A new research project proposal

ARP-3590: Studies on the efficacy of phototrophic bacterial extracts as feed supplement for management of diseases in silkworm, *Bombyx mori* L.

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Submitted to -
Central Silk Board
Ministry of Textiles: Government of India
Bangalore

By
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PROFORMA – I

PROFORMA FOR SUBMISSION OF PROJECT PROPOSALS ON RESEARCH AND DEVELOPMENT, PROGRAMME SUPPORT

(To be filled by the applicant)

PART I: GENERAL INFORMATION

1.	Name of the Institute/ University/ Organisation submitting the Project Proposal	**:**	Central Sericultural Research & Training Institute, Central Silk Board, Ministry of Textiles, Govt. of India, Berhampore, Murshidabad, W.B.-742101, India.
2.	Status of the Institute(s)	**:**	Govt.
3.	Name and designation of the Executive Authority of the Institute/University forwarding the application	**:**	Dr. Kanika Trivedy, Director
4.	Project Title	**:**	Studies on the efficacy of phototrophic bacterial extracts as feed supplement for management of diseases in silkworm, *Bombyx mori* L.
5.	Category of the Project	**:**	Research and Development
6.	Specific area	**:**	Silkworm Pathology & Physiology
7.	Duration	**:**	03 years
8.	Total cost	**:**	Rs. 18.00 Lakh
9.	Is the project Single Institutional or Multiple-Institutional (S/M):	**:**	Single - Institutional
10.	If the project is multi-institutional, please furnish the following: Name of Project Co-ordinator Affiliation & Address	**:**	Not applicable
11(a)	Project Summary	**:**	The success of Sericulture is reliant on two prime factors: **a) Larval nutrition:** Nutrition plays a significant role in improving the growth and development of silkworm *Bombyx mori*. Although mulberry leaves is complete diet for silkworm but sometimes it is possible that some deficiencies occur due to different reasons. **b) Larval resistance to diseases:** Like many other insects, *B. mori* is also susceptible to a large number of micro-organisms. Several disease prevention options are available but are found to be insufficient to control the diseases. So, promising alternatives such as fortified diet which improves the immunity and disease resistance are being extensively probed. Enriching mulberry leaves by nutrient supplementation is one of the ways to improve overall development and disease resistance in *B. mori*. In recent years, many attempts have been made to improve the

quality and quantity of silk through enhancing the leaves with nutrients, spraying with antibiotics, vitamins, hormones and hormone analogues. Owing to the high cost of synthetic food, introduction of these techniques in India is very limited. Hence, an economically cheaper technique which could still improve cocoon character and production, disease resistance is needed (Pallavi *et al.*, 2011). Hence the present project aims at investigating the efficacy of phototrophic purple bacterial extracts as feed supplements for a cost effective and eco- friendly disease management in silkworm with improved cocoon character and production.

Phototrophic bacteria are a group of microorganisms containing abundant nutrient materials and functional factors. Compared to microalgae and yeasts, phototrophic bacteria have many advantages such as a more digestible bacterial cell wall, rich in protein, carotenoids, biological cofactors and vitamins. They can also serve as probiotics. The carotenoids of these bacteria have antimicrobial and antioxidant activity. All these factors make them the best source to be probed as feed supplement for overall development and disease resistance in silkworm. Comprehensive research studies have been carried out on the potential of photosynthetic bacterial extracts (purple non sulfur bacteria) as feed supplements in aquaculture and it is being successfully practiced in various regions of the world (Kobayashi and Kurata, 1978; Sasikala and Ramana, 1995; Qi *et al.*, 2009). The role of photosynthetic bacterial extracts as feed supplement in silkworm is yet to be probed and hence the present project is proposed.

Anoxygenic phototrophic bacteria (APB) are physiologically and phylogenetically diverse group of photosynthetic bacteria which can perform photosynthesis without producing oxygen and in the absence of air. They are Gram negative; contain several types of bacterio-chlorophylls and a variety of carotenoids as pigments, which function in the transformation of light into chemical energy. The various photosynthetic pigments give the cell cultures a distinct coloration of green, brown, red, pink and purple depending on the pigment content. The bacterio-chlorophylls and carotenoids of the spirilloxanthin, rhodopinal, spheroidene and okenone series are present in APB.

Profiling metabolites, particularly the factors that are expressed or suppressed when fed on the phototrophic bacterial extracts or a combination of extracts will also be studied by Gas chromatography mass spectroscopy (GC-MS) analysis. This will provide information related to the bio-chemical and physiological changes in the silkworm when fed on phototrophic bacterial extracts as feed supplement. Metabolic profiling provides

			information regarding lead bioactive compounds (produced as metabolites on feeding the fortified diet) resisting the pathogen infection, which can be exploited further. The silkworms gut microbiome is an intricate ecosystem that harbors diverse groups of microorganisms. The interactions of the silkworm with its gut microbiome play an important role in its development and immune responses. The structure of gut microbiome of silkworm reared with normal diet and the enriched diet will be studied by a culture-independent molecular approach as the traditional culture-based technique possesses rather low sensitivity for measuring the composition, structure of microbiota colonizing the gut of silkworm.
11(b)	Aims and objectives	:	1) To screen the efficacy of phototrophic bacterial extracts as feed supplements for disease management in silkworm. 2) To prepare metabolite profiling of silkworm when fed on normal and phototrophic bacterial extract enriched diet

PART II: PARTICULARS OF INVESTIGATORS

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Number of Research projects being handled at present: Several

13. No. of Projects being handled by each investigator at present: Mentioned in 12.1 & 12.2

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PART III: TECHNICAL DETAILS OF PROJECT

15. Introduction

Silkworm and nutrition: Mulberry silkworm *Bombyx mori* L. is a monophagous lepidopteron insect which derives the required nutrients for its growth from mulberry leaves. Success of sericulture i.e silkworm development, cocoon crop and silk yield is mainly dependent on the mulberry leaf quality during rearing. So, in sericulture, food is a factor of paramount importance that regulates growth, development, and silk yield. Nutritional studies in silkworm with respect to moulting, cocoon productivity, economic characters, silk production, silk protein and body weight gain have elucidated their dependency on quantitative and qualitative variations of mulberry leaves used as feed (Sumoika *et al*., 1982). Major bio-molecules such as proteins, carbohydrates and lipids of mulberry leaf play an important role in biochemical process underlying growth and development of silkworm (Ito and Horie, 1959, Kumar *et al*., 2011). **Significant seasonal variations occur in the nutritional value and composition of mulberry leaves depending on factors such as weather, pests and diseases and agricultural practices which in turn have a great impact on growth and development of silkworm.**

Silkworm and diseases: Grasserie, flacherie, muscardine and pebrine are the common silkworm diseases. Success of sericulture also depends on proper management and protection of silkworm crops from diseases. Diseases are the major yield limiting factors in silkworm crop. Several disease prevention options are available. These include biological and chemical prevention based on direct antibiotic principle and usage of different bed and room disinfectants. Due to the prolonged exposure of disinfectants and antibiotics, most of the pathogens are becoming resistant and their efficacy is decreasing even with higher doses. The biological / chemical control measures and development of disease tolerant silkworm breeds are found to be insufficient to control the diseases.

The primary defense of the silkworm against pathogens is the prevention of the infection by possible structural barriers like the integument and the peritropic membrane that surrounds the food bolus

and protects the midgut epithelium. Secondary defense is provided by the haemolymph through cellular and humoral responses. According to Charles (2004) lower animals do not have well developed humoral immunity and under such circumstances vaccine development may not be of much use and in these lower animals and **factors that achieve immune-stimulation could be very useful for disease resistance** (Zafar *et al*., 2013).

Fortification of mulberry leaves: A technique involving supplementation of mulberry leaves with nutrients. Feeding of nutritionally enriched leaves showed better growth and development of silkworms as well as improve the economic value of cocoons (Pallavi *et al*., 2011). The growth, development and disease resistance in silkworm is profoundly influenced when fed on nutritionally enriched mulberry leaves. Previous studies have been concentrated on enriching mulberry leaves with vitamins, minerals, hormones, antibiotics, probiotics, sugars and botanicals (Konala *et al*., 2013).

