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A COMPARATIVE STUDY OF HEMOLYMPH PROTEIN PROFILES OF NORMAL AND INFECTED LARVAE OF MUGA SILKWORM *ANTHERAEA ASSAMA* WW

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ABSTRACT

Repertoire of hemolymph proteins of normal and infected larvae of *Antheraea assama* were estimated by performing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) resulting in a series of protein bands in the gel. These protein bands in both normal and infected larvae were compared depending on the number of observed bands, and their molecular weight. From the study it was found that the hemolymph of infected larvae had fewer proteins as compared to the normal healthy larvae. This may be attributed to the overwhelming of the humoral immune system of the larvae due to infection.

KEYWORDS: *Antheraea assama*, Hemolymph, Immune System, Protein Profile, SDS-PAGE

INTRODUCTION

Insects, like most other invertebrates have an open circulatory system. Blood and interstitial fluid are indistinguishable and are collectively referred to as hemolymph, which bathes all internal tissues, organs and hemocytes, and facilitates the transport of nutrients, waste products and metabolites (Tsakas and Marmaras, 2010). Hemocytes are the cellular inclusions (analogous to blood cells) that circulate in the hemolymph of insects and other invertebrates groups (Arnold, 1974; Baishya et al, 2015). The hemolymph also includes various proteins produced and secreted mainly by the fat body and the epithelia. These blood proteins together with the hemocytes play important role in the physiology of the organism to which they belong. Most hemolymph proteins are expressed by genes present in the fat body (analogous to liver) of the insect which are then secreted into the hemolymph (Dimopoulos, 2003). Infection of larvae (either natural or induced) triggers the expression of different genes leading to the production of many different proteins required for defense reactions. But, overwhelming of the immune system, which happens in case of severe infection, might slow and gradually stop the expression of proteins (Sarma et al, 1994) either during transcription or translation stages. It might also affect the expression of proteins working as transcription factors involved in the expression of the genes of these important hemolymph proteins.

MATERIALS AND METHODS

Insects: 5th instar larvae of *A. assama* were collected from two different farms, viz., Central Silk Board Farm, Tura, West Khasi Hills, Meghalaya and Central Silk Board Farm, Nongpoh, Meghalaya.

Infection of larvae: 5th instar larvae were infected with the bacteria E. coli, cultured in the laboratory, centrifuged and then suspended in physiological saline (final concentration of bacteria 1.9x10^8 cells/ml approx.). 10 µl of the bacterial

suspension was injected sub-dorsally between the prolegs at the seventh segment of the larvae using a Hamilton microsyringe. Those not injected were considered normal/healthy for the study.

Collection of hemolymph: Hemolymph samples were collected by severing one of the prolegs of the larvae allowing maximum blood to flow out from the wound into the microcentrifuge tubes. Hemolymph samples from infected larvae were collected 24 hrs after injection, because reported works do not suggest significant change in protein synthesis within 12-18 hours of injection/infection, rather first few hours of infection merely activates the immune system (Hoffmann, 1995). The hemocytes were then removed from hemolymph by centrifugation at 10,000 rpm for 10 mins at 4^0C using Refrigerated Table Top centrifuge (make: Eppendorf). The cell-free hemolymph samples were then stored at -20°C (deep freezer; make: Cellfrost)

Sodium Dodecyl Sulphate Polyacrylamide gel Electrophoresis: SDS-PAGE was performed according to Lammelli (1970) using electrophoresis apparatus from BIO-RAD and SDS-PAGE chemicals from Merck. A 12% separating gel was prepared followed by a 4.5% stacking gel.

Preparation of samples for SDS PAGE: 20ul of cell-free hemolymph sample of both normal and infected larvae was mixed with 5µl of 5X SDS-PAGE sample loading buffer, and boiled in a water bath at 95^0C for 10 minutes. Once cool, the samples were loaded into the wells of the gel together with a protein molecular marker (PMM).

Electrophoresis: The electrophoresis apparatus was connected to a power pack (make: BIO-RAD) and the gel was run at constant 100V for 120 mins.

Staining: Once electrophoresis was over, the gel was removed from the apparatus and stained in a staining box with Ezee Blue stain (Merck). Overnight staining of the gel produces clear protein bands in the gel. Destaining of gel is not required.

Gel Documentation: The protein bands in the gel were then observed under a gel documentation system, software UVITECH.

Protein Profile Study: After SDS-PAGE electrophoresis, the protein profiles of normal and infected hemolymph samples of 5^{th} instar larvae in each experiment set was compared to investigate the difference in the expression of proteins in the hemolymph of normal and infected silkworms.

