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The Digestive Lipase and Protease in the Dung Beetle, *Chironitis Arrowi* (Janssens)

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Abstract: ***Characteristics of lipase and protease from mid gut (MG) and hind gut (HG) of adult dung beetle; Chironitis arrowi were studied. The pH maxima, the optimal temperature and Km of these enzymes were determined. Lipase was showing the pH maxima at 8.0, while protease interestingly showed two pH optima at 7.0 and 10.0 in both sexes and guts. Temperature optima for lipase were occurred at 40^0C in both guts and sexes. However protease was showing higher temperature optima at 55^0C in both guts of male and female. The Km values of lipase were$3.175x10^{-4}$M and $2.413x10^{-4}$ M in male and female (MG) respectively. For protease the Km values were 0 .0312% in male (MG) and 0.0357% in female (MG). The 50% inhibition of lipase occurred at 50^0C within 9.5 minutes in male (MG) and 9.7 minutes in female (MG). The half life of protease at 65^0C was occurred within 38.5 minutes and 31.50 minutes, in male and female (MG) respectively. The digestion periods of 10 minutes (for lipase in both sexes, MG) and 20 minutes (for protease in both sexes, MG) were fitted very well within the linear part of enzymatic action.***

Keywords: Dung beetle, characteristics of lipase, protease enzymes, Km values, thermolability.

1. Introduction

The dung beetles play a vital role in natural ecosystems. Bornemissza (1960) suggested that dung beetles could help to remove dung pads in an efficient manner. Dung beetles are an important component of dung fauna. In many areas they are the dominant species present at vertebrate dung. They exhibit a wide range of ecological, morphological and behavioral adaptations that have helped them to establish in various regions of the world (Hanski and Cambefort, 1991). ***Chironitis arrowi*** a common true dung beetle found in Maharashtra. Adult dung beetles have specialized mouthparts for dung feeding (Halffter and Matthews 1966). We are presently engaging the studies related to digestive physiology of dung beetles. The adult beetles 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 protein and fat digesting enzymes of alimentary canal of ***Chironitis arrowi***.

2. Materials and Methods

2.1. Insect Collection

The dung beetles were collected from 2 to 3 days old cattle dung pads from the grazing fields of Phaltan region, Maharashtra, India. The collected beetles were maintained in the laboratory in earthen pots.

2.2. Preparation of Enzyme Extract

After acclimatization the male and female beetles were sacrificed separately for preparation of mid gut (MG) and hind gut (HG) enzyme extracts. The homogenates of pooled tissues were prepared in 0.9% chilled NaCl, which were cold, centrifuged for 15 min. at 10,000 rpm. Aliquots of supernatants were used as enzyme source. The homogenates were stored in freezer until used.

2.3. Lipase Assays

The activity of lipase was measured according to Hayashi and Tappel (1970). The assay system consisted of 0.25% ml emulsion of trioline in appropriate buffer, 0.25 ml of supernatant and 1.5 ml of appropriate buffer followed by incubation at appropriate temperature for 10 minutes using metabolic shaker. The reaction was terminated by adding 2 ml of ATC (mixture of acetic acid; tri-ethanol amine and copper nitrate in ratio of 1:9:1) reagent and then 10 ml of chloroform the reaction mixture was then vigorously shaken and allowed to stand for 10 minutes. Then 2 ml of the chloroform layer was pipette out in well stopper tube. In this tube 1.5 ml of lipase colouring reagent (25 mg diphenylcarbazone and 475 mg of diphenylcarbazide in 100 ml methanol) was added. The liberated free fatty acids in chloroform produce pink colour with colouring reagent, were measured colorimetrically according to Itaya (1977) at 550 nm. The standard curve was obtained by using palmitic acid under similar assay conditions. The activity was expressed as µg palmitic acid/ mg protein/hr.

2.4. Protease Assays

The protease activity was determined by using Birk *et. al.* (1962) procedure as used by Ishaaya *et. al.* (1971).The absorbency of the reaction mixture was read as O.D. units using quartz cuvettes Hitachi U. V. spectrophotometer at 280 nm against a control in which the enzyme was substituted by preheated supernatant. The reaction mixture consisted of 1 ml of1% Caesin (Hammerstein) prepared in appropriate 0.1 M buffer solution and 0.5 ml of supernatant. The reaction mixture was incubated at appropriate temperature for 20 minutes. The reaction was terminated by adding 3ml of 5%TCA. The precipitate of unreacted protein was removed by centrifugation. The supernatants thus obtained were read at 280 nm against the blank. The

standard curve was obtained by using different tyrosine concentrations, keeping conditions constant. The activity was expressed in terms of µg tyrosine/mg protein/hr.

