Experimental Parasitology
Volume 106, Issues 3-4, March-April 2004, Pages 75-84

doi:10.1016/j.exppara.2004.03.010

Induced immunity in *Antheraea assama* Ww larvae against flacherie causing *Pseudomonas aeruginosa* AC-3

Arundhati Choudhury, Arijit Guha, Archana Yadav, Jyotsna Kumari, Bala G. Unni, and Monoj K. Roy

Biochemistry Division, Regional Research Laboratory, Jorhat 785 006, Assam, India

Received 22 August 2002; Revised 26 March 2004; accepted 26 March 2004. Available online 4 May 2004.

Abstract

This study reports for the first time the induction of immunity in *Antheraea assama* Ww larvae against bacterial flacherie. In silkworms group of disease caused by bacteria are collectively called “flacherie.” This refers to the flaccid condition of the larvae due to the infections of bacterial strains pathogenic to muga silkworm. Antibacterial activity against pathogenic *Pseudomonas aeruginosa* AC-3 causing flacherie, was induced by injection of heat-killed cells of the same strain. Experiments on larval survivability and viable cell count revealed peak immune response on third day. Comparison of the amount of food ingested, excreta produced and larval weight of the saline-injected control, live bacteria-challenged larvae and heat-killed bacteria-injected larvae “(vaccinated)” confirmed the development of immunity against bacterial infection in the “vaccinated” set. The

haemolymph of *A. assama* larvae was analyzed for proteins associated with bacterial infection. Out of the total 32 detected proteins, eleven (A1–2, A15–20, A22–23, and A29) were constitutively synthesized in both the control and live bacteria-injected larvae. Four inducible proteins A4, A9–10, and A21 were detected in the haemolymph of the live bacteria-injected larvae. Synthesis of rest of the proteins varied between the control and their live bacteria-injected counterparts. General protein profile of “vaccinated” larvae injected with live bacteria were found to be similar to that of the saline-injected control.

Author Keywords: *Antheraea assama*; Flacherie; *Pseudomonas aeruginosa*; Immunity; Haemolymph proteins

Article Outline

1. Introduction

The term ‘flacherie' refers to the infection of silkworms by pathogenic bacteria. The muga silkworm, *Antheraea assama* Ww (which produces golden silk) is indigenous to north-east India and has a high incidence of flacherie (Choudhury, 1981). Outdoor rearing of the silkworm makes it more susceptible to diseases. The causal organism of flacherie was identified to be *Pseudomonas aeruginosa* strain AC-3 (Choudhury et al., 2002).

Extensive work on induced immunity in insects has been carried out (Abraham et al., 1995; Dunn, 1986; Hoffmann and Reichart, 1997 and Hoffmann and Reichart, 2002; Hughes et al., 1983; Karp, 1990;

Steiner et al., 1981; Yamano et al., 1998).

This communication reports the impact of induced immunity in *A. assama* larvae by 'heat-killed *P. aeruginosa* AC-3' on the development of flacherie and 'insect' feeding efficiency and a comparison of protein profile of haemolymph of saline-injected control, live bacteria-injected larvae, and "vaccinated" live bacteria-challenged larvae.

2. Materials and methods

2.1. Rearing of silkworms

Eggs of *A. assama* Ww were collected from Muga Training and Research Institute, Lahdoigarh, Assam, India. Newly hatched larvae were reared on some plants (*Persea bombycina* Kost) at the RRL campus following conventional methods (Unni et al., 1997). Fifth instar larvae (2–3 days old) weighing 2.75 g were used for all the experiments in this study.

2.2. Induction of disease symptoms and immunity

Pseudomonas aeruginosa AC-3 was grown overnight at 30 °C in Luria broth (g/L; tryptone 10, yeast extract 5, NaCl 5) in a rotary shaker (200 rpm, Adolf Kuhner AG). Bacteria were washed by centrifugation (4 °C, 5000 rpm, Sorvall RC 5B Plus SS 34), and resuspended in sterile saline (bacterial concentration, 1.9×10^8 cells/ml). Two microliters from this suspension was injected for induction of disease. All injections were made at the muscular base of the third abdominal leg using a 10 μl Hamilton syringe. A portion of the stock suspension was heated at 100 °C for 30 min in a water bath to kill the bacteria. An aliquot of the heated suspension was streaked on Luria agar plates to confirm absence of viable cells. Two microliters of heat-killed bacterial suspension was injected for induction of immunity. The consequences of challenging immune and non-immune larvae with live *P. aeruginosa* were observed until either silk spinning or 100% mortality occurred.

