amylase were occurred at 60^0C within 23 minutes (MG) and 40 minutes (HG) in male and 29 minutes (MG) and 41.30 minutes (HG) in female. The half life of invertase at 60^0C was occurred within 16 minutes (MG) and 20 minutes (HG) in male and 10 minutes (MG) and 17.75 minutes (HG) in female beetle. The digestion periods of 60 minutes (for amylase in both sexes and guts) and 80 minutes (for invertase in both sexes and guts) were fitted very well within the linear part of enzymatic action.

Key words: *Chironitis arrowi,* Characteristic of Amylase and Invertase, Km values.

1. Introduction

Dung beetles are an important component of dung fauna. ***Chironitis arrowi*** is a common true dung beetle found in South-Western Maharashtra. These dung beetles require dung as a food source during some stage of their lifecycle. Some species are generalists, attracted to and feeding on many types of dung. Others are specialized to groups of animals or, in extreme cases, use the dung of only one species (stenophagy) (Halffter and Matthews 1966). Generally, these dung beetles are more attracted to cattle dung, then to omnivore dung (Fincher et al. 1970).

We are presently engaging the study of digestive physiology of dung beetles. The adult beetles and grubs feed on the liquid and colloidal content of dung. The alimentary canal is adapted for coprophagy. The activity of most digestive enzymes is reflected with degree of adaptation to food components. Therefore, we presently have worked on starch and sucrose digesting enzymes of alimentary canal of ***adults*** of ***Chironitis arrowi.***

2. Materials and Methods

Insect Collection

The adults of ***Chironitis arrowi*** beetles were collected from the dung pads (3 to 4 days old) from the grazing fields of Phaltan region, Maharashtra, India. These adults were maintained in the laboratory under constant condition.

Enzyme Preparation:

The adult male and females were obtained from the laboratory stock for the preparation of mid gut (MG) and hind gut (HG)

enzyme extracts. Homogenates of the pooled tissues were prepared in 0.9% chilled NaCl, which were cold, centrifuged for 15 minutes at 10000 rpm. Aliquots of supernatants were used as enzyme source. Homogenates were stored in freezer until used.

Assay

The activities of amylase and invertase were determined by using 3-5 dinitrosalicylic acid (DNSA) reagents (Bernfeld, 1955). The aldehyde group formed due to enzymatic action on substrate reduces the DNSA reagent which was measured spectrophotometrically at 540 nm (Ishaaya and Swirski, 1970).

The assay mixture for enzymes consists of 1 ml appropriate substrate, 1 ml 0.1 M buffer of appropriate pH and 0.5 ml supernatant. The test-tubes were incubated at appropriate temperature and period of time. The reactions were terminated by adding 2 ml of DNSA followed 2 ml of distilled water. The test –tubes were heated in boiling water bath exactly for 5 minutes. Then tubes were cooled immediately. The activities for invertase, trehalase, cellulase, inulinase and salicinase are expressed as µg glucose / mg protein / hr. and for amylase as µg maltose / mg protein / hr.

Thermolability:

The grubs were dissected in 0.9% saline and their mid and hind guts were taken out for the enzyme extract preparation. A portion of enzyme extract was immediately stored in refrigerator for control purpose. The remaining portion of enzyme extract was then subjected to high temperature treatment by keeping the test-tubes containing enzyme in water both maintained at 60^0C for different period time. The various heat treated enzyme extracts were stored in the refrigerator, until they were used for experiment.

The activities of residual enzymes left after heat treatments were determined by the procedures as described earlier for respective enzymes.

Protein estimation: The soluble protein content of the enzyme extract was determined by Lowry *et. al*. (1951) method using bovine serum albumin as standard.

Assay mixture consisted of 0.5 ml of homogenate, made to 1 ml with double distilled water, to this added 5 ml of Lower's 'C'solution. Then after 10 to 15 minutes, 0.5 ml of Folin-Ciocalteus regent was added. The optical density was read at 640 nm after 20 minutes.

3. Results:

1) Effect of pH. : Both enzymes were showing the pH maxima at 7.2 in both sexes of MG and HG.Gr.No.1 and 2.
2) Effect of Temperature: Gr. No. 3 and 4 shows the temperature optima for these enzymes were occurred at 45^0C in both sexes and guts.
3) Effect of substrate concentration: The relationship between substrate concentration and rates of hydrolysis for MG and HG amylase and invertase were studied in both sexes. The Km values were obtained through line weaver burk's plot for these enzymes which are shown in Gr. No. 7and8
4) Effect of Time: The Gr. No.5and 6 shows the digestion periods of 60 minutes (for amylase in both sexes and guts) and 80 minutes (for invertase in both sexes and guts) were fitted very well within the linear part of enzymatic action.
5) Thermolability: The effect of higher temperature on the stability of amylase and invertase are shown in Gr. No. The 50% inhibition of amylase were occurred at 60^0C within 23 minutes (MG) and 40 minutes (HG) in male and 29 minutes (MG) and 41.30 minutes (HG) in female. The half life of invertase at 60^0C was occurred within 16 minutes (MG) and 20

minutes (HG) in male and 10 minutes (MG) and 17.75 minutes (HG) in female beetle.