Anoxygenic phototrophic bacteria (APB): Anoxygenic phototrophic bacteria (APB) are physiologically and phylogenetically heterogeneous group of bacteria with a common property to perform photosynthesis under anoxic conditions in the presence of light with the help of bacteriochlorophylls without the liberation of oxygen. Unlike oxygenic phototrophs (cyanobacteria, algae and green plants), they use only one photosystem and are unable to use water as an electron donor (Imhoff, 1995). They are widely distributed in terrestrial and aquatic ecosystems including extremes of light, temperature, pH and salinity. The various photosynthetic pigments give the cell cultures a distinct coloration of green, yellowish-green, brown, brownish green, brownish red, red, pink, purple, pink to red and purple violet depending on the pigment content. The bacteriochlorophylls *a*/*b*/*c*/*d*/*e*/*g* and carotenoids of the spirilloxanthin, rhodopinal, spheroidene (yellowish brown in the absence of O_2 and reddish brown in the presence of O_2) and okenone series are present in APB. APB are comprised of the members of the groups of purple sulfur bacteria (PSB), purple nonsulfur bacteria (PNSB), green sulfur bacteria (GSB), green nonsulfur bacteria (GNSB), Heliobacteria and aerobic anoxygenic phototrophic bacteria (Imhoff, 1995; Yurkov and Beatty, 1998). All validly published species of purple sulfur bacteria (62) are included in 27 different genera, which come under two major families, *Chromatiaceae* and *Ectothiorhodospiraceae* of the order *Chromatiales* of class *Gammaproteobacteria*. Purple non-sulfur bacteria (75) are positioned in 25 different genera that are distributed in 8 major families, *Rhodospirillaceae Acetobacteiaceae, Rhodobacteriaceae, Bradyrhizobiaceae, Hypomicrobiaceae, Rhodobiaceae, Commamonadiaceae, Rhodocyclaceae*, belong to 5 major orders of class *Alpha (α)* and *Beta (β) Proteobacteria.*

Role of Anoxygenic phototrophic bacteria as feed supplements in aquaculture: The most exploited group in aquaculture is the purple non-sulfur bacteria. PNSB as food additives stimulated the growth of shrimp and fish (Zhang *et al*., 1988), enhanced the survival rate of fish larvae and improved the production of scallop seed (Huang *et al*., 1990; Wang *et al*., 1994). Nowadays, using photosynthetic bacteria as probiotics is common practice in many fish or shellfish hatcheries and farms in China. Instead of using homemade photosynthetic bacterial products, many farmers today are using concentrated and encapsulated commercial photosynthetic bacterial products. Many commercial photosynthetic bacterial products are labeled as either single or multiple species at concentrations higher than 10^9 ml^{-1}, and are often combined with growth promoters or conditioners, and are claimed to have multifunctional effects such as improvement of water quality, enhancement of growth rate and prevention of disease (Qi *et al*., 2009).

Metabolite profiling: Metabolites are the intermediate products of metabolic reactions catalyzed by various enzymes that naturally occur within cells. Metabolites have various functions including fuel, structure, signaling, stimulatory and inhibitory effects on enzymes, catalytic activity of their own (usually as a cofactor to an enzyme), defense, and interactions with other organisms (e.g. pigments, odorants, and pheromones). A primary metabolite is directly involved in normal growth, development and reproduction. Secondary metabolites play an important role in ecological functions. Examples include antibiotics and

pigments such as resins and terpenes etc. Profiling metabolites is of prime importance as it offers essential information regarding the biochemical aspects and metabo dynamics involved in an organism when fed on a specified diet.

Gut Microbiome studies: Insect guts present unique environments for microbial colonization, and especially bacteria in the gut potentially provide many beneficial services to their hosts. Insects exhibit a wide range in degree of dependence on gut bacteria for basic functions. The composition and structure of gut microbiome are dynamic, which will vary with respect to changes in nutrient availability and physiological environment. This study increases our understanding of the change of silkworm gut microbiota in response to diet. We can use the dominant populations to make probiotic formulations for better nutrient absorption and disease prevention in the silkworm.

So, the present study focuses on using phototrophic purple bacterial extracts as feed supplements in boosting the overall growth and development of silkworm besides providing disease resistance. Besides, metabolite profiling and gut microbiome studies in normal conditions and when fed on different bio-fortified diet will also be studied.

15.1 Definition of the Problem

In India, considerable seasonal fluctuations occur in the nutritional value and composition of mulberry leaves depending on factors such as weather, pests and diseases and agricultural practices which have an immense impact on growth and development of silkworm which in turn results in crop loss (Ito 1978, Ito *et al*., 1966a, 1966b). Crop loss due to diseases is also a common scenario in sericulture. The above shortcomings should be addressed in an effective way in order to achieve a successful sericulture practice. Fortification of mulberry leaves by using supplementary nutrients and feeding to the silkworms is a constructive modern technique to increase economic value of cocoon besides offering disease resistance (Kumaraj, 1972). Mulberry leaves sprayed with antibiotics, vitamins, hormones and hormone analogues are tested and found to have a better influence on development of silkworm. But, owing to the high cost of synthetic food, introduction of these techniques in India is very limited (Pallavi *et al*., 2011). Hence an economically cheaper technique which could still improve cocoon character and production, disease resistance is needed. So modern research is being concentrated on using plant extracts, probiotics, bacterial extracts as feed supplements in improving the disease resistance and development of silkworm in a cost effective mode. In this direction, phototrophic purple bacterial extracts have been extensively used in aquaculture. **Hence, the present project is being proposed to investigate the efficacy of phototrophic purple bacterial extracts as feed supplements for a cost effective and eco-friendly disease management in silkworm with improved cocoon character and production.**

15.2 Origin of the Proposal / Rationale of the Study

Anoxygenic phototrophic purple bacteria are an important group of phylogenetically diverse Gram negative photosynthetic prokaryotes. They are capable of autotrophic growth with CO_2 as sole carbon source. The purple bacteria possess photosystem II, bacteriochlorophyll *a* or *b* and contain carotenoids of spirilloxanthin, rhodopinal, spheroidene or okenone series as the major photosynthetic pigments. According to phylogenetic analyses, the purple bacteria are present interspersed with their non-phototrophic counterparts. Based on morphological, physiological and molecular data, the purple bacteria form an extremely heterogeneous group and are classified into three subgroups: purple non sulfur bacteria (PNSB) and purple sulfur bacteria (PSB); aerobic anoxygenic purple bacteria (AAPB). While the PNSB fall in α and β subclasses of *Proteobacteria*, the PSB are restricted to the γ *Proteobacteria* (Imhoff, 1995).

Biotechnological potential of Anoxygenic phototrophic purple bacteria:

1. As feed supplements in aquaculture, poultry (Zhou *et al*., 2007)
2. As probiotics (Zhou *et al*., 2007)
3. Carotenoids possesing antioxidant activities (Sasikala and Ramana, 1995)
4. SCP (Sasikala and Ramana, 1995)
5. Vitamins, hormones, quinones, enzymes (Sasikala and Ramana, 1995)
6. Plant growth promoting activities
7. Bio-colorants
8. In biodegradation & bioremediation (Sasikala and Ramana, 1995)

Advantages in using photosynthetic bacterial extracts as feed supplements

1. More digestible bacterial cell wall, rich in protein, carotenoids having antimicrobial property, biological cofactors and act as probiotics (Kobayashi and Kurata, 1978; Qi *et al.*, 2009).

Anoxygenic phototrophic bacteria as feed supplements in aquaculture: Anoxygenic phototrophic bacteria represent such a group that has the potential to be used in the aquaculture as feed supplement. The bacterial biomass is not only rich in protein, fat and vitamin but also contain a significantly amount of carotenoids and biological cofactors that are useful in fish feed formulation (Jalal, *et al*., 2001; Shapawi *et al*., 2012). Phototrophic bacteria are one of the naturally available carotenoid rich sources. Carotenoids have shown extensive applications in pharmaceutical, aquaculture and animal feed industries. Carotenoids are a class of unsaturated hydrocarbons and their oxygenated derivatives, which consist of eight isoprenoid units. The source of carotenoids obtained from phototrophic bacteria is much cheaper, safe and easy to acquire from them. The inclusion of carotenoids in the fish diets is not only found to improve skin color but also improve growth, metabolism, reproduction and increase the value of ornamental fishes (Gupta *et al*., 2007). Phototrophic bacteria also contain biological cofactors that improve the survival and growth of fish larvae (Kobayashi and Kobayashi 1995)

Status of dietary supplements used in silkworm: Many people in India are involved in sericulture, which involves a great deal of labor and intensive care. In order to make sericulture more economically viable, it has become important to study and analyze various factors that improve growth, yield, fiber quality, and larvae's resistance to pathogens. Larval nutrition and resistance to diseases play a prominent role in sericulture (Konala *et al.*, 2013).

Application of potential dietary supplements is being extensively studied as it plays an important role in growth, development, disease management in silkworm. The effect of mulberry leaves enriched with amino acids on the growth of *B. mori* has been studied (Khan and Saha 1995; Nirwani and Kaliwal 1998; Radjabi, 2010). The protein content of the silk gland, fat body, and muscles was found to increase significantly when larvae were fed with ascorbic acid (Quraiza *et al*., 2008). Different combinations of mineral nutrients were found to improve larval growth and silk production (Ahmad, 1993). Mulberry leaves enriched with nickel chloride and/or potassium iodide has increased cocoon weight at low concentrations (Islam *et al*., 2004). The presence of casein in a diet has enhanced the growth rate of *Manduca sexta* caterpillars and was observed to stimulate the feeding efficiency of *B. mori* (Ito, 1960; Woods, 1999). Diet supplements containing fatty acids and carbohydrates were shown to have a regulatory effect on fatty acid synthesis in larval stages of *B. mori* (Horie and Nakasone, 1970). Sterols including cholesterol had a positive influence on dietary efficiency in *B. mori* (Ito *et al*., 1963).

