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RESEARCH ARTICLE

A STUDY ON IMMUNOMODULATION AND HEALTH ENHANCEMENT IN *BOMBYX MORI* SUBJECTED TO SUPPLEMENTARY FEEDING WITH A PROBIOTIC CONSORTIUM

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ABSTRACT

A study was carried out to assess the health enhancement and immunomodulation in silkworm *Bombyx mori* reared on probiotic consortium, added as a supplement to mulberry leaves. The consortium was coated on mulberry leaves and fed to experimental animals. Third instar larvae of *Bombyx mori* were segregated into three sets: first set was maintained on normal mulberry leaves, second set of diseased animals also maintained with normal mulberry leaves and the third set on mulberry leaves coated with the probiotic consortium. Morphometric characters like body weight, body length and cocoon weight of the three groups were assessed. Among the three sets of *Bombyx mori,* probiotic supplemented animals showed maximum body weight (2.93g in IV instar, 4.5g in V instar), body length (5.8 cm in IV instar, 7.6cm in V instar) and cocoon weight (2.93g). Total blood count and differential blood count was carried out in all the three groups. Among the three sets of animals there were statistically significant variations in each type of haemocytes. Normally the major haemocyte type was granulocytes (64.8%) followed by semigranulocyte (15.4%), large granulocyte (11.5%) and hyaline cells. Under diseased condition, there was an overall increase in haemocyte-types. Prophenol oxidase activity, an index of immunity enhancement in arthropods was maximum in the probiotic supplemented silkworms. The study showed that feed supplementation with probiotic consortium had a definite role in the growth and immune enhancement of silkworm.

INTRODUCTION

Gut of an insect is a very dynamic environment, providing plenty of opportunity for bacteria to flourish, thus resulting in extreme competition among bacteria to utilize the nutrients and survive within it. Many of the gut commensal bacteria have evolved to inhabit this environmental niche and thus are able to colonize the gut successfully. In doing so, they provide effective protection against invasion by pathogenic microbes by a process called colonization resistance (CR), the exact mechanisms of which are just about being revealed

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(Bignell, 1983). In many insect species, the gut possesses different types of bacteria, which are transient and do not remain in the gut during all life stages. In mulberry silkworm, *Bombyx mori,* the presence of different types of bacteria in the gut have been reported (Roy *et al,* 2000; Kodama, 2001). Most of the observed strains belonging to the genus *Streptococcus* are considered to be pathogenic to *Bombyx mori* larvae, while bacteria of the genera *Pediococccus*, *Leuconostoc* and *Lactobacillus* do not produce any infection (Steinhaus, 1949 ; Kodama, 2001). The precise mechanism of imparting beneficial effect on host by gut bacteria or interaction among the different bacterial strains present as micro-flora is not known. A variety of permanent microorganisms present in several families of insects, supply essential nutrients to their host (Bridges, 1981; Breznak, 1982; Cruden and Markovetz, 1984). Nutritional contributions may take several forms such as improved ability to live on suboptimal diets, improved digestion efficiency, acquisition of digestive enzymes and provision for vitamins (Tanada and Kaya, 1993). The purpose of this paper is to reassess other subtle, but nonetheless potentially important, ways in which microbes benefit an insect host, *Bombyx mori*.

Probiotics are the live microbial food supplements beneficially affecting the host by improving the enteric microbial balance (Fuller, 1991; Austin *et al.,*1995).Several researchers have reported the beneficial role played by probiotics in humans (Chan *et al.,*1985; Brigidi *et al.,*2000 and 2001), ruminants, aquaculture (Douillet and Langdon, 1994; Gildberg *et al.,*1997) and insects (Dillon and Dillon, 2004). Therefore, products containing probiotic bacteria are gaining popularity, increasing the importance of their accurate speciation (Yeung *et al.,*2002). Nutritional contributions and the probiotic benefits offered by insect gut-dwelling bacteria (Dillon and Dillon, 2004; Yuan *et al.,*2006) can substantially modify and promote the health and silk production capacity of *Bombyx mori,* although this field has attained limited attention only in the sericulture-research scenario. All the past investigations in the search of nutritional probiotics in the gut of host animals, have revealed that these bacteria along with the non-beneficial microbes enter the animal's gut through food, and get colonized in specific areas of intestine. These bacteria express their probiotic capacity including immunostimulation all through the life of the hosts, while living as commensals in their gut.

Insects seemingly lack any adaptive immune response that operate analogously to the well documented antibody or histocompatibility adaptive immune responses as in vertebrates (Hoffmann, 2003). Apoptosis or programmed cell death is one of the phenomena evolved by certain vertebrates and invertebrates lacking humoral immunity to function as antiviral defense mechanism (Narayan, 2004). This mechanism is a controlled biochemical pathway distinguishable from cell necrosis by characteristics that include cellular shrinkage, membrane blebbing, chromatin condensation, apoptotic body formation and fragmentation (Wyllie *et al.,*1980). Insect immunity plays an important role in the interaction between the host and pathogen as a part of survival strategy including physical blockades such as cuticle and peritrophic matrix, epithelial barriers, protease cascades leading to coagulation and melanization, cellular responses such as phagocytosis and encapsulation, and also the production of certain antimicrobial peptides (Vernick *et al.,*1995; Lehane, 1997; Lehane *et al.,*1997; Tzou *et al.,*2000; Lavine and Levashina *et al.,*2001; Strand, 2002; Ligoxygakis, 2002; 2004; Meister, 2003;). Haemocytes are of special interest because they are the mediators of the cellular defense reactions in insects that involve phagocytosis and encapsulation of foreign intruders (Gillespie *et al.,*1997; Trenczek, 1998). During metamorphosis, the haemocytes can phagocytose, store, and transport various cellular and humoral components (Crossley, 1968; Nardi and Miklasz,1989; Kobayashi *et al.,*1991; Kiger *et al.,*2001; Lanot *et al.,*2001). Certain types of haemocytes have an immunomodulatory molecular mechanism associating several proteins: the prophenoloxidase system (pro-po-system) (Smith and Soderhall, 1983; Johansson and Soderhall, 1989). Mechanisms of cell mediated immunity in arthropods have been reviewed by Jiravanichpaisal et al. (2006). An attempt has been made at present to clarify the relationship between the probiotic supplementation and immunity of

