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Comparision of Midgut Trehalase Characteristics in Bivoltine and Multivoltine *Bombyx* mori L.

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ABSTRACT : The optimal pH of midgut trehalase from CSR2 and Kolar gold (PM X CSR2) were 5.5 the 50% inactivation time for trehalase at 60°C were 3 min. in CSR2 and 10 min. in Kolar gold. The optimal temperature for both races was 50°C. The Km value for CSR2 was 10.57 × 10^{-3} M and for Kolar gold was 3.0 × 10^{-3} M.

Keywords : Midgut trehalase, *Bombyx mori* bivoltine race.

INTRODUCTION

In many insect trehalase is the major haemolymph sugar and serves as an indispensable substrate for energy production and biosynthesis of macromolecules (Wyatt 1967). The trehalose play vital role in silkworm carbohydrate metabolism by hydrolysis of trehalose into two glucose moieties. It is localized in midgut, haemolymph, ovary, pupal midgut, fat bodies, thorasic muscles etc. were studied by various workers (Saitto 1960, Derr 1966, Dahlman 1970, Zebe 1959) in various insects. In silkworm pharate adult the trehalase activity is extremely high in midgut than other tissue (Yamashita, 1974). The membrane bound trehalase is reported in *B. mori* midgut (Sumida, 1974).

The trehalase level in the haemolymph of Vth instar larvae of three different varieties of bivoltine, multivoltine and cross breed were investigated (Sowri and Sarangi 2002). In this article, information is presented on midgut trehalase enzyme characteristics such as specific activity, effect of pH, temperature, effect of time, thermolability and substrate concentration in bivoltine race i. e. CASR2 and multivoltine race i.e. Kolargold (PM × CSR2) comparatively.

MATERIAL AND METHODS

The DFLs of bivoltine race CSR2 and multivoltine race Kolargold of *B. mori* were procured from Director Sericulture Government Grainage centre, Gadhinglaj, District Kolhapur. The eggs were incubated at 25°C and 85% humidity and worms were reared as per recommended regimen of Krishnaswami (1978, 1979) in rearing house of Shivaji University, Kolhapur.

The V^{th} instar larvae of both races were dissected in chilled insect ringer. The midgut was homogenized in 0.8% insect saline. Homogenate was centrifuge at 3000 rpm for 15 min. The supernatant used as enzyme source during characterization study of trehalse.

In enzyme assay contained 0.5 ml supernatant, 1ml appropriate buffer, 1ml 0.25% trehalose as substrate. The mixture incubated for 1 hr at 40°C. The reaction was terminated by adding 2.5ml DNSA. The reaction mixture was heated in boiling water bath for 5 min. Then 2.5ml distilled water was added. The developed colour was measured on spectrophotometer at 540 nm.

The pH optima determined by using appropriate buffers. The optimum temperature was determined by incubating at 10 to 70°C temperature. To determine Km values 0.125% trehalase used as substrate and concentration of substrate varied from 0.0125 to 0.125 mg/ml. The thermolability studies by killing the enzyme extract at various temperature. For each experiment three observations were maintained.

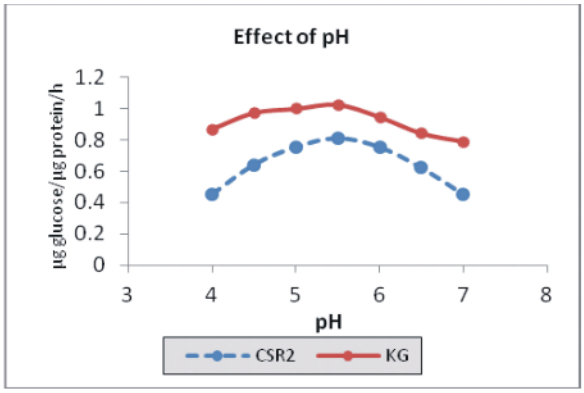
The protein concentration of enzyme extract was determined as per Lowry *et al.*, (1951).