2.3. Determination of time period for peak immune response

Two hundred and fifty healthy larvae were randomly selected from the stock colony, "vaccinated" and divided into five groups, each containing 50 larvae.

These groups were injected with live bacteria on different days after “vaccination.” The groups received live bacteria after 0, 1, 2, 3, and 4 days of “vaccination” and were named as groups I, II, III, IV, and V, respectively. Controls of each group were injected with live bacteria on the same day as their corresponding treated ones. A saline-injected (2 μl) control was also maintained. All the treated and control groups were allowed to feed on leaves ad libitum.

2.4. Feeding efficiency

One hundred and fifty larvae were randomly divided into 3 groups each containing 50 larvae. One group was “vaccinated” with heat-killed cells as mentioned earlier. On third day of “vaccination” the same group was injected with live bacteria. For induction of disease the second group was injected with live bacteria on the same day as the “vaccinated” ones. Survivability and other parameters observed in the case of feeding efficiency experiment were same in the case of both saline-injected control and “vaccinated” larvae. Hence the group containing saline-injected (2 μl) larvae were considered as control for this experiment. From day 0 onwards each group was allowed to feed on known amount of leaves. From day 1 onwards average weight of live larvae, left over leaves and excreta were recorded. Dry weight of food consumed per larvae per day was calculated. Data were analyzed statistically using the technique of analysis of variance.

2.5. Haemolymph preparation

Haemolymph was collected from 10 larvae on 1, 2, 3, 4, 5, and 6 days after “vaccination” and pooled in a tube containing a few crystals of phenylthiourea at 4 ° C by cutting off an abdominal leg. The haemolymph was immediately centrifuged in the cold at 10,000 rpm to remove haemocytes. Haemolymph was also collected from control larvae (saline-injected) in the same way.

2.6. Comparative antibacterial activity of immune and normal haemolymph

Antibacterial activity of normal and immune haemolymph raised against *P. aeruginosa* strain AC-3 was tested against *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and

Staphylococcus aureus (Institute of Microbial Technology, Chandigarh, India) and *P. aeruginosa* strain AC-3 itself. Reaction mixture for assay of antibacterial activity was prepared as described by Hoffmann (1980). A mixture of 1 volume of haemolymph (immune or control) and 5 volumes of 0.5% NaCl was inoculated with 50 μl of inoculum (0.19 × 105 cells/μl) of different bacteria in sterile test tubes. The tubes were incubated at 30 °C (200 rpm, Adolfe Kuhener, AG) and growth was monitored by A_{600} measurement in a spectrophotometer (Shimadzu A260 UV–VIS spectrophotometer).

2.7. SDS–PAGE of haemolymph

Haemolymph was collected as described earlier from control (saline-injected) and live bacteria-injected larvae at different time period (0–4 days) to study the effect of bacterial infection on protein profile. Haemolymph was stored at −20 °C after removal of haemocytes. The basic procedure for SDS–PAGE (10%) was followed as described by Laemelli (1970). Haemolymph samples were diluted in SDS–sample buffer containing 5% mercaptoethanol and heated to 100 °C for 3 min, before application to the gel (1 mm thick). Equal amount of protein (200 μg) was loaded in each well.

Comparative protein profile of haemolymph of saline-injected control, live bacteria-injected larvae and heat-killed bacteria-injected “(vaccinated)” larvae challenged with *P. aeruginosa* was also studied. For this experiment a set of larvae was injected with heat-killed cells and on the third day live bacteria was injected. To another set of larvae of same age, only live bacterial cells were injected to create disease symptoms. Control set received only saline. Haemolymph was collected on the third day of live bacteria injection. Procedure of haemolymph collection and gel electrophoresis has been described earlier.