silkworm, concentrating mainly on aspects such as total and differential haemocyte counts and prophenoloxidase activity.

MATERIALS AND METHODS

Mulberry leaves

Mulberry leaves selected for the present study belonged to Mysore local variety M5, raised in the Zoology Department garden of Periyar E.V.R. College, Tiruchirappalli-23. Fresh leaves were collected daily and fed to the silk worms, ad libitum, all through the experiment.

Bombyx mori larvae

The silk worm, Bombyx *mori* (Lepidoptera) LxNB4D2, a crossbreed of a local and multivoltine variety was used in this study. Third instar larvae were obtained from the Tamilnadu State Government Silk Rearing Centre, Manikandam, Tiruchirappalli, and were maintained in bamboo trays lined with newspaper sheets, and covered by wiremesh-lids. The trays were kept on moistened gunny bags to maintain cool, humid conditions (27±2°C; relative humidity: 70±5%). Care was taken to keep away natural enemies like rats and lizards.

Experimental Protocol

Silk worm larvae of the third instar stage were procured and separated into two duplicate sets. Mulberry leaves were fed to *Bombyx mor*i larvae as per rearing methods suggested by Krishnaswami *et al.* (1978). Experimental as well as control groups had 2 replications consisting of 100 larvae each. Several of the third instar larvae developed 'Flacherie' like symptoms and these larvae were separated and were treated as the 'infected' group, although their number was lesser than the other two groups. Mulberry leaves of equal weight and approximately same number were used for feeding *B. mori* larvae in control and experimental groups. Infected larvae were fed on mulberry leaves, proportionate to their number. Experimental groups were fed with mulberry leaves coated the leaves with the suspension of probiotic consortium and partially dried at room temperature. The control group of *B. mori* larvae and the infected larvae were fed with mulberry leaves without probiotic coating.

Probiotic composition

A commercial probiotic consortium manufactured by Biostadt Agrisciences (Wockhardt, India) was used in the present study. The composition of the probiotic per kilogram of the substance was as given below: *Lactobacillus sporogenes* (45,000 million cfu), *Lactobacillus acidophilus* (45,000 million cfu), *Bacillus licheniformis* (30,000 million cfu), *Bacillus subtilis* (30,000 million cfu), *Saccharomyces cerevisiae* (1,25,000 million cfu. Mulberry leaves were enriched by the probiotic at 1% of feed weight.

Feed Coating

Appropriate amount of chosen probiotic consortium was taken and mixed thoroughly with molten 'China grass' (food grade agar) at about 50°C and coated rapidly on both the sides of mulberry leaves with a sterile paint-brush. The coated leaves were torn into appropriate sizes prior to feeding the larvae.

Total Haemocyte count

Larvae at the late 4th instar were surface-sterilized for 30 seconds with 75% ethanol and then washed with sterilized distilled water. Haemocyte morphotypes were classified based on previously established morphological criteria (Strand and Noda, 1991; Pech *et al.,*1994). For in vitro assays, haemocytes were collected by the procedure of Pech *et al.* (1994). Larvae were partially anaesthetized and surface sterilized with 95% ethanol, and bled from a proleg into 500 µl of anticoagulant buffer (0.098 M NaOH, 0.186 M NaCl, 0.0017 M EDTA and 0.041 M citric acid; pH 4.5). Total haemocyte count was made using an improved Neubauer hemocytometer following the method as described for counting WBC (Garvey *et al.,*1979).

Prophenol oxidase activity

Phenoloxidase activity in the haemolymph was measured spectrophotometrically by recording the formation of dopachrome produced from L-dihydroxy phenylalanine (L-DOPA, Hi Media,

Mumbai) (Hernandez-Lopez *et al.,*1996; Sahoo *et al.,*2007). The buffer-diluted haemolymph was centrifuged at 300g at 4 °C for 10 min. The supernatant fluid was discarded and the pellet was rinsed and re-suspended gently in 1 ml cacodylate-citrate buffer (sodium cacodylate 0.01 M, sodium chloride 0.45 M, trisodium citrate 0.10 M: pH 7.0), and then centrifuged again. The pellet was then re-suspended in 200 ml cacodylate buffer (sodium cacodylate 0.01 M, sodium chloride 0.45 M, calcium chloride 0.01 M, magnesium chloride 0.26 M: pH 7.0). One hundred ml of the cell suspension was incubated with 50 ml of trypsin (1 mg ml^{-1}), which served as an elicitor, for 10 min at 25 °C. To this 50 ml of L-DOPA (3 mg ml^{-1}) was added, followed by 800 ml of cacodylate buffer, added 5 min later. The optical density was measured at 490 nm using a spectrophotometer. The control solution, which consisted of 100 ml of cell suspension, 50 ml cacodylate buffer (to replace the trypsin) and 50 ml of L-DOPA, was used for the background phenoloxidase activity in all test conditions. The background phenoloxidase activity optical density values were in the range of 0.03 to 0.09. The phenoloxidase activity in terms of optical density was expressed as dopachrome formation per 50 ml haemolymph.

