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CHARACTERISTICS OF MIDGUT TREHALASE IN SILKWORM *BOMBYX MORI* L. A COMPARATIVE ANALYSIS IN BIVOLTINE RACES CSR2, CSR2×4 AND CSR4

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ABSTRACT

In present study, the comparative study on midgut trehalase in fifth instars larvae of bivoltine races CSR2, CSR2×4 and CSR4 were done. The maximum activity of trehalase showed at pH 5.5 in all the races at maximum temperature 50^0C in CSR2 and CSR2×4 while in CSR4 at 45^0C. The digestion time required for 30 minutes in race CSR2 and CSR2×4 and 20 minutes for CSR4.The Km values calculated from the graph. The Km values for CSR2, CSR2×4 and CSR4 were $10.57x10^{-3}$M, $2.57\ x10^{-3}$M and $0.77x10^{-3}$M respectively. It indicates that CSR4 showed maximum efficient trehalase activity as compared to other two races.

KEYWORDS: Midgut trehalase, Bivoltine, Silkworm.

INTRODUCTION

The trehalase is an enzyme present in midgut of major species. It hydrolyse the non reducing sugar disaccharide, trehalose (a-D-glucopyranosyl-1, 1-a-D-glucopyranoside) into monosaccharide i.e. glucose.Trehalose is present in all organisms from yeast to higher animals. It is also present in insect as a major haemolymph sugar. It is storage of metabolic energy.[1,2,3,4,5] Trehalose of insect is indispensible substrate for energy production and macromolecules biosynthesis.[2,6,7]The amount of Trehalase present in all species is depends on method of extraction and source of tissue.[8,9,10,11] Trehalase is of two different forms viz, one is pupal midgut soluble form and other is membrane bound.[12] The membrane bound trehalase is associated with uptake and utilization of haemolymph trehalose in cells.[13] The physiological function of the soluble trehalase, characterization and purification of trehalase have been worked out in several insects.[14,15]The trehalase activity has been determined in larva, pupa and adult of *Pissodes notatus*,[16] *Bombyx mori*[17], *Pieris brassicae*.[18]

From the above literature survey it showed that, much more information is available on the insect anatomy, histology, physiology of digestive system, salivary gland and their physiology, silk gland and their secretion in silk worm but the information on characteristics of midgut enzyme trehalase in bivoltine races of mulberry silkworm was not documented. Therefore, in the present investigation, work was carried out on detailed study on characterization of midgut enzymes trehalase in bivoltine races of *Bombyx mori.* The biochemical study may helps in output of silk in different racial organisms.

MATERIAL AND METHODS

The rearing of silkworm CSR2, CSR2×CSR4 and CSR4 of bivoltine races were done in the rearing house, Department of Zoology, Shivaji University, Kolhapur by the rearing technique.[19] The larvae were fed on mulberry leaves which were collected from mulberry garden of Department.

Midgut Enzyme Extract: (Homogenate preparation)

For the enzymological study, crude homogenate of midgut of V^{th} instar larva was used as an enzyme source. The larva was anesthetized by chloroform soaked cotton plug and dissected in chilled insect ringer's solution. The gut was removed from the body and washed in 0.8% saline. The midgut content was flashed and weighed the tissue on electronic balance for homogenate preparation. The weighed tissue homogenized by mortar and pestle. The extract made in insect saline and centrifuged at 3000 rpm for 5 min. The supernatant used a crude enzyme extract for enzymatic assay.

Enzyme assay

Determination of trehalase enzyme activities measured by using 3, 5 dinitrosalicylic acid (DNSA) reagent[20] which produces reducing sugar. The colorimetric estimation was done to measure the reducing sugars which formed in the reaction with 3, 5 dinitrosalisylic acid. The reaction mixture contained 0.5ml crude enzyme extract, 1ml appropriate pH and 1ml 0.25% trehalose solution as substrate. The reaction mixture was incubated at 40^oC for 1 hour. The incubation of reaction mixture was terminated by addition of 2.5 ml DNSA reagent. The mixture was boiled in boiling water bath for 5 min. The reaction mixture contain test tubes were