2.5 Thermolability

To study the thermolability of these enzymes, the adults were dissected in 0.9% saline and their mid 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 bath maintained at 50° C (for lipase) and at 65° C (for protease) for different period of 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. The mean activities of control (without heat treatment) were taken as 100% activity of lipase and protease enzymes.

2.6 Protein Estimation

Lowery *et.al. (*1951) method was used for determining soluble protein content of the enzyme extract. Assay mixture consisted of 0.5 ml of enzyme extract made to 1 ml with distilled water to this 5 ml of Lowery's 'C' Solution was added. Then after 10 to 15 minutes 0.5 ml of Folin-Ciocalteus regent was added. The optimal density as read at 640 nm after 30 minutes.

3. Results

The characteristics of lipase and protease in mid guts and hind guts of adult dung beetle ***Chironitis arrowi*** were studied. The results are as follows.

3.1Effect of pH

The lipase showed the maximum activity at pH 8.0 in both sexes and guts(Gr.1),while the protease interesting showing two pH optima ,one at pH 7.0 and other at pH 10.5 in both gut sections of male and female beetles(Gr.2)

3.2. Effect of Temperature

The lipase enzyme showed comparatively low temperature optima of 40°C irrespective of sexes and guts (Gr.3) while protease showed higher temperature optima of 55°C in both sexes and guts (Gr.4).

3.3. Effect of Time

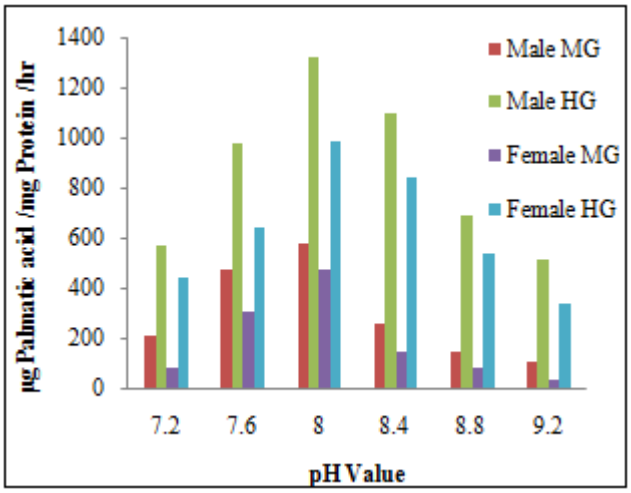
The digestion period of 10 minutes was found to be fit within the linear part of enzymatic activity curve for mid gut lipase of both sexes. The digestion period of 20 minutes for protease in mid gut of both sexes was found to be fit very well within the linear part of enzymatic activity curve.

3.4. Thermolability

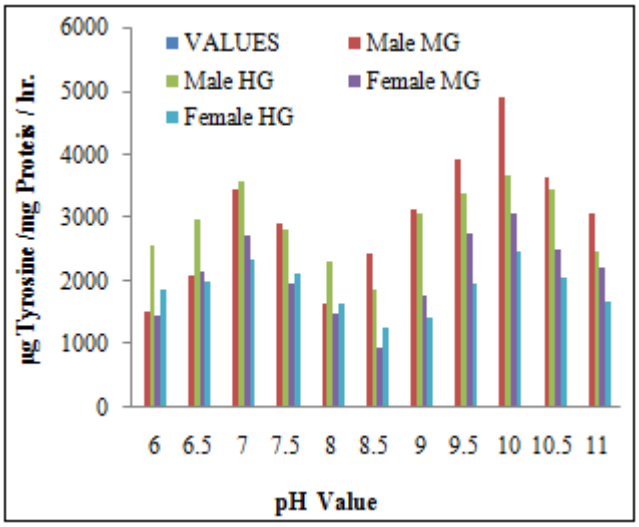
The effect of high temperature on the stability of lipase (at 50°C) and protease (at 65°C) were studied in mid gut sections of this dung beetle species. The 50% inhibition of lipase occurred at 50°C within 9.5 minutes in male (MG) and 9.7 minutes in female (MG) (Gr.5). The half life of protease at 65°C was occurred within 38.5 minutes and 31.50 minutes, in male and female (MG) respectively (Gr.6).

