



Metabolic Modulations in the Haemolymph of silkworm *Bombyx mori* L during the progress of fungal pathogen *Beauveria bassiana* (Bals) Vuill

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Abstract: Metabolic changes play an important role in understanding the interaction between the host and pathogen as a part of a survival strategy. Day-to-day biochemical changes in haemolymph were examined in the 5th instar silkworm *Bombyx mori* L during the development of fungal pathogen *Beauveria bassiana*. Inoculation of fungal pathogen resulted gradual reduction of carbohydrates and lipids in the haemolymph 5th instar silkworm larvae (4.12 to 6.13 mg/ml and 31.06 to 27.39 mg/ml) from 1st day to 6th day of inoculation. Significant elevation of trehalose content (3.49 to 8.98 mg/ml) was noticed in the inoculated larvae.

Keywords: Silkworm; *Beauveria bassiana*; Haemolymph; Biochemical variations

Introduction

Pathogens were reported to induce several biochemical and physiological alterations in insects (Bergold, 1963, Benz, 1963, Martignoni, 1964). Through the association of the pathogen with host cells, both the pathogen and the host cell lose their individual identity and form a new unit with distinct physiological and characteristics. Haemolymph is the only extracellular fluid of insects with diverse functions and reservoir for the products required for every physiological activity of the insect body, thus changes in the composition of haemolymph reflect the physiological and biochemical transformations taking place in the insect tissues (Pawar and Ramakrishnan 1977). Changes in the levels of important biomolecules in infected larvae would envisage the metabolic stress that the insect experience during the development of pathogen. In view of the significance of major biomolecules and its crucial involvement in various metabolic pathways, many compensative and reductive processes involving biological stresses, the study was carried out.

Materials and Methods

Silkworm hybrid of PM x CSR₂ was selected for the study. Silkworm larvae were brushed and reared in the laboratory under optimum conditions according to Dandin (2003). Immediately after fourth moult i.e. on the first day of the fifth instar, the larvae were inoculated, by dipping in fungal spore suspension (2.15 x 10⁶ spores/ml @ 50 ml/100 worms for 45 sec). The larvae treated with double distilled water were used as control. After 24 hours of the induction of pathogen, day to day biochemical variations occurring in the haemolymph were examined. Haemolymph was collected by clipping third pair of abdominal legs of larvae. The haemolymph was drained into pre-chilled centrifuge tubes with a pinch of thiourea. The parameters examined and methods employed were as follows

- a) Carbohydrates – Carroll *et al* (1956)
- b) Trehalose – Saito (1963)
- c) Lipids – Folch *et al* (1957)

RESULTS AND DISCUSSION

In the present investigation biochemical changes were examined in haemolymph of silkworm during infection by the fungal pathogen *Beauveria bassiana*.

Carbohydrates:

Gradual elevation of total carbohydrate content was noticed in haemolymph of inoculated (3.99 mg/ml to 7.62 mg/ml) and control (4.12 mg/ml to 6.13 mg/ml) batches. Enhancement of carbohydrate content was recorded up to 4th day of inoculation (4.12 mg/ml to 6.24 mg/ml) but the elevation was not significant compare to control (3.99 mg/ml to 5.90 mg/ml) and on 5th and 6th day significant reduction of the carbohydrate content was recorded in the experimental larvae (6.41 mg/ml and 6.13 mg/ml) compare to control (7.12 mg/ml and 7.62 mg/ml).