Statistical Analysis

For all the animals under study, the mean value of selected parameters both in control, infected and experimental worms were estimated and standard deviations were calculated. Two way ANOVA of the results and post-hoc (SNK) test were carried out using a statistical package (SPSS version.10).

RESULTS

Body weight

After supplementing the silkworm fourth instar larva, with the probiotic consortium a three fold increase was observed in body weight (2.93 grams) than the normally fed larvae (0.73grams). Whereas in the infected animals, much lower body weight was observed. In the fifth instar larvae also a similar trend was observed: probiotic supplemented group recorded maximum body weight of 4.5 grams, followed by control group (3.2 grams) and the lowest was observed in infected animals (2.3grams) (Table -1). Analysis of variance revealed significant variation among the three groups and SNK post hoc analysis showed significant gradation in weight variation.

Table 1. Body weight of *Bombyx mori* larvae fed on mulberry leaves coated with probiotic consortium (mean values ± Standard deviation)

Stage of silkworm	Feed supplemented	Normal	Infected
V instar	2.93 ± 0.5^{a}	0.73 ± 0.2^{b}	0.50 ± 0.1^{c}
V instar	4.5 ± 0.5^{a}	3.2 ± 0.3^{b}	2.3 ± 0.2^{c}

Note: Dissimilar superscripts indicate significantly different (SNK test) values, while similar superscripts indicate similar values

Table 2. Body length of *Bombyx mori* larvae fed on mulberry leaves coated with probiotic consortium (mean values ± Standard Deviation)

STAGE OF SILKWORM	Feed supplemented	Normal	Infected
IV instar	5.8 ± 0.6^{a}	3.9 ± 0.2^{b}	2.5 ± 0.3^{c}
V instar	7.6 ± 0.2^{a}	6.1 ± 0.7^{b}	4.5 ± 0.3^{c}

Body length

The body length of fourth instars larvae showed significant variation among three groups-probiotic supplemented, normal and infected animals. Increased body length was observed in the probiotic supplemented groups (5.8 cm), whereas in the control group the increase was 3.9 cm and the lowest body length was in the infected group (2.5 cm). In fifth instars larvae, also body length of silkworm, increased in all the three groups. Maximum growth was observed in probiotic coated group (7.6cm), and the minimum growth in infected group (4.5 cm) (Table –2). Two ways ANOVA revealed statistically significant variation among all the three groups. SNK post hoc analysis also showed the distinctive nature of length variation among the groups.

Cocoon weight

The contributory effect of probiotic leaf coating of mulberry leaves was clearly evident in the cocoon weight of the silkworm. In feed supplemented silkworms cocoon weight was maximum (2.9 gm), whereas in the control animals (1.9 gm) and in the infected ones cocoon weight were low (1.1gm)

(Table 3). Analysis of Variance revealed significant variations among the cocoons from feed supplemented, control and infected animals. SNK post hoc analysis showed significant disparity among all the three groups and the beneficial effect of probiotic supplementation was revealed through the high weight gain in cocoons.

Table 3. Cocoon weight of *bombyx mori* fed on mulberry leaves coated with probiotic consortium (mean values ± standard deviation)

S. No	Feed supplemented	Normal	Infected
1	2.9 ±0.5[a]	1.9 ±0.1[b]	1.1 ±0.5[c]

Note: Dissimilar superscripts indicate significantly different (SNK test) values, while similar superscripts indicate similar values

Table 4. Blood cell variation in *Bombyx mori* fed on mulberry leaves coated with probiotic consortium (mean values ± standard deviation) ; percentage given in paranthesis

S. No	Blood cell types	Normal	Infected	Feed supplemented
1	Large granulocytes	10.0 ±2.5[a] (11.5)	17.6 ±4.3[a] (15.3)	13.6 ±4.9[a] (13.5)
2	Granulocytes	56.4 ±3.0[b] (64.8)	65.8 ±6.2[a] (57.3)	65.8 ±4.2[a] (65.7)
3	Semigranulocytes	13.4 ±2.3[a] (15.4)	21.4 ±9.3[a] (18.6)	17.6 ±5.5[a] (17.6)
4	Hyaline cells	7.2 ±2.5[a] (8.3)	10.0 ±3.0[a] (8.7)	3.2 ±1.0[b] (3.2)

Note: Dissimilar superscripts indicate significantly different (SNK test values, while similar superscripts indicate similar values

Table 5. Prophenol oxidase activity in haemolymph of *Bombyx mori* fed on mulberry leaves coated with probiotic consortium(Phenoloxidase activity[a] Units/min/mg of protein)

Number of silkworm	Feed supplemented	Normal	Infected
15	6.9	3.1	2.1

Note: Dissimilar superscripts indicate significantly different (SNK test) values,while similar superscripts indicate similar values

Blood cell enumeration

Differential count of haemocytes of the three groups of silkworms, showed variation in the composition of different types of haemocytes. Elevated level of granulocytes was observed in the feed supplemented groups. Maximum number of 66.0 cells per cu.mm was observed in probiotic supplemented animals and the lowest number in normal control animals (56.0 cells per cu.mm). Equal number of granulocytes in infected and probiotic supplemented could also be observed (Table 4). Other types of haemocytes like large granulocytes, granulocytes and hyaline cells recorded maximum number in infected animals, while in probiotic supplemented worms, the increase of these cells (except hyaline cells) was negligible. Hyaline cells were in meagre number (3.2 cell/cu.mm) in probiotic supplemented silkworms. In general granulocytes formed the major haemocyte (57.3-65.7%). Other haemocytes such as semigranulocytes (15.4-18.6%), large granulocytes (11.5-8.7%) and hyaline cells (3.2-8.7%) were observed in lesser numbers. F-test and SNK revealed the uniformity of distribution of small granulocytes and large granulocytes irrespective of the experimental contexts, while granulocytes were significantly high in infected and probiotic supplemented sets of larvae.