3.5. Effect of Substrate Concentration

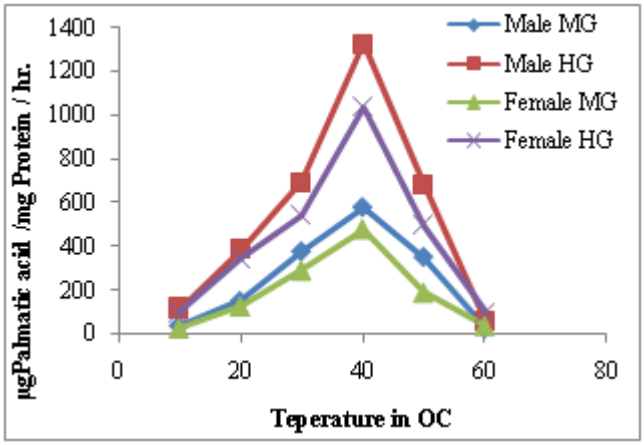
The relationship between substrate (triolene for lipase and casein for protease) concentration and rate of hydrolysis of these enzymes were studied in mid and hind guts of adult *C. arrowi* .Line weaver- Burk's plots were plotted so as to obtain Michaeli's constant(km) , by using reciprocals of substrate concentration (1/S) and reciprocals of velocity concentration (1/V). Such Line weaver Burk's plots are shown in Gr.7 (for lipase) and Gr.8 (for protease). The Km values of lipase were$3.175x10^{-4}$M and $2.413x10^{-4}$ M in male and female (MG) respectively. For protease the Km values were 0 .0312% in male (MG) and 0.0357% in female (MG).



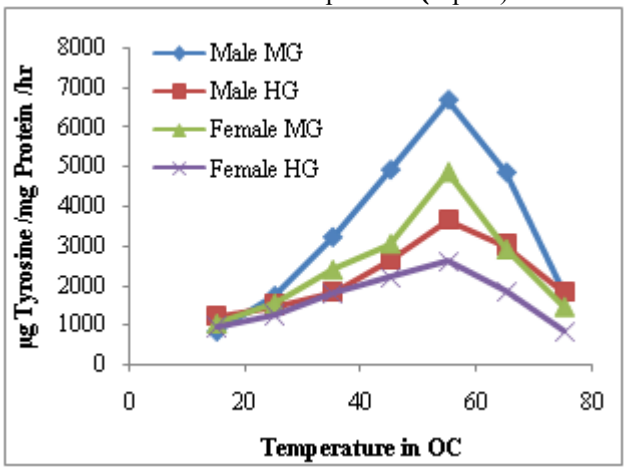
Gr.1. Effect of pH (Lipase)



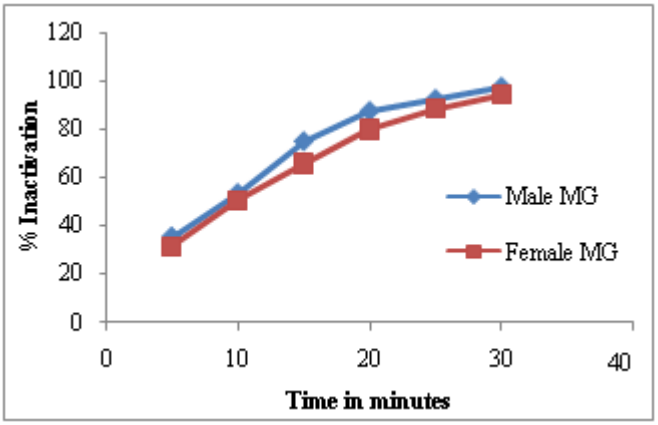
Gr.2. Effect of pH (Protease)