The initial elevation in carbohydrate content in the haemolymph indicated that the carbohydrate store exists for deriving the chemical energy (for synthesis of ATP) essential for sustaining life activities. However, the

elevation was not significant but the increase in total carbohydrate content is indicative of preponderance of carbohydrate metabolism for energy supply to both host and pathogen. Accumulation of carbohydrates in haemolymph could be due to infection in the host may be unable to metabolize the available carbohydrates for its growth because of low amylase activity in haemolymph compared to control which is supported by the reports of Ambika (1990). Infection by *Beauveria bassiana* induces an increased accumulation of total sugars, trehalose and glucose in the haemolymph, the accumulation of various carbohydrates may contribute to an increased osmotic pressure of haemolymph, which in turn alters the osmotic relations of haemolymph with other tissues, thus making it hypertonic in nature. The same author noticed the increased levels of sugar in the infected haemolymph with advancement of disease and it is maximum in susceptible breeds and she also observed increased glucose levels in haemolymph of silkworm infected with *Beauveria bassiana*. She suggested that the increased glucose level in haemolymph indicates that the condition of hyperglycemia has resulted due to infection, which may be a consequence of failure of neurosecretory or neuroendocrine regulations.

In the present investigation significant reduction of carbohydrate content was noticed on 5th and 6th day (6.41 mg/ml and 6.13 mg/ml) of inoculated larvae compared to control (7.12 mg/ml and 7.62 mg/ml), it may be due to utilization of carbohydrates by the pathogen and food intake is low due to the advancement of disease. Secondly increased mobilization of carbohydrates into different tissue from the haemolymph as energy demands were stepped up in the physiology of the host is altered to combat the disease as a natural response. This is in accordance with the earlier findings of Satake et al (2000). He reported that the insect haemolymph has a large pool of sugars from which all the tissues of the insect body withdraw fuel resources to meet their immediate metabolic needs. Sarma et al (1994) reported a steady decrease in carbohydrate content as infection of NPV progresses upto the 5th day of the 5th instar. They attributed this is due to utilization of carbohydrates as an energy source required for the biosynthesis of viral constituents. Similar results were observed by Kobayashi and Kawase (1981); Bhosale and Kallapur (1990); Horie (1961); Saito (1963) under stress conditions. Gururaj (1996) reported a decrease in food consumption and food assimilation from 96 hrs onwards in fifth instar larvae inoculated with BmNPV whereas, decrease in the level of glycogen, reducing sugars and protein in the midgut and fat body starts much earlier (48 hrs). This suggests that at least during the early stages of infection, the decrease in the levels of biomolecules in the target tissues is not associated with starvation. Reduced food consumption and assimilation during the advanced stage of infection, however, may be contributing to the decrease in the levels of these biomolecules. It is reasonable to assume that the depletion of glycogen in the target tissues may be the result of its utilization as a source of energy required for the increased metabolism (Kobayashi and Kawase, 1981; Sujak et al., 1978) in the host due to infection. Mallikarjuna et al (2002) noticed that the total carbohydrate content was decreased steadily as the disease developed and it is reasonable to assume that the carbohydrates was used as a source of energy required for the growth and development of fungus. Siraj et al (2007) reported decreased carbohydrate content in haemolymph of silkworm infected with Densonucleus virus type1 (BmDNV1), and suggested that as energy is a vital force in the biological system, breakdown of organic constituents mainly carbohydrates is required to meet the energy under the stress condition. The decreased carbohydrate levels in haemolymph and mid gut tissue can be attributed to excessive utilization of carbohydrate to meet the demand of energy to cope with BmDNV1 infection.

Trehalose:

Increased trend of trehalose was noticed in both the haemolymph of experimental (3.49 mg/ml to 8.98 mg/ml) and control (3.29 mg/ml to 7.20 mg/ml) batches from 1st day to 6th day of inoculation in 5th instar of silkworm larvae. Significant elevation of trehalose content was noticed in the inoculated larvae compared to control.