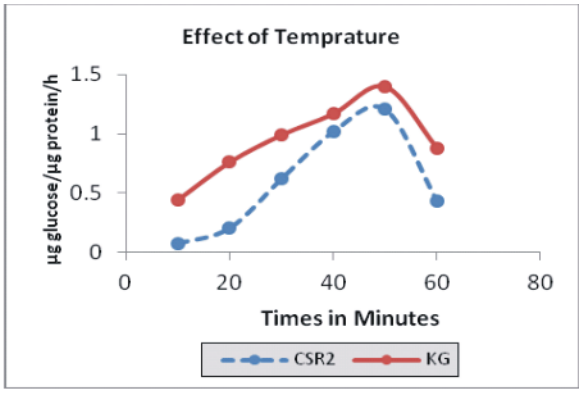
RESULT AND DISCUSSION

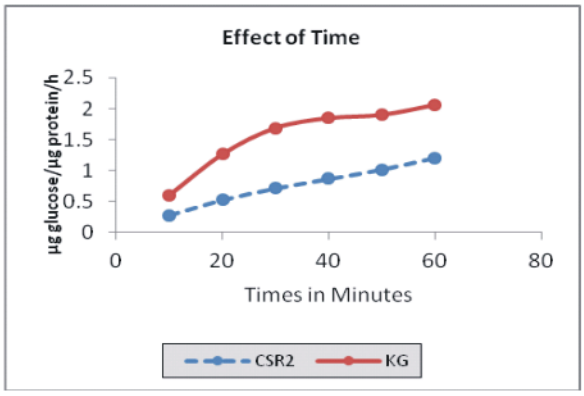
The activity of midgut trehalase is associated with food intake in B. mori. The last two days of Vth instar trehalase level was maximum observed at VI and VII day in *Bombyx mori* L. (Sawri and Sarangi 2002). The pH optima of midgut trehalase in bivoltine race CSR2 and multivoltine race Kolar gold were 5.5. The present observation is consistent with Sumida and Yamashita (1977) studied in *B. mori.* It is quite close to the optimal pH of House cricket (5.2 pH). In gypsi moth pH optima 6.0 studied by Algimantas *et. al.*, (1992) in *Rhychosciora americana* larvae pH optima is 6.0 (Terra *et. al.*, 1994).

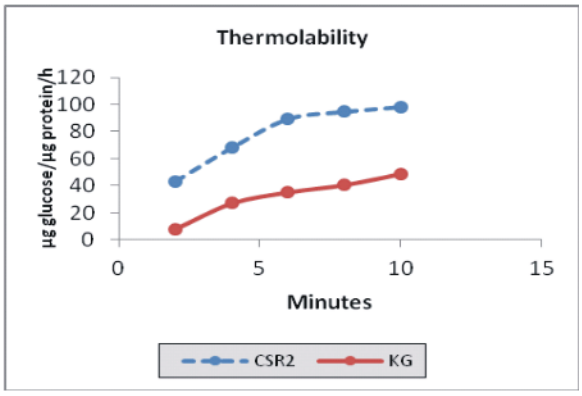
The trehalase activity in both races increased linearly as temperature rose from 10–50°C and fall down significantly at 60°C to 70°C. The temperature for both races was same at 50°C. The temperature optima in other insects were studied at 45°C in *Leucopholis lepidophora* (Bhawane *et. al.*, 1991), in Tobacco hornworm larvae 37.50 (Dahlman 1971), 40°C in *Pantala flarescents* (Bhawane *et. al.*, 1998).

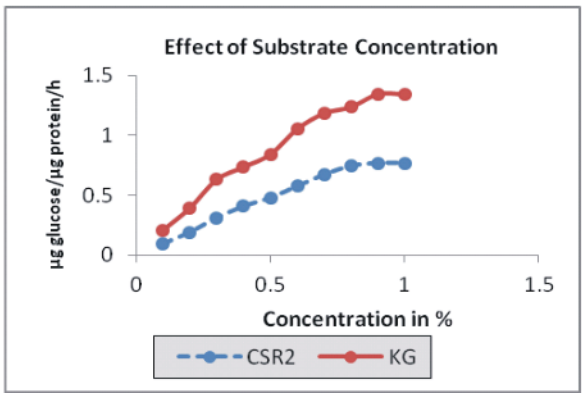
The observation in both the races shows higher optimal temperature but in order to maximize yield and to minimize any potential heat induced changes in enzyme when *B. mori* reared at low temperature (Algimantas *et. al.*, 1992).

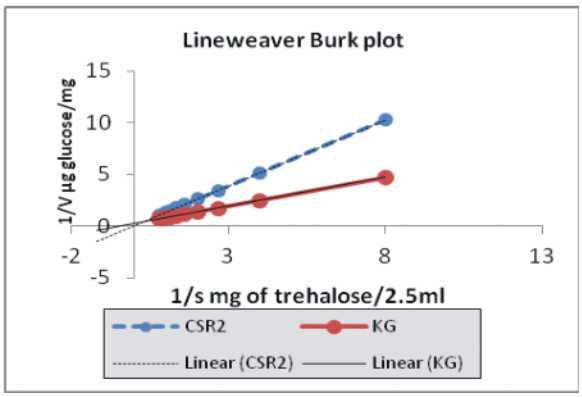












The half life period for midgut enzyme trehalase in CSR2 is 2 minutes while in Kolar gold is 10 minutes when enzyme heated at 60°C. In *Valanga nigrocornis* half life period of 132 min at 60°C, while in *Holotricia serrata* 50% loss of activity showed at 60°C for 17 minutes in midgut and 18 minutesin hindgut of male (Bhawane and Mandlik, 1992).

In Tobacco hornworm larvae the half life period of trehalase showed 50 minutes at 50°C and 57°C was 9 minutes only (Dalhman, 1971). The Km values were reported by various workers in various insect for midgut trehalase. The Km value for *Leucopholis lepidophora* is 1.6×10^{-4}M (Bhawane *et al*., 1991), in *Rhyncocera americana* 6.7×10^{-4}M (Terra, 1978), *Holodermis mossambicus* 9.7×10^{-3}M (Retief, 1973), *Locusta migratoria* 9.7×10^{-3}M (Worm, 1981) were recorded. In present study the Km value for CSR2 was 10.57×10^{-3}M and in Kolar gold was 3×10^{-3}M. This comparison showed that the midgut trehalase is more efficient in the Kolar gold than CSR2.

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