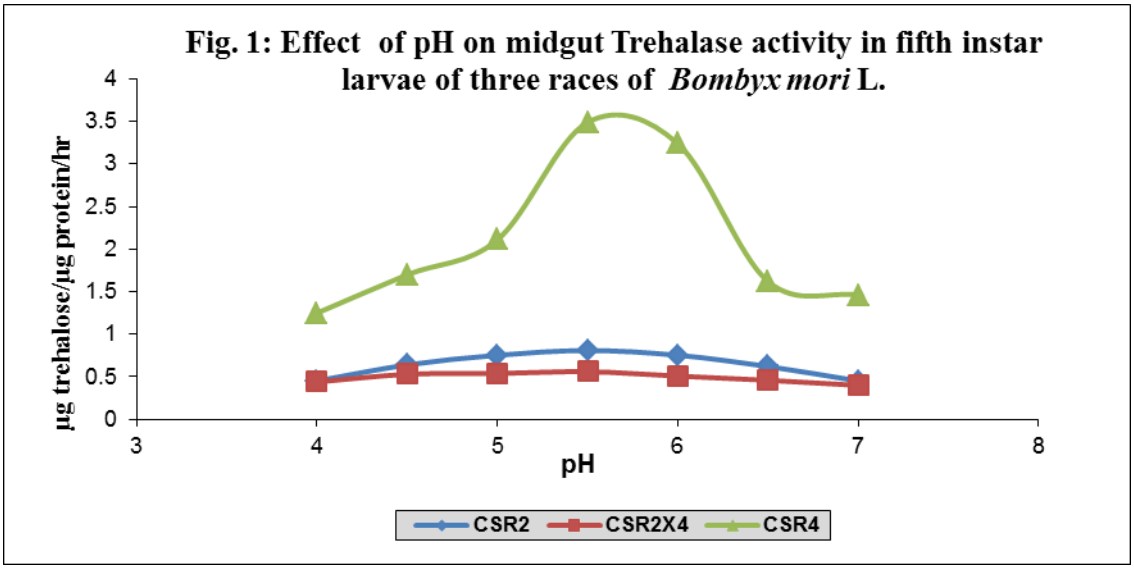
cooled immediately in ice bath. After immediate cooling, 2.5 ml distilled water was added in each tube. The developed colour was measured on spectrophotometer at 540 nm.[21]

The standard graph was obtained by glucose following the same protocol. The specific activity was expressed in µg glucose/µg protein/hour.

RESULTS

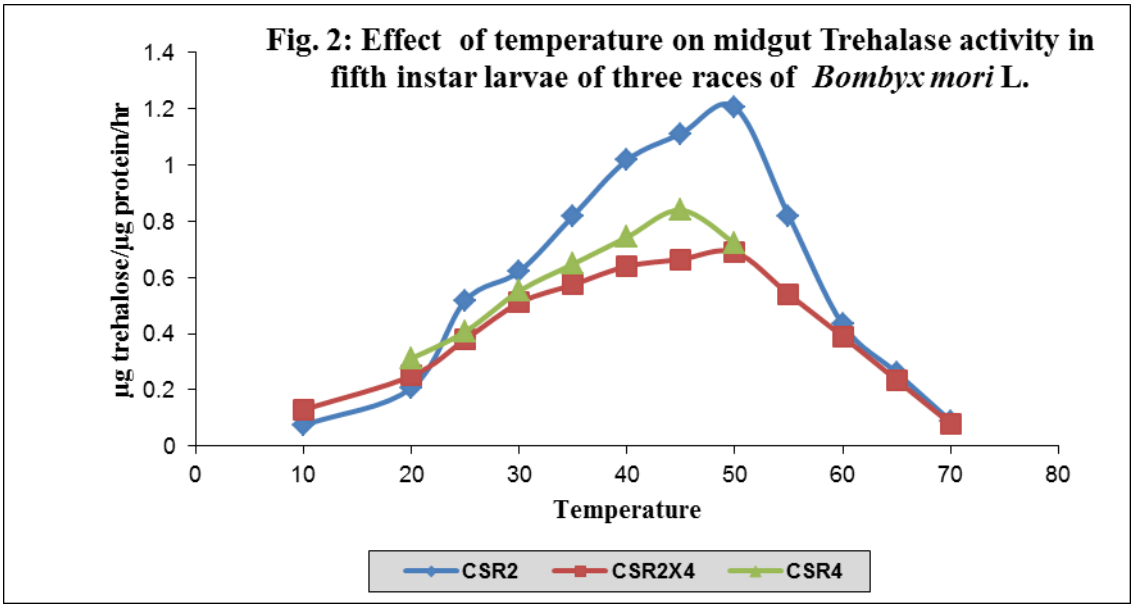
(a) Effect of pH

The maximal activity of trehalase in mid gut of *B. mori* under study in bivoltine races showed at pH 5.5 **(Fig. 1).**



