वार्षिक प्रतिवेदन
Annual Report 2015-16

रेशमकीट जैव प्रौद्योगिकी अनुसंधान प्रयोगशाला

केन्द्रीय रेशम बोर्ड, वस्त्र मंत्रालय, भारत सरकार

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Cover Page : Transgenic CSR4 moth of Bombyx mori with higher NPV tolerance developed at SBRL through conventional breeding technique using Nistari with NPV transgenes

CONTENTS

Dr. A. K. Awasthi, Director (I/c) SBRL receives the ISO 9001: 2008 Certificate of Registration during the year 2015-16.

प्रस्तावना

रेशम उत्पादन लिए आण्विक जैव विज्ञान का समर्थन आजकल अनिवार्य हो गया है क्योंकि रेशम उन्पादन क्षेत्र को परजीवियों से ही नहीं, बल्कि वैश्विक तापन, वर्षा की कमी तथा नगरीकरण के कारण नई समस्याओं का सामना करना पड़ रहा है। समय की माँग है कि कृषकों की क्षति कम करने के लिए उच्च रेशम उत्पादन तथा प्रतिबल प्रबंधन के साथ नए रेशमकीट नस्लों का विकास किया जाए। रेशम जैव प्रौद्योगिकी अनुसंधान प्रयोगशाला में हमने भारतीय पृथक्कारी डेन्सोन्युक्लियोसिस विषाणु के लक्षण-वर्णन, प्रतिजीवाणुक पेपटाइड़ के साथ रेशम विशेषकों का संयोजन करने तथा रेशमकीट की असंक्राम्यता को रोकने वाले कवक, लघुबीजाणु तथा ऊज़ी मक्खी का आणिवक लक्ष्य पहचाना है और अनुसंधान परियोजनाओं के माध्यम से भविष्य की चुनौतियों के प्रबंधन के लिए नए आण्विक वैज्ञानिक अभिगम को प्रारंभ किया है। विशेष रूप से केन्द्रीय रेशम बोर्ड तथा जैवप्रौद्योगिकी विभाग (डीबीटी, भारत सरकार) ने शहतुत तथा वन्य रेशम क्षेत्रों में रेशम जैव प्रौद्योगीकी में नई अनुसंधान प्रणाली के उपयोगार्थ उनका वित्तीय समर्थन बढ़ाया है। भारत के स्थानीय मूंगा रेशमकीट का संजीनी डीएनए अनुक्रम तथा ट्रान्स्क्रिप्टोम विश्लेषण केरेबो के संपूर्ण समर्थन से प्रारंभ किया गया है ताकि जीनोम स्तर पर वन्य तथा वाणिज्यिक संख्या को अलग किया जा सके तथा आण्विक लक्षण-वर्णल के आधार पर सुनहरे मूंगा रेशम के वस्त्र लक्षणों की भूमिका को स्पष्ट किया जा सके।

चिहनक की सहायता से चयल के जरिए नए संकरों के विकास से एनपीवी संक्रमित दशा में भी अधिक कोसा उत्पादन हुआ। जैवप्रौद्योगिकी विभाग (डीबीटी) समर्थन से पूरे देश में इन नए संकरों का बड़े पैमाने पर कीटपालन प्रस्तावित है जो रेशम उत्पादन क्षेत्रों में विभिन्न जलवायु दशा में संकर की सहनशीलता की पुष्टि करेगा। बी. मोरी (सीएसआर4 तथा सीएसआर 27) के उच्च उत्पादक सुग्राहय प्रजातियों के एनपीवी सहनशीलता को बढ़ाने के लिए पारजीनी निस्तरी से उक्त उत्पादक रेशमकीट प्रजातियों में एनपीवी पारजीनों को हस्तांतरित करते हुए आरएनए हस्तक्षेप तथा परंपरागत प्रजन्न तकनीक का प्रयोग किया गया और इन सीएसआर प्रजातियों को परजीनी सीएसआर के रूप में परिवर्तित किया गया हैं। इस तकनीक से सामान्य सीएसआर प्रजातियों में 5% की तुलना में एनपीवी संक्रमण के बाद पारजीनी सीएसआर प्रजातियों की उत्तरजीविका दर में 50% तक वृद्धि हुई, जिससे परंपरागत तथा आण्विक आनुवंशिक अभिगमें के सफल सम्मिश्रण से उन्नत रेशम उत्पादन के परिणाम की पुष्टि की जा सके।

प्रयोगशाला के वैज्ञानिक योगदान का प्रकाशन उच्चस्तरीय जर्नलों में होता है, नई अनुसंधान परियोजनओं की सरगर्भित योजना बनाते हुए इसका कार्यान्वयन किया जाता है । इसके अलावा अनुसंधान तथा स्नातकोत्तर विद्यार्थियों को मार्गदर्शन तथा प्रशिक्षण भी दिया जाता है। मैं उन सभी अनुसंधान विद्यार्थियों को बधाई देता हूँ जिन्हें मैसूरू विश्वविद्यालय से पीएचडी की उपाधि मिली। विभिन्न अनुसंधान परियोजनाओं के सफल तथा समय पर निष्पादन के लिए मैं सभी वैज्ञानिकों, अनुसंधान छात्रों / साथियों तथा अन्य सभी कर्मचारियों को उनकी समार्पित सेवा की सराहना करता हूँ।

मैं अनुसंधान सलाहकार समिति के प्रति उनकी सतत सहायता के लिए आभार व्यक्त करता हूँ तथा इस रिपोर्ट में और सुधार के लिए सभी से महत्वपूर्ण निवेश का स्वागत करता हूँ।

डॉ. प्रदीप कुमार मिश्र

FOREWORD

Molecular Biology support to sericulture development became imminent these days as the industry faces lot of challenges in the field not only from parasites but from newer encounters due to global warming, rain scarcity and urbanization. Development of new silkworm breeds with high silk production and stress management became need of the hour to cut short the loss ensued to the farmers. We at SBRL rolled out new molecular biology approaches to manage the challenges ahead by opting research projects to characterize Indian isolates of densonucleosis virus, to generate fusion of silk traits with antibacterial peptides and to identify molecular targets of fungus, microsporidia and uzifly that suppress silkworm immunity. Notably Central Silk Board and Department of Biotechnology (Government of India) enhanced their financial support to utilize new research methodologies in seri-biotechnology both in mulberry and *Vanya* silk sectors. Genomic DNA sequencing and Transcriptome analysis of the muga silkworm, endemic to India has been initiated with complete support from CSB to differentiate wild and commercial populations at genome level as well as to elucidate the role of textile characters of golden muga silk based on molecular characterization.

The development of new breeds through Marker Assisted Selection led to increased cocoon production even under NPV infected conditions. Large scale rearing of these new breeds is scheduled throughout the country with DBT support which will confirm hardiness of the breed under different climatic conditions in the sericultural belt. In order to enhance the NPV tolerance of high yielding susceptible races of *B. mori* (CSR4 and CSR27), RNA interference and conventional breeding techniques were applied by transferring NPV transgenes from transgenic Nistari to the said productive silkworm races and these CSR races were transformed to transgenic CSR. The technique enhanced the survival rate of transgenic CSR races to 50% after NPV infection compared to 5% in normal CSR races, thereby confirming improved sericultural output through successful amalgamation of conventional and molecular genetic approaches.

The scientific contributions of the laboratory are supported by research publications in fairly high impact factor journals, precise planning and execution of new research projects including guidance and training of research scholars and postgraduate

students. I congratulate our research scholars who were awarded with Ph.D from the University of Mysore. I appreciate the hard work and scientific inputs of all the scientists and research fellows as well as the dedicated services of all other supporting staff for the successful timely execution of different research projects.

I thank the Research Advisory Committee for their persistent support and welcome critical inputs from one and all on this report for further improvement.

Dr. Pradeep Kumar Mishra
Director

VISION AND MISSION

VISION

To become a Centre of Excellence in Seribiotechnology.

MISSION

To achieve excellence in research in frontier areas of modern biology to transform Indian Sericulture Industry into a competitive commercial production base.

MANDATE

- To conduct research in silk biotechnology towards improvement in silk productivity
- To interact with reputed R&D institutions in sericulture and allied activities
- To develop and disseminate technology to other R&D organizations

OBJECTIVES

- Conduct scientific research in frontier areas of modern biology for developing potential applications in improving silk productivity
- Conduct research on silk for biomaterial and biomedical applications
- Development and patenting of products/technologies
- Capacity building in biotechnology
- Strengthening institutional framework to support research programmes
- Publication of R&D outcome
- Collaborative research programmes with other R&D organizations in India and abroad including industry
- Efficient functioning through RFD System
- Improving internal efficiency / responsiveness / service delivery of the institute
- Training for employable manpower development

FUNCTIONS

- To formulate and implement research projects in frontier areas of modern biology
- To take up collaborative projects with other institutions in applied research
- To develop and disseminate the products/technologies
- To generate Human Resource in seribiotechnology