RESULTS AND DISCUSSIONS

The protein profile study of normal and infected larvae collected from Tura and Nongpoh showed a good number of clear protein bands. The normal hemolymph samples from Tura showed around 12-14 protein bands (Figure 1), with the one with molecular weight 77.26kD showing exaggerated thickness (thickness of band indicating amount of the specific protein in the hemolymph) (Xylander, 2009). However, infected hemolymph showed visible difference as compared to the normal; few of the proteins were absent as evidenced by the lack of bands of respective molecular weights in the gel, whereas those present showed lower band intensity as compared to the normal. Proteins with molecular weight lower than 24.91kD were found to be totally absent in the infected larvae.

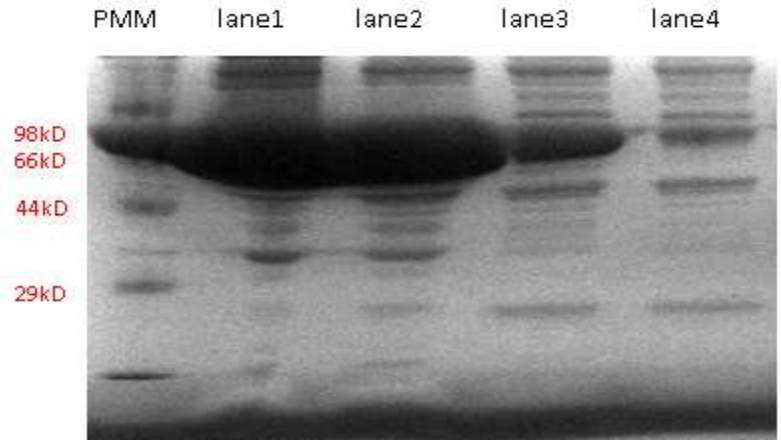

Figure 1: Protein Profile of Normal and Infected Silkworm hemolymph TURA; PMM Indicates Protein Molecular Marker; 1st and 2nd lane from Left show Normal hemolymph Protein Profile; 3rd and 4th Lane Indicate Infected hemolymph Protein Profile

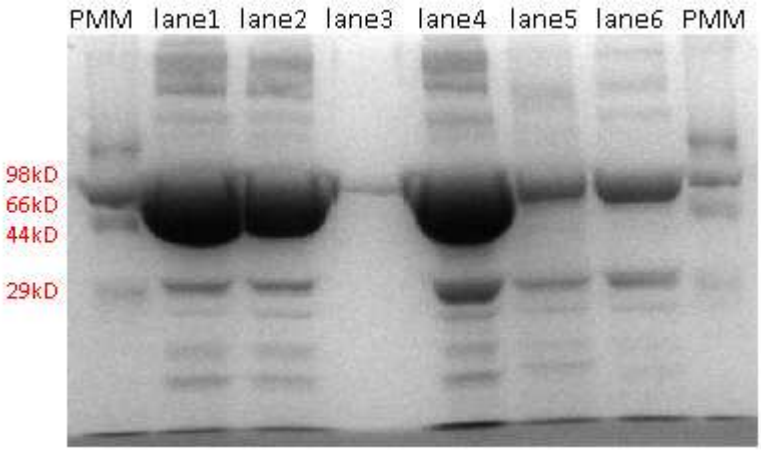

Figure 2: Protein Profile of Normal and Infected Silkworm hemolymph NONGPOH; PMM Indicates Protein Molecular Marker; 1st, 2nd, and 4th Lane from Left Show Normal hemolyph Protein Profile; 5th and 6th Lane Indicate Infected hemolymph Protein Profile

Protein profile study of larvae collected from Nongpoh, Meghalaya (Figure 2) showed similar results with that of Tura. The normal protein profile showed protein bands in the range of 12kD to 145D. However, the infected protein profile showed much lesser number of protein bands; proteins bands in the range of 30kD to 50kD were very faint or completely absent in the infected hemolymph samples.

Thus it is evident from the study that protein expression differs in normal and infected larvae. As observed in this study, infection tends to decrease or terminate hemolymph protein production and secretion. This can be explained as that-the onset of infection triggers the production of various immune proteins which are secreted into the hemolymph for their participation in various immune related reactions. But as infection gradually increases and overpowers the various life systems of the larvae/insect, protein synthesis machinery is affected, thereby overwhelming the humoral immune system. This results in gradual decrease and sometimes termination of the expression of various proteins, including those of the hemolymph (Sarma, et al 1994, Smitha and Bhaskara Rao, 2010). Moreover, during stress, proteolysis is dominated over protein synthesis, i.e., proteins in hemolymph are broken down to constituent free amino acids (Kumar et al, 1998). Another explanation may be that as available resources are being diverted to provide immune defense, the various physiological functions fail to work at par and make the organism weak; one of the symptoms being reduced proteins in the

hemolymph (Wanatabe et al., 1968).

Therefore, it can be assumed that the study of protein expression during infection can provide us an insight into the strength and co-ordination between the various subsystems of the immune system, and the threshold of infection at which it fails to deliver its purpose of defense. Moreover, retrieval of hemolymph samples at short, regular intervals from the time of infection and studying the protein profiles would provide us the rate of expression of different proteins at different intervals of time.

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