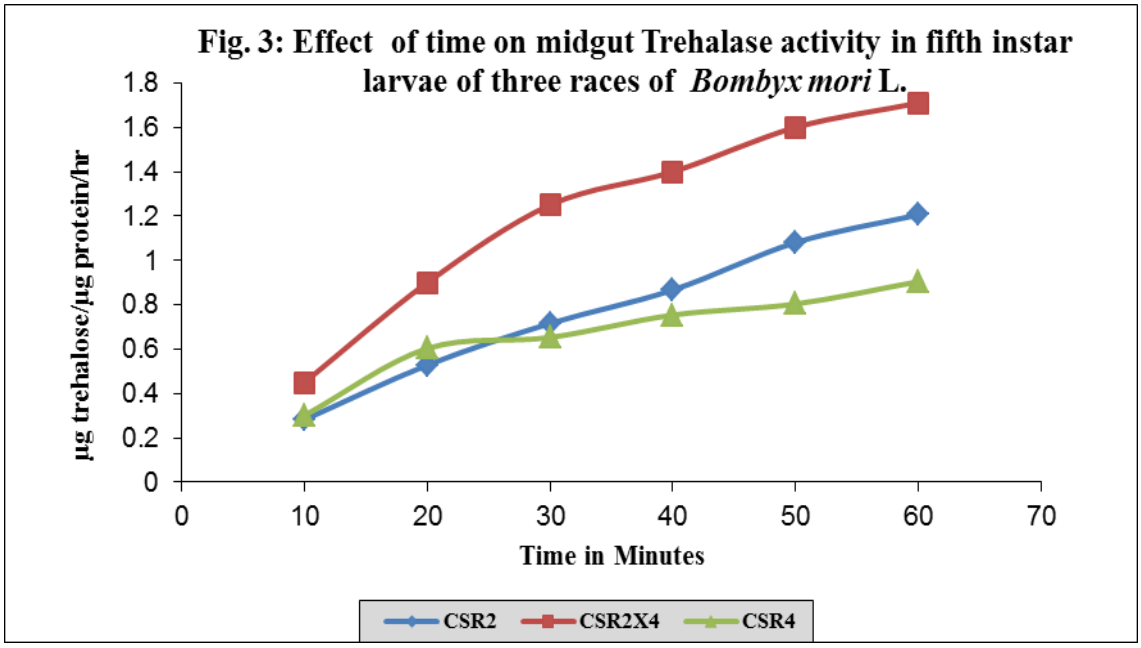
(b) Effect of Temperature

The maximal activity of trehalase in mid gut of *Bombyx mori* was observed at temperature 50°C in race CSR2 and CSR2×CSR4, while temperature optima in race CSR4 was at 45°C **(Fig. 2).**



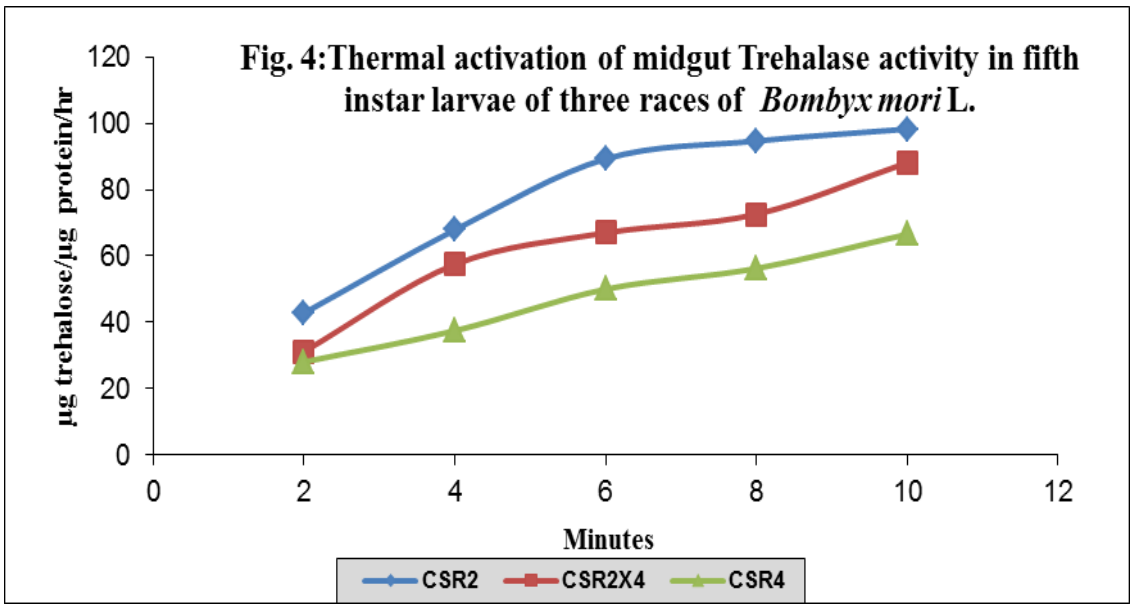
(c) Effect of Time

The digestion period for the linear activity of midgut trehalase of *Bombyx mori* of bivoltine race CSR2 and CSR2×CSR4 was 30 minutes and in CSR4 was found 20 minutes to be fit **(Fig. 3).**