अनुसंधान की मुख्य विशेषताएँ

- रोग प्रतिरोधी क्षमता में शामिल जीन नामत: रेलिश, सेक्रोपिन बी, ड्रोसोमाइसिन तथा उसके प्रवर्तकों को पीसीआर द्वारा संवर्धन किया गया और टीए वेक्टरों में संयोजित किया गया। रचनाओं को रेशमकीट अंडे में उनके मैक्रोइंजेक्शन (microinjection) के लिए पारजीनी वेक्टर, पिग्गी बैक (piggy bac) में स्थानांतरित किया गया।
- हेमोसाइट में कोशिका मध्यस्थ रोधक्षमता (immunity) सक्रिय हुई जो हयूमरल रोधक्षमता पद्धति के सक्रियण से समर्थित हैं। दूसरी ओर, परजीवी परपोषी रोधक्षमता पद्धति पर विजयी होती है और रक्षा यंत्रावली को पार कर लेते हैं। हयूमरल लेक्टिन (Humoral lectin), हेमोसाइटिन (Hemocytin) तथा बीजीआरपी 2 बॉम्बिक्स मोरी में लघुबीजाणु संक्रमण के बाद अधिक अभिव्यक्ति स्तर दर्शित हुआ जबकि मेंलानाइज़ेशन (melanization) जीन लघुबीजाणु संक्रमण के बाद के दिनों में कम अभिव्यक्ति देखी गयी। अनिर्धारित लक्षणवाले प्रोटीन को ट्रान्समेम्ब्रेन परिवाहक के रूप में लक्षण निर्धारित किया गया जो लघुबीजाणु संक्रमण के बाद होमोसाइट में बड़ी मात्रा में विद्यमान हैं।
- एक्सओरिस्ता बोम्बईसिस के संक्रमण से पिस्दम्ब्रे में ऑक्सीकरण / रिडक्शन प्रतिक्रियाओं और प्रतिक्रियाशील ऑक्सीजन प्रजातियों के उत्पादन और बॉम्बिक्स मोरी में एंटीऑक्सीडेंट एंजाइम (Antioxidant) गतिविधियों का प्रेरित हुआ हैं।
- आरआरएनए (rRNA) सार्वत्रिक प्राइमर के आधार पर नोसीमा जीनस के लघुबीजाणु के दो नये प्रभेद की पहचान की गई।
- चार विभिन्न लघुबीजाणु प्रभेदकों से निकाले गये डीएनए को पीसीआर के माध्यम से 16_S आरआरएनए तथा आईजीएस प्राइमर के साथ संवर्धन किया गया। आईजीएस क्षेत्र नोसीमा बोम्बिक्स के मानक प्रकार की जाति की तुलना में अधिक न्यूक्लियोटाईड विविधता दर्शाई गई। लघुबीजाणुओं का पता लगाने के लिए पारिवर्तनीय क्षेत्रों को लक्ष्य बनाया जाएगा।
- तसर रेशमकीट से एंथेरिया माइलिटा फ्लेचरी सम्बद्ध रोगजलक की पहचान की गई, नामत: दो प्रकार के विषाणु, साइटोप्लास्मिक पॉलिडेड्रोसिस विषाणु (सीपीवी) इन्फेक्शियस फ्लेचरी विषाणु (आईएफवी) तथा फ्लेचरी रोग से सम्बद्ध जीवाणु प्रजातियों के चार प्रकार जैसे एन्टरोबैक्टर, प्रोटेस, स्टफालिकोकस तथा एन्टरोकोकस जाति की पहचान की गई, तसर रेशमकीटों में दोनों विषाणु और जीवाणु के कारण फ्लेचरी होता है।
- F_{14} पीढ़ी (ग्रीष्म कीटपालन) में सीएसआर4 तथा सीएसआर 27 के पारजीनी वंश के कवच भार और कवच अनुपात में बहुत कम मूल्य देखा गया जबकि डिम्भकीय तथा प्यूपा भार तुलनात्मक रूप में उच्च रहा। F_{15} पीढ़ा में, कोसा लक्षणों की प्रतिप्राप्ति हुई जिससे पारजीनी वंशों में अनुकूल विशेषकों पर उच्च तापमान का अधिक प्रभाव देखा गया।
- सीएसआर 4 (CSR4) तथा सीएसआर 27 (CSR27) के पारजीनी वंश के F_{12} तथा F_{14} पीढ़ी ने एनपीवी संक्रमण के बाद उत्तरजीविता दर 50-55% दर्शायी।
- 12 प्रतिकवक जीन के तुलनात्मक अभिव्यक्ति विश्लेषण एपीएम 2 (AMZ) में अधिकतर जीन में अधिक और एपीएम 3 (APM3) में सबसे कम अभिव्यक्ति दर्शायी। कवक ब्यूवेरिया बेसियाना का प्रगुणन एपीएम 3 (APM3) में सबसे अधिक और प्रतिकवक जीन की उच्च अभिव्यक्ति के साथ साथ कम कवक प्रगुणन के सुझाव के साथ एपीएम 2(APM2) में कम रहा।
- बीएम बीहीवी (BmBDV) के भारतीय पृथक्कारी लक्षण निर्धारण से छ: ओआरएफएस (ORFs) का पता चला। विषाणु का प्रगुणन उत्प्रेरित करने के डीएलए पॉलिमिरेस जीन के लिए ओआरएफ-4 कोडिंग सम्पूरक रेखा पर स्थित है और यह पृथक्कारी के बीडीवी स्थिति की पुष्टि करती है। अस्थायी एनसीबीआई (NCBI) अभिगम के साथ बीएमबीडीवी के भारतीय पृथक्कारी का संपूर्ण संजीन विश्लेषण बीएमबीडीवी के जापानी पृथक्कारी के साथ अधिकतम सादृश्य देखा गया।
- बारंबारता सहित रेशम फाइब्रॉइन जीन के विखण्डन तथा जीवाणुरोध प्रोटीन, सेक्रोपिन बी का पीसरीआर संवर्धन किया गया। जीन के साथ बनाई गई रचना इन प्रोटीन एवं पॉलीहिस्टिडीन टैग की एनकोडिंग करता है। इसे पिचिया पास्टरीस वेक्टर में क्लोन किया गया। पुन: संयोजन अभिव्यक्ति के लिए अनूक्रमण से रचना की पुष्टि की गई।

RESEARCH HIGHLIGHTS

1. In the transgenic lines on CSR4 and CSR27, shell weight and shell ratio showed significantly low value in F_{14} generation (summer rearing) whereas larval and pupa weight is comparatively higher. In F_{15} generation, the cocoon characters were regained indicating larger influence of high temperature on the adaptive traits in the transgenic lines.

2. In F_{12} and F_{14} generations of transgenic lines of CSR4 and CSR27,survival rate after NPV infection showed 50-55%.

3. A comparative expression analysis of 12 antifungal genes revealed higher expression of most of the genes in APM2 and least in APM3. Multiplication of the fungus *Beauvaria bassiana* was highest in APM3 and lowest in APM2 suggesting a lower fungal multiplication is accompanied with higher expression of antifungal genes.

4. Characterization of the Indian isolate of BmBDV revealed six ORFs. ORF-4 coding for DNA polymerase gene that catalyse multiplication of the virus is located on the complimentary strand and it confirm the BDV status of the isolate. Whole genome analysis of the Indian isolate of BmBDV with temporary NCBI accession showed maximum homology with that of Japanese isolate of the BmBDV.

5. Fragments of silk fibroin gene with repeats and an antibacterial protein, cecropin B were PCR amplified. Constructs were made with genes encoding these proteins and polyhistidine tags and cloned in to *Pichia pastoris* vectors. Constructs were confirmed by sequencing for recombinant expression.

6. Genes involved in immunity, viz., Relish, Cecropin B, Drosomycin and their promoters were amplified by PCR and ligated into TA vectors. The constructs were transferred in to the transgenic vector, piggyBac for their microinjection into silkworm eggs.

7. Infection of *Bombyx mori* larva with *Nosema bombycis* activated cell-mediated immunity in hemocytes which is supported by activation of humoral immune system. On the other hand, the parasites surmount the host immune system and overcome the defense mechanisms. Humoral lectin, hemocytin and BGRP2 showed increased expression level after microsporidian infection in *B. mori* whereas melanization genes showed lower expression in later days of

microsporidian infection. An uncharacterized protein is characterized as transmembrane transporter which is present in large quantity in hemocytes after microsporidian infection

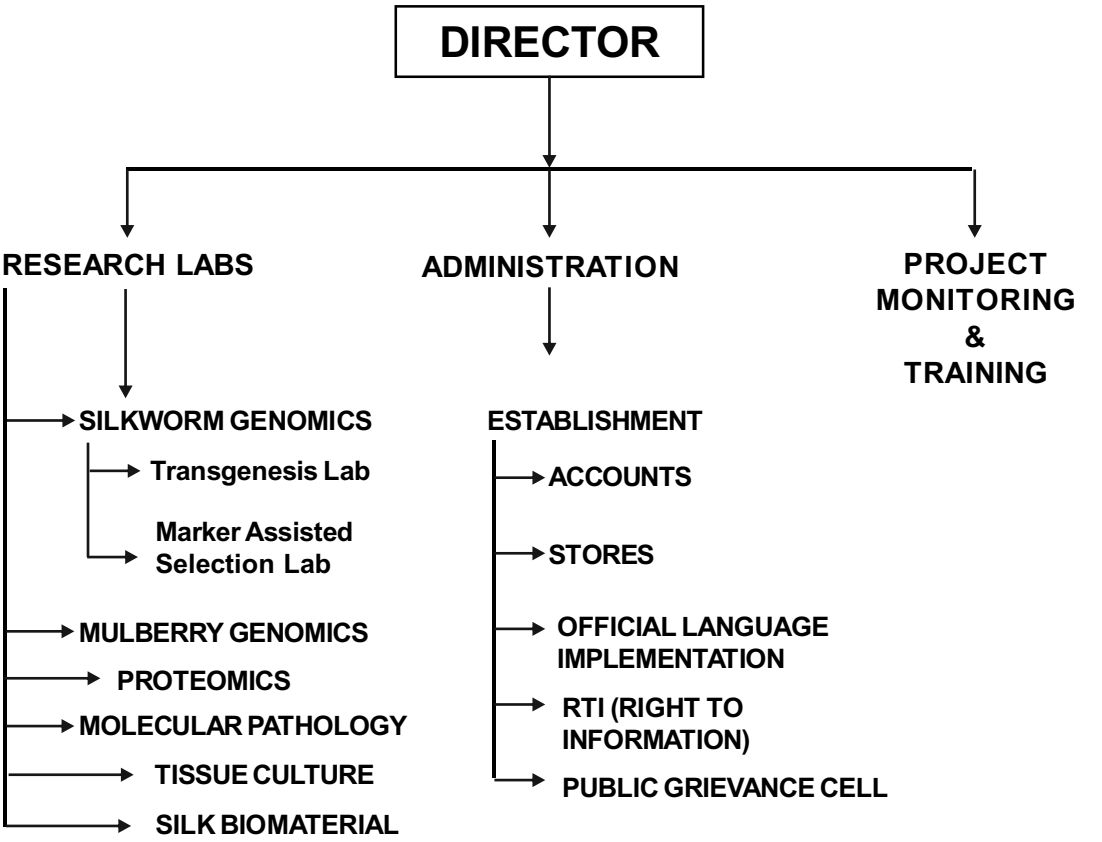
8. Infection by *Exorista bombycis* induced oxidation / reduction reactions in hemocytes and production of reactive oxygen species and antioxidative enzyme activity in *B. mori* larva.

9. Based on rRNA universal primers, two new strains of microsporidians of *Nosema* genus were identified.

10. DNA was extracted from four different microsporidian strains and were amplified with 16S rRNA and IGS primers by PCR. The IGS region exhibited more nucleotide variations when compared with standard type species of *Nosema bombycis*. These variable regions will be targeted for early detection of microsporidians.

11. Flacherie associated pathogens were identified from tasar silkworm, *Antheraea mylitta*, *viz.,* two types of viruses Cytoplasmic Polyhedrosis Virus (CPV), Infectious Flacherie Virus (IFV) and four types of bacterial species associated with flacherie disease like *Enterobacter*, *Proteus*, *Staphalycoccus* and *Enterococcus spp.* have been identified indicating that flacherie disease is caused by both virus and bacteria in tasar silkworms.

