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COMPARITIVE ACCOUNT OF ENZYME B FRUCTOFURANOSIDASE ACTIVITY & PROTEIN CONTENT IN DIFFERENT RACES OF 5TH INSTAR LARVAE OF *BOMBYX MORI* L.

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ABSTRACT

The silkworm *Bombyx mori* L is known for the production of therapeutic bioactive enzymes. Studies were conducted to analyse the total protein content and enzyme activity of β- fructofuranosidase from the midgut region of five different races namely PM, NK_2, CSR_2, MH_1 and $RP_9 \times NK_2$. Protein content was determined by Lowry's method and enzyme activity was determined by DNS method. On analysis, the protein content and enzyme activity was found to be highest in $RP_9 \times NK_2$ and lowest in PM, in comparison to other races.

KEYWORDS: **Silkworm races, protein content, enzyme activity, midgut, β fructofuranosidase, DNS Method Lowry's method**

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INTRODUCTION

Bombyx mori has a unique ability to eat toxic mulberry leaves without becoming sick. This is because the silkworm contains a special digestive enzyme that is not affected by toxic chemicals which is present in mulberry. Mulberry leaves contain very high amount of alkaloids that inhibit the enzymes to break down sucrose and thus are potentially quite toxic. One type of sucrase called β fructofuranosidase is not affected by these alkaloids. In the silkworm *Bombyx mori* the proteolytic enzymes are predominantly present in the alimentary canal[8]. β-fructofuranosidase is an unique enzyme for silkworm because of which the worm feeds on mulberry leaves. This enzyme can be isolated from the mid gut region of the larvae. Mulberry latex contains extremely high concentrations of alkaloidal sugar mimic glucosidase inhibitors such as 1, 4-dideoxy-1, 4-imino D-arabinital (D-AB1) and 1-deoxyohirimycin (DNJ). Although these compounds do not harm *Bombyx mori* – the mulberry specialist, they are highly toxic to insects that do not feed on mulberry leaves. Though D-AB1 and DNJ are strong inhibitors of α-Glucosidases, they do not affect the activity of β-fructofuranosidase[4,5]. The present study describes a comparative account of β fructofuranosidase in five different races of *Bombyx mori.*

MATERIALS AND METHODS

100 worms of each race of *Bombyx mori* (5th instar) were collected from Bidadi rearing unit, Bangalore, India .The entire mid gut regions of 100 worms were dissected pooled and homogenized with 50mM phosphate buffer, pH 6.5 in cold condition. The homogenate was ultra-centrifuged at 4 degree centigrade clarified by centrifugation in cold condition. The clear supernatant was taken for further analysis. β-fructofuranosidase activity was determined by measuring reducing sugar released by hydrolysis. By using high concentration of sucrose solution the enzyme activity was determined[4]. 0.5 ml of the supernatant solution (clarified liquid) and 0.5ml of (m/V) sucrose in 50mM phosphate buffer. (pH 6.5) was mixed at 30^{o}C in an orbital shaker at 200 rpm. After 10 min the reaction was stopped by boiling in a water bath for 10 min. Blanks were used with heat inactivated enzyme sample (100^{o}C) for 10min. The mixture was treated with DNS reagent, boiled in a water bath for 2 min and then cooled to ambient temperature. It was then diluted to 25 ml of distilled water and the absorbance was read at 540nm[7]. The amount of glucose released was determined from a standard curve prepared under identical conditions. Total activity was calculated by the given definition "One unit of β-fructofuranosidase activity is defined as the amount of enzyme required to release 1µM of reducing sugar per minute under the above described conditions"[2,6,10].Protein concentration of the crude enzyme extract was determined by Lowry's method using BSA as a standard[3].

RESULTS

Table 1
β-fructofuranosidase enzyme activity in Units (U)

Insect races	β-Fructofuranosidase enzyme activity For 100 worms in Units
Pure Mysore	5100
NK2	12620
CSR 2	13460
MH 1	10780
RP9XNK2	16065

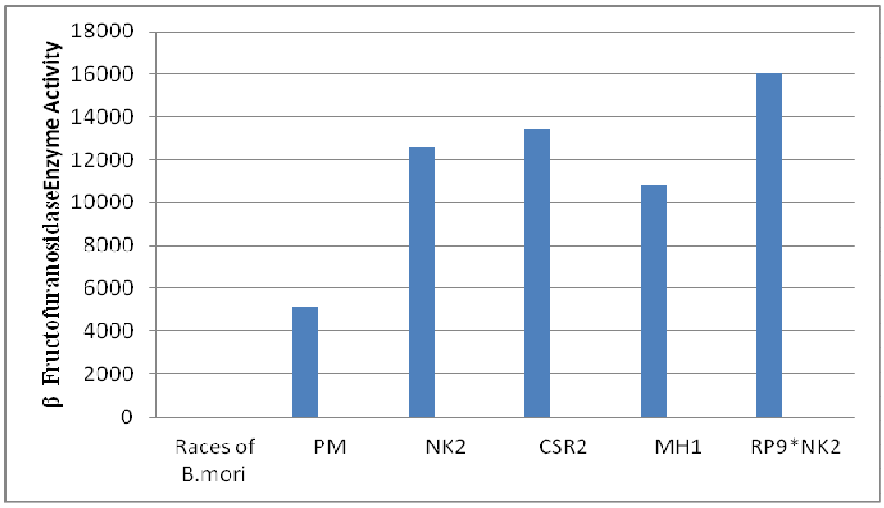

Figure 1
β-fructofuranosidase enzyme activity in Units (U)