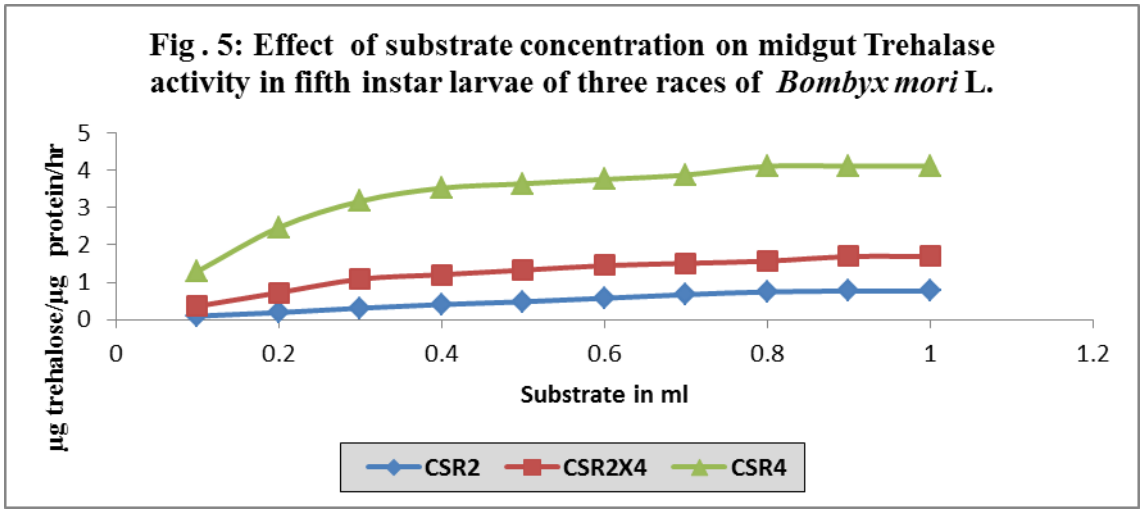
(d) Thermolability

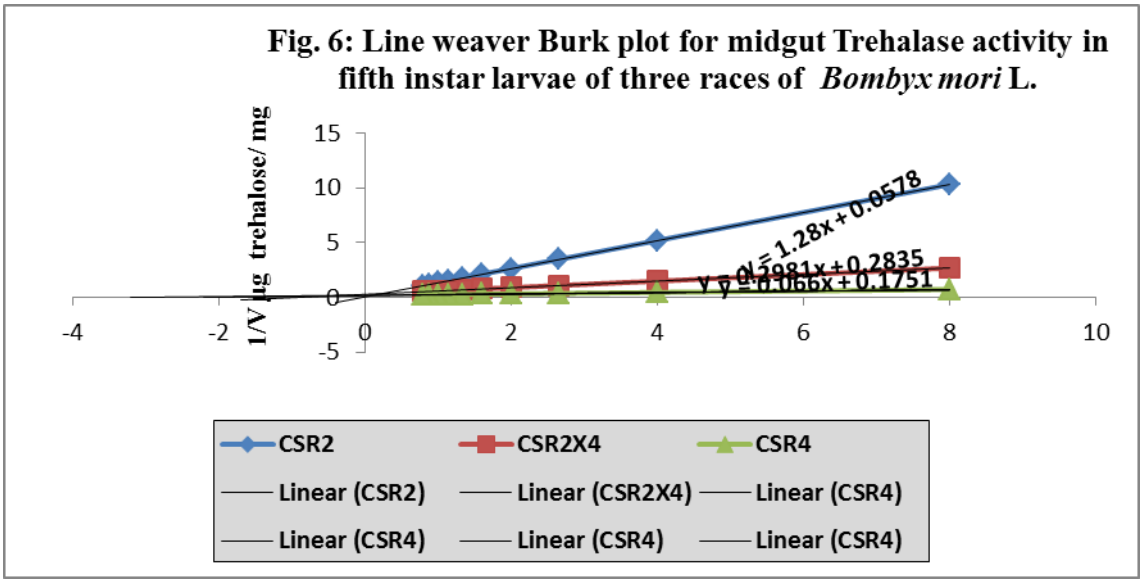
The high temperature tolerance capacity was tested in present study. The 50% loss of enzyme trehalase activity at what temperature and how many time required for half life activity of enzyme is determined here. For CSR2 and CSR2×CSR4 and CSR4 minutes at temperature 55 ^{0}C were 3 min, 5 min, and 6 min respectively.CSR4 was high thermal stability at 55 ^{0}C temperature than CSR2 and CSR2×CSR4 **(Fig. 4).**