ORGANIZATION CHART



LIST OF RESEARCH PROJECTS

ONGOING RESEARCH PROJECTS

1. **AIT 3468 (DBT):** Development of RNA interference (RNAi) based nuclearpoly hedrosis virus resistance transgenic silk moth. (Jointly with CDFD, Hyderabad, APSSRDI, Hindupur) (Under Center of excellence on Genetics and Genomics of Silk moth to CDFD Hyderabad-Phase II) (Sept. 2011- Aug. 2016)

2. **ARP 3518**: Expression profiling of genes associated with resistance to *Beauvaria bassiana* in *Bombyx mori* silkworm strains (Oct. 2014 - Sept. 2017)

3. **AIT 3494 (DBT)**: Host-parasite interaction: Transcriptome responses to parasitism in the silkworm *Bombyx mori* (Jan. 2013 - Dec. 2016).

4. **ARP 3495:** Development of immuno-molecular techniques for early diagnosis of different microsporidians infecting silkworm, *Bombyx mori* L. (Jan.2013-Dec.2016).

5. **AIB 3503:** Identification of autumn-specific silkworm breeds/hybrids suitable for subtropical zones of North and North West India **(in collaboration with CSR&TI Pampore, NSSO Bangalore and CSTRI Bangalore)**(Nov.2013-Oct. 2016)

6. **AIT 3538:** Development of Fibroin Fusion Silk with Antioxidant and Anti-bacterial Properties. (Jun 2015- May 2019)

7. **AIT 3540:** Development of Transgenic Silkworms for the Over-expression of Disease-Resistant Genes for Enhanced Immunity. **(In collaboration with IISc Bangalore)** (Aug 2015 - July 2018)

8. **AIT 3544:** Validation of Vitellogenin Receptor (VgR) Gene Expression Levels as Molecular Indicator for Fecundity and Fertility in Silkworm Races. **(In Collaboration with CSGRC Hosur)** (July 2015 - June 2016)

9. **CFC 7064:** Sericin for cosmetic applications **(collaboration with CSTRI and M/s. Unilever Industries, Bangalore)** (May 2015- April 2017)

10. **ARP3522.** Isolation, cloning, and characterization of antibacterial proteins from silkworm, *Bombyx mori* **(Collaborative project of CSR&TI, Berhampore)** (May 2015 - April 2018)

DST-JSPS Indo-Japan Collaborative Project

1. **ARP-3513:** Molecular characterization of Indian isolate(s) of Densovirus (DNV) and viral resistance gene in the host silkworm *Bombyx mori* (June 2014 - May 2016)

PILOT STUDIES

1. **SBRL002** - Male accessory gland proteome analysis and characterization of oviposition stimulating substances (oss) from tasar silkworm, *Antheraea mylitta* (Oct. 2014 - Mar. 2016)
2. **SBRL 003** - Identification of uzifly maggot tissue protein that induces toxicity in silkworm *Bombyx mori* (Dec. 2014 - March 2016)
3. **SBRL 005** – Evaluation of Long duration line of *Bombyx mori* Nistari in different seasons at Berhampore (April 2015 – March 2016)

CONCLUDED RESEARCH PROJECTS

1. **ARP 3453:** Development of Immuno molecular techniques for early diagnosis of different microsporidians infecting silkworm, *Bombyx mori* L (Feb. 2013 - Jan. 2016)
2. **ARP 3489:** Isolation and molecular characterization of major pathogens associated with flacherie disease in *Anthereaea mylitta* D. **(in collaboration with CTR&TIRanchi)** (Oct. 2012- Sept. 2015).

PROGRESS OF ONGOING RESEARCH PROJECTS

AIT 3468 (DBT): Development of RNA interference (RNAi) based Nuclear Polyhedrosis Virus resistant transgenic silk moth (Jointly with CDFD, Hyderabad and APSSRDI, Hindupur under Center of Excellence for Genetics and Genomics of Silk moth to Center for DNA Fingerprinting and Diagnostics, Hyderabad]

Duration: Sep. 2011– Aug.2016

Awasthi A. K., A. R. Pradeep and Kanika Trivedy [SBRL, Bangalore]
Ms. Varada Burdekar (JRF, SBRL, Bangalore)

The project is implemented under the aegis of the Centre for Excellence for "Genetics and Genomics of Silk moth" funded to CDFD, Hyderabad by DBT and second phase of the project was initiated during 2011. The project is based on the RNA silencing of NPV genes that will be activated to initiate multiplication of NPV. Four NPV genes viz *ie1, lef1, lef3* and *p74* will be silenced by double stranded RNA through RNA interference technology. In an earlier program of CDFD, transgenic Nistari race of the silkworm *Bombyx mori* was synthesized through germline transformation by implanting a vector carrying short sequences of four essential BmNPV genes, *ie1, lef1, lef3* and *p74* in tandem, either in sense or antisense or in inverted-repeat arrangement (Subbaiah et al 2013, Genetics 193: 63-75) along with reporter gene, red fluorescent protein (RFP). On NPV infection, the transgenic Nistari has the capability to silence the NPV genes by RNA interference, thereby preventing the NPV multiplication and increase the chances of survival of the larvae. It is found that the transgenes are integrated into the silkworm genome and the transgenes are mapped in three chromosomes 13, 18 and 20. Expression of RFP will be visible in the ommatidia of the moths which is used as the reporter.

In the present Phase II of the project, inheritance property of the transgene is exploited to transfer the transgene in to high yielding but NPV susceptible races, through conventional breeding programs by crossing with transgenic Nistari. High yielding races CSR4 and CSR27 are used in the study. Crosses were made between transgenic Nistari female x CSR4 male and transgenic Nistari female x CSR27 male. The F_1 is crossed with CSR4 or CSR27 to develop back cross (BC_1). Further crosses were made and developed upto BC_4 generations. The BC generations were developed to improve the yield traits to CSR race level. The BC_4 was used for sibmating and BC_4F_1 generation was created. Followed by 14 generations (BC_4F_{15}) by sibmating

were developed. All generations were tested for red fluorescence in the eyes of each individual and confirmed the expression of (RFP). Different intermittent back cross filial generations were exposed to NPV infection and tested the survival rate. During last year BC_4F_{11} to BC_4F_{15} were reared and the data showed consistent yield traits among the transgenic lines. In F_{14}, the traits showed lower level, probably due to high environmental temperature (37 ± 2°C) during February to April 2015. Mean traits of F_{15} showed improvement. The shell weight and shell ratio showed significantly low value in F_{14} generation whereas larval and pupa weight is comparatively higher **(Fig. 1)** indicating the larger influence of high temperature on the adaptive traits in the transgenic lines. However the cocoons showed uniformity in all transgenic lines (**Fig.2**)

On experimental NPV infection of day 0 third instar larvae with 2 x 10^5 polyhedra/ larva, the transgenic lines showed 50 - 80% survival when compared with non-transgenic lines (10-20%) (**Fig.3)** revealing the effectiveness of inherited transgenes on curtailing NPV multiplication and increasing survival of the worms.

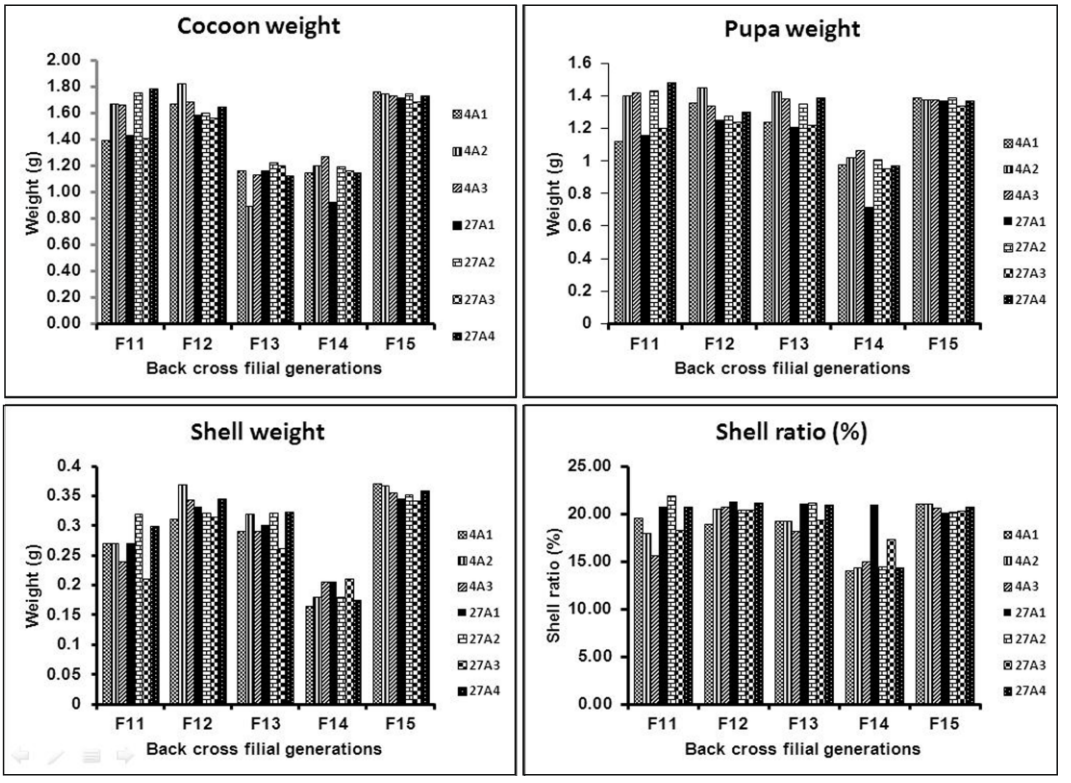


Figure 1: Mean yield traits of transgenic lines developed from crosses of transgenic Nistari with CSR4 and CSR27.