4. Discussion

The pH optima of amylase and invertase in both gut sections of male and female beetles' falls within the pH range of haemolymph and alimentary canal. The pH of alimentary canal content is ranging from 7 to 7.5 while haemolymph have pH of 7.0 (determined by p H paper method).It is interesting that amylase and invertase enzymes in present dung beetle, in both sexes and gut sections, showed optimal pH at 7.2.These enzymes showed maximum range activity in between p H 6.8 to7.6.This range of maximum activity of these enzymes is very close to the p H of the alimentary canal, indicating that pH conditions in mid gut and hind gut are suitable for these enzyme activity. Similar range of pH for these enzymes were observed for larvae and adults of *Holotrichia, Leucopholois, Onthophagus, Chironitis, Onitis* (Bhanot, 1992; Patil, 1996; Gaikwad et.al., 1997; Gaikwad, 1998) .However in other insects like pulse beetle, *Tribollium* and *Tenebrio*, acidic pH range for amylase (4.6 to 5.8) was reported (Poddler and Applebaum, 1971; Applebaum and Conigan, 1965 and Bounocor et. al., 1976),for Invertase (5.0 to 6.5) was reported in the insects like cockroach, cabbage butterfly, khapra beetle and locust (Wigglesworth,1927;Nishide and Kusano,1976;Krishna,1958 and Evans and Payne,1964).

Temperature optima for these enzymes in both guts sections of this dung beetle showed at 45^0C.Similar higher temperature optima also recorded by Terra *et. al.* (1977);Day and Powing,1949;Kusano and Tanabe(1986);Teo and Heng (1987) and Gaikwad(1998).(for *Richnocera americana, Bombyx mori, Biattella germonica, Valanga nigriconis, Chironitis arrowi* and *Onthophagus catta).*

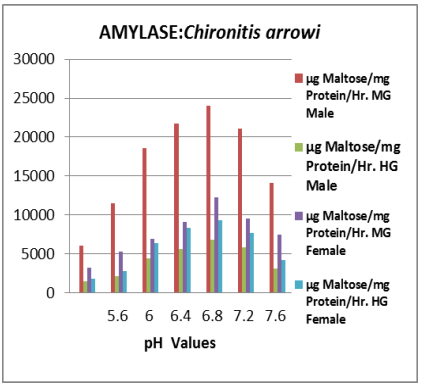
The Km values of amylase and invertase were calculated from Line weaver –Burk's plots. It is important parameter as it provides valuable information regarding the mode of action of an enzyme catalyzing the reaction (Conn and Stumf 1963). In present dung beetle the Km values of amylase were 0.47040% (MG) and 0.7272% (HG) in male and 0.266 %(MG) and 0.888 % (HG) in female dung beetle. This indicates MG amylase is more efficient having greater affinity towards the substrate. In present dung beetle male mid gut amylase Km value (0.266% of starch) is lowest then in male hind gut and both the gut sections of female. In ***Drosophila melanogaster*** different verities showed different Km values for amylase such as 0.9% and 0.15% of starch (Droste and Zebe, 1974).In ***Tenebrio moliter*** and ***Calasobruchus chinesis*** 0.18% AND 0.23% OF STARCH AS Km values were observed by Poddler and Applebaum(1971). Similar Km values were recorded in other dung beetle grub (Gaikwad *et. al.,* 1998).

The Km values for invertase were 4.247 X 10^{-3}M (MG) and 4.58 X 10^{-3}M (HG) of sucrose in male and 8.49 X 10^{-3}M (MG) and 3.895 X 10^{-3}M (HG) of sucrose in female. These values indicate that hind gut invertase in female is much more efficient than invertase of male mid gut and hind gut. Only in few insects the Km values for gut invertase were determined. These are 3.92 X10^{-3} M for cabbage butterfly larvae (Nishide and Kusano,1973); 3.04 X 10^{-2} M for Valanga (Teo,1973); 2.337 X 10^{-3} M in larval mid gut and 7.789 X 10^{-3} M in male mid gut of *Holotrichia serrata* (Patil, 1996) 2.43 X 10^{-3} M and 7.12 X 10^{-4} M for Chiloloba orientalis (Kumbhar,1996); 8.65 X 10^{-3} M and 6.49 X 10^{-3} M for *Onthophagus catta*(Gaikwad, 1998).