The role of photosynthetic bacterial extracts as feed supplements in aquaculture and poultry has been extensively probed and it is being used successfully. Microbial feeds produced through biotechnological processes have been actively investigated as alternative or unconventional feed supplement as well as probiotic resources for aquaculture. Phototrophic bacteria have the potential to be used as an aquaculture feed supplement since they have been found to be nutritious and non-toxic (Getha *et al.,* 1998). Extensive studies have been carried out on the potential of photosynthetic bacteria as feed supplements in aquaculture and the role of phosynthetic bacteria as feed additives in sericulture is not probed. So, the present project is proposed to explore the role of phosynthetic bacterial extracts as feed supplements in silkworm. Another objective of the project is also to study the metabolite profile of silkworm in normal and enriched diet conditions.

Metabolite profiling: Earlier studies concentrated on profiling of various physiological aspects in terms of upregulation and down regulation of biomolecules and enzymes as a part of defense mechanism in silkworm. But complete metabolite profiling (qualitative & quantitative) was not studied. So, the present study aims for the first time to profile the complete list of metabolites (carbohydrates, aminoacids, lipids, nucleic acids, organic acids, alcohols, amines, terpenes or any other compounds synthesized as a part of metabolism) in normal and photosynthetic bacterial extract enriched diet of silkworm.

Gut microbiome: The effect of dietary components on the gut microbiota of silkworm has not been catalogued. This study offers essential information with respect to influence of diet on gut flora alterations. Dominant populations inhabiting the gut aiding overall growth and development of silkworm can be studied further and formulations can be developed.

15.3 Relevance to the current issues and expected outcome

The success of sericulture depends on larval nutrition and protection of silkworm crops from diseases. Significant seasonal variations occur in the nutritional value and composition of mulberry leaves depending on factors such as weather, pests and diseases and agricultural practices (Ito 1978, Ito *et al*., 1966a, 1966b). Enrichment of mulberry leaves by nutrient supplementation is an emerging field to combat diseases and also to increase the quality and quantity of silk. There is suggestive evidence that several strategies of enriching mulberry leaves with vitamins, minerals, hormones, antibiotics, probiotics, sugars and botanicals have profound influence on boosting the immune response in silkworm and also enhanced growth and development in silkworm. Application of phototrophic bacterial extracts as feed supplements in aquaculture is being widely practiced in various parts of the world and it has been exponentially growing (Qi *et al*., 2009). The major advantages of using phototrophic bacterial extracts is that they have a more digestible bacterial cell wall, and are rich in proteins, carotenoids, biological cofactors and vitamins. They can also serve as probiotics which boost the immune system and carotenoids of phototrophic bacteria have antimicrobial activity which aid in combating diseases. Hence the present attempt is a very good approach on *B.mori* to strengthen their immunity to resist the microbial pathogenic attack and to promote good yield when fed on photosynthetic bacterial extracts enriched diet. In addition, metabolome profiling and gut microbiome of silkworm when fed on phototrophic bacterial extract enriched diet will be studied. Metabolic profiling provides information regarding lead bioactive compounds resisting the pathogen infection, which can be exploited further. Gut microbiome study increases our knowledge with respect to silkworm gut microbiota in response to diet. We can use the prevailing populations to make probiotic formulations for better nutrient absorption and disease prevention in silkworm.

The novelties of the project are:

- **Photosynthetic bacterial extracts as feed supplements for disease management, overall growth and development in silkworm is being probed for the first time**
- **Metabolite profiling of silkworm under normal as well as when fed on phototrophic bacterial extract enriched diet is being probed for the first time**
- **Gut microbiome of silkworm under normal as well as when fed on phototrophic bacterial extract enriched diet is being probed for the first time**

Expected outcome:

- A Photosynthetic bacterial extract formulation as a feed additive can be developed which aids in boosting the immunity and over all development of silkworm.
- Metabolite profile of silkworm in normal and phototrophic bacterial extract enriched diet can be developed
- Gut microbiome of silkworm in normal and phototrophic bacterial extract enriched diet can be developed
- A good number of publications is expected
- Patents are possible

15.4 Objectives

1) To screen the efficacy of phototrophic bacterial extracts as feed supplements for disease management in silkworm.
2) To prepare metabolite profiling of silkworm when fed on normal and phototrophic bacterial extract enriched diet

16. Review of status of Research and Development on the subject.

16.1. International Status

Nutrition plays an important role in improving the growth and development of silkworm *Bombyx mori* like other organisms (Legay, 1958). Silk production is dependent on larval nutrition and nutritive value of mulberry leaves play an effective role in producing good quality of cocoons. In recent years attempts have been made in sericulture with feed supplement of nutrients, such as proteins, carbohydrates, amino acids, vitamins, hormones, and antibiotics etc. for better performance of silkworm leading to good quality of cocoons. Fortification of mulberry leaves with complementary compounds was found to increase the larval growth and post cocoon characteristics. Etebari *et al*., 2004 have reported different aspects of mulberry leaves supplementation with various nutritional compounds in sericulture. Some nitrogenous compounds are involved in digestion and palatability of plants eaten by insects and improve many biological performances of the insects. Zaman *et al*., 1996 showed that adding 0.2 % nitrogen to silkworm diet can increase the larval weight. Physiological changes of Silkworm (*Bombyx mori* L.) larvae fed on Mulberry leaves supplemented with nitrogenous compounds were studied by Etebari *et al*., 2007. Growth and development of the mulberry silkworm, *Bombyx mori* L. on vitamin B and C supplemented diet was studied by Ahsan *et al*., 2013.

Elevation of dietary protein to an optional level (Horie *et al*., 1971) and supplementation of low nutritive proteins with their limiting amino acids (Ito and Arai, 1965) have found to accelerate the growth of the silkworm. Ito (1980) stated that rich sources of dietary proteins like soyaprotein are known to promote growth and to improve the economic characters of the silkworm. Horie and Watanabe (1983) showed that the supplementation of soybean protein increased the protein and amino acid content in the larval haemolymph of the silkworm *B.mori.* Artificial diets rich in amino acids are required for optimal growth of an insect, and casein has been widely used as it contains all amino acids (Panizzi and Parra 1991). Casein contains fatty acids, cholesterol, sugars, vitamins, and minerals (Vanderzant, 1966). The presence of casein in a diet has enhanced the growth rate of *Manduca sexta* caterpillars and was observed to stimulate the feeding efficiency of *B. mori* (Ito, 1960; Woods, 1999). Diet supplements containing fatty acids and carbohydrates were shown to have a regulatory effect on fatty acid synthesis in larval stages of *B. mori* (Horie and Nakasone, 1970). Sterols including cholesterol had a positive influence on dietary efficiency in *B. mori* (Ito *et al*., 1963).

The use of phototrophic bacteria as feed supplements was extensively studied in the International arena. Currently, photosynthetic bacteria are found in five bacterial phyla, i.e. Chlorobi, Cyanobacteria, Chloroflexi (filamentous anoxygenic phototrophs), Firmicutes (heliobacteria) and Proteobacteria (purple sulfur and purple non-sulfur bacteria) (Bryant and Frigaard, 2006). Traditionally in Chinese aquaculture, the photosynthetic bacteria refer to the photosynthetic bacteria in the proteobacteria alpha subdivision, i.e. the purple non-sulfur bacteria. Purple non-sulfur bacteria are widely distributed in freshwater, marine, soil and hot-spring environments. They have various metabolic pathways for the degradation of organic wastes. They also have a more digestible bacterial cell wall, and are rich in proteins, carotenoids, biological cofactors, and vitamins (Kobayashi and Kurata, 1978). The species currently used in Chinese aquaculture are *Rhodopseudomonas palustris*, *Rubrivivax gelatinosa*, *Rhodobacter capsulata*, *R. spaheroides*, *Phaeospirillum fulvum*. Those are probably the earliest and the most widely used probiotics in China since the 1980s. It was reported that the addition of photosynthetic bacteria as food additives stimulated the growth of shrimp and fish (Zhang *et al*., 1988), enhanced the survival rate of fish larvae, and improved the production of scallop seed (Huang *et al*., 1990; Wang *et al*., 1994). They were also found to increase the population growth rate of live food such as *Brachionus plicatilis* (Xu *et al*., 1992). Phototrophic bacteria as feed supplement for rearing *Penaeus monodon* Larvae was studied by chong and vikineswary, 2002. Phototrophic bacteria as a fish feed supplement was studied by Banarjee *et al*., 2000. Phototrophic purple bacteria as feed supplement on the growth, feed utilization and body compositions of Malaysian Mahseer, *Tor tambroides* Juveniles was studied by Chowdhury *et al*., 2016. Inclusion of Purple non sulfur bacterial biomass in formulated feed to promote growth, feed conversion ratio and survival of Asian Seabass *Lates calcarifer* juveniles was studied by Shapawi *et al*., 2012. In vitro assessment of gastrointestinal viability of two photosynthetic bacteria, *Rhodopseudomonas palustris* and *Rhodobacter sphaeroides* was studied by Xia *et al*., 2007.The role of carotenoids from phototsynthetic bacteria as potential feed additives towards food and pharmaceutical industries and advantages of photosynthetic bacteria as fish feed supplement to enhance growth, survival and coloration of cultured fishes was demonstrated by Jalal *et al*., 2014.