(e) Effect of Substrate concentration

The relationship between sucrose concentration and rate of hydrolysis is shown in **Fig. 5**. The Lineweaver Burk plot is employed by using regression equation Y= ax + b (**Fig. 6**) and the regression line obtained were Y = 1.28x + 0.057 for CSR2; Y = 0.298x + 0.283 for CSR2×CSR4 and Y = 0.066x + 0.175for CSR4. The Km values calculated from the graph for CSR2 was $10.57x10^{-3}$M, CSR2×CSR4 was 2.57 $x10^{-3}$M and $0.77x10^{-3}$M in CSR4 which indicates that CSR4 showed maximum efficient trehalase activity comparatively than CSR2 and CSR2×CSR4. The specific activity of midgut trehalase in CSR2 was 0.810 µg glucose/ µg protein /hour, CSR2×CSR4 was 0.560 µg glucose/ µg protein /hour and CSR4 was 3.490 µg glucose/ µg protein /hour recorded.





DISCUSSION

The characteristics of midgut trehalase were compared in between three bivoltine races showed optimum pH 5.5. Similar result observed in cocoon floss trehalase of *Bombyx mori*.[22]The pharate adult of *Bombyx mori* midgut trehalase reported pH optima is 5.4[14] and pupal midgut trehalase of *Bombyx mori* recorded optimum pH 5.2.[9]

These differences were probably occurred because of racial differences. The trehalase activity also observed at different temperature ranges. There was gradually increase in activity upto 50^{0}C and later on the activity falls down as increased in temperature observed in all the races under study. The temperature optima for trehalase 30^{0}C in pupa of *Bombyx mori*[9], due to difference in climatic conditions the Japanese races recorded low temperature optima than the Indian races of tropical climate. The observation in all mentioned races showed 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. The tropical climate showed high temperature optima. In *Leucopholis lepidophora* grubs and tobacco hornworm larvae trehalase optimum temperature was found 45^{0}C and 37.7^{0}C respectively.[23, 24]

The time required for digestion by trehalase recorded in bivoltine race CSR2 and CSR2×CSR4 30 minutes and in CSR4 was found 20 minutes to be fit. In pupa of *Bombyx mori* the digestion period 15 minutes reported fit for linear activity of trehalase.[9] The high temperature tolerance capacity was tested in present study. The 50% loss of enzyme trehalase activity at what temperature and how many time required for half life activity of enzyme is determined here. For CSR2 and CSR2×CSR4 and CSR4 minutes at temperature 55 ^{0}C was 3 min, 5 min, and 6 min respectively.CSR4 was high thermal stability at 55 ^{0}C temperatures than CSR2 and CSR2×CSR4. In *Valanga nigrocornis* half life period of 132 min at 60^{0}C, while in *Holotrichia serrata* 50% loss of activity showed at 60^{0}C for 17 minutes in midgut and 18 minutes in hindgut of male.[25]

The thermolability of trehalase in tobacco hornworm *M. sexta* showed above 57^{o}C and it was heat stable at that

temperature[23] The Km values calculated from the graph for CSR2 was 10.57×10^{-3}M, in CSR2×CSR4 was2.57 $x10^{-3}$M and 0.77×10^{-3}M in CSR4 which indicates that CSR4 showed maximum efficient trehalase activity comparatively than CSR2 and CSR2×CSR4. The specific activity of midgut trehalase in CSR2 was 0.810 µg glucose/ µg protein /hour, CSR2×CSR4 was 0.560 µg glucose/ µg protein /hour and CSR4 was 3.490 µg glucose/ µg protein /hour recorded. Km value of 0.46mM was recorded in the pharate adult midgut of *Bombyx mori.*[14] Trehalase of cocoon floss of *Bombyx mori* had the Km value 1.41mM.[22] The Km values of midgut trehalase in other insects such as in *Leucopholis lepidophora* was $1.6x10^{-3}$[24], in *Holotricha serrata* it was 2.11×10^{-3} for anterior gut and 3.52×10^{-4} for posterior gut[25] and 6.7×10^{-4} in *Rhyncocera Americana*.[26] Km value of *Papilio polytes polytes* 0.302×10^{-3}M.[2] In *E. cloacae* trehalase reported Km of 1.47 (±0.05) mM and Vmax 0.2 (±0.002) mm/min at 55 °C.[5]

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