Prophenol oxidase activity

We evaluated the presence of phenoloxidase activity of silkworm in all the three sets which was read as optical density, indicating the formation of dopachrome per 50 µl of haemolymph. The result showed that phenoloxidase activity was enhanced in probiotic supplemented animals than in normal mulberry leaves-fed silkworms. Infected silkworms showed low prophenoloxidase activity than the normal ones (Table 5). a- Phenoloxidase activity estimates were taken spectrophotometrically at 490nm using L-dopa as substrate. One unit equals the amount of enzyme showing an increase in absorbance at 490nm of 0.001 per min per mg.

DISCUSSION

Mulberry leaves are the staple food of the silkworm, *Bombyx mori*. Development of the worm depends entirely on the nutritional quality of mulberry leaves. Food protein is known to influence the synthesis of new cuticle, moulting and thereby maintains the duration of silkworm – life cycle. Therefore, it can be rightly assumed that the duration of the larval period of *Bombyx mori* is inversely related to the protein content of mulberry leaves (Vanisree, 1998). Because of its phyllophagous nature, feeding exclusively on mulberry leaves, the options for improving the

growth and productivity of silkworms are largely restricted to the improvement of mulberry varieties. Beneficial effects of bacteria on their host organism had been a topic of research among microbiologists and health scientists for a very long time, and this field–the uses of probiotics is gaining popularity in diverse biomass-production establishments. Different species of lactic acid bacteria have been extensively studied (Bruno *et al* .,1993; Bernet-Camard *et al.,*1997; Gibson *et al* 1998; Kodama, 2001) and found to be beneficial as probiotics (Fuller, 1991;Bernet-Camard, 1997; Sakamoto *et al* ; 2001). Studies on the effects of probiotics in sericulture are yet to come in a big way, and we have the recent reports pertaining to the economic quality enhancement in *Bombyx mori* as a result of feed supplementation with *Lactobacillus plantarum* (Singh *et al.,*2005) and *Streptomyces noursei* (Subramanian *et al.,*2009). In the past Sukumar (1983) reported the enhancing effect on silk yield using mulberry phylloplane yeast *Sporobolomyces roseus*. Slansky and Scriber, (1985) opined that a fundamental shift in the microbial profile in silkworm larval gut is beneficial to the host which in turn may significantly contribute to increased silk production. Therefore it was now planned to study the effect of a probiotics consortium on economic parameters of and immunity enhancement in *Bombyx mori*. In the present study, it was observed that facts such as larval length, weight and cocoon weight significantly increased in the probiotic supplemented worms, while in the infected worms, these factors significantly decreased. These observation may be attributed to increased efficiency in digestion brought about by bacterial enzymes, and assimilation of food materials leading to increased protein synthesis and subsequent accumulation of storage proteins in the body. Comparatively, the food consumption in the control category with indigenous micro flora reflects the comparatively low silk production ability; Feeding quality-leaves influences the synthesis of total RNA and translation of fibroin messenger RNA and DNA synthesis (Chavancy and Flournier, 1979). Conversely the retrogressive nature of development was evident in 'flacherie' infected worms. Gut mucosal degeneration and associated physiological changes like loss of appetite, sluggishness and retardation of growth are significant among the primary symptoms of flacherie (Ganga and Sulochanachetty, 1997). Because of the intestinal mucosal degeneration even the indigenous microbiota would have been sloughed off, and hence even the health and digestive benefits presumably available for the normal animals might have been denied to the infected animals.

Insect haemocytes, like vertebrate leucocytes, are a mixture of cell types with different morphological and biological functions. The haemocytes have the ability to discriminate stranger agents, mediate phagocytosis, cytotoxicity, encapsulation, wound repair and coagulation. These defense reactions are observed against pathogens, parasites and other foreign bodies, which enter in the haemocoel (Ratcliffe *et al.,*1985; Lackie, 1986; Ratcliffe and Rowley, 1987; Brookman *et al.,*1989; Rahmet-Alla and Rowley, 1989; Falleiros described in various insects: prohaemocytes, plasmatocytes, granulocytes, spherulocytes, adipohemocytes, oenocytoids and coagulocytes (Jones, 1979; Gupta, 1985; Brehélin and Zachary, 1986). With the possible exception of some Diptera (Gotz, 1986), encapsulation in most insects clearly depends on a cooperative response between haemocytes. What remains unclear is whether this response is mediated by a functionally uniform population of haemocytes or through an interaction between functionally different subpopulations of cells. When haemocytes called granular cells contact a foreign target, they lyse or degranulate, releasing material that promotes attachment of plasmatocytes. Multiple layers of plasmatocytes then form the capsule (Schmit and Ratcliffe, 1977, 1978). In contrast, Brehelin et al. (1975) report that only a single class of cells, designated as granular haemocytes, mediates encapsulation by *Locusta migratoria* (Orthoptera) and *Melolontha melolontha* (Coleoptera). Jiravanichpaisal et al. (2006) opine that granular and semigranular hepatocytes are involved in nodulation and encapsulation, the main protective actions against invading microorganisms in Crustacea and in Drosophila, while hyaline cells are chiefly involved in phagocytosis. In infected silkworms granulocytes and semigranulocytes were found to increase, probably to encounter the invading bacteria, causing flacherie-symptoms.