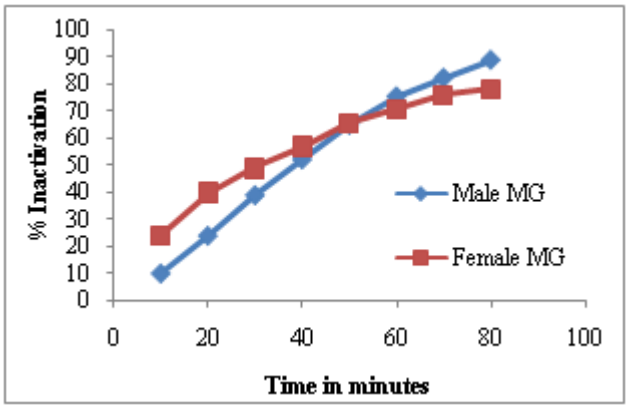
Gr.3. Effect of Temperature (Lipase)



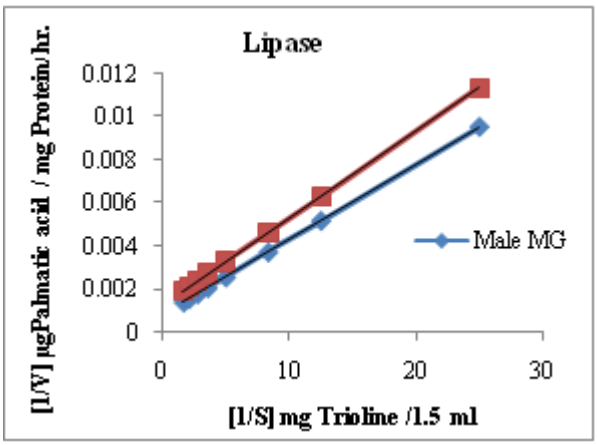
Gr.4. Effect of Temperature (Protease)



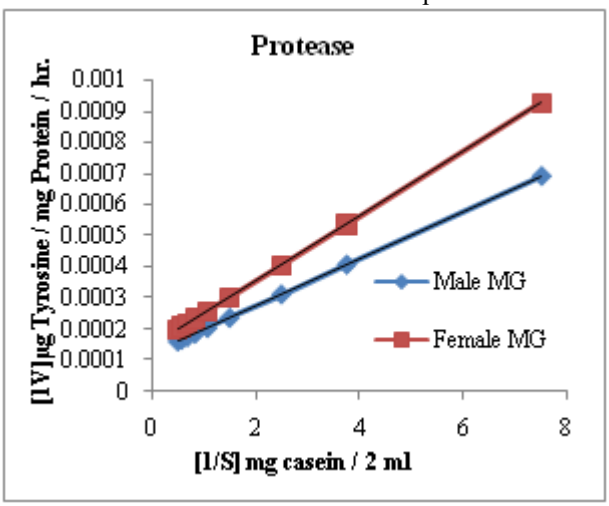
Gr.5. Thermolability (Lipase)



Gr.6 Thermolability (Protease)



Gr.7. Line weaver Burk's plot



Gr.8 Line weaver Burk's plot

Male MG: Y= 0.000827+0.000348X Male MG: Y= 0.0001261+ 0.0000749X
Female MG: Y= 0.001273+0.0004036X Female MG: Y= 0.0001481 + 0.0001034X

4. Discussion

Lipase showed pH optima at 8.0 in both sexes and gut sections of alimentary canal. Similar findings were obtained for the some Scarabaeid species by earlier workers (Bhanot, 1992, Patil, 1996 and Kumbhar, 1996). Scant information exists on the insect lipase (Terra *et.al.*, 1979; Male and Storey, 1981; Thomas and Nation, 1984; Bhawane and Bhanot, 1989; Pol and Sawant, 1990 and 1995). They reported pH optima in the range of 8.2 to 8.8 in the insects.

Very interestingly the protease enzymes showed two peaks of activity at two different pH. The adults of *C. arrowi* in both gut section showed maximum activity of protease at pH 7.0 and 10.5 for this enzyme. Thus the enzyme protease is active either neutral or at highly alkaline pH. Similar condition exit in the some species of Scarabaeid studied earlier (Bhanot 1992; Patil 1996 and Kumbhar 1996). Above observations indicates that there exists iso-enzymes of protease in the mid and hind guts of Scarabaeidae. But the activity of iso enzymes having pH 10.0 more pronounced. Optimum pH values for the protease in most of the insect studied are either neutral or alkaline and they are found to be

slightly higher than the pH of gut contents, (Ishaaya, *et.al.,* 1971; Eguchi, *et, al.,* 1982; Eguchi and Iwamoto, 1976).