Metabolome profiling in various insects was studied by GCMS analysis. Metabolomics is one of the newest '-omics' technologies, and has rapidly expanded over the last decade, providing an integral new approach to the study of biological systems (Dettmer & Hammock, 2004; Rochfort, 2005). Although this field was first defined by Oliver *et al*., 1998 as 'the quantitative measurement of the dynamic multi-parametric metabolic response of living systems to pathophysiological stimuli or genetic modification', some entomological investigations employing a recognizably metabolomic approach pre-date the adoption of the term (Thompson *et al*., 1990). The profound interplay between environment and genetics requires that metabolic studies be undertaken with careful attention to genetic background, diet, stock maintenance, and statistical analysis of data. The metabolic profile of organism could provide valuable

insights on the factors that cause their disturbance and furthermore, could be used to define the status of an organism as normal / healthy or not (Fiehn, 2002). Thus making metabolites a robust bio-analytical tool for diagnostic and monitoring purposes (Aliferis and Jabaji, 2011; Simpson *et al*., 2011). The dietary interventions, measurements of triglycerides, cholesterol, glucose, trehalose, and glycogen in terms of stress and normal conditions in *Drosophila* was studied by Tennessen *et al.*, 2014. Bacterial disease diagnosis in *Bombyx mori* L. by Thiodiglycol analysis using gas chromatography / mass spectrometry was studied by Taha and Kamel (2012). Bacterial infection to silkworm larvae cause behavioral and physiological alterations, leading to a new metabolic profile was reported by Taha (2007). Gas chromatography–mass spectrometry metabolite profiling of worker honey bee (*Apis mellifera* L.) hemolymph for the study of *Nosema ceranae* infection was studied by Aliferis & Jabaji (2012). A handful of reports had focused on hemolymph metabolome-based toxicity studies conducted on the *Daphnid*, *Daphniae magna* (Poynton *et al*., 2011; Taylor *et al*., 2010) and disease studies of hemolymph of the Atlantic Blue Crab (Schock *et al*., 2010). A review on how metabolomic analyses can reveal associations between an organism's metabolome and further aspects of its phenotypic state as an attractive prospect for many life-sciences researchers was authored by Snart *et al*., 2015. Homeo domain protein Scr regulates the transcription of genes involved in Juvenile hormone biosynthesis in the Silkworm by GCMS analysis was studied by Meng *et al*., 2015. GC-MS determination of bioactive constituents of Giant African Snail (*Archachatina maginata*) haemolymph was studied by Lawal *et al*., 2015. Quantitative assessment of hemolymph metabolites in two physiological states and two populations of the land snail *Helix pomatia* was studied by Nicolai *et al*., 2012.

Limited survey is conducted on gut microbiome studies in insects. Broderick *et al*., 2004 and Colman *et al*., 2012 indicated that the gut of most insects harbours a rich and complex microbial community, typically 10^7–10^9 prokaryotic cells. Broderick *et al*., 2004 studied the bacterial community of the gypsy moth by using culturing and culture-independent methods. The gut bacteria of insects with respect to nonpathogenic interactions were studied by Dillon & Dillon (2004). Mutualism between the desert locust *Schistocerca gregaria* and its gut microbiota was analysed by Dillon & Charnley (2002). Brinkmann *et al*., 2008 studied the gut bacteria of *Manduca sexta.* However information on gut microbiome of silkworm is scanty and has to be studied.

References

Ahsan Md. Kamrul, Ataur Rahman Khan and Tasnima Ferdous (2013). Growth and development of the mulberry silkworm, *Bombyx mori* L. on vitamin B and C supplemented diet. Bangladesh J. Zool. 41(2): 199-206.

Aliferis, K.A. and S. Jabaji, (2012). Gas chromatography–mass spectrometry metabolite profiling of worker honeybee (*Apis mellifera* L.) heamolymph for the study of *Nosema ceranae* infection Journal of Insect Physiology 58:1349–1359

Aliferis, K.A. and S. Jabaji, (2011). Metabolomics – A robust bioanalytical approach for the discovery of the modes of action of pesticides. A review of pesticide biochemistry and physiology, 100: 105-117.

Banerjee, S., Azad SA, Vikineswary S, Selva Raj, OS., Mukherjee, TK (2000). Phototrophic bacteria as fish feed supplement. Asian-Aus Jou Anim Sci. Vol 13. No 7; 991-994.

Brinkmann N, Martens R, Tebbe CC. (2008). Origin and diversity of metabolically active gut bacteria from laboratory-bred larvae of *Manduca sexta* (*Sphingidae, Lepidoptera, Insecta*). Appl Environ Microbiol. 74:7189-7196.

Broderick NA, Raffa KF, Goodman RM, Handelsman J. (2004). Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture-independent methods. Appl Environ Microbiol. 70:293-300.

Bryant, D.A., Frigaard, N.U., (2006). Prokaryotic photosynthesis and phototrophy illuminated. Trends Microbiol. 14: 488–496.

Charles, T.J.B., (2004). Probiotics in agriculture. Advanced Biotech.,January pp27-30.

Chong VC & Vikineswary S (2002). Phototrophic Bacteria as Feed Supplement for Rearing *Penaeus monodon* Larvae. Journal of the world aquaculture society Vol. 33, No. 2 158-168.

Chowdhury AJK, Nor Hafizah Zakaria, Zaima Azira Zainal Abidin & Mohammad Mustafizur Rahman (2016). Phototrophic Purple Bacteria as Feed Supplement on the Growth, Feed Utilization and Body Compositions of Malaysian Mahseer, *Tor tambroides* Juveniles. Sains Malaysiana 45(1): 135–140

Colman DR, Toolson EC, Takacs-Vesbach CD. (2012). Do diet and taxonomy influence insect gut bacterial communities? Mol Ecol. 21:5124-5137.

Dettmer K & Hammock BD (2004). Metabolomics – a new exciting field within the "omics" sciences. Environmental Health Perspectives 112: 396–397.

Dillon R, Charnley K. (2002). Mutualism between the desert locust *Schistocerca gregaria* and its gut microbiota. Res Microbiol. 153:503-509.

Dillon RJ, Dillon VM. (2004). The gut bacteria of insects: nonpathogenic interactions. Annu Rev Entomol. 49:71-92.

Etebari, K., Kaliwal, B.B., Matindoost, L., (2004). Different aspects of mulberry leaves supplementation with various nutritional compounds in sericulture. *International Journal of Industrial Entomology,* 9:15-28.

Etebari K, Rahim Ebadi, Leila Matindoost (2007). Physiological Changes of Silkworm (*Bombyx mori* L.) Larva Fed on Mulberry Leaves Supplemented with Nitrogenous Compounds J. Ent. Res. Soc. 9(2): 1-15.

Fiehn, O., (2002). Metabolomics – the Link Between Genotypes and Phenotypes. Plant Molecular Biology, 48: 155-171.

Horie Y, Watanabe K and Shinohara (1971). Effect of dietary composition on growth, silk glands and components in haemolymph of the silkworm, Acta. Sericologia, Japan, 78: 44-50.

Horie Y and Watanabe K (1983). Effect of various levels of dietary protein and supplementation with limiting amino acids on growth, haemolymph components and uric acid excretion in silkworm, *Bombyx mori*, J. Insect Physiol., 29:187-199.

Horie Y, Nakasone S. (1990). Effects of the levels of fatty acids and carbohydrates in a diet on the biosynthesis of fatty acids in larvae of the silkworm, *Bombyx mori*. Journal of Insect Physiology 17(8): 1441–1450.

Huang, H., Yao, Z., Zheng, J., (1990). The study of the applications of photosynthetic bacteria to aquaculture the value of using photosynthetic bacteria as the initial feed for fish larvae. J. Zhanjiang Fish. College 10: 58–61 (in Chinese, With English abstract).

Imhoff, J. F. (1995): Taxonomy and physiology of phototrophic purple bacteria and green sulfur bacteria. In *Anoxygenic photosynthetic bacteria*‖ (Blankenship, R. E., Madigan, M. T., and Bauer, C. E. (eds), Kluwer Academic Publishers, The Netherlands. (1995), 1-15 (PP).

Ito, T., & Horie, Y. (1959). Carbohydrate metabolism of the midgut of the silkworm *Bombyx mori* L. *Archives of biochemistry and biophysics.,* 80: 174-176.

Ito T. (1960). Effect of sugars on feeding of larvae of the silkworm, *Bombyx mori*. Journal of Insect Physiology 5: 95–107.

Ito T, Kawashima K, Nakahara M, Nakanishi K, Terahara A. (1963). Effect of sterols on feeding and nutrition of the silkworm, *Bombyx Mori* L. Journal of Insect Physiology 10: 225– 238.

Ito T & Arai N (1965). Nutrition of the silkworm, *B.mori*., VIII, amino acid requirements and nutritive effects of various proteins, Bulletin of Sericulture Experimental station, Japan, 19 (1965): 345-373

Ito, T; Niminura, M (1966a) Nutrition of silkworm *Bombyx mori*. Its specific requirement and its nutrition in relation the mineral nutrition of its host plant mulberry. *Morus indica* L Indian J. Exptl. Biol. 4: 31-36.