3. Results

From Fig. 1 it can be seen that of all the five groups of “vaccinated” larvae, group IV that had been injected with live bacteria after 3 days of “vaccination” displayed the best response against induced bacterial infection. While all larvae belonging to its live bacteria-injected control were dead by day 7, 45% of the group IV larvae survived and spun cocoons. The

percent survivability of groups I, II, III, and V were found to be 0, 0, 0, and 65%, respectively, while that of their respective live bacteria-injected controls were 0, 0, 0, and 50%. The saline-injected control displayed 100% survivability. Food intake (g/day/larva) of control larvae was maximum on day 7 (Fig. 2), which declined gradually up to day 9 (when spinning of silk commenced). The “vaccinated set” (live bacteria injected after day 3 of “vaccination”) exhibited peak food intake on day 6. In contrast, peak food intake of the live bacteria-injected larvae was observed on day 4 which declined sharply up to day 8 when survivability was 0%. The difference in food intake among the three groups was statistically insignificant till day 5. On day 6, the difference was significant between control and live bacteria-injected larvae but not between control and “vaccinated” larvae. Moreover the difference between the live bacteria-injected larvae and “vaccinated” larvae was also significant. From day 7 onwards the three groups exhibited significant differences among themselves. However, the pattern of decline in food intake of “vaccinated” larvae was similar to that of the control.

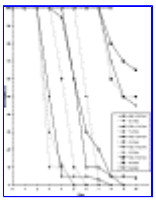

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Fig. 1. Survival percentage of control, “vaccinated” larvae (Vac) challenged with live bacteria and live bacteria-injected larvae at different time period (treatments on 0, 1, 2, 3, and 4 days represent groups I, II, III, IV, and V, respectively).

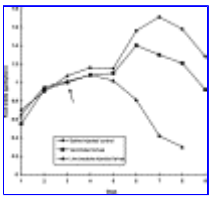

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Fig. 2. Average food intake of control, “vaccinated” larvae challenged with live bacteria and live bacteria-injected larvae (arrow indicates injection of live bacteria). SED (±) for the 3 SETS=0.93, CD (*P*=0.05) =0.19.

The average body weight of all the above mentioned three sets of larvae are depicted in Fig. 3. No difference was observed among the three sets till day 2, however, the difference was significant from day 4 onwards. In general, body weight of both control and “vaccinated” sets increased sharply with age reaching maximum on day 8. On the other hand, the body weight of live bacteria-injected larvae declined steadily from day 4 onwards.

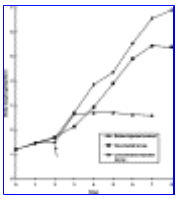 Full-size image (9K)

Fig. 3. Average larval body weight of control, “vaccinated” larvae challenged with live bacteria and live bacteria-injected larvae (arrow indicates injection of live bacteria). SED (±) for the 3 SETS=0.187, CD (*P*=0.05)=0.37.

Fig. 4 shows dry weight of excreta produced per larva per day of all the three sets of larvae. It can be seen that till day 4, control and “vaccinated” larvae produced equal amount of excreta showing no significant difference. Subsequently, the amount of excreta produced by the control set significantly increased till day 9, whereas the amount of excreta of the “vaccinated” larvae gradually declined. On the other hand the amount of excreta produced by the live bacteria-injected larvae significantly declined from day 4 and the level remained almost steady from day 5 to 8 (100% mortality).

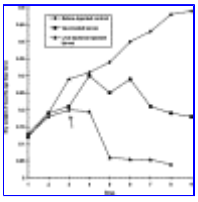 Full-size image (10K)

Fig. 4. Amount of excreta (dry weight) produced by control, “vaccinated” larvae challenged with live bacteria and live bacteria-injected larvae (arrow

indicates injection of live bacteria). SED (±) for the three SETS=0.057, CD (*P*=0.05)=0.10.

Antibacterial activity of the immune plasma raised against *P. aeruginosa* strain AC-3 was effective (Figs. 5A–C) against *P. aeruginosa* strain AC-3, *E. coli* and *B. subtilis*, while it was ineffective against the other 3 bacteria tested. In general, the activity of immune haemolymph increased from day 1 to day 3.

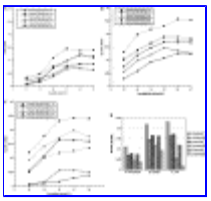
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Fig. 5. (A) Effect of normal and anti-*P. aeruginosa* strain AC-3 plasma collected at different time intervals on growth of *P. aeruginosa* strain AC-3. (B) Effect of normal and anti-*P. aeruginosa* strain AC-3 plasma collected at different time intervals on growth of *B. subtilis*. (C) Effect of normal and anti-*P. aeruginosa* strain AC-3 plasma collected at different time intervals on growth of *E. coli*. (D) Growth of different bacteria after 18 h incubation in normal and anti-*P. aeruginosa* strain AC-3 plasma collected on 1–3 days after vaccination.