Even hyaline cells were on the rise (Table-4), probably indicative of the increased phagocytic activity.

In probiotic supplemented silkworms there was a general increasing trend among the haemocyte subpopulations, probably making the animals more immunocompetent, even during the apparent absence of pathogens. Phagocytes, the culminative action of all immune responses also would have been at a very low level, and hence the low percentage of hyaline cells. Morphological features of the cellular types in *Bombyx mori* larvae-the granulocytes, semigranulocytes and hyaline cells, were similar to those found in other lepidopteran species (Akai and Sato, 1973; Lai-Fook, 1973; Beaulaton and Monpeyssin, 1976; Raina, 1976; Beeman *et al.,*1983; Essawy *et al.,*1985; Wago and Kitano, 1985; Saxena, 1992; Ribeiro *et al.,*1996), except Wago and Kitano (1985) who recognized only three types of haemocytes present in the haemolymph of *Pieris rapae crucivora*. (Boisduval).

Prophenoloxidase (PO) is activated as part of the innate immune response of insects and other arthropods. PO catalyzes the formation of quinones that are reactive intermediates for cuticle sclerotization, melanin synthesis, wound healing, and sequestering killing of invading pathogens (Nappi and Vass, 2001). Insect PO is synthesized as an inactive zymogen (proPO), which can be activated by proteolytic cleavage at a specific site near its amino-terminus (Ashida and Brey 1998). Biosynthesis of prophenoloxidase in haemocytes of larval haemolymph of silkworm, *Bombyx mori*, was observed by Iwama and Ashida (1996). Granular cells are observed to be the storage cells for PPO system (Jiravanichpaisal *et al.,*2006). In several groups of insects have purified and characterized PPO from hemolymph of the lepidopterans *B. mori* (Yasuhara *et al.,*1995), *Manduca. sexta* (Hall *et al.,*1995), *Heliothis cecropia* (Andersson *et al.,*1989), *G. mellonella* (Kopacek *et al.,*1995), and *Spodoptera littoralis* (Lee and Anstee, 1995); the Dipteran *Drosophila. melanogaster* (Fujimoto *et al.,*1993); the cockroach *Blatta discoidalis* (Dunn, 1986); the locust *Locusta migratoria* (Cherqui *et al.,*1996) and the crayfish *Pacifastacus leniusculus* (Aspan and Soderhall, 1991). Feeding with probionts elicited an augmentation of prophenoloxidase activity in *Bombyx mori*, while its activity was reduced in infected worms, probably an indication of compromising the immunity of the organism.

Present preliminary study indicates the beneficial effects of a probiotic consortium on economic traits of silkworm *B. mori*, however the mechanism of interaction of host native microbiota with the probiotic bacteria influencing directly and indirectly on food assimilation, physiology and innate immunity of silkworm require further investigation.

REFERENCES

Akai, H. and Sato. S., 1973. Ultrastructure of the larval hemocytes of the silkworm, *Bombyx mori* L. (Lepidoptera : Bombycidae) . Int. Insect. Morphol.& Embryo1. 2, 207.

Andersson K, Sun S-C, Boman HG, Steiner H. 1989. Purification of the prophenoloxidase from *Hyalophora cecropia* and four proteins involved in its activation. Insect Biochem. 19:629–37.

Ashida M, Brey PT 1998. Recent advances on the research of the insect prophenoloxidase cascade. In: Brey PT, Hultmark D, eds. Molecular Mechanisms of Immune Responses in Insects. London: Chapman & Hall: 135–172.

Aspan A, Huang T-S, Cerenius L, S¨oderh¨all K. 1995. cDNA cloning of prophenoloxidase from the freshwater crayfish *Pacifastacus leniusculus* and its activation. Proc. Natl. Acad. Sci. USA 92:939–43.

Aspan A, Soderhall K. 1991. Purification of prophenoloxidase from cray- fish blood cells, and its activation by an endogenous serine proteinase. Insect Biochem. 21:363–73.

Austin B. L., F. Stuckney, P. A. W. Robertson, I. Effandi and D.R.W. Griffith (1995). A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida*, *Vibrio angullarum* and *Vibrio ordalli* J. Fish Dis. 18: 93-96.

Beaulton, J and M, Monopeyssin, 1976. Ultrastructure and cytochemistry of the haemocytes of *Anthereae peryinii* (Lepidoptera) during the 5 instar larva, prohaemocyte,

plasmatocytes and granulocytes. J. Ultrastruc.Res. 55: 143-146.

Beeman, S. C.; Wilson, M. E.; Bulla, L. A. et al., 1983. Structural characterization of the haemocytes of *Plodia interpunctella.* Journal of Morphology, 175, 1-16.

Bernet Camarad M.F., V. Lievin, D. Brassert, J. R. Nesser A. L. Servin S. Hudault, (1997). The human *Lactobacillus acidophilus* strain LA1secretes a non bacteriocin antibacterial substances active in vitro and vivo Appl. Environ. Microbiol. 63 (7) 2747-53.

Brehelin M, Zachary D, Hofmann JA. 1978. A comparative ultrastructural study of blood cells from nine insect orders. Cell Tissue Res 195:45–57.