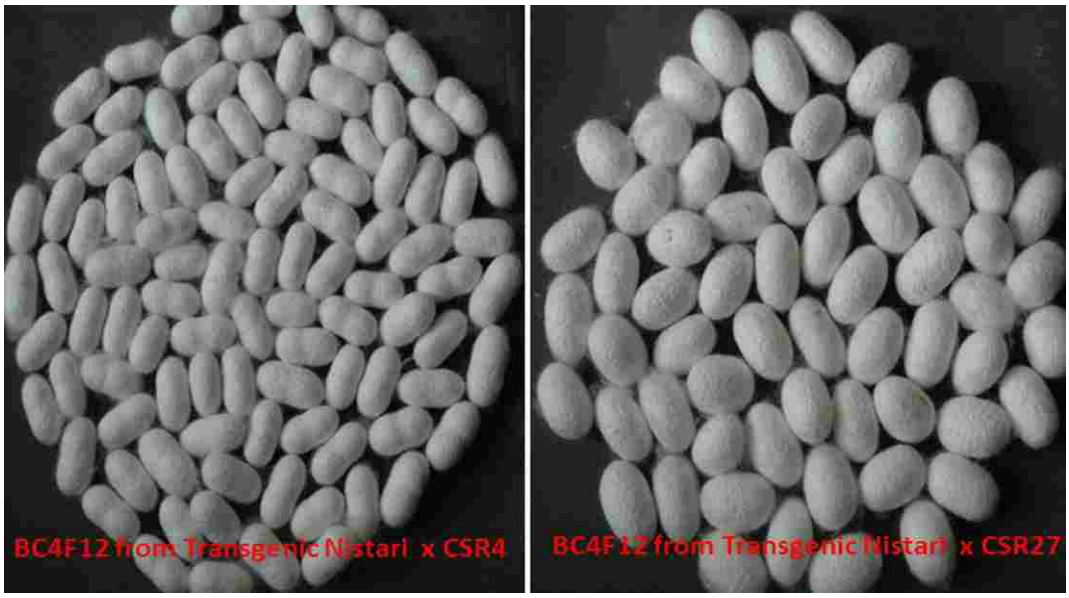


Figure 2: Cocoons of BC_4F_{12} generation of transgenic lines developed from crosses of transgenic Nistari with CSR4 and CSR27

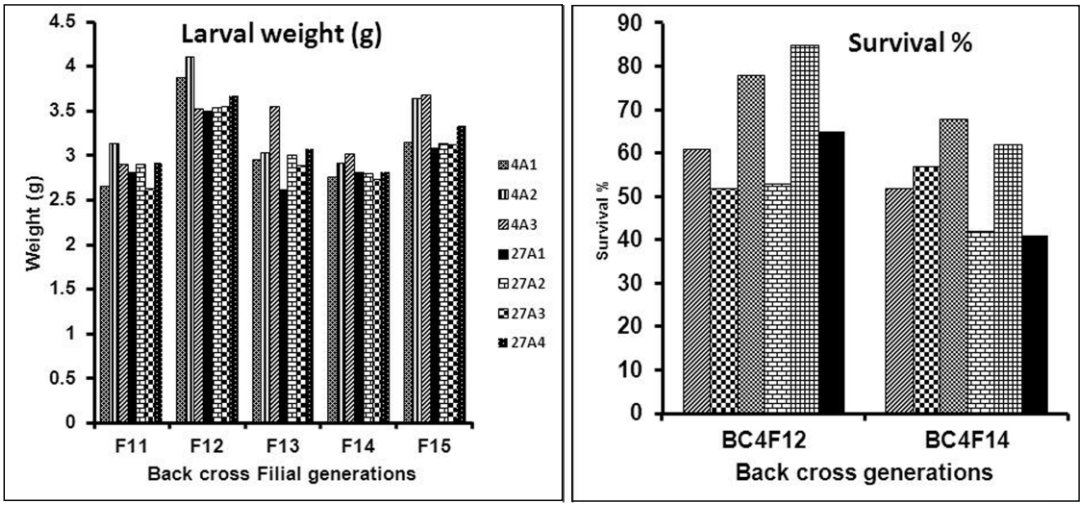


Figure 3: Mean larval weight and survival (%) of transgenic silkworms developed from crosses of transgenic Nistari with CSR4 and CSR27 after NPV infection.

ARP 3518: Expression profiling of genes associated with resistance to *Beauvaria bassiana* in *Bombyx mori* silkworm strains (In Collaboration with CSGRC Hosur)

Duration: Oct. 2014 - Sep. 2017

Geetha N. Murthy and K. M. Ponnuvel [SBRL, Bangalore]
P. Somasundaram and N. Balachandran [CSGRC, Hosur]

Beauvaria bassiana causes white muscardine in *Bombyx mori* resulting in substantial cocoon crop loss especially during humid rainy and winter seasons. Reports reveal up and down regulations of several antifungal genes on *B.bassiana* infection in *B.mori*. Some of these genes have also been validated through Real time PCR. Hence, a project was taken up in collaboration with CSGRC Hosur to investigate the differential expressions of the anti-fungal genes and its correlation with fungal proliferation among different *B.mori* breeds. The resultant outcome could indicate breed specific response of the antifungal genes and their probable feasibility as markers for susceptibility / resistance to muscardine diseasein *B.mori* breeds.

During the period under report, maintenance of *B.bassiana* was continued on Potato Dextrose Agar [PDA] mediumthrough regular sub-culturing. Further, larvae of multivoltine and bivoltine *B.mori* breeds obtained from CSGRC Hosur were inoculated with *B.bassiana* fungal spores. Integument samples from around 10 larvae were collected by dissection at each time point i.e. 0h, 24h control, 24h post inoculation from each breed. These samples were then processed for extraction of DNA and RNA. The RNA samples were utilized for synthesis of cDNA. The cDNA thus obtained were confirmed through semi-quantitative PCR with beta actin primers and stored at -20°C.

A comparative expression analysis of 12 antifungal genes *viz.* Lysozyme, LIM protein, Transferrin, Arylphorin, Glucose transporter, Peptidoglycan precursor, Ecdysone induced protein, Chemosensory protein, Bm8 interacting protein, Troponin C, Amidase and Vacuolar ATP synthase was carried out in *B.bassiana* infected and control samples of four silkworm breeds viz. MH1, APM2, APM3 and APDR15. Results revealed higher expression of maximum genes in APM2 and least in APM3. The multiplication of *B.bassiana* was highest in APM3 and lowest in APM2 suggesting a lower fungal multiplication with higher gene expression **(Fig. 4)**

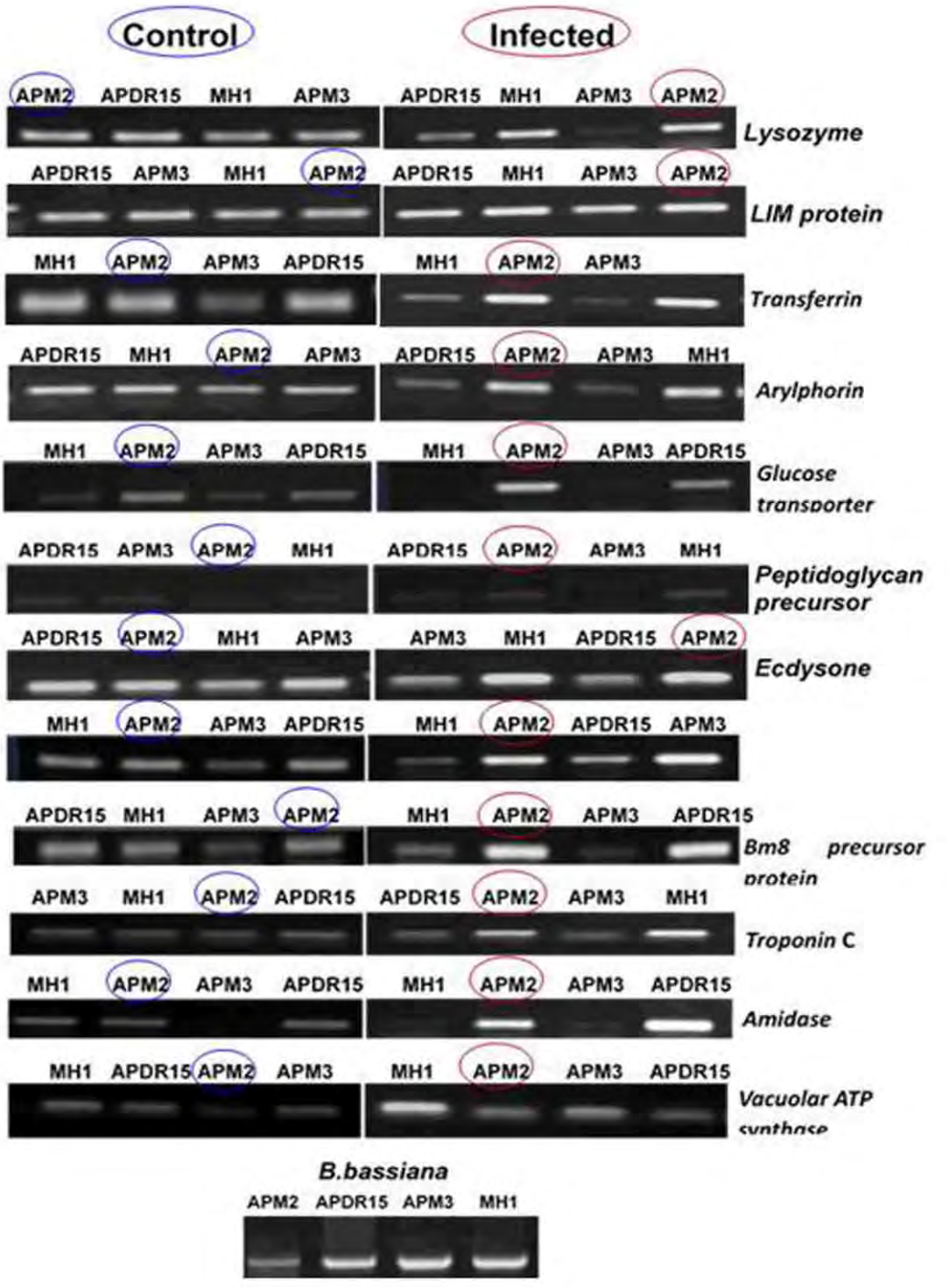


Figure 4: Comparative expression analysis of antifungal and *B.bassiana* specific genes in *B.bassiana* infected and control *B.mori* integument samples.

AIT 3538: Development of Fibroin Fusion Silk with Antioxidant and Antibacterial Properties.

Duration : July 2015 – June 2019

G. Ravikumar, [SBRL, Bangalore]
K. Vijayan [CSB, Bangalore]
Mrs Chitra, M[JRF, SBRL]
Mrs. Dyna Susan Thomas [Research Fellow, SBRL]

This project is aimed to develop novel fibroin fusion protein for new generation dressing materials and tissue engineering applications. Fragments of silk fibroin gene with repeats and an antibacterial protein, cecropin B were PCR amplified. Constructs were made with these proteins and polyhistidine tags and cloned in to *Pichia pastoris* vectors. Constructs were confirmed by sequencing and are being used for the recombinant expression in *P. pastoris*. *P. pastoris* strains were propagated and cells were made for the transformation of fusion construct and its expression.