Ito T ; Niminura, M. (1966 b). Nutrition of silkworm *Bombyx mori*. XII Nutritive effects of minerals Bul.Sericult. Expt Sta. 20: 373.

Ito, T.(1978). Silkworm Nutrition in the silkworm an important laboratory tool. Tazima, Y. (ed), pp. 121- 157, Ko.Ltd. , Tokyo.

Ito T (1980). Dietary requirements of the silkworm, *Bombyx mori* L. and its artificial diets., *XVI Int.Cong. Entomol. Abr.,* Tokyo, Japan, 448.

Jalal, K.C.A., Sorgeloos, P., Mohd. Zaki, M. S. and Saad, C.R (2001). Effects of Spirulina on the growth and carotenoid position in tropical sport fish Tor *Tambroides fry*. J. of Euro. Aqua. Soc. 30: 258-261.

Jalal K.C.A., Zaima Azira, Z.A., Nor Hafizah, Z, M. M. Rahman, B.Y. Kamaruzzaman & Noor Faizul, H. N.(2014). Carotenoid Contents in Anoxygenic Phototrophic Purple Bacteria, *Marichromatium* sp. and *Rhodopseudomonas* sp. of Tropical Aquatic Environment, Malaysia. OJCHEG 2014, Vol. 30, No. (2): Pg. 607-613

Kobayashi, M. and M. Kobayashi. (1995). Waste remediation and treatment using anoxygenic photosynthetic bacteria. Page 1269-1282 in R. E. Blankensh, M. T. Madigan, and C. E. Bauer, editors. Anoxygenic photosynthetic bacteria. Kluwer Academic Publisher, Boston, Massachusetts, USA.

Kobayashi, M., Kurata, S., (1978). The mass culture and cell utilization of photosynthetic bacteria. Process Biochem. 13:27–30.

Lawal B, Oluwatosin K. Shittu, Tawakaltu AbdulRasheed-Adeleke, Prince C. Ossai, and Aisha M. Ibrahim (2015) GC-MS determination of bioactive constituents of giant African Snail (*Archachatina maginata*) haemolymph. Volume 10, Issue 2 Ver. IV (Mar -Apr. 2015), PP 59-64

Legay, J.M.(1958). Recent advances in silkworm nutrition. Ann.Rev.Ent., 3:75- 86.

Meng Meng, Chun Liu, Jian Peng, Wenliang Qian, Heying Qian, Ling Tian, Jiarui Li, Dandan Dai, Anying Xu, Sheng Li, Qingyou Xia, and Daojun Cheng (2015). Homeodomain protein Scr regulates the transcription of genes involved in juvenile hormone biosynthesis in the Silkworm. Int. J. Mol. Sci. 16: 26166–26185

Nicolai Annegret, Juliane Filser, Roman Lenz, Carole Bertrand, Maryvonne Charrier (2012). Quantitative assessment of hemolymph metabolites in two physiological states and two populations of the land snail *Helix pomatia*. Physiological and Biochemical Zoology 85(3):274–284.

Oliver SG, Winson MK, Kell DB & Baganz F (1998). Systematic functional analysis of the yeast genome. Trends in Biotechnology 16: 373–378.

Panizzi AR, Parra JRP. (1991). Ecologia nutricional de insetos esua aplicacao no manejo de pragas. Manole/CNP.

Poynton, H.C., Taylor, N.S., Hicks, J., Colson, K., Chan, S., Clark, C., Scanlan, L., Loguinov, A.V., Vulpe, C., Viant, M.R., (2011). Metabolomics of microliter hemolymph samples enables an improved understanding

of the combined metabolic and transcriptional responses of *Daphnia magna* to cadmium. Environmental Science and Technology 45:3710–3717.

Qunlan Zhou, Kangmin Li, Xie Jun, Liu Bo (2009). Role and functions of beneficial microorganisms in sustainable aquaculture. Bioresource Technology 100: 3780–3786

Radjabi R. (2010). Effect of Mulberry leaves enrichment with amino acid supplementary nutrients on Silkworm, *Bombyx mori* L. at North of Iran. *Academic journal of Entomology* 3(1): 45–51.

Rochfort, S. (2005). Metabolomics reviewed: a new "Omics" platform technology for systems biology and implications for natural products research. Journal of Natural Products 68:1813–1820.

Schock, T., Stancyk, D., Thibodeaux, L., Burnett, K., Burnett, L., Boroujerdi, A., Bearden, D., (2010). Metabolomic analysis of Atlantic blue crab, *Callinectes sapidus*, hemolymph following oxidative stress. Metabolomics 6: 250–262.

Shapawi, R., Ting, T. E., and Azad, S. A (2012). Inclusion of purple non sulfur bacterial biomass in formulated feed to promote growth, feed conversion ratio and survival of asian *Seabass Lates* calcarifer juveniles. J. of Fish. and Aqua. Sci. 7: 475–480.

Simpson A.J., McNally D.J. and Simpson, M.J. (2011). NMR Spectroscopy in environmental research from molecular interactions to global processes. Progress in Nuclear Magnetic Resonance Spectroscopy, 58: 97-175.

Snart CJP, Hardy ICW & Barrett DA (2015). Entometabolomics: applications of modern analytical techniques to insect studies. Entomologia Experimentalis et Applicata 155: 1–17.

Sumoika, H., Kuroda, S., & Yoshitake, N. (1982). Relationships among food ingestion, food digestion and body weight gain in the silkworm larvae *Bombyx mori* L. under restricted feeding by indices. J. Seric. Sci. Jpn., 51: 52-57.

Taylor, N.S., Weber, R.J.M., White, T.A., Viant, M.R., (2010). Discriminating between different acute chemical toxicities via changes in the *Daphnid* metabolome. Toxicological Sciences 118: 307– 317.

Taha, R.H., (2007). Early diagnosis and genetic mapping of viruses causing flacherrie and grasserie diseases in gamma radiated and non irradiated Mulberry Silkworm, *Bombyx mori* L. Ph.D. Thesis, Faculty of Science, Ain Shams Univ.

Taha RH & Kamel WM (2012). Bacterial disease diagnosis in *Bombyx mori* L. by Thiodiglycol analysis using gas chromatography / mass spectrometry. Journal of Applied Sciences Research, 8(8): 4668- 4671.

Tennessen JM, Barry WE, James Cox, Carl S. Thummel (2014). Methods for studying metabolism in Drosophila. Methods 68:105–115.

Thompson SN, Lee RWK & Beckage NE (1990). Metabolism of parasitized *Manduca sexta* examined by nuclear-magnetic-resonance. Archives of Insect Biochemistry and Physiology 13:127–143.

Vanderzant ES. (1966). Development, significance and application of artificial diet for insect. Annual Review of Entomology 19: 139–405.

Wang, X., Sun, Z., Liu, X., Ma, J., Wang, B., Song, X., Zhu, Z., Shi, J., Wang, F., (1994). Application of photosynthetic bacteria in scallop artificial seed breeding. J. Fish Chin. 18, 65–68 (in Chinese, With English abstract).

Woods HA. (1999). Patterns and mechanisms of growth of fifth-instar *Manduca sexta* caterpillars following exposure to low- or high-protein food during early instars. Physiological and Biochemical Zoology 72(4): 445–454

Woolf, C.M (1968). Bioassay- Reed and Muench statistical method of estimation of LD 50 for biologists. Principles of Biochemistry, D. Van Nostrand Company Ltd., USA, pp.288-297.

Xia ZX, Pan Yuan-jiang, Wang Yan-bo, Li Wei-fen (2007). In vitro assessment of gastrointestinal viability of two photosynthetic bacteria, *Rhodopseudomonas palustris* and *Rhodobacter sphaeroides*. Sci B 8(9):686-692

Xu, B., Ding, M., Mao, J., Xu, H., (1992). The food value of *Rhodopseudomonas sphaeroides* for *Brachionus plicatilis*. Trans. Oceanol. Limnol. 2:17–22 (in Chinese, With English abstract).

Yurkov, V. V. and Beatty, J. T. (1998): Aerobic anoxygenic phototrophic bacteria. *Microbiol. Mol. Biol. Rev.* 62:695-724.

Zaman, K., Ashfaq, M., Akram, K., (1996). Effect of feeding Mg and N treated mulberry leaves on larval development of silkworm *Bombyx mori* L. and silk yield. Pakistan Entomology, 18:78-79.

Zhang, D., Sun, Q., Naihong, C., Qiao, Z., (1988). Isolation and cultivation of photosynthetic bacteria of *Rhodospirillaceae* and its application as additive in feeds of fish and prawn. J. Fish. Chin. 12:367–369 (in Chinese).

Zizhong Qi, Xiao-Hua Zhang, Nico Boon, Peter Bossier (2009). Probiotics in aquaculture of China — Current state, problems and prospect. Aquaculture 290:15–21

16.2. National Status

Research activities with relation to role of fortified diet in silkworm rearing have been studied at this institute (CSRTI, Berhampore) and also by other groups.

Dr. S. Chakrabarty has worked on fortification of mulberry leaves with different nutrient supplements in controlling silkworm diseases. A project entitled "Studies on synbiotics (combination of probiotic and prebiotic) induction for control of common diseases of silkworm, *Bombyx mori* L." (ARP-3516) is on the way towards formulation of a synbiotic to be used as a fortifier for control of silkworm diseases.

Chakrabarty *et al.*, (2012) has also worked on immunological impact of some chemicals, botanicals, antibacterial proteins and live nonpathogenic bacteria on silkworm, *Bombyx mori* L. to control bacterial disease. The outcome of these studies indicates advantage of humoral immunity derived components as well as their application in the field to control the silkworm diseases through the feed supplement along with mulberry leaf. In the study, it was observed that mortality % was recorded lowest in all the treatments supplemented with ascorbic acid.

A study has also been conducted on prevention and control of nuclear polyhedrosis and white muscardine diseases of silkworm, *Bombyx mori* L. using botanicals (ARP3286) at the institute.

Recent publication of this Institute on the subject is depicted below:

1) Chakrabarty, S., Manna,B., Mitra,P., Saha, A.K. and Bindroo, B.B. (2012). Studies on immunological impact of some chemicals, botanicals, antibacterial proteins and live non-pathogenic bacteria in silkworm, *Bombyx mori* L. to control bacterial disease. Proceeding of the 22nd Indian Congress of Parasitology, *'Advances in Parasitology: A novel approach towards a disease free world'*, Dept of Zoology, University of Kalyani, West Bengal, India. p- 244 - 251.

Studies on fortified diet to silkworm by other groups in India

Krishnan *et al.*, 1995 showed that the hydrolyzed soyaprotein (P-soyatase) supplementation decreased the larval duration, increased the accumulation of haemolymph protein (SP-1: female specific protein and SP-2; an arlyphorin), larval weight and cocoon characters. The effect of mulberry leaves enriched with amino acids on the growth of *B. mori* has been studied (Khan and Saha 1995; Nirwani and Kaliwal 1998). The protein content of the silk gland, fat body, and muscles was found to increase significantly when larvae were fed with ascorbic acid (Quraiza *et al.*, 2008). Different combinations of mineral nutrients were found to improve larval growth and silk production (Ahmad, 1993). Mulberry leaves enriched with nickel chloride and/or potassium iodide has increased cocoon weight at low concentrations (Islam *et al.*, 2004). The growth and development of larvae, and subsequent cocoon production are greatly influenced by the nutritional quality of mulberry leaves (Masthan *et al.*, 2011). The effect of bovine milk on the growth of *Bombyx mori* has been studied by Konala *et al.*, 2013. Role of food additives on young age Silkworm (*Bombyx mori* .L) rearing was studied by Pallavi *et al.*, 2011. Supplementary effect of Spirulina on lipids and enzymes in silk gland of silkworm, *Bombyx mori* (L.) was studied by Kumar *et al.*, 2014. Impact of probiotic *Saccharomyces cerevisiae* on the enzymatic profile and the economic parameters of silkworm *Bombyx mori* L. was studied by Esaivani *et al.*, 2014. Voluminous literature has been documented in the area of using fortification techniques (using sugars, aminoacids) for overall development and disease resistance in silkworm. (Sengupta *et al.*, 1972 and Sheeba *et al.*, 2007). Growth rate pattern and economic traits of silkworm, *Bombyx mori* L under the influence of folic acid administration was studied by Rahmathulla *et al.*, 2007. Evaluation of antibacterial efficacy of certain botanicals against bacterial pathogen *Bacillus* sp. of Silkworm, *Bombyx mori* L. has been studied by Pachiappan *et al.*, 2009. The potentiality of antibiotics with reference to growth of silkworm was studied by Venkatesh Kumar *et al.*, 2010. The effect of nutritional supplementation with *Amaranthus hybridus* Linn. extract on economic performance of Mulberry Silkworm, *Bombyx mori* L. is studied by Pardeshi *et al.*, 2014.

Studies on the mass culture of photosynthetic sulfur bacteria (PSB) and their role as diet in aquaculture is studied by Palanichamy (2001). Getha *et al.*, 1998 studied the potential use of the phototrophic bacterium, *Rhodopseudomonas palustris*, as an aquaculture feed. The phototrophic bacterial strain *Rhodovulum sulfidophilum* as an aquaculture feed supplement for tilapia was studied by Banerjee *et al.*, 2000. Phototrophic bacteria are one of the naturally available carotenoid rich sources. Carotenoids have shown extensive applications in pharmaceutical, aquaculture and animal feed industries. Carotenoids are a class of unsaturated hydrocarbons and their oxygenated derivatives, which consist of eight isoprenoid units. The source of carotenoids obtained from phototrophic bacteria is much cheaper, safe and easy to acquire from them. The inclusion of carotenoids in the fish diets is not only found to improve skin color but also improve growth, metabolism, reproduction, disease resistance and increase the value of ornamental fishes (Gupta *et al.*, 2007).

The development and economic production of sericulture largely and greatly depends on the metabolic modulations and molecular mechanism of silkworm, besides its genetic composition and immunological resistance (Babu *et al.*, 2009). So, metabolome profiling plays a major role in understanding the physiological and biochemical aspects of an organism. GC-MS analysis of fatty acid constituents from various tissues of prawn, such as haemolymph, muscle tissue, gonad and hepato pancreas revealed that there were 14 types of bioactive components based on retention time, molecular formula, molecular weight and peak area (Athiyaman *et al.*, 2014). Qualitative and quantitative fluctuations of primary metabolites and allelo chemicals such as phenols, flavanoids and volatile profiles of diets exert a strong influence on the fitness of *probergrothius sanguinolens* in terms of post embryonic development, adult longevity, egg out put, egg hatchability, total growth index, adult emergence and fecundity was studied by GC analysis (Gurusubramanian *et al.*, 1996). GCMS based metabolome

approach in India was mostly restricted to Microorganisms but extensively worked in insects in the international arena which was mentioned above (in international status).

The importance of studying insect gut microbiome in general and in terms of biotechnological point was highlighted by Krishnan *et al.*, 2014.The quantitative and qualitative changes of bacterial flora associated with the silkworm (*Bombyx mori*) at different stages of its life cycle were studied by Kalpana *et al.*, 1994 who indicated that the bacterial flora of the silkworm digestive tract showed a gradual increase in the later instars which may be due to the higher feeding activity of the instars. Assessment of colonization resistance in silkworm, *Bombyx mori* L. using molecular marker tagged *Escherichia coli* was studied by Mohan Raj *et al.*, 2009.They demonstrated the role of microflora especially *Streptomyces* in conferring colonization resistance to invading pathogens in the guts of silkworms. However reports on gut microbiome with respect to change in diet are scarce and needs to be studied.

References

Ahmad Z. (1993). Effect of nitrogen, phosphorous and potassium treated mulberry leaves on larval development and silk yield of *Bombyx mori* L. M.Sc. Thesis, Department of Agricultural Entomology. University of Agriculture, Faisalabad.

Athiyaman R & Rajendran K (2014). GC-MS evaluation of Fatty acid constituents from various tissues of *Macrobrachium scabriculum* Int. J. of Res. in Pharmacology & Pharmacotherapeutics Vol-3(2):97-101

Babu, Mahesh S.,Gopalaswamy, G. and Chandramohan, N. (2005). Identification of an antiviral principle in *Spirulina platensis* against *Bombyx mori* nuclear polyhedrosis virus (bmnpv), Indian Journal of Biotechnology,.4, pp. 384-388.

Banerjee, S., Azad, S.A., Vikineswary, S., Selvaraj, O.S. and Mukherjee, T.K. (2000). Phototrophic bacteria as fish feed supplement. Asian Australasia Journal of Animal Science 13: 991-994.

Esaivani C, Vasanthi K, Bharathi R, Chairman K (2014). Impact of probiotic *saccharomyces cerevisiae* on the enzymatic profile and the economic parameters of silkworm *Bombyx mori* L. Advances in Biology &BioMedicine 1 (1):1-7.

Getha, K., Chong, V.C. and Vikineswary, S. (1998). Potential use of the phototrophic bacterium, *Rhodopseudomonas palustris*, as an aquaculture feed. Asian Fisheries Science, 10(3): 179-268.

Gupta, S. K.; Jha, A. K.; Pal, A. K. and Venkateshwarlu, G. (2007) Use of natural carotenoids for pigmentation in fishes. Ind. J. of Nat. Prod. and Res. 6(1): 46–49.

Gurusubramanian G; Prakash DS, JeyaKumar A. (1996). Chemical ecology of *probergrothius sanguinolens* in relation to herbivory and canivory.Proc. Indian natl sci Acad B62 No 2: 81-90

Islam Md R, Ali Md AO, Paul DK, Sultana S, Banu NK, Islam Md R. (2004). Effect of Salt,Nickel chloride supplementation on the growth of silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). Journal of Biological Sciences 4(2): 170–172.

Kalpana S, Hatha AAM, Lakshmanaperumalsamy P. (1994). Gut microflora of the larva of silkworm, *Bombyx mori* Insect Sci. Applic. Vol. 15, No. 4/5, 499-502.

Khan MD, Saha BN. (1995). Growth and development of the mulberry silkworm, *Bombyx mori* L. on feed supplemented with alanine and glutamine. Sericolgia 35: 657–663.

Krishnan M, Subburathinam KM and Janarthan S (1995). Effect of hydrolyzed protein (PSoyatose) on haemolymph protein profile, larval and pupal characters of the silkworm, *Bombyx mori* L. (lepidoptera:Bombycidae), Sericologia, 35:227-235.

Krishnan M, Bharathiraja C, Pandiarajan J, Prasanna VA, Rajendhran J and Gunasekaran P (2014). Insect gut microbiome - An unexploited reserve for biotechnological application. Asian Pac J Trop Biomed 2014; 4(Suppl 1): S16-S21

Krishnaswami (1978). New technology of silkworm rearing. Bulletin No.2, CSR&TI, Mysore, India

Kumar. K, Balasubramanian. U (2014).Supplementary effect of Spirulina on lipids and enzymes in silk gland of silkworm, *Bombyx mori* (L.) Journal of Entomology and Zoology Studies 2014; 2 (4): 279-282

Kumar, D., Pandey, J. P., Jain, J., Mishra, P. K., & Prasad, B. C. (2011). Qualitative and quantitative changes in protein profile of various tissues of tropical tasar silkworm, *Antheraea mylitta* Drury. Int. J. Zool. Res*., 7*, 147-155.

Kumaraj, S; Vijayaraghavan, S. ; Krishnaswami, S. (1972). Studies on Fortification of mulberry leaves for feeding silkworms. Indian J. Seric., 11: 68 -72.

Konala N, Abburi P, Bovilla VR, Mamillapalli A. (2013). The effect of bovine milk on the growth of *Bombyx mori*. Journal of Insect Science 13:98.

Masthan K, Kumar TR, Narsimha Murthy CV. (2011). Beneficial effects of blue green algae Spirulina and yeast *Saccharomyces cerevisiae* on cocoon quantitative parameters of silkworm *Bombyx mori* L. Asian Journal of Microbiology Biotechnology 13(1): 205–208.

Mohanraj P, Subramanian S, Muthuswamy M. (2009). Assessment of colonization resistance in silkworm, Bombyx mori L. using molecular marker tagged *Escherichia coli*. Karnataka J. Agric. Sci., 22(3-Spl. Issue): 519-520

Nirwana RB, Kaliwal BB. (1998). Effect of thiamine on commercial traits and biochemical contents of the fat body and haemolymph in the silkworm *Bombyx mori* L. Sericolgia 38: 639–646.

Pachiappan P, Mahalingam C Aruchamy1 and Shashidhar Kaluvarahalli Ramanna (2009). Evaluation of antibacterial efficacy of certain botanicals against bacterial pathogen *Bacillus* sp. of Silkworm, *Bombyx mori* L. Int. J. Indust. Entomol. Vol. 18, No. 1, pp. 49-52.

Palanichamy S. (2001). Observations on the mass culture of photosynthetic sulfur bacteria (PSB) and their role as diet in production of rotifer and brine shrimp. J. mar. biol. Ass. India, 43 (1&2) : 155 – 160.

Pallavi, M., Muthuswami, R.N., Bhaskar and V. Naveen (2011). Role of food additives on young age Silkworm (*Bombyxmori* .L) rearing *Int. J. Pure Appl. Sci. Technol.*, 7(2) : 132-140

Pardeshi A. B., Bajad P. N.(2014). The effect of nutritional supplementation with *Amaranthus hybridus* Linn. extract on economic performance of Mulberry Silkworm, *Bombyx mori* L. Sch. Acad. J. Biosci., 2(4): 272-276

Quraiza MTF, Rathi CR, Muthu PT, Das SM, Bai MR. (2008). A study on the effect of ascorbic acid in modifying tissue protein content and economic traits of *Bombyx mori* L. Journal of Basic and Applied Biology 2(3-4): 32–35.

Rahmathulla V. K. , Priyabrata Das, M. Ramesh and R . K. Rajan (2007). Growth rate pattern and economic traits of silkworm, *Bombyx mori* L under the influence of folic acid administration Journal of Applied Sciences and Environmental Management, Vol. 11, No. 4, pp. 81-84.

Rajitha K & Savithri G (2014). Day to day analysis of amylase and trehalose activity in the haemolymph of silkworm *Bombyx mori* L. infected with fungal pathogen *Beauveria bassiana* (bals.) vuill. Vol. 3, No. 1, 225-230.

Sasikala Ch & Ramana Ch.V (1995); Biotechnological Potentials of Anoxygenic Phototrophic Bacteria. Production of Single-Cell Protein, Vitamins, Ubiquinones, Hormones, Enzymes and Use in Waste Treatment Adv Appl Microbiol. 41:173-226.

Sengupta, K., Singh, B.D. and Mustafi, J.C.(1972). Nutrition of silkworm *Bombyx mori* L.1, studies on enrichment of mulberry with various sugars, proteins, amino acids and vitamins for vigorous growth of worms and increased cocoon production. Indian J.Seric., 11(1) : 11-19.

Sheeba Rajakumari, D.V. Padamalatha, C., Jeyapaul. C., Sam Manohar doss, and S., Ranjit Singh, AJ.A. (2005). Leucine and valine on the economic characters of silkworm *Bombyx mori* L. (Bombycidae : Lepidoptera). Journal of theorectical and experiemental Biology 1(4).

Sheeba Rajakumari, D.V., Padmalata, C., Das, S.M.C. and Singh, R.(2007). Indian J. Seric., 46(2):179-182.

Venkatesh Kumar R. and Amit Srivastava (2010). Relevance of antibiotics with reference to sericulture industry. I.J.S.N., VOL. 1(2): 97-100

Zafar Buhroo, Shabir A. Wani, Malik, M. A. and Ganai, M. A (2013). A review: disease resistance in mulberry silkworm *Bombyx mori*.L Asian Journal of Science and Technology Vol. 4, Issue 11, pp.157-166,.

16.3 Importance of the proposed project in the context of current status:

- Economic benefit resulting from reduced mortality of silkworms due to silkworm diseases by adopting the recommended formulation of phototrophic bacterial extracts as feed supplement
- Economic benefit resulting from the overall growth and development of silkworm which in turn aids in better yield and quality produce by adopting the recommended formulation of phototrophic bacterial extracts as feed supplement

16.4 Anticipated Products, processes / Technology, Packages / Information or other outcome from the project and their expected utility:

Photosynthetic bacterial extract formulations that assist in ecofriendly disease management, overall growth and development in silkworm.

16.5. Expertise available with proposed investigation group/institution on the subject of the project:

Name of the Scientists	Desig.	Experience
K. Rahul	Scientist- B	Isolation, cultivation, polyphasic characterization of bacteria (aerobic and anaerobic). Bacterial diversity studies by culture independent approaches: DGGE and BTEFAP analysis. Bioinformatics tools in phylogenetic analysis. Metabolite profiling by GCMS and LCMS analysis.
Zakir Hossain	Scientist D	Pathogenicity studies: Preparation of silkworm pathogenic suspensions and artificial induction of pathogenic suspension to *Bombyx mori*.

17. **Work Plan**

17.1 **Methodology**

Culturing of Anoxygenic phototrophic purple bacteria

The cultures of anoxygenic phototrophic purple bacteria will be cultured in Biebl and Pfennigs mineral salts media at 2400 lux under tube light illumination as the light source.

Authenticity of the culture, physiological and biochemical characterisation of the cultures

The authenticity of the pure cultures will be checked by 16S rRNA gene sequence analysis which will be outsourced. Physiological & biochemical characterisation of the isolates will be done as it is essential to know the growth conditions of an organism in order to culture them in bulk. Physiological & Biochemical characters of the type strains of anoxygenic phototrophic purple bacteria will be taken from literature and those of strains (which are not validly described) will be characterised. The physiological characterization of anoxygenic phototrophic purple bacteria involves checking the growth ranges and optimum of each bacterial isolate with respect to temperature, salinity and pH. The biochemical characterisation involves optimisation of growth of various bacterial strains with respect to various nitrogen, carbon, sulfur and vitamin sources. The physiological & biochemical characterisation helps us to know the optimum growth conditions of a bacterial strain which in turn helps us to culture the bacteria in bulk for biomass production. All the APB strains will be cultured in Biebl and Pfennigs mineral salts media at 2400 lux under tube light illumination as the light source.

Silkworm rearing

Silkworm *Bombyx mori* will be reared at Silkworm pathology laboratory on fresh mulberry leaves *ad lib* under ambient conditions as per the standard rearing methods. Care will be taken to ensure that all insects are healthy and robust at the time of experiment. Silkworms will be fed with untreated leaves until the end of 3rd instar.

Anoxygenic purple bacteria feed supplement preparation & feeding to the silkworm:

The different strains of selected anoxygenic purple bacteria will be grown under standard conditions. The cells will be harvested by centrifugation at 10,000 rpm for 10 min at the log phase (after studying the growth kinetics of bacteria spectrophotometrically). The cell pellet will be washed thrice with sterile water to remove traces of media components and spent wastes. The pellet will be laminar air dried or with a drier. The pellets will be diluted in different concentrations in sterile water and will be sprayed on the mulberry leaves and allowed to air dry for 15 minutes. The formulation coated leaves will be fed to the silkworm on the first day of 4th instar up to third day of 5th instar. The innocuous nature of the biomass will be checked. The leaves to be fed to the control worms will be sprayed with water and the water will be dried before feeding. Three replications will be maintained for each treatment.

Preparation of pathogenic suspension:

Polyhedra of *Bm*NPV will be extracted from 'grasserie' infected silkworm gut and will be purified by multi-layer gauze and centrifuged @ 6000 rpm and then re-suspended to 10^8 polyhedra per ml (Sun *et al*., 2016). Bacterial suspensions (10^7 cells per ml), measured in Neubauer haemocytometer, will be used for bioassay studies (Pachiappan *et al.*, 2009). Fungal conidia suspension will be prepared (2.15 x 10^6 conidia / ml @ 50 ml per 100 larvae) following standard methods (Rajitha *et al.*, 2014). The Lethal Concentration LC50 / LC90 shall be measured following standard procedure (Woolf, 1968).

Artificial induction of pathogenic suspension to *Bombyx mori*

Mulberry leaves will be freshly collected and dipped in pathogen suspension of selective dose as mentioned above and the leaves will be allowed to shade dry for 10 minutes. The silkworm larvae fed on mulberry leaves sprayed with distilled water serves as control. They will be fed to the freshly moulted fifth instar larvae after two hour starvation. Disease incidence (larval resistance to diseases) will be monitored.

Collection of haemolymph

Haemolymph samples will be collected at appropriate intervals (6h, 12h, 24h) after feeding the silkworm with anoxygenic purple bacteria feed supplement and haemolymph from silkworm fed on mulberry leaf without formulation will be used as control.

Metabolite profiling

Metabolites in the haemolymph samples will be checked by GCMS analysis after deravatization. **(This will be performed after initial screening of the extracts. Analysis will be performed only with extracts showing the best activity in terms of overall growth of silkworm and disease resistance).**

Recording of disease incidence, cocoon and reeling parameters:

Disease incidence and economic characters like larval weight, cocoon weight, pupal weight, shell weight, shell ratio and silk characters including filament length, non-breakable filament length and denier will be recorded.

Cocoon Parameters:

Larval weight: V^{th} stage (ten numbers) mature larvae will be weighed by using an electronic balance.

Shell weight: Ten shells of the good cocoon devoid of pupa (five male and five female) will be weighed using an electronic balance.

Pupal weight: Ten pupa (five male and five female) will be weighed using an electronic balance.

Cocoon weight: Summation of both shell weight and pupal weight.

Shell %: The Shell % will be calculated by using the following formula and expressed in percentage.

$$\text{Shell \%} = \frac{\text{Shell weight}}{\text{Cocoon weight}} \times 100$$

Reeling parameters

Filament length: It is the total length of silk filament, unwound from a single cocoon measured in meters. Cocoons from each replication will be stifled in boiling water and threads from individual cocoons will be reeled using an epprouvette and observed for silk filament length

Non-breakable filament length: It is the average length of filament that can be unwound from the cocoons without a break. Non-breakable filament length will be calculated by using the formula as follows.

$$\text{Average Non-breakable filament Length} = \frac{\text{Total filament length}}{1 + \text{No. of breaks}}$$

Denier: Denier is the unit, used to denote the thickness of silk filament. It is the weight of 9,000 m length of silk expressed in grams.

$$\text{Denier} = \frac{\text{Weight of the filament (g)}}{\text{Length of the filament (m)}} \times 9000$$

Gut microbiome studies: Gut contents of silkworm fed under normal diet and the enriched diet will be removed using sterile forceps under aseptic conditions and will be homogenized by shaking in sterile tube containing glass beads. Genomic DNA will be extracted by using standard genomic DNA isolation kits according to the manufacturers recommended protocol. 16S rRNA/18S rRNA gene will be amplified and pyrosequencing (culture-independent molecular approach as the traditional culture-based techniques possesses rather low sensitivity for measuring the composition, structure of microbiota colonizing the gut of silkworm) of the 16S rRNA/18S rRNA gene will be outsourced. **(This will be performed after initial screening of the extracts. Analysis will be performed only with extracts showing the best activity in terms of overall growth of silkworm and disease resistance).**

The economic feasibility of the proposed feed supplement will also be studied.

This will be performed after initial screening of the extracts. The feasibility of the extracts showing the best activity in terms of overall growth of silkworm and disease resistance will be evaluated. The extracts to be used in the study are cost-effective and it is expected that the result of the study will also be cost-effective and affordable by the farmers. The same will be calculated at the end of the study.

17.2 Organization of Work Elements

Name of Scientists	Desig.	Time	Organization of work elements
K. Rahul	Sci-B	70 %	Culturing of various strains of anoxygenic phototrophic purple bacteria, determining their authenticity and their biochemical and physiological characterisation, anoxygenic purple bacteria feed supplement preparation & feeding to the silkworm, metabolite profiling, gut microbiome studies, recording of disease incidence and economic characters.
Zakir Hossain	Sci-D	30 %	Zakir Hossain, Sci-D will be assisting in preparation of pathogenic suspension, artificial induction of pathogenic suspension to *Bombyx mori*, collection of haemolymph samples and overall monitoring of the project.

17.3 **Proprietary / Patented items, if any, expected to be used for this Project**: No.

17.4 **Suggested plan of action for utilization of the expected outcome from the project:**

Photosynthetic bacterial extract formulations that assist in eco-friendly disease management, overall growth and development in silkworm may be developed.

17.5 Time Schedule of activities giving milestones

#	Organization of work/Milestone /Activity	Period of study	
		Starting	**Completion**
1	Procurement of chemicals / equipments, establishment of illuminated culture room, preparation of the stock cultures or reviving the cultures from lyophilised vials and initiation of culturing of anoxygenic phototrophic purple bacterial strains	October, 2016	December, 2016
2	Purification of anoxygenic phototrophic purple bacterial strains, confirming the authenticity by 16S rRNA gene sequence analysis and physiological and biochemical characterisation of the selected anoxygenic phototrophic purple bacterial strains.	January 2017	March, 2017
3	Bulk growth of the anoxygenic phototrophic purple bacterial strains (purple non sulphur bacteria), harvesting of biomass, drying of pellets (feed supplement preparation) and *in vivo* screening / testing of preparation, Assessment of cocoon / reeling performance and disease incidence in breeds / hybrids	April, 2017	March, 2018

4	Bulk growth of the anoxygenic phototrophic purple bacterial strains (purple sulfur bacteria), harvesting of biomass, drying of pellets (feed supplement preparation) and In vivo screening / testing of preparation, Assessment of cocoon / reeling performance and disease incidence in breeds / hybrids	April, 2018	September, 2018
5	Combinatorial studies: Bulk growth of the anoxygenic phototrophic purple bacterial strains showing the best results in terms of overall growth and disease resistance in silkworm, harvesting of biomass, drying of pellets (feed supplement preparation), Making combinations of the pellets and in vivo screening / testing of preparation, Assessment of cocoon / reeling performance and disease incidence in breeds / hybrids	October, 2018	March, 2019
6	Metabolite profiling of haemolymph samples (by GCMS analysis) and gut microbiome studies (by pyrosequencing analysis) from silkworm when fed on normal diets and phototrophic bacterial extracts enriched diet (This will be performed after initial screening of the extracts. Analysis will be performed only with extracts showing the best activity in terms of overall growth of silkworm and disease resistance) and analysis of results. The economic feasibility of the proposed feed supplement will also be studied. Final report preparation and submission	April, 2019	September, 2019

17.6 **Project Implementing Agency / Agencies**

Name of the agency	Address of the agency	Proposed Research Aspects	Proposed Amount	Cost Sharing %
Central Silk Board, Bangalore	BTM Layout Madiwala Bangalore	Silkworm Crop protection	18.00 lakh	100 %

PART-IV: BUDGET PARTICULARS

18. BUDGET (in Lakhs): [In case of multi-institutional projects, the budget details should be provided separately for each of the Institute]

TOTAL: 18.00 Lakh

A. Non-Recurring (e.g. equipments, accessories, etc.) *
[Total – 7.50]

#	Item	Year 1	Year 2	Year 3	Total (Rs in lakh)
1.	Refrigerator	0.50			0.50
2.	Spectrophotometer	3.00			3.00

3.	Gel documentation system	4.00			4.00
	Subtotal A	**7.50**			**7.50**

* -If the procurement of equipment is not completed within the time frame mentioned (first 3 months from the start date of project) it will be carried forward to the next quarter .

B) Recurring
[Total – 10.50]

B.1. Manpower: The help of Sectional staff (**One Technical Assistant and one SFW**) will be taken for the smooth running of the project.

#	Position No.	Consolidated Emolument	Year 1	Year 2	Year 3	Total (Rs)
1	Technical Assistant	Nil	Nil	Nil	Nil	Nil
2	SFW	Nil				

B.2 Consumables

#	Item	Qty	Year 1	Year 2