Brehélin, M. and Zachary, D. 1986. Insect haemocytes: a new classification to rule out the controversy. In: Brehélin, M. (ed.). *'Immunity invertebrates*, *cells*, *molecules and defense reactions'*. Heidelberg: Spring Verlag. pp. 37-48.

Breznak JA. 1982. Intestinal microbiota of termites and other xylophagous insects. Annual Review of Microbiology 36: 323-343.

Bridges JR. 1981. Nitrogen-fixing bacteria associated with bark beetles. Microbial Ecology 7: 131-137.

Brigidi P. B. Vitali, E. Swennen, G. Bazzocchi, D. Matteuzzi (2001). Effect of probiotic administration upon the composition and enzymatic activity of human faecal micro-biota in patients with irritable bowel syndrome or functional diarrhea Res. Microbiol. 152 (8): 735- 41.

Brigidi P., B. Vitali, E. Swennen, L. Altomare, M. Rossi, D.Matteuzzi (2000). Specific detection of *Bifidobacterium* strains in a pharmaceutical probiotic product and in human feces by polymerase chain reaction Appl. Microbiol. 23(3):391-9.

Brookman, J. L.; Ratcliffe, N. A. and Rowley, A. F. (1989), Optimization of a monolayer phagocytosis and its applications for studying the role of prophenoloxidase system in the wax moth, *Galleria mellonella*. Journal of Insect Physiology, 34, 337-346.

Bruno M. E. C. and T.J. Montville (1993). Common mechanistic action of bactreiocins from lactic acid bacteria. Appl. Environ. Microbiol.59: 3003-3010.

Chan R. C. Y., G. Reid, R. T. Irvin, A. W. Bruce and J. Costerson, (1985). Competitive exclusion of uropathogens from human epithelial cells by Lactobacillus whole cells and cell wall fragments Infect. Immun. 47; 84- 89.

Chavancy G. and A. Flournier (1979). Effect of starvation on t- RNA synthatase activities in the posterior silk gland of *Bombyx mori* L. Biochimie 61: 229-243

Cherqui A, Duvic B, BrehClin M. 1996. Purification and characterization of prophenoloxidase from the haemolymph of *Locusta migratoria.* Arch. Insect Biochem. Physiol. 32:225– 35.

Crossley A.C. 1968. The fine structure and mechanism of breakdown of larval intersegmental muscles in the blowfly *Calliphora erythrocephala*. J Insect Physiol 14:1389–1407.

Cruden DL, Markovetz AJ. 1987. Microbial ecology of the cockroach gut. Annual Review of Microbiology 41: 617-643.

Dillon, R.M. and V.M. Dillon. 2004. The gut bacteria of insects : Nonpathogenic interactions; Annu.Rev.Entomol.49.79-92.

Douillet, P. and Langton, C.J., 1994. use of probiotic for the culture of larvae of the pacific oyster (*Crassostrea gigas* Thunberg). Aquaculture, 119: 25-40.

Dunn PE. 1986. Biochemical aspects of insect immunity. Annu. Rev. Entomol. 31:321–39

Essawy, M. A.; Maleville, A. and Brehélin, M. 1985. The hemocytes of *Heliothis armigera*. Ultrastructure, function, and evolution in the course of larval development. Journal of Morphology, 186, 225-264.

Falleiros, A. M. F. and Gregório, E. A. (1995). Hemócitos fagocitários em larvas de *Diatraea saccharalis* (Fabricius) (Lepidoptera, Pyralidae). Revista Brasileira Zoologia, 12, 751-758.

Fujimoto K, Masuda K, Asada N, Ohnishi E. 1993. Purification and characterization of prophenoloxidase from pupae of *Drosophila melanogaster.* J. Biochem. 113:285–91.

Fuller, R, 1991. Probiotics in human medicine. Gut Apr; 32(4):439-42.

Ganga, G and J. Sulochana Chetty,1980. An introduction to sericulture. In : Biology of *Bombyx mori*. Oxford and IBH publishing co Pvt. Ltd. New Delhi. 111-122.

G¨otz P. 1986. Encapsulation in arthropods. In *Immunity in Invertebrates:Cells,* Molecules and Defense Reactions, ed. M Breh`elin, pp. 153–70.

Gibson, L, J. Woodworth and A. George, 1998. Probiotic activity of *Aermonas* media on the pacific Oyster, *Crassostrea gigas*, when challenged with *Vibrio tubishii.* Aquaculture, 169:111-120.

Gildberg A., H. Mikkelsen, E. Sandker and E. Ringo, (1997). Probiotic effect of lactic acid bacteria in the feed on the growth and survival of fry of Atlantic cod (*Gadu*s *morhua*) Hydrobiologia 352: 279- 285.

Gillespie JP, Kanost MR, Trenczek T. 1997. Biological mediators of insect immunity. Annu Rev Entomol 42:611–643.

Gupta, A. P. (1985. Cellular elements in the hemolymph In: '*Comprehensive insect physiology biochemistry and pharmacology'*. Kerkut, G.A., Gilbert, L. I. Eds. Oxford: Pergamon Press. pp. 402-444.

Hall M, Scott T, Sugumaran M, S¨oderh¨all K, Law JH. 1995. Proenzyme of *Manduca sexta* phenol oxidase: purification, activation, substrate specificity of the active enzyme, and molecular cloning. Proc. Natl. Acad. Sci. USA 92:7764–68.