AIT 3540: Development of Transgenic Silkworms for the Over-expression of Disease-Resistant Genes for Enhanced Immunity (In collaboration with IISc., Bangalore)

Duration : June 2015 – July 2018
G. Ravikumar [SBRL, Bangalore] and
Upendra Nongthomba [IISc, Bangalore]
Sandhya Rasalkar [JRF, IISc]
Mrs. Chitra M. [JRF, SBRL]
Mrs. Dyna Susan Thomas [Research Fellow, SBRL]

The aim of the project is to develop silkworm lines with enhanced disease resistance to multiple pathogens to increase silk productivity. Genes involved in immunity, viz., Relish, Cecropin B, Drosomycin and their promoters were amplified by PCR and ligated in to TA vectors. The inserts were checked for errors by sequencing. Error free constructs were transferred in to the transgenic vector, piggyBac for their microinjection into silkworm eggs. The optimization of microinjection process is under progress.

AIT 3544: Validation of Vitellogenin Receptor (VgR) Gene Expression Levels as Molecular Indicator for Fecundity and Fertility in Silkworm Races

(In Collaboration with CSGRC Hosur).

Duration : July 2013 – June 2016

G. Ravikumar [SBRL, Bangalore]

Ashok Kumar [CSGRC, Hosur]

Mrs. Chitra M. [JRF]

Mrs. Dyna Susan Thomas [Research Fellow, SBRL]

The project is aimed to develop a molecular tool to identify high yielding breeds of silkworm using VgR gene in terms of fecundity and fertility.

Pupal ovaries were dissected from 20 silkworm strains (bi- and multivoltine strains) collected from CSGRC, Hosur. Primers were designed for the semi quantitative and quantitative Taqman real time PCR analysis. The project is under progress.

AIT 3494: Host - parasite interaction: Transcriptome responses to parasitism in the silkworm *Bombyx mori.*

Duration : 2013 January – 2016 July

Pradeep A.R., A.K. Awasthi and K.M. Ponnuvel [SBRL, Bangalore]

Ms. Pooja M. and Ms. Shambhavi P.H. [JRFs, SBRL, Bangalore]

Sericulture is one of the major sources of income for thousands of farmers in India and in different states, mulberry and non-mulberry silkworms are reared to produce commercially valuable silk. All silkworm species are exposed to pathogens such as bacteria, virus, microsporidia and dipteran parasites, uzi fly etc. However different parasites attack silkworms causing diseases and incurring a loss of 20-30%. Pebrine is the most dreaded disease of the mulberry silkworm, *B. mori* caused by the microsporidian, *N. bombycis*. It is known that various genes have been activated in the Chinese strain of *B. mori* larva after *Nosema* infection but there are no reports on the study of immune reactions at molecular level in tropical strains of *B. mori* available in India.

Though early observations have been made at organismal level after infection by different parasites, only recently molecular level variations have been revealed in the *B.mori*–parasite interaction. Our studies showed immuno-competence of *B. mori* integument against uzi fly, *Exorista bombycis* attack. Cell mediated immunity is also activated in hemocytes which is supported by activation of humoral immune system.

On the other hand, the parasites surmount the host immune system and overcome the defense mechanisms.

Aim of this project is to identify host-response proteins and transcriptome responses in the hemocytes of the *B. mori* against invasion of the parasites, *Exorista bombycis* and *Nosema bombycis.*

Identification of host-response proteins activated in hemocytes of *Bombyx mori* after uzifly and microsporidian infection

Host – response proteins and immune – associated proteins were identified by mass spectrometry from hemocytes collected from the mulberry silkworm *Bombyx mori* larvae infected by uzi fly, *Exorista bombycis* and the microsporidian, *Nosema bombycis*.Among the proteins identified, five uncharacterized proteins were identified from hemocytes after infection by *N. bombycis* and no detailed information is available except that two of them are putatively known to bind with proteins. However, preliminary analysis showed that the two uncharacterized proteins showed putative functions of protein binding (H9JAZ8) and lipid transporter activity (H9JJ98). Hence these two proteins were considered for further analysis. Twenty two peptides were identified from H9JAZ8 and fourteen peptides from H9JJ98 suggesting its presence in larger level in the hemocytes after the *N. bombycis* infection.

These two uncharacterized proteins with Uniprot accession H9JJ98 and H9JZ8 has a molecular weight of 127.7kDa and 125.7kDa respectively, and were partially characterized by *in silico* analysis and expression studies using RT-PCR. Both the accessions H9JJ98 and H9JAZ8 showed secondary structure composition consisting of largest proportion of the loop followed by strand and helices. Kyte - Dolittle hydropathy plots prepared from the amino acid sequence of the accessions H9JJ98 and H9JAZ8 showed large number of hydrophilic amino acids with several negative peaks which indicates the presence of globular proteins.

DAS prediction server identified three potential transmembrane segments from H9JJ98 and two from H9JAZ8 whereas PredProt revealed two transmembrane regions from H9JJ98 only **(Fig.5)**. The transmembrane helices in the uncharacterized protein H9JJ98 showed orientation from ‘outside to inside’ with a larger score of 751 and 1286.Similarly ‘inside to outside’ orientation of helix that corresponds to ‘outside to inside’ orientation is positioned at 722 amino acid in H9JJ98. No signal proteins were identified from amino acid sequences of both the proteins.

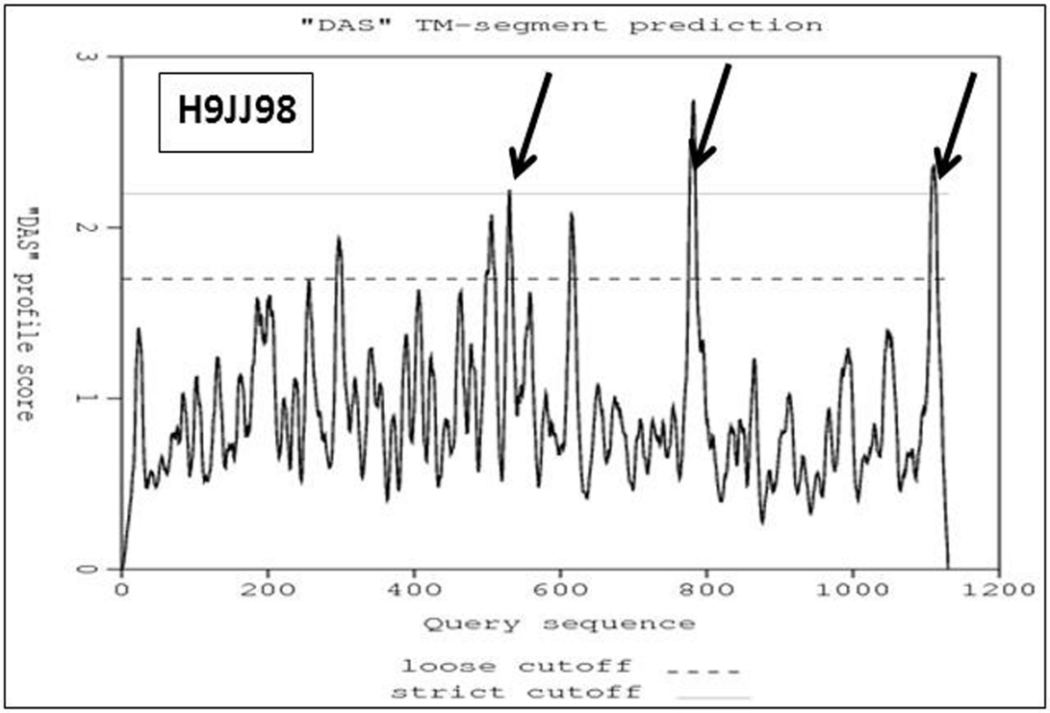


Figure 5: Transmembrane segments predicted from uncharacterized protein.

Three potential post translational modification sites, N Glycosylationsites were predicted from H9JAZ8 whereas eight potential N Glycosylation sites were predicted from H9JJ98. In the accession number H9JJ98, seven protein binding sites with short protein length were identified and in the accession number H9JAZ8, five protein binding sites were identified by Predict Protein program.

On similarity search using BLASTP, H9JAZ7 showed close similarity with hemocytin and its isoforms of lepidopteran insects. The accession H9JJ98 showed similarity with uncharacterized protein from different insects. During the search, NCBI-conserved domain (CDD) search identified three conserved domains viz von Willebrand factor (VWD), cysteine 8 (C8) and trypsin inhibitor like (TIL) from H9JAZ8 whereas epidermal growth factor like (EGF) was also identified by PROSITE. From the accession, H9JJ98, three conserved domains, Vitellinogen (superhelical), lipid transport protein (N- terminal) and VWD factors were identified.

Expression profiles of both genes was analysed by semi quantitative RT-PCR. The BGIBMGA009599 gene encoding H9JJ98 showed enhanced expression at 10th day after microsporidian infection than on day 8 after the infection. However expression of BGIBMGA006693 encoding H9JAZ8 showed reverse pattern in which enhanced expression was on 8th day after infection, probably due to difference in ensuing physiological functions of the encoding protein.

Transcriptome responses of hemocytes of *B. mori* after the parasitic infection.

Transmission electron microscopy (TEM) observations showed appearance of phagocytic characters on granulocytes such as presence of pseudopodia on cell membrane and secondary lysosomes after microsporidian infection. This indicates activation of phagocytosis in the granulocytes. In order to confirm the activation of phagocytosis, four specific genes associated with phagocytosis were selected from *Drosophila* as the same is not known in *B. mori*. The genes *Eater* and *Thioester containing protein* (TEP) were amplified using the template cDNA of hemocytes of CSR2 strain. The amplicons showed enhanced expression in the early stages of microsporidian infection indicating the activation of genes associated with phagocytosis in hemocytes after the microsporidian infection.

Expression profile of a set of candidate genes associated with host- response, immune reactions, recognition and antimicrobial protein was examined in hemocytes of larvae infected with the microsporidia. Genes encoding SERPIN and its family proteins showed delayed but enhanced expression **(Fig. 6)**. On testing expression of four Toll genes (BmToll, Toll 9, 11, 18), only BmToll 11 amplified with the Toll specific primers. Humoral lectin, hemocytin and BGRP2 showed increased expression level whereas BGRP4 was down regulated. CTL-11, chitinase and Beta glucosaminidase showed decreased expression in hemocytes after the infection showing differential expression in hemocytes induced by the infection.