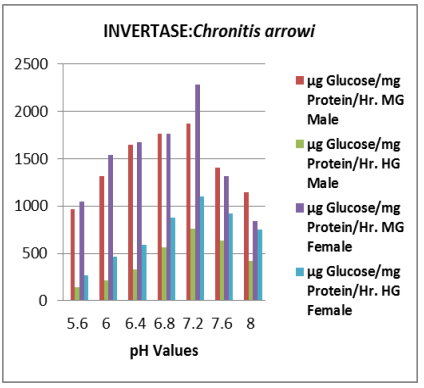
The digestion periods of 60 minutes (for amylase in both sexes and guts) and 80 minutes (for invertase in both sexes and guts) were fitted very well within the linear part of enzymatic action. Gaikwad (1998) reported 30 minutes (in *Onthophagus catta*).

The effect of higher temperature on the stability of amylase and invertase were studied in this dung beetle. The half life of amylase was occurred at 60^0C within 23 minutes (MG) and 40 minutes (HG) in male and 29 minutes (MG) and 41.30 minutes (HG) in female. The 50% inhibition of invertase at 60^0C were occurred within 16 minutes (MG) and 20 minutes (HG) in male and 10 minutes (MG) and 17.75 minutes (HG) in female beetle. The result indicates that Amylase and Invertase enzymes of hind gut are more heat stable than mid gut.

Effect of pH

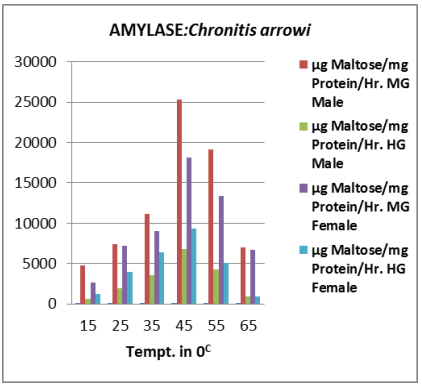


Gr.No. 1

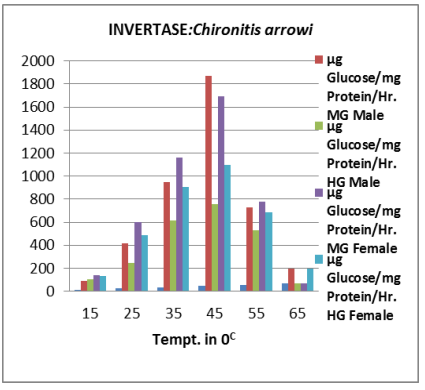


Gr.No.2

Effect of Temperature

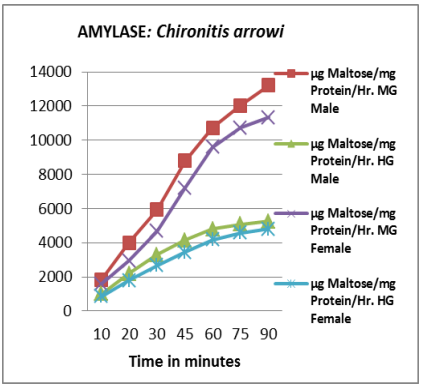


Gr. No. 3

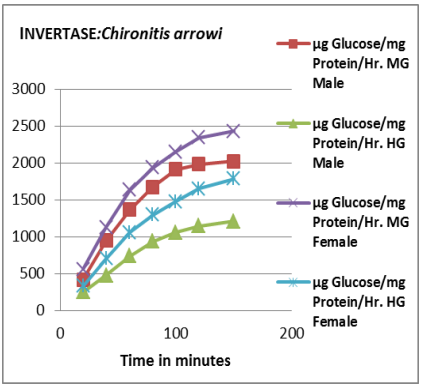


Gr. No.4

Effect of Time

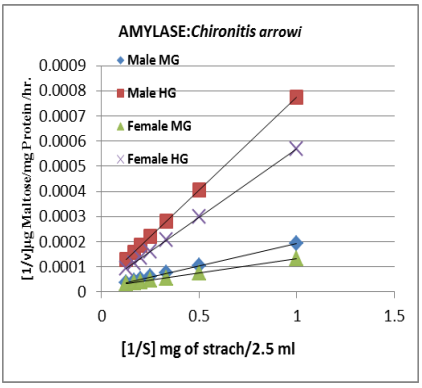


Gr. No. 5

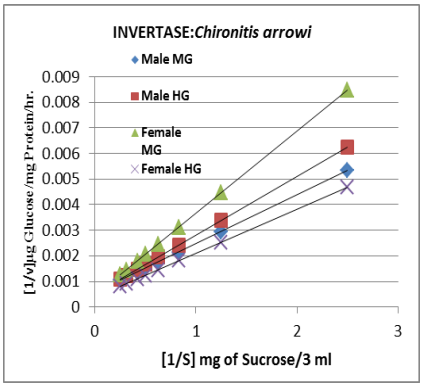


Gr. No. 6

Line weaver –Burk's plots



Gr. No. 7



Gr. No. 8

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