Hernandez-Lopez, J., Gollas-Galvan, T., Vargas-Albores, F., 1996. Activation of the prophenoloxidase system of the brown shrimp *Penaeus californiensis* Holmes haemolymph. Comp. Biochem. Physiol. 104B, 407–413.

Hoffmann, J.A., 2003. The immune response in *Drosophila*. Nature, 426(6962):33-35 [doi:10.1038/nature02021]

Iwama R, Ashida M. Biosynthesis of prophenoloxidase in hemocytes of larval hemolymph of the silk worm, *Bombyx mori*. Insect Biochem 1996;16:547–555.

Jiravanichpaisal P, Lee BL, Söderhäll K (2006). Cell-mediated immunity in arthropods: hematopoiesis, coagulation, melanization and opsonization. Immunobiol. 211: 213-236.

Johansson MW, Söderhäll K, 1989. Cellular immunity in crustaceans and the proPO system. Parasitol. Today 5: 171-176.

Jones, J. C. 1979. Pathways and pitfalls in the classification and study of insect hemocytes. In: Gupta, A. P. (ed.). '*Insect hemocytes: development*, *forms*, *functions and techniques'*. Cambridge: Cambridge University Press. pp. 279-300.

Kiger JA, Natzle JE, Green MM. 2001. Hemocytes are essential for wing maturation in *Drosophila melanogaster*. Proc Natl Acad Sci USA 98:10190–10195.

Kobayashi H, Kurata S, Natori S. 1991. Purification of the 200 kDa hemocyte membrane protein of *Sarcophaga peregrina* and its specific interaction with fat body. Insect Biochem 21:517–522.

Kodama R. (2001) Bacterial diseases and countermeasures chapter IV Silkworm rearing and artificial diet Editor Hamamura Y Oxford and IBH publishing Co. Pvt. Ltd. New Delhi Calcutta.

Kopacek P, Weise C, G¨otz P. 1995. The prophenoloxidase from the wax moth *Galleria mellonella*: purification and characterization of the proenzyme. Insect Biochem. Mol. Biol. 25:1081–91.

Krishnaswami S. (1978) Sericulture Manual Vol. 2: Silkworm rearing. FAO Bulletin, Rome. Srirampura, Mysore.16-18 November ABSTRACT NO.MMSPP/0-7 P.87.

Lackie AM. 1986. Transplantation; the limits of recognition. In *Hemocytic and Humoral Immunity in Arthropods*, ed. AP Gupta, pp. 191–223. New York: Wiley.

Lai-Fook, 1973. The structure of the haemocytes of *Calpodes ethlius* (Lepidoptera) Journal of Morphology, 139, 1, 79–103.

Lanot R, Zachary D, Holder F, Meister M. 2001. Postembryonic hematopoiesis in *Drosophila*. Dev Biol 230:243–257.

Lavine, M.D., Strand, M.R., 2002. Insect hemocytes and their role in immunity. Insec. Biochem. Mol. Biol., 32(10): 1295-1309. [doi:10.1016/S0965-1748(02)00092-9].

Lee MJ, Anstee JH. 1995. Phenoloxidase and its zymogen from the haemolymph of larvae of the lepidopteran *Spodoptera littoralis* (Lepidoptera:

Noctuidae). Comp. Biochem. Physiol. 110B:379–84.

Lehane, M.J., 1997. Peritrophic matrix structure and function. Annu. Rev. Entomol., 42(1):525-550. [doi:10.1146/annurev. ento.42.1.525].

Lehane, M.J., Wu, D., Lehane, S.M., 1997. Midgut-specific immune molecules are produced by the blood-sucking insect *Stomoxys calcitrans*. PNAS, 94(21):11502-11507. [doi:10.1073/pnas.94.21.11502]

Levashina, E.A., Moita, L.F., Blandin, S., Vriend, G., Lagueux, M., Kafatos, F.C., 2001. Conserved role of a complement-like protein in phagocytosis revealed by dsRNA knockout in cultured cells of the mosquito, *Anopheles gambiae*. Cell, 104(5):709-718. [doi:10.1016/S0092-8674(01)00267-7]

Ligoxygakis, P., Pelte, N., Ji, C., Leclerc, V., Duvic, B., Belvin, M., Jiang, H., Hoffmann, J.A., Reichhart, J.M., 2002. A serpin mutant links toll activation to melanization in the host defence of *Drosophila*. EMBO J., 21(23):6330-6337. [doi:10.1093/emboj/cdf661]

Meister, M., Lagueux, M., 2003. *Drosophila* blood cells. Cell. Microbiol., 5(9):573-580. [doi:10.1046/j.1462-5822.2003. 00302.x]

Nappi AJ, Vass E. 2001. Cytotoxic reactions associated with insect immunity. Adv Exp Med Biol 484:329–348.

Narayan, K., 2004. Insect resistance: its impact on microbial control of insect pests. Current Science, 86(6):800-814.

Nardi JB, Miklasz SD. 1989. Hemocytes contribute to both the formation and breakdown of the basal lamina in developing wings of *Manduca sexta*. Tiss Cell 21:559–567.

Pech LL, Trudeau D, Strand MR. 1994. Separation and behavior in vitro of hemocytes from the moth, *Pseudoplusia includens*. Cell Tissue Res. 277:159–67.

Rahmet-Alla, M. and Rowley, A. F,1989. Studies on the cellular defense reactions of the madeir cockroach, *Leucophaea maderae*: in vivo phagocytosis of different strains of *Bacillus cereus* and either effect on hemocyte viability. Journal of Invertebrate pathology, 54, 200-207.