Lipase showed maximum activity at temperature 40^{o}C in studied gut sections of dung beetles. Similar temperature optima for lipase were observed in some insects by Patil (1996); Pol, (1984); Pol and Sawant (1995).Temperature optima of 30^{o}C was observed for the lipase in crickets by Thomas and Nation (1984). In *Acheta domesticus* adults the temperature optima of lipase are at 37^{o}C (Teo and Woodring, 1988).

Protease showed maximum activity at Temperature 55^{o}C; .more or less similar temperature range was reported in other insects by Bhanot (1992); Baker (1977); Gooding ;(1977); Gooding and House ;(1969); Ishaaya and Swirski (1970) and Patil (1996).

Digestion period of 10 minutes was found to be fit very well within the linear part of enzymatic activity curve for lipase. Similar result was also obtained in *C. orientalis* (Kumbhar, 1996) and *H serrata* (Patil, 1996) for this enzyme. The digestion period of 20 minutes was found to be fit well within the linear part of enzymatic activity curve for protease in studied dung beetle species. In other insects in which this enzyme was studied showed same digestion period (Kumbhar, 1996; Patil, 1996).

The 50% inhibition of lipase occurred at 50^{0}C within 9.5 minutes in male (MG) and 9.7 minutes in female (MG). The results co-related with that of *C. orientalis* (Kumbhar, 1996); and in *H. serrata* Patil (1996).This indicates that lipase is most heat labile enzyme. The half life of protease at 65^{0}C was occurred within 38.5 minutes and 31.50 minutes, in male and female (MG) respectively. Papin is most heat stable Proteolytic enzyme which lost of its activity after 120 min. of heat treatment at 70% (Powing *et.al.*1951).

In the present dung beetle species, the Km values of lipase were3.175x10^{-4}M and 2.413x10^{-4} M in male and female (MG) respectively. The km values for lipase of *Chrysomia rufifacies* (4.0 x 10^{-4} M of triolene); for *Valanga* (2.68 x 10^{-2} M of triolene) ; for *Valanga* (2.68 x 10^{-2} M of tributryn); for *H. serrata*(6.284 x 10^{-4} M and 1.5 x 10^{-3} M of triolene) ;for *C. orientalis* (2.28 x 10^{-4} M of triolene and 2.15 x 10^{-4} M of triolene) (Teo, 1973, Pol, 1984; Pol and Sawant, 1990; Patil, 1996 and Kumbhar, 1996).

Protease showing km values 0.0315% and 0.0357% of casein in mid gut of male and female respectively. This indicates that in *C. arrowi* protease is equally efficient in both sexes. Very little is known about the km values of the gut proteases in insects. In Valanga, km values for protease were 19.358 mg/ml for casein and 9.025 mg/ml for gelatin (Teo, 1973). The km vale for larval blowfly protease was 71.9 mg/ml (Evans, 1958); for larval and adult mid gut of *H. serrata* were 0.028% and 0.069% of casein respectively (Patil, 1996) and larval and adult mid gut of *C. orientalis were* 158% and 0.476% of casein respectively (Kumbhar, 1996).

The mid gut is the measure source for these digestive enzymes in this dung beetle species. These results agree with the general view that mid gut is the chief site of digestive enzyme secretion (Dadd, 1970; Law, *et.al*, 1977; Engelmann and Gerarets, 1980). However, hind gut also contributes significantly in the secretion of digestive enzymes in larvae and adults of the dung beetle species. This indicates that the hind gut also plays important role in the process of digestion of coprophagous larvae and adults of *O. philemon* and *O. catta (*Gaikwad, 1998). Similar results were also obtained by Thomas and Nation, (1984) in the hind gut of *G. rubens* and *S. acletus*; by Bhawane and Bhanot (1989) in hind gut of white grub, *H. serrata* by Bhanot (1992) in *L. lepidophora* larvae; by Patil, 1996 in larvae and adults of *H. serrata* by Kumbhar (1996) in larvae of *C. orientalis*.

5. Acknowledgement

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