In order to test the activation of detoxification after the microsporidian infection, expression of glutathione S transferase (GST), super oxide dismutase (SOD) and catalase was tested. SOD and GST did not show any variation in expression profile whereas catalase expression was decreased. The flavin-dependent monoxygenase (FMO) showed differential expression of alleles in hemocytes of *B. mori* larva after infection by *N. bombycis*. However, assays of SOD and catalase enzymes as well H_2O_2 and nitrous oxide did not show any

significant variation from control indicating low physiological role for detoxification after microsporidian infection in hemocytes.

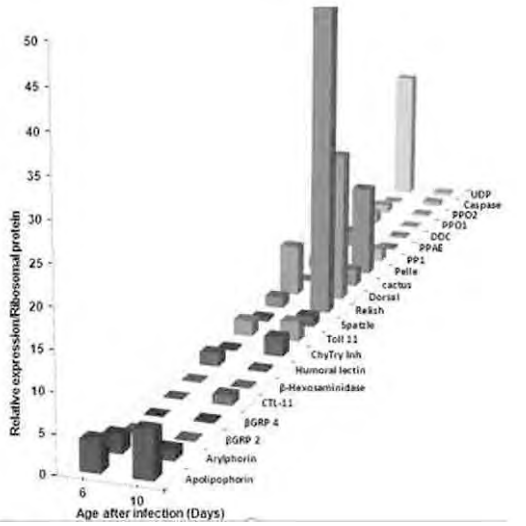


Figure 6: Quantitative expression of candidate genes amplified in hemocytes of *B.mori* larva after microsporidian infection.

COLLABORATIVE PROJECTS OF OTHER INSTITUTES

CFC7064: Sericin for cosmetic applications (Collaboration with CSTRI, Bangalore and M/s Unilever Industries, Bangalore)

Duration: May 2015 – April 2017

G. Ravikumar [SBRL, Bangalore]
Mrs. Chitra M. JRF [SBRL]
Mrs. Dyna Susan Thomas, Research Fellow [SBRL]
M.A.Joseph, [CSTRI, Bangalore]
Jyoti Tiwary, [M/s Unilever Industries, Bangalore]

Aim: To characterize sericin proteins from cocoons and silk gland.

Progress

Sericin was extracted from silk glands of CSR2 at different days (Vth instar day 1 to 5) and subjected to SDS-PAGE. Sericin fragments of varying molecular weights were observed. Sericin samples (powder made from cocoons) from CSTRI, CSB, Bangalore were subjected to SDS-PAGE. They were found as smears of different

molecular weights whereas discrete banding patterns were observed from protein extracted from the silk glands.

ARP 3522: Isolation, cloning and characterization of antibacterial proteins from silkworm, *Bombyx mori* (In Collaboration with CSRTI, Berhampore)

Duration: May 2015 – April 2018

Kanika Trivedy and Satadal Chakrabarty [CSRTI, Berhampore]
G. Ravikumar [SBRL, Bangalore]
Mr. Gourab Roy [JRF, CSRTI, Berhampore]

Aim: To isolate and clone antibacterial genes from silkworm *Bombyx Mori*

Progress

Silkworm *Bombyx mori* Vth instar larvae were infected with *Staphylococcus vitulinus* with controls. Proteins were extracted from the haemolymph and fat body at 12, 24, 48 hr after inoculation. Proteins were subjected to gel filtration followed by SDS-PAGE. Proteins of different molecular masses were isolated and subjected to Mass Spec analysis. The analysis of the peptide sequences is under progress.

DST-JSPS INDO-JAPAN COLLABORATIVE PROJECT

ARP-3513: Molecular characterization of Indian isolate(s) of Densovirus (DNV) and viral resistance gene in the host, silkworm *Bombyx mori*

Duration: June 2014 – May 2016

Ponnuvel K. M.[1], Tania Gupta[1], V. Sivaprasad[1], Keiko Kadono-Okuda[2]
Yumiko Nakajima[3], Katsuhiko Ito[4]

[1]Seribiotech Research Laboratory, Bangalore 560035, [2]Insect Genome Research Unit, National Institute of Agrobiological Sciences, Tsukuba, Japan, [3]Division of Functional Genomics, University of the Ryukuys, Okinawa, Japan, [4]Department of Biological Production, Tokyo University of Agriculture and Technology, Japan.

Bombyx mori bidensovirus (BmBDV) previously known as *B. mori* densovirus type 2 (BmDNV-2) was earlier grouped under the subfamily *Densovirinae* of the family *Parvoviridae*. BmBDVs are one of the causative agents of the silkworm disease, 'flacherie' which causes immense economic loss to the sericulture industry. Structurally, BmBDVs have a resemblance with *Bombyx mori* densovirus type 1 (BmDNV-1) and other parvoviruses wherein BmBDVs exhibit a similar non-enveloped,

spherical, icosahedral structure of approximately 22 nm diameter. However, unlike the monopartite genomes of BmDNV-1 and other parvoviruses, the BmBDVs have a bipartite genome. Two non-homologous, single-stranded, linear DNA molecules (VD1 and VD2) encapsulated in separate virions, constitute the bipartite genome of BmBDVs. The VD1 and VD2 DNA segments of BmBDVs have inverted terminal repeats (ITRs) and share a common terminal sequence (CTS). However, unlike BmDV-1 and other parvoviruses, BmBDVs do not possess any common terminal palindromic sequences that can form terminal hairpins. Adding up to its uniqueness, BmBDV is the only virus that possesses a single-stranded DNA that encodes for the DNA polymerase enzyme. Considering all these specificities of the virus, the International Committee on the Taxonomy of Viruses (ICTV), in 2011, established a new family of viruses, *Bidnaviridae* and assigned BmBDV as the type species in the new genus, *bidensovirus*.

To date, three isolates of BmBDV have been reported causing the commonly occurring fatal silkworm disease, 'flacherie'. They are (1) *Bm*DV-1(Ina isolate), (2) *Bm*DV-2 (Saku isolate and Yamanashi isolate) and finally (3) *Bm*DV-Z (Zhenjiang isolate). While most of the strains of *B. mori* are susceptible to BmBDV, few races have been found to be completely resistant to the virus. Studies have shown both dominant and recessive alleles to be responsible for the resistance. So far four genes have been reported conferring resistance against *BmBDV*-1 and *BmB*DV-2. These are the *Nid-1* and *nsd-1* genes against *BmBDV*-1 and *nsd-2* and *nsd-Z* genes against *BmBDV*-2.

With this backdrop, BmBDV flacherie diseased silkworm samples were collected from different sericultural areas of Kolar district. The Indian isolate of BmBDV has been found to have a closer homology with the Japanese Yamanashi isolate (BmBDV). Based on these reports several primer sets were designed as per the sequence of the Japanese isolate of BmBDV. The BmBDV DNA polymerase gene located on VD1 strand was made the first target for cloning. Primers were designed to amplify the DNA polymerase gene (3348bp) of BmBDV. This was followed by the cloning and sequencing of the subsequent regions. The amplified products were successfully cloned through pGEM-T vector and were sequenced. The cloned plasmid DNA samples were sequenced using M13 primers at Eurofins Genomics India Pvt.Ltd., Bangalore. The multiple sequence alignment and phylogenetic relationship of the Indian isolate of BmBDV with Chinese and Japanese BmBDV isolates were

analysed using ClustalW and MEGA 5.1.

The complete nucleotide sequence of the Indian isolate of BmBDV has been determined and submitted to NCBI. The VD1 and VD2 fragments of DNA of Indian isolate of BmBDV were found to be of 6542 bp and 6023 bp length respectively. The temporary gene accession numbers assigned to VD1 and VD2 segments are KX760110 and KX779526, respectively. Since the DNA polymerase gene sequence of Indian isolate of BmBDV was cloned first, its sequence was submitted to NCBI database earlier and the accession number assigned to the gene is KP886818.1. The sequence analysis of VD1 and VD2 DNA fragments of Indian isolate of BmBDV revealed that it contained six ORFs. Four ORFs were located on the VD1 DNA fragment while two were on the VD2 fragment. The ORF of Indian isolate of BmBDV is represented in **(Fig 7)**. The VD1 DNA fragment of Indian isolate of BmBDV was found to have 5' 1-224 and 3' 6318-6542 repeat regions. The ORF-1 (5'-310-690-3'), ORF-2 (5'-473-1423-3') and ORF-3 (5'-1416-2915-3') were all found to code for a non-structural protein. However, ORF-4 coding for DNA polymerase gene was found to be on the complimentary strand (3'-6286-2939-5'). On the other hand, the VD2 DNA fragment of BmBDV was found to have two ORFs. ORF- 1 (5'-684-4163-3') was found to code for a minor structural protein while ORF-2 (3'-5437-4769-5') was for a non-structural protein obtained from the complement strand.

Dr. Katsuhiko Ito, a member of the Japanese counterpart of the project visited the laboratory during the study tenure. During his stay, a survey was conducted wherein diseased silkworm samples were collected from various sericultural areas. The samples were checked for Densovirus infection. Genomic DNA as well as RNA extraction was carried out from the Densovirus positive samples. cDNA was further processed from the RNA samples for checking Densovirus infectivity.

Real-time primers have also been designed to quantify the Densovirus infection. The RNA was extracted and first strand cDNA was synthesised for RT-PCR analysis. Results revealed high copy numbers of BmBDV in the midguts of the flacherie diseased silkworms at the end of the reaction. This confirmed presence of viable BmBDV in quite high numbers in the field and its widespread association with flacherie disease in *B.mori*. Simultaneously, studies were also carried out to screen the Indian *B. mori* germplasm races for *nsd-2*gene. The aa-trans1 forward primer (5'-TCTACGTGCTTTCATACTACGTATC) designed to have a binding site within exon 4 and the reverse primer (5'-TTCCTCACGTTTCTGAATTTCTCTTG) within exon 14

was used to screen the Densovirus resistant races. On the other hand, the aa-trans3 forward primer (5'- GGTAAGAGGTCCAACGCTGTTAAGTT-3') designed to have a binding site at exon 13, 3' flanking region and reverse primer (5'-TTCCTCACGTTTCT-GAATTTCTCTTG) within exon 14 was used for the detection of Densovirus susceptible races.