Table 2
Total protein assay for different races of Bombyx mori

Insect races	Protein content (g/100ml)
Pure Mysore	7.7
NK2	14.2
CSR 2	16.82
MH1	15.2
RP9XNK2	16.9

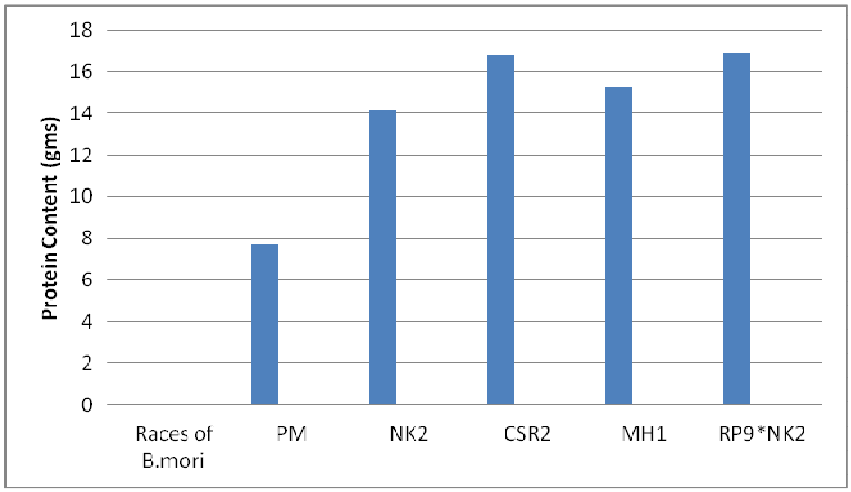

Figure 2
Total protein assay for different races of Bombyx mori

(The quantitative analysis showed highest activity of β-fructofuranosidase in RP9×NK2 having 16065 units and the least activity of the enzyme in PM having 5100 units (Table 1. & Fig.1) Further the protein analysis showed the highest content in RP9XNK2 with 16.9 g in comparison with PM race having 7.7 g (Table 2 & Fig.2).

DISCUSSION

Production of β-fructofuranosidase by *Arthobacter sp.* and its applications has been reported by Zhong et al[11]. As mentioned before, the silk yielding capacity of different races varies considerably based on the production and activity of the enzyme β-fructofuranosidase. Since it is known that the 5th instar stage of silkworm is a crucial stage in the development of larvae in any race of silk worm and is also considered as

a transit period before metamorphosis into an adult. In this stage there will be the development of silk glands which produces the silk. Due to high rate of physiological activity the worms require more energy for which they consume more food. This excess food consumption is related to higher requirement of the enzyme β fructofuranosidase. Hence the result showed the highest metabolic activity in the quantitative estimations of RP9×NK2 which produces more yields. The lowest enzyme activity and protein content was seen in PM race. Phylogenetically far related species like Yeast also has β-fructofuranosidase activity of 74.29 U and protein content 9.0 g[4].

The beneficial aspects can be attributed to the races RP9×NK2 and CSR2 to get better yield in the silk industry. This enzyme helps in developing regional and seasonal robust silkworm races as it directly influences the protein synthesis in silkworm. Invertases or β-fructofuranosidases catalyze the release of β-fructose from the non reducing termini of various β -D-fructofuranoside substrates. Microbial β -fructofuranosidases, in general are dimeric or multimeric enzymes that may also catalyze the synthesis of short chain fructooligosaccharides (FOS), in which one to three fructosyl moieties are linked to the sucrose skeleton by different glycosidic bonds depending on the source of the enzyme.[1,9].

Fructooligosaccharides act as probiotic's and they exert beneficial effects on human health participating in the prevention of cardiovascular diseases or osteoporosis. Thus purification of this enzyme may in future be used in probiotic formulations in the food industry.

CONCLUSION

From the above studies it is inferred that 5th instar stage of any race of silkworm is a crucial stage in the development which is a transitory period prior to metamorphosis into an adult stage. The comparison of the enzyme β-fructofuranosidase activity in different races showed that the highest activity of this enzyme and protein content was seen in RP9×NK2 race which can be substantiated for its high yield of silk compared to the least activity of the enzyme and protein content in PM race.

Such relevant information of this enzyme details are also helpful in product diversification for value addition in sericulture and their applications. The application aspect of this enzyme in total contributes for better productivity, production and quality of silk in Sericulture.

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