Figs. 6A and B show the protein profile of haemolymph of saline-injected control and live bacteria-injected larvae at different time periods. A total of 32 (A1–A32) proteins were easily detected in the un-photographed gels, and therefore designated for discussion in this study, some of which may not be discernible in the photographs. Table 1 shows the molecular weights of the proteins A8–A24 and their presence or absence in the control and bacteria-injected larvae.

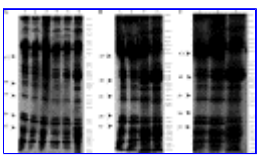
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Fig. 6. (A) SDS–PAGE protein profile of *A. assama* haemolymph of saline-injected control and live bacteria-injected larvae. Lanes 1–3 represent protein profile of larvae at 0, 1, and 2 days post saline injection, respectively, and lanes 4–6 represent protein profile of larvae at 0, 1, and 2 days post live bacteria injection, respectively. Molecular weight standards are noted on the left (kilodaltons) and numerical designations of major protein bands are noted on the right. (B) SDS–PAGE protein profile of *A. assama* haemolymph of saline-injected control and live bacteria-injected larvae. Lanes 1–2 represent protein profile of larvae at 3 and 4 days post saline injection, respectively, and lanes 3–4 represent protein profile of larvae at 3 and 4 days post live bacteria injection, respectively. Electrophoresis was the same as that in A. (C) SDS–PAGE protein profile of *A. assama* haemolymph of saline-injected control, "vaccinated" larvae challenged with live bacteria and live bacteria-injected larvae. Lane 1, control; lane 2, "vaccinated" larvae 3 day after live bacteria-injection; and lane 3, larvae 3 days after live bacteria-injection. Electrophoresis was the same as that in A.

Table 1. Characteristics of some major haemolymph proteins in control and live bacteria-injected larvae (from Figs. 6A and B)

Haemolymph proteins		
Protein	Apparent molecular weight (kd)[a]	Contr
8	65.5	+
9	58	–
10	56	–
11	54	+
12	51	+
13	49	+
14	47	+
15	45.4	+
16	42.9	+
17	41.6	+
18	37.2	+
19	34.1	+
20	31.6	+
21	29.1	–
22	22.2	+
23	19	+
24	15.9	+

Full-size table (<1K)

+, Present; −, Absent.

When the haemolymph of the control was compared to the haemolymph of the live bacteria-challenged larvae several differences were observed. Proteins A1–2, A15–20, A22–23, and A29 were always present in the latter (0–4 days) at intensity same as their corresponding controls. Other than the above mentioned proteins, the rest were either present only in the live bacteria-injected larvae or their level differed between the latter and the control. A4 though not well visualized in the photograph, was detectable from 0 to 2 days in the bacteria-injected larvae. This protein was absent in the controls. Proteins A5–8 increased in the bacteria-injected larvae but the degree of increase was less in comparison to the control. Proteins A9–10 and A21 which was absent in the latter could be detected in the bacteria-injected larvae from day 2 onwards. A3, A11–14, A24–28, and A30–32 which displayed a uniform level in the control increased gradually in the bacteria-injected larvae from day 2.

Fig. 6C shows the protein profile of the haemolymph of saline-injected larvae, “vaccinated” larvae challenged with live bacteria and live bacteria-injected larvae (day 3).

4. Discussion

In this study we report for the first time the induction of immunity in *A. assama* larvae by injection of heat-killed cells of *P. aeruginosa* strain AC-3. Use of heat-killed *P. aeruginosa* cells for innate immune response have been reported (Hoffmann, 1980).