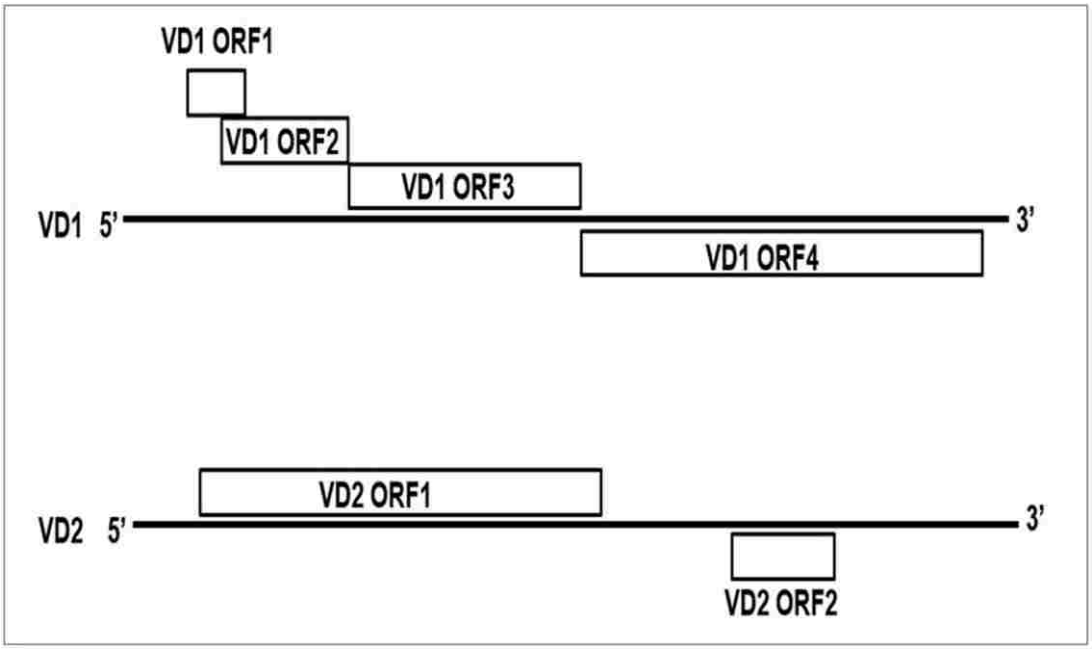


Figure 7: Details of ORFs position on the BmBDV genome

PILOT STUDIES

SBRL002: Male accessory gland proteome analysis and characterization of oviposition stimulating substances (oss) from tasar silkworm, *Antheraea mylitta.*

Duration: Oct. 2014 – March 2016

G. Ravikumar [SBRL, Bangalore]

Objective: To characterize male accessory gland proteins

Male accessory gland proteins were separated by SDS-PAGE and analysed by Mass spectrometer. Homology search indicates heat shock proteins and uncharacterized proteins.

SBRL003: Identification of uzifly maggot tissue protein that induces toxicity in silkworm *Bombyx mori*

Duration: Dec. 2014 - March 2016

A.R. Pradeep [SBRL, Bangalore]

Objective: Identification of uzifly protein that induces toxicity in hemocytes.

Uzifly maggot tissue protein was extracted and syringe filtered (0.22micron). The protein was resolved by HPLC using a reverse phase C18 column. The chromatogram showed 17 peaks between 10mts to 25mts and between 35mts to 55mts. The protein from the peaks were collected and used for *in vitro* bioassay using hemocytes. Fractions 2 and 7 induced cell rupture and degranulation of hemocytes and were analyzed by mass spectrometry (C-CAMP). Degranulation was assessed by hexosaminidase assay which showed increased hexosaminidase activity.

Mass spectrometry of Fragment 7 showed presence of 44 peptides which showed presence of hydrolase, histidine kinase, ceramide kinase, methyl transferase, tyrosine phosphatase, glutamate-1-semialdehyde aminotransferase, Polyprenyl synthetase, hemocyte aggregation inhibiting factor and pyridoxamine-phosphate oxidase.

Genes of two proteins, hemocyte aggregation inhibiting factor and pyridoxamine-phosphate oxidase showed amplification in integument of uzifly maggot.

SBRL005: Evaluation of Long duration line of *Bombyx mori* Nistari in different seasons at Berhampore.

Duration: April 2015 – March 2016

A.R. Pradeep [SBRL, Bangalore]

A.K. Verma (CSRTI, Berhampore)

Objective: To examine the performance of *Bombyx mori* Nistari to artificial selection in different seasons at Berhampore (West Bengal) conditions

Nistari larvae were reared under selection pressure for long larval duration four generations and the fifth generation was without selection. The cocoon showed 10-15% increase in weight and fecundity was in the range of 370-420 per DFL. The DFL was send to Berhampore for rearing. The Berhampore rearing was in summer (April – May 2016). In the first rearing, the yield parameters did not show significant difference from control. For each rearing, directional selection should be performed till stabilization of the trait.

REPORTS ON CONCLUDED RESEARCH PROJECTS

ARP3453: Development of Immuno molecular techniques for early diagnosis of different microsporidians infecting silkworm, *Bombyx mori* L

Duration: Feb 2013 – Jan 2016

B. Surendra Nath and Geetha N. Murthy [SBRL, Bangalore]

Objectives:

1. Survey and identification of major unidentified microsporidians through molecular techniques.
2. Development of different PCR based diagnostic approaches for detection of different microsporidians as well as virulent and non-virulent species/ strains.
3. Identification of specific proteins as well as induced host specific proteins using SDS-PAGE, 2D Gel electrophoresis and their characterization for developing antibodies and development of polyclonal antibodies for identification of microsporidians (for protein based approach)

CONCLUSION REPORT

Survey conducted in potentially important sericultural zones of Karnataka. Based on spore morphology, SEM and TEM, three new different microsporidians have been identified and their pathogenicity and tissue specificity studied.

Identification by PCR technique

Based on rRNA universal primers, the identified two microsporidians belong to the genus *Nosema*. Species specific as well as universal primers have been developed for detection of *Nosema* and *Vairimorpha* by PCR and Real Time PCR.

Two microsporidians from Karnataka were multiplied and purified by using Percoll gradient centrifugation. The purified mature spore yield is found to be $1x10^1$. DNA was extracted from four different microsporidian and were amplified with 16S rRNA and IGS primers by PCR. The IGS region exhibited more nucleotide variations when compared with standard type species of *Nosema bombycis*. These variable regions will be targeted for detecting microsporidians

Spore protein identification by SDS-PAGE analysis showed specific microsporidian spore wall proteins. Unique spore wall protein markers from different microsporidians have been identified. Two types of antibodies have been developed for simple detection of microsporidian species.

ARP3489: Isolation and molecular characterization of major pathogens associated with flacherie disease in *Antheraea mylitta* D. (in collaboration with CTR&TI Ranchi)

Duration: 2012 October – 2015 September

B. Surendra Nath and Kanika Trivedy (SBRL)

A.K. Sinha and K.P. Kiran Kumar (CTR&TI, Ranchi)

Objectives

1. To identify the pathogens associated with flacherie disease in *A. mylitta*

CONCLUSION REPORT

Collection:

Diseased tasar silkworms, *Antheraea mylitta* were collected by CTR&TI, Ranchi and were of flacherie disease samples from tasar culture areas. Pathogen infected guts, culturing of bacterial pathogens and their purification using standard agar culture and purification methods were done. Various bacterial strains causing rectal

protrusion, anal lip sealing and bacteriosis were cultured, sub-cultured and purified.

Flacherie associated pathogens were identified from tasar silkworm, *Antheraea mylitta*, *viz.,* two types of viruses Cytoplasmic Polyhedrosis Virus (CPV), Infectious Flacherie Virus (IFV) and four types of bacterial species associated with flacherie disease like *Enterobacter*, *Proteus*, *Staphalycoccus* and *Enterococcus spp.* have been identified. The results clearly indicate that the flacherie disease is caused by both virus and bacteria in tasar silkworms.

From the findings it is clearly evident that, different viruses and bacteria were associated with the Flacherie. A suitable prophylaxis measures to be developed to control the flacherie disease in order to save the tasar sericulture industry.

SILKWORM STOCK MAINTENANCE

1. Transgenic CSR4 and CSR27.
2. Silkworm breeds for NPV resistance developed through marker assisted selection.

PUBLICATIONS

1. Lekha G, Gupta T, Awasthi A.K, Murthy, G.N., Trivedy K. and Ponnuvel K.M. (2015) Genome wide microarray based expression profiles associated with BmNPV resistance and susceptibility in Indian silkworm races of *Bombyx mori*. ***Genomics***106: 393–403
2. Rati Sudha, Geetha N. Murthy, Arvind K. Awasthi, Kangayam M. Ponnuvel (2015). Attacin gene sequence variations in different ecoraces of tasar silkworm *Antheraea mylitta* ***Bioinformation*** 11(10): 481-483
3. Pradeep AR, Anitha J, Panda A, Pooja M, Awasthi AK, Geetha NM, Ponnuvel KM and Trivedy K (2015). Phylogeny of host response proteins activated in silkworm *Bombyx mori* in response to infestation by Dipteran endoparasitoid revealed functional divergence and temporal molecular adaptive evolution. ***Journal of Clinical & Cellular Immunology*** 6: 370. doi:10.4172/2155-9899.1000370
4. Chandrakanth, N., Ponnuvel, K. M., Moorthy, S., Sasibhushan, S., Sivaprasad, S. (2015) Analysis of transcripts of heat shock protein genes in silkworm, *Bombyx mori*, ***European Journal of Entomology*** 112: 1-14.

5. Lekha G.,Gupta T., Trivedy K., Ponnuvel K.M. (2015) Paralogous gene conversion, allelic divergence of attacin genes in silkworm *Bombyx mori*, ***Invertebrate Survival Journal,*** 12: 214-224.

6. Gupta, T.,Kadono-Okuda,K., Ito, K.,Trivedy K., Ponnuvel, K.M., (2015) Densovirus infection in silkworm *Bombyx mori* and genes associated with disease resistance. ***Invertebrate Survival Journal***, 12: 118-128.

7. Dyna Susan Thomas, Chitra Manoharan, KanikaTrivedy, Kunjupillai Vijayan, Ravikumar Gopalapillai 2016. Lipophorin and its Immunological Properties of Eri Silkmoth, *Samia ricini.* ***Sericologia*** (Accepted).

Book Chapters

1. Ponnuvel K. M., Sasibhushan S., Geetha N. Murthy and Rao C.G.P. (2015). Diapause-Related Gene Expression in Eggs of Multivoltine *Bombyx mori* L. Silkworm Races. Chapter in ***New Horizons in Insect Science: Towards Sustainable Pest Management,*** A. K. Chakravarthy (ed.), pp 187-198.