Significant elevation in trehalose content in infected haemolymph might be due to the conversion of glycogen into trehalose and its subsequent release into the haemolymph by the fat body during the starvation due to infection. And the another reason for accumulation of trehalose at the end of the instar may be the efficiency of infected larvae to utilize the available haemolymph trehalose for deriving energy to put forth growth with the progress of the age in fifth instar might have been reduced. Same results were observed by Ambika (1990) during fungal infection by *Beauveria bassiana* and she also observed the decreased glycogen content in fat body during the infection. Elevation in trehalose may also be due to defense mechanism in host haemolymph by the formation of protective membranes for structural support. Trehalose is a multifunctional molecule, and diverse functions like structural support, transport role (Takayama and Armstrong, 1976), signaling and protection of membranes and proteins against heat or cold have been attributed to the sugars. The major source of trehalose in the haemolymph appears to be from the breakdown of glycogen in the fat body (Steels 1963; Mathews and Downer 1974). The higher concentration of trehalose in the haemolymph may be due to the release of trehalose as a result of histolysis of various tissues or release from the fat body by Unni et al (1997). As per the report of Shigeru Saito (1963) the first possibility is that trehalose is formed directly from dietary carbohydrates and is released into the body fluid. Secondly is that the sugars is supplied from carbohydrate-rich tissues such as fat body and mid-gut. The amount of trehalose present in the haemolymph is directly related to the glycogen content of the fat body which is influenced by a number of endogenous organic and inorganic factors (Kochi

and Kaliwal 2006). In contrast to the study Jabbar and Mahamed (1990) suggested that trehalose diffuses from the haemolymph according to the concentration into the gut, and then degrades into glucose to meet the energy needs

Lipids:

No significant alteration was noticed in the lipid content till 48 hours of inoculation (31.06 mg/ml to 31.53 mg/ml), then significant reduction of lipid levels was observed from the 3rd day to 6th day of inoculation in 5th instar (30.13mg/ml to 27.39mg/ml) compared to control (33.08 mg/ml to 34.8 mg/ml).

The study showed insignificant elevation of lipid content in the initial stage of infection. This may be due to mobilization of lipids to the haemolymph in response to immune challenge for membrane biogenesis in the sites of infection (Cheon et al, 2006; Dettloff et al, 2001; Mullen and Goldsworthy 2003). Then significant decrease of lipid content was observed during the course of pathogen infection. It might be due to the utilization of lipids in metabolic activity of host to combat against the infection and by the pathogen. Secondly increased lipase activity in infected haemolymph may be the reason for decreased content of lipid in haemolymph. Arrese et al (1996) and Beenackers et al (1985) reported that the trehalose reduces the lipid concentration in haemolymph, implying that an inverse relationship exists between the haemolymph concentration of lipid and trehalose. Arrese et al (1996) suggested that Trehalose has an inhibitory effect on the lipolytic activity of the fat body as judged by the disease of *M.sexta* fat body lipase activity. Mallikarjuna et al (2002) noticed the steady decrease of lipid levels with the advancement of the disease and it is reasonable to assume that the lipids are used as a source of energy required for the growth and development of fungus.

Jadhav et al (1990) suggested that Free Fatty Acid (FFA) content in haemolymph was decreased significantly in uzi infested larvae and it may be due to rapid uptake by fat-body of the FFA that disappears from the blood. Rajasekhar et al (1992) reported decreased lipid content in the infected silkworm and attributed this may be due to the utilization of lipid components by the pathogen. Similar observations were made by Sarma et al (1994) and Bennett and Shotwell (1972) and they explained that the infested larvae may produce enzymes that utilize lipids in an effort to remove the invading organism.

In contrast to the study increased levels of lipid content was recorded by several authors. Aboul-ela et al (1991) recorded double folded elevation of lipid levels in the haemolymph of *Plodia interpunctella* larvae after treatment with *Bacillus thuringiensis* and suggested that the increase of lipid may be due to the conversion of some proteins to fats (Raina, 1980; Rostom et al 1972) during the course of starvation. Increased lipid content in infected silkworm larvae with NPV were reported by Govindan et al (1998). Siraj et al (2007) reported that there was a significant increase in total lipid content in both haemolymph and mid gut tissues after inoculation of BmDNV1 in susceptible breeds and conclude that lipids serve as a source of metabolic energy as well as essential for structural components of cells.