Experiments on survivability of heat-killed bacteria-injected larvae upon injection of live bacteria were carried out to determine the optimum time period for the larvae to reach peak immunity. Of all the “vaccinated” groups, larvae belonging to group IV showed the best response with 45% survivability while survivability of its bacteria-injected control was 0%. Fifty percent of the latter group died on day 6 and the rest died on day 7. It is interesting to note that the “vaccinated” group V larvae showed a higher percentage of survivability (65%) than group IV, but this enhancement of survivability can't be attributed to increased immunity as its live bacteria-injected control too exhibited a remarkably high survivability (50%). The difference in the survivability value between the

these two sets was statistically significant. The fifth instar of silkworms is characterized by a high rate of metabolism and growth. Thus it might be presumed that the bacteria-injected control of group V had reached such a developmental stage in which, injection of the same dosage of bacteria as the bacteria-injected controls of group I–IV, failed to create the desired disease symptoms. In general, the susceptibility of insects to pathogens seems to decrease as the insects advance in age (Tanada, 1967). From this experiment we could conclude that the immune response in *A. assama* fifth instar larvae reaches a maximum level after 3 days of "vaccination." The "vaccinated" larvae of groups I–III either died or exhibited a negligible survivability, in general, their life span was prolonged by 3–4 days as compared to their live bacteria-injected controls suggesting possible development of immunity. Time taken to reach peak immunity seems to vary among various insect species (Boman et al., 1981; Dickinson et al., 1988; Qu et al., 1982). Further, injection of vaccines of pathogenic bacteria into individuals of several insect species have been reported to elicit an acquired immunity that may persist for several days depending on species (Dunn, 1986).

"Vaccinated" larvae challenged with live bacteria showed a similar pattern of food intake and increase in body weight as the control showing immune response against bacterial infection. Lower amount of food intake and low body weight in the "vaccinated" larvae as compared to the control was expected as the population contained live bacteria in their system as well. The significant rapid decline in food intake and loss of body weight in the live bacteria-injected larvae is similar to that observed under the influence of bacterial infection in natural environmental conditions.

The "vaccinated" larvae showed similar patterns of excreta production as the control till 4th day, after which the amount of excreta produced gradually, declined. Excreta produced by the live bacteria-injected larvae rapidly declined from day 4 to 5, which maintained a steady low level till death. In general, amount of food intake was directly proportional to excreta production in all the three sets of larvae. The control curve (excreta) showed a characteristic rise at the later stage.

For further confirmation of immune response of the

larvae, immune response raised against *P. aeruginosa* strain AC-3 was tested for antibacterial activity against *E. coli*, *B. subtilis*, *K. pneumoniae*, *P. vulgaris*, and *S. aureus* by Inhibition zone assay method (diameter in mm) which are reported to be virulent for insects (Steinhaus, 1967). The activity was also tested against *P. aeruginosa* strain AC-3. Anti-bacterial proteins raised against *P. aeruginosa* strain AC-3 was found to be effective not only against the same organism but also against *E. coli* and *B. subtilis*, while it was ineffective in suppressing the growth of *K. pneumoniae*, *P. vulgaris*, and *S. aureus*. Anti-*P. aeruginosa* plasma of *Locusta migratoria* has been reported to depress the growth of *E. coli* and *Bacillus thuringiensis* (Hoffmann, 1980). In this study, in all the three susceptible bacteria the activity of anti-bacterial proteins was found to increase from day 1 to day 3. The kinetics of appearance of antibacterial activity in haemolymph was similar to that observed under in vivo condition, where the least mortality was observed in the set of larvae injected with live *P. aeruginosa* strain AC-3 on the third day after “vaccination.” It was interesting to note that the growth of the three above mentioned bacteria in normal haemolymph gradually decreased from day 1 to day 3 (Fig. 5D) suggesting the appearance of natural antibacterial factors in the haemolymph of normal larvae with the progression of age as the larvae approached the silk spinning stage. In general, it is an acknowledged fact that the chrysalis stage is quite resistant to diseases. We ignored the difference in bacterial growth in normal and immune haemolymph as a measure of efficiency of antibacterial activity, because this difference though being almost same in some cases e.g., 1, 2, and 3 days in *P. aeruginosa* strain AC-3, the growth of the bacteria was relatively high in the early days after “vaccination.”