International Conference attended and paper presentation

Pradeep A.R., Pooja M, Anitha J, Shambhavi P.H., Awasthi A.K, Geetha NM, Ponnuvel K.M and Trivedy K (2015) Immune responses of silkworm, *Bombyx mori* against infection by an endoparasitoid. Oral presentation by Dr. A.R.Pradeep in the **IV International Conference on Analytical Proteomics at Caparica, Lisbon, Portugal,held on 7 -9 September, 2015 (Invited lecture).**

Foundation Training Programme by CSB for young scientists

Dr. (Mrs.) K.S. Tulsi Naik, Dr. A Ramesha and Dr. (Mrs.) Sailaja Bandam, Scientists B attended 15 days foundation training programme at CSB complex, BTM layout Bangalore, CSR&TI Mysore and CMER&TI Jorhat, Assam during Feb- march 2016. The training was intended to introduce the young scientists about CSB Structure, R&D programme and various sectors of CSB.

ACKNOWLEDGEMENTS

SBRL would like to acknowledge the following organizations/Institutes for their support and co-ordination in R&D activities of this laboratory

Sl. No	Name of the Institute
1.	Department of Biotechnology, Government of India
2.	Department of Science & Technology, Government of India
3.	Japanese Society for the promotion of Science, (JSPS) Tokyo, Japan
4.	IISc, Bangalore
5.	IIT, Guwahati
6.	CDFD, Hyderabad
7.	APSSRDI, Hindupur
8.	M/s Unilever Industries, Bangalore
9.	C-CAMP, Bangalore
10.	NIMHANS, Bangalore
11.	SSTL, Kodathi, Bangalore
12.	RSRS, Kodathi, Bangalore
13.	NSSO, Bangalore
14.	CSTRI, Bangalore
15.	CSR&TI, Mysore
16.	CSR&TI, Berhampore, West Bengal
17.	CSGRC, Hosur
18.	CTR&TI, Ranchi
19.	CMER&TI, Lahdoigarh
20.	CSR&TI, Pampore
21.	BTSSO, Bilaspur

RESEARCH ADVISORY COMMITTEE

Sl.No.	List of RAC Members	Remarks
1	Prof. H.S. Savithri, Dept. of Biochemistry, Indian Institute of Science Bangalore-560 012.	CHAIRPERSON
2	Prof. Usha Vijayaraghavan, Dept. of Microbiology & Molecular Biology, Indian Institute of Science, Bangalore-560 012.	Member
3	Dr. R. Ashokan, Principal, Scientist, Dept. of Biotechnology IIHR, Hesaraghatta Bangalore-560 089.	Member
4	Prof. P.H. Ramanjini Gowda, Professor, Dept. of Biotechnology, UAS, GKVK, Bangalore-560 065.	Member
5	Prof. H.E. Shashidhar, Professor, Dept. of Biotechnology, UAS, GKVK, Bangalore-560 065.	Member
6	Dr. Arunkumar K. P., Staff Scientist & Group Head, Lab of Molecular Genetics Center for DNA Fingerprinting & Diagnostics [CDFD] Bldg. 7, Gruhakalpa, 5-4-399/B, Nampally Hyderabad-500 001.	Member
7	The Director (Tech), Central Silk Board, Ministry of Textiles, Govt. of India, CSB Complex, BTM Layout, Madiwala, Bangalore-560 068.	Member
8	The Director, Central Sericultural Research & Training Institute, Central Silk Board, Ministry of Textiles, Govt. of India Srirampura, Manandavadi Road, Mysore-570 008.	Member
9	The Director, Central Sericultural Research & Training Institute, Central Silk Board, Ministry of Textiles, Govt. of India, Berhampore-742 101. West Bengal.	Member
10	The Director, Central Sericultural Research & Training Institute, Central Silk Board, Ministry of Textiles, Govt. of India, Gallandar Pampore-192 121. (J&K)	Member
11	The Director, Central Tasar Research & Training Institute, Central Silk Board, Ministry of Textiles, Govt. of India, PO- Piska-Nagri , Ranchi-835 303. Jharkhand.	Member
12	The Director, Central Muga Eri Research & Training Institute, Central Silk Board, Ministry of Textiles, Govt. of India, P.O. Lahdoigarh, Jorhat-785 700. Assam.	Member
13	The Director, Central Sericultural Germplasm Resources Centre P.B. 44, Thally Road, Krishnagiri Dist., Hosur-635 109. T.N.	Member
14	The Director, Seri-Biotech Research Laboratory, Kodathi, Carmelram Post, Sarjapur Road, Bangalore-560 035.	Member- Convener

INSTITUTE BIOSAFETY COMMITTEE

Sl.No.	Name	Address	Remarks
1	Prof. Usha Vijayaraghavan	Department of Microbiology & Cell Biology, Indian Institute of Science, Bangalore -12.	Member-Secretary
2	The Director	Seri Biotech Research Laboratory, Bangalore - 35.	Chairman
3	Dr. R. Ashokan	Principal Scientist, Dept. of Biotechnology IIHR, Hesaraghatta Bangalore-89.	Member
4	Dr. B.L. Mohan Kumar, MBBS	AMA-CSB Employees Dr Mohan Nursing Home Sarjapura Road, Bangalore-35.	Member
5	Prof. H.P. Puttaraju	Dept, of Life Sciences, Bangalore University	Member
6	Dr. V.V. Satyavati	Molecular Genetics LabCDFD, Hyderabad	Member External Expert
7	Dr. A.K. Awasthi	Scientist-D & PI Seribiotech Research Laboratory,	Member Internal Expert
8	Dr. A.R. Pradeep	Scientist-D & CI Seribiotech Research Laboratory, Bangalore-35.	Member Internal Expert

HUMAN RESOURCES

Sl No	Name	Designation
1	Dr. (Mrs.) Kanika Trivedy	Director (February 2015 - October 31st 2015)
2	Dr. A. K. Awasthi	Scientist-D & Director I/C (from 1st Nov. 2015)
3	Dr. M. Venkateswarulu	Scientist-D
4	Dr. (Mrs.) Geetha N. Murthy	Scientist-D
5	Dr. B. Surendranath	Scientist-D (till 28th February 2016)
6	Dr. K. M. Ponnuvel	Scientist-D
7	Dr. G. Ravikumar	Scientist-D
8	Dr. A. R. Pradeep	Scientist-D
9	Mr. S. Ramesh Kumar	Scientist-C
10	Dr Tulsi Naik K S	Scientist-B (Joined service on 23-10- 2015)
11	Dr Sailaja Bandam	Scientist-B (Joined service on 29-10- 2015)
12	Dr A Ramesha	Scientist-B (Joined service on 09-11- 2015)
14	Mr. S. N. Gundurao	Technical Assistant
15	Mr. Venkateshaiah	Technical Assistant
16	Mr. G. Sumant Kumar	Technical Assistant

RESEARCH FELLOWS

Sl.No.	Name	Designation	Division
1.	Mr. Wazid Hussain	Senior Research fellow	Molecular pathology
2.	Mrs. G. Bhuvaneswari	Research scholar	Molecular pathology
3	Ms. Pooja Makwana	Senior Research fellow (till Dec 2015)	Proteomics
4	Mrs Varada Burdekar	Senior Research Fellow	Genomics
5	Mrs. Lekha G	Junior Research Fellow	Genomics
6	Ms Tania gupta	Junior Research Fellow	Genomics
7	Mrs Vijaya Gowri	Junior Research Fellow	Genomics
8	Ms Shambhavi P Hungund	Junior Research Fellow	Proteomics
9	Mrs Dyna Susan Thomas	Junior Research Fellow	Genomics
10	Mrs Chitra Manoharan	Junior Research Fellow	Genomics
11	Mrs. Sandhya Rasalkar	Junior Research Fellow	Genomics

ADMINISTRATION

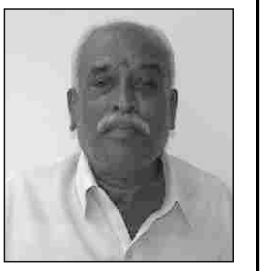
Sl.No.	Name	Designation
1	Mrs. M. Jayalakshmi	Superintendent
2	Mr. Mohan Raj	Cashier
3	Mr. Chandrashekhar Rao	Assistant
4	Mr. A. Mallesha	Assistant Technician
5	Mr. Nagaraja	Attender (Retired on 30th June 2015)
6	Mr. S. Nagesh	Driver
7	Mr. Kenchappa	Multi-tasking Staff

SUPPORTING STAFF

Sl.No.	Name	Designation
1	Mr. Shankar Shetty	SFW
2	Mr. Basavalingaiah	SFW
3	Mr. L.P Sampangi Rao	SFW
4	Mr. Hombalaiah	SFW
5	Mr. M. Krishnappa	SFW
6	Mr. N Pillappa	SFW
7	Mr. E Shivanna	SFW
6	Mrs. Lakshmamma	TSFW

SUPERANNUATION:

Mr. Nagaraja, Attender, retired from service of Central Silk Board (Government of India) on attaining superannuation at the age of 60 years in the financial year (2015-16). The fraternity of SBRL thanking him for his esteemed service and wish him a happy retired life.



FINANCIAL PROGRESS (2015-16)

During the year under report, an expenditure of Rs.257.163 lakhs was incurred from Grants-in-Aid sanctioned by CSB and Department of Biotechnology, Government of India

Sl.No.	PARTICULARS	Amount (Rupees in lakhs)
1	Salary and allowances	169.087
2	Wages & EPF	8.088
3	Travelling expenses	3.623
4	Contingent expenses	39.543
5	Assets	10.995
6	Others	15.946
7	Sub Total	247.284
8	DBT assistance	9.879
	Total	**257.163**

EVENTS

Anti-terrorism Day was celebrated on 21 may 2015 as per instruction of Central Silk Board on the occasion oath taking was also done by all the Scientists and staffs.

International yoga day was observed on 21 June 2015 as per instruction of Central Silk Board.

Blood Donation Day was observed on 1 october 2015 and awareness on blood donation was created and on this occasion few scientists, staff and research scholars voluntarily donated blood.

Hindi Fortnight

Hindi fortnight was observed from 15th - 30th September 2015 and conducted workshops for scientists. Scientists presented technical papers on research program in Hindi. Various competitions were organised for staff to reveal proficiency in Hindi.

Vigilance Awareness week

Vigilance Awareness week was organized from 26th October to 31th October 2015 as per instruction of Government of India and Central Silk Board. An debate competition on increasing corruption in different fields of life was also conducted, and prizes were distributed accordingly.

Communal Harmony week

Communal Harmony week was celebrated from 20th to 26th August 2015 as per instruction of Central Silk Board.

रेशमकीट जैव प्रौद्योगिकी अनुसंधान प्रयोगशाला

केन्द्रीय रेशम बोर्ड, वस्त्र मंत्रालय, भारत सरकार
कार्मेलराम पोस्ट, कोड़ति, बेंगलूर - 560 035.
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