Raina, A.K. 1976. Ultrastructure of the larval hameocyte of pink bollworm *Pectinophora gossypiella* (Lepidoptera). Int. J. Insect. Morphol. Embryol. 5: 167-185.

Ratcliffe NA, Rowley AF. 1979. Role of hemocytes in defense against biological agents. In *Insect Hemocytes: Development, Forms, Functions and Techniques*, ed. AP Gupta, pp. 331–414. London: Cambridge Univ. Press.

Ratcliffe, N.A., Rowley, A.F., Fitzgerald, S.W., Rhodes, C.P., 1985. Invertebrate immunity: basic concepts and recent advances. Int. Rev. Cytol. 97, 183–350.

Ribeiro, C.; Simões, N. and Brehélin M. 1996. Insect immunity: the haemocytes of the armyworm *Mythimna unipuncta* (Lepidoptera: Noctuidae) and their role in defence reactions. In vivo and in vitro studies. Journal of Insect Physiology, 42, 815-822.

Roy D. K., D. N.Sahay., S. Rai, B.R.R.Pd. Sinha and K. Thangavelu, 2000. National conference on strategies for sericulture research and development at Central sericultural research and training institute central silk board (Ministry of textiles) Govt. of India.

Sakamoto I., M. Igarashi, K. Kimura A.Takagi, T. Miwa, and Y Koga, 2001. *Helicobacter pylori* infection in humans J. Antimicrob. Chemother. 47(5): 709- 10.

Saxena, B. P. 1992. Comparative study of three lepidopterans by light and scanning electron microscopy. Acta Entomologica Bohemoslovaca, 89, 323-329.

Schmit,A.R.and Ratcliffe , N . A . The encapsulation of araldite implants and recognition of foreignness in *Clitumnus extradentatus*. J . Insect Physiol. 24, 511,1978.

Schmit, A. R.and Ratcliffe, N . A . The encapsulation of foreign tissue implants in *Galleria mellonella* larvae . J . Insect Physiol. 23,175,1977.

Singh, K.K, R.M.Chauhan, A.B.Pande, S.B.Gokhale and N.G.Hegde, 2005. Effect of Use of *Lactobacillus plantarum* as a Probiotics to improve Cocoon Production of Mulberry Silkworm, *Bombyx mori* (L.). J. Basic Appl. Sci. Vol. (1), June.Nov. :1-8.

Slansky F. Jr., and Sacriber J. M, 1985. Food consumption and utilization. In: comprehensive Insect physiology Biochemistry and Pharmacology.. (Eds. Kerkut G. A. and gilbert L. I.) Vol.4 pp 87-163.Pergamon press, New York.

Smith V. J, Söderhäll K, 1983. â-1-3-glucan activation of crustacean hemocytes *in vitro* and *in vivo*. Biol. Bull. (Woods Hole, Mass.), 164: 299-314.

Steinhaus E.A.,1949. Principles of Insect Pathology. McGrow and Hill book Co. Inc.New York pp 757.

Subramanian, S, P. Mohanraj and M. Muthusamy, 2009. Newparadigm in silkworm disease management using probiotic application of *Streptomyces noursei.* Karnataka J. Agric. Sci., 22 (3-Spl. Issue) : 499-501.

Sukumar J, 1983. Studies on the phylloplane microflora of mulberry (*Morus indica* L.).Ph.D. thesis, Univ. Mysore, India.

Tanada, Y., and H. K. Kaya. 1993. Insect Pathology. Academic Press, New York, NY.

Trenczek T. 1998. Endogenous defense mechanisms of insects. Zoology 101:298–315.

Tzou, P., Ohresser, S., Ferrandon, D., Capovilla, M., Reichhart, J.M., Lemaitre, B.,

Hoffmann. J.A., Imler, J.L., 2000. Tissue-specific inducible expression of antimicrobial peptide genes in *Drosophila* surface epithelia. Immunity, 13(5):737-748. [doi:10.1016/S1074-7613(00)00072-8]

Vanisree, V. 1988. Differential synthesis and sequestration of storage proteins by various fat body tissues during development of silkworm *Bombyx mori*. Ph.D. Thesis , Bharathidasan University. India.

Vernick, K.D., Fujioka, H., Seeley, D.C., Tandler, B., Aikawa, M., Miller, L.H., 1995. *Plasmodium allinaceum* A refractory mechanism of ookinete killing in the mosquito, *Anopheles gambiae*. *Exp. Parasitol.*, 80(4):583-595. [doi:10.1006/expr.1995.1074]

Wago, H. and Kitano, H. 1985. Morphological and functional characterization of the larval haemocytes of the cabbage white butterfly *Pieris rapae crucivora*. Applied Entomology and Zoology, 20, 1-7.

Wyllie, A.H., Kerr, J.F.R., Currie, A.R., 1980. Cell death: the significance of apoptosis. Int. Rev. Cytol., 68:251-306.

Yasuhara Y, Koizumi Y, Katagiri C, Ashida. M. 1995. Rexamination of properties of prophenoloxidase isolated from larval hemolymph of the silkworm *Bombyx mori.* Arch. Biochem. Biophys. 320:14–23

Yeung P. S. M., M. E. Sanders, C. L. Kitts, R. Cano and P. S. Tong, 2002. Species Specific Identification of Commercial Probiotic Strains J. Dairy Sci Association 85: 1039-1051.

Yuan ZH, Lan XQ, Yang T, Xiao J, Zhou ZY., 2006. Investigation and analysis of the bacteria community in silkworm intestine. Acta Microbiol Sinaca, 46:285–291.

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