Studies with insect haemolymph have revealed the presence of a variety of proteins formed in response to injury or bacterial challenge (Abraham et al., 1995; Dickinson et al., 1988; Hughes et al., 1983; Hultmark et al., 1980; Karp, 1990; Yamano et al., 1998). Apparent molecular weight of 17 proteins was determined and proteins associated with disease development were also identified. As our acrylamide gel was not a linear concentration gel and molecular markers used were from 17 to 67 kDa, we could determine the molecular weights of the proteins occurring only within this range. When the protein profile of the live bacteria-injected larvae was

compared to that of the control, 11 proteins were found to be constitutively synthesized in both the sets. Proteins A5–8 increased at a lower rate than their corresponding controls suggesting possible hindered metabolic process during disease development or may be due to proteolytic activity of the bacteria on host proteins. These four proteins (mw>67 kDa) showing similarity to *Manduca sexta* proteins (M4–7) as regards to banding pattern in gel, may be component of HDL (Hughes et al., 1983) or larval storage proteins. Molecular weight comparison with those of other workers suggest that *A. assama* protein A13 may correspond to *M. sexta* protein M11 (Hughes et al., 1983) and *Hyalophora cecropia* protein P4 (Rasmuson and Boman, 1979). Enhanced biosynthesis of the proteins A24–28 and A30–32 was observed in live bacteria-injected larvae as compared to the control. Proteins A9–10 and A21 were found to be synthesized after 2 days of injection which were totally absent in the controls. The protein A4 (mw>67 kDa) showed a different pattern of synthesis in the sense that it was detected only from 0 to 2 days after injection of live bacteria, synthesis of which ceased afterwards. This protein was totally absent in the control. The synthesis of new haemolymph proteins in response to infection has been demonstrated in both larval and pupal stages of several species (Abraham et al., 1995; Hughes et al., 1983; Hurlbert et al., 1985).

Haemolymph protein profile of control, live bacteria-injected larvae and heat-killed bacteria-injected larvae challenged with live bacteria “(vaccinated)” was compared. It was observed that the latter were indeed efficient in resisting the biochemical changes caused by live cells of *P. aeruginosa* strain AC-3 which caused death in the live bacteria-injected larvae. This conclusion was drawn based on similarity of the haemolymph protein profile of the control and live bacteria-challenged “vaccinated” larvae. Though the presence of different proteins in “vaccinated” larvae is expected, these could not be detected as the concentration of the acrylamide gel used was not suitable for this purpose. From this study it is clearly evident that peak immune response was achieved in *A. assama* larvae against *P. aeruginosa* strain AC-3 on third day of vaccination with heat-killed cells of the same strain as observed from the in vivo and in vitro experiments. Comparison of the haemolymph protein of normal and induced diseased larvae revealed both quantitative and qualitative variations. Protein profile

of “vaccinated” larvae challenged with live bacteria was similar to that of the control suggesting unhindered metabolism in the former even after being challenged with live bacteria.

Acknowledgements

AC, AG, and JK are thankful to Council of Scientific and Industrial Research, Govt. of India, New Delhi for providing Research Associateships and Junior Research Fellowship, respectively. Part of this project work is funded by GB Pant Institute of Himalayan Environment and Development (under Ministry of Environment and Forests, Govt. of India).

References

Abraham, E.G., Nagaraju, J., Salunke, D., Gupta, H.M. and Datta, R.K., 1995. Purification and partial characterization of an induced antibacterial protein in the silkworm, *Bombyx mori*. *J. Invert. Pathol.* **65**, pp. 17–24. Abstract | PDF (562 K) | View Record in Scopus | Cited By in Scopus (10)

Boman, H.G., Boman, A. and Pigon, A., 1981. A molecular approach to immunity and pathogenicity in aninsect-bacterial system. *Insect Biochem.* **11**, pp. 33–42. Abstract | PDF (1107 K) | View Record in Scopus | Cited By in Scopus (0)

Choudhury, S.N., 1981. Diseases, pests and parasites. In: Choudhury, S.N. (Ed.), Muga Silk Industry, Directorate of Sericulture and Weaving. Govt. of Assam, Guwahati (Assam), India, pp. 74–81

Choudhury, A., Guha, A., Yadav, A., Unni, B.G. and Roy, M.K., 2002. Causal organism of flacherie in the silkworm *Antheraea assama* Ww: isolation, characterization and its inhibition by garlic extract. *Phys. Res.* **15**, pp. 1–2.

Dickinson, L., Russell, V. and Dunn, P.E., 1988. A family of bacteria-regulated, cecropin D-like peptides from *Manduca sexta*. *J. Biol. Chem.* **263**, pp. 19424–19429. View Record in Scopus | Cited By in Scopus (37)

Dunn, P.E., 1986. Biochemical aspects of insect immunity. *Ann. Rev. Entomol.* **31**, pp. 321–339.

