# Bacterial induced immunity studies in selected silkworm (*Bombyx mori* L.) germ plasm strains

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#### ABSTRACT

The present investigation was an attempt to understand the immune mechanism prevailing in the selected multivoltine and bivoltine silkworm accessions. The silkworm accession were subjected to study after injecting with attenuated preparation of microorganism. Viz., *E.coli* to the late age (Vth instar) silkworms. The cellular and humoral responses were studied through haemocyte counts and protein profiles expressed in control and treated silkworm larvae through SDS-PAGE followed by antimicrobial assay. The present study demonstrates that silkworm can be best used to produce several antibacterial proteins useful for the human beings in addition to its main role for the production of silk.

Key words: Multivoltine and Bivoltine silkworm, Haemolymph, Cellular and Humoral responses.

#### INTRODUCTION

Insects posses innate immunity. Injection of microorganism (*E.coli*) induced a combination of humoral and cellular response (Boman and Hultmark 1987). Humoral reaction involves synthesis and release of several antibacterial proteins of 4 types Viz., cecropin, attacin, lectin and moricin have been identified in B.mori (Gillespie *et al* 1997). In *B.mori* cellular responses involves synthesis of six major groups of haemocytes. These cells reacts by phagocyting in case of small organism or nodulating and encapsulating the large objects. (Miller and Ratcliffe 1994).

## MATERIAL AND METHODS

In the present study, 11 silkworm genetic resources comprising 5 multivoltine and 6 bivoltine germplasm accessions were utilized. The study was conducted with 2 sets of 10 numbers of larvae for temperature treatment. In the proleg of the multivoltine larvae 2.5cc of log phase DH5á *E.coli*  was injected. After 6 hours incubation hemolymph was collected from the larvae by cutting the legs. To the one set of 6 accession of bivoltine larvae 2.5cc of log phase DH5a E.coli was injected and kept it for 1-5 days. To the second set of bivoltine larvae the heat shock treatment was given that is at 41æ%c for 6 hours. The hemolymph was collected daily from day 1 to day 5 from the bivoltine larvae. The total haemocytes were counted by using haemocytometer from the collected hemolymph. ( Bala venkatasubbaiah et al 2001). Antibacterial activity of hemolymph was assayed by measuring the inhibition zone in the LB plates. (Minoru et al 1995). To confirm the induction of immunity in the bacteria treated multivoltine and bivoltine accessions, SDS-PAGE was performed to see the formation of extra protein band (Madhavan and Velpandi 1988)

### RESULTS

There was a gradual increase of THC count in all the bacteria treated larvae, the maximum

Day	Acc. No	- 173	Acc. No	- 176	Acc. No	- 197	Acc. No	- 265	Acc. No	- 271	Acc. No	- 275
	Bacterial treated	Treated at 41°C	Bacterial treated	Treated at 41°C	Bacterial treated	Treated at 41°C						
-	13.296	24.919	7.554	36.686	24.144	1.447	31.238	37.668	19.812	29.273	10.406	30.100
2	39.474	30.794	17.516	38.424	24.877	27.754	38.878	33.541	44.114	18.398	27.930	39.787
ო	35.096	32.674	36.022	45.725	56.885	45.661	51.870	31.089	61.899	36.144	71.922	53.022
4	44.510	25.448	84.586	57.278	73.648	46.258	44.510	40.877	59.229	57.057	99.115	26.296
5	62.186	38.961	70.949	54.089	76.826	7.17	62.186	41.506	86.453	61.771	72.995	45.576

Table 2: T value of total haemocyte count
in bacteria injected mv accession and
temperarure treated multivoltine accession

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S. No	Races	T.values
1	MV-0001	38.537
2	MV-0027	15.400
3	MV-0045	24.222
4	MV-0056	23.866
5	MV-0066	6.881

count was observed on the 5<sup>th</sup> day larvae. There was increase of THC counts in temperature treated bivoltine accession also but not as bacterial treated (Table1). The results obtained with bacteria treated and thermal treated larvae were statistically analyzed. Among 6 bivoltine, maximum inhibition zone of 2.1 CMs diameter was found in BBI-275 followed by BBI-197 with 2 CMs and other inhibition zone of 1.7 CMs BBI-173, 1.5CMs in BBI-176, 1.4 CMs in BBI-265 and 1.35 CMs in BBI-271. In case of multivoltine, maximum inhibition zone of 2.1 CMs was found in BMI-0045 followed by 1.9 CMs in BMI-0001. The results obtained in MV accessions were given in the table 2

The gel electrophoresis study indicated that the response varied among 6 bivoltine accessions maximum number of 4 bands were observed in BBI-197, followed by 3 bands in BBI-176.

#### DISCUSSION

The increase in the haemocyte counts in the bacteria injected larvae indicated the cellular responses to the foreign bodies. Similar observation were reported by Venkatasubbaiah et al 2001 in the BV larvae infected with Bombyx mori nuclear poly hochoris virus (BmNPV). In the present study race A has indicated 4 extra protein band, which are directly correlated with maximum inhibition zone (2.0 CMs) in that particular accession. From the above study, it can be inferred that among multivoltine accession BMI-0045 are considered as resistant to bacterial infection based on inhibition zone assay. Among bivoltine BBI-0197 and BBI-0197 are considered as resistant to bacterial infections based on the appearance of extra protein observed in the electrophoresis study.

## REFERENCES

- Balavenkatasubbaiah..B, V.Natraj, U.Thiyagarajan and R.K. Datta., Hemocyte count in the different breeds of silkworm, Bombyx mori L. and their changes during the progressive infection of BmNPV. *Indian. J. Seric.*, 40(2): 158-162 (2001).
- Boman, H.G and D. Hultmark., Cell-free immunity in insects. Ann. Rev. Microbial., 41: 103-126 (1987).
- Gillespie, J.R, M.R.Kanost and T.Trenczek., Biological mediators of insects immunity. *Annual review of entomology*, **42**: 611-643 (1997).
- 4. Madhavan.S, and A,Velpandi, Bacteria induced protein synthesis in the hemolymph

of Bombyx mori L.larvae. *Sericologia.*, **28**(2): 201-209 (1988).

- Millar, D.A and N. A. Ratcliffe., Invertebrates In : Immunology: a comparative approach. (Turner R.J.ed.) John wiley and sons ltd. Chichester. 29-68 (1994).
- Minoru.Y, K.O.Keiko, T.Kiyoko and K.Yusuku, B.M.Cecropin B an antibacterial protein: structure regulation of the gene expression and antibacterial spectra. *Indian.J.Serc.*, 34(1): 1-5 (1995).
- Nittono. Y., Studies on the blood cells in the silkworm, bombyx mori L. bull seric expstn. Tokyo., 16: 171 (1960).