The study emphasized to understand the metabolic modulations in silkworm larvae infected with fungal pathogen *Beauveria bassiana* in biochemical perspective. Biochemical alterations denotes the existence of pathological condition and aberrations induced by the fungal pathogen in the metabolism of host. The progress of a fungal pathogen can monitor by studying the degree of variation in several functions and biochemical constituents in the haemolymph, as the fungus confines to haemolymph till the silkworm larvae approach death. Proper understanding of host-pathogen interaction, biochemical and physiological manipulations in the host body in response to infection is necessary before stepping into other aspects like disease control. Therefore knowledge on the biochemical alterations in haemolymph due to infection by fungal pathogen *Beauveria bassiana* in the larvae of *Bombyx mori* will be useful for evolving suitable prophylactic and control measures against the disease in turn to enhance the commercial characters of cocoon crops.

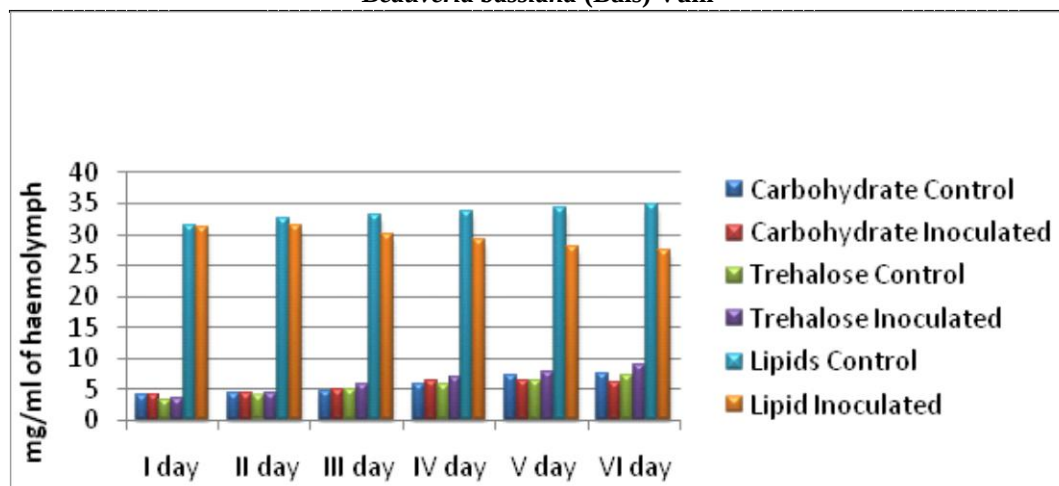
Table 1. Day to day biochemical changes in Carbohydrate, Trehalose, and Lipid (mg/ml) content in haemolymph of silkworm *Bombyx mori* L. inoculated with fungal pathogen *Beauveria bassiana* (Bals) Vuill

Carbohydrates						
V instar	I day	II day	III day	IV day	V day	VI day
Control	3.99±0.26	4.23±0.29	4.65±0.39	5.90±0.37	7.12±0.38	7.62±0.73
Inoculated	4.12±0.40 NS	4.31±0.46 NS	4.81±0.39 NS	6.24±0.70 NS	6.41±0.42 *	6.13±0.86 *
Trehalose						
Control	3.29±0.18	3.93±0.13	5.04±0.18	5.66±0.16	6.43±0.11	7.2±0.12
Inoculated	3.49±0.11 NS	4.32±0.20 ***	5.79±0.31 ***	6.88±0.29 ****	7.71±0.15 ****	8.98±0.25 ****
Lipids						
Control	31.44±1.55	32.58±0.99	33.08±1.11	33.85±0.73	34.41±1.04	34.8±1.24

Inoculated	31.06±1.49 NS	31.53±0.98 NS	30.13±1.10 ***	29.08±1.11 ****	28.09±1.33 ****	27.39±0.91 ****
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* = $P \leq 0.05$, ** = $P \leq 0.02$, *** = $P \leq 0.01$, **** = $P \leq 0.001$,

Figure 1 Histogram showing day to day biochemical changes in Carbohydrate, Trehalose, and Lipid (mg/ml) content in haemolymph of silkworm *Bombyx mori* L. during the development of fungal pathogen *Beauveria bassiana* (Bals) Vuill



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