Hoffmann, D., 1980. Induction of antibacterial activity

in the blood of the migratory locust *Locusta migratoria* L. *J. Insect Physiol.* **26**, pp. 539–549. Abstract | **Article** | PDF (1264 K)

Hoffmann, J.A. and Reichhart, J.M., 1997. Drosophila immunity. *Trends Cell Biol.* **7**, pp. 309–316.

Hoffmann, J.A. and Reichart, J.M., 2002. *Drosophila* innate immunity: an evolutionary perspective. *Nat. Immunol.* **3** 2, pp. 121–126. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (334)

Hughes, J.A., Hurlbert, R.E., Rupp, R.A. and Spence, K.D., 1983. Bacteria-induced haemolymph proteins of *Manduca sexta* pupae and larvae. *J. Insect Physiol.* **29**, pp. 625–632. Abstract | **Article** | PDF (1280 K) | View Record in Scopus | Cited By in Scopus (15)

Hultmark, D., Steiner, H., Rasmuson, T. and Boman, H.G., 1980. Insect immunity. Purification and properties of three inducible bactericidal proteins from haemolymph of immunized pupae of *Hyalophora cecropia*. *Eur. J. Biochem.* **106**, pp. 7–16. View Record in Scopus | Cited By in Scopus (186)

Hurlbert, R.E., Karlinsey, J.E. and Spence, K.D., 1985. Differential synthesis of bacteria induced proteins of *Manduca sexta* larvae and pupae. *J. Insect. Physiol.* **31**, pp. 205–215. Abstract | **Article** | PDF (2734 K) | View Record in Scopus | Cited By in Scopus (7)

Karp, R.D., 1990. Inducible humoral immunity in insects: does an antibody-like response exist in invertebrates?. *Res. Immunol.* **141**, pp. 932–934. Abstract | PDF (231 K) Abstract | PDF (213 K) | View Record in Scopus | Cited By in Scopus (1)

Laemelli, U.K., 1970. Cleavage of structural proteins during the assembly of head of the bacteriophage T4. *Nature (London)* **227**, pp. 680–685.

Qu, X., Steiner, H., Engstrom, A., Bennich, H. and Boman, H.G., 1982. Insect immunity: isolation and structure of cecropins B and D from pupae of the Chinese oak silk moth, *Antheraea pernyi*. *Eur. J. Biochem.* **127**, pp. 219–224. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (34)

Rasmuson, T. and Boman, H.G., 1979. Insect immunity V. Purification and some properties of

immune protein P4 from haemolymph of *Hyalophora cecropia* pupae. *Insect Biochem.* **9**, pp. 259–264. Abstract | PDF (767 K) | View Record in Scopus | Cited By in Scopus (11)

Steiner, H., Hultmark, D., Engstrom, A., Bennich, H. and Boman, H.G., 1981. Sequence and specificity of two antibacterial proteins involved in insect immunity. *Nature* **292**, pp. 246–248. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (397)

Steinhaus, E.A., 1967. Immunity in insects. In: Steinhaus, E.A. (Ed.), Insect Microbiology. Hafner Publishing Company, New York and London. pp. 555–572

Tanada, Y., 1967. Microbial pesticides. In: Kilgore, W.W. and Doutt, R.L., Editors, 1967. *Pest Control. Biological, Physical and Selected Methods*, Academic Press, London, pp. 31–38.

Unni, B.G., Choudhury, A., Khanikor, D. and Ghosh, A.C., 1997. Studies on trehalase and trehalose in the fat body, haemolymph, silk gland and head region of muga silkworm, *Antheraea assama*, during the spinning period. *Sericologia (France)* **37**, pp. 31–33.

Yamano, Y., Matsumoto, M., Sasahara, K., Sakamoto, E. and Morishima, I., 1998. Structure of genes for cecropin A and an inducible nuclear protein that binds to the promoter region of the genes from the silkworms, *Bombyx mori*. *Biosci. Biotechnol. Biochem.* **62**, pp. 237–241. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (12)

Corresponding author. Fax: +91-376-237-0011

Experimental Parasitology
Volume 106, Issues 3-4, March-April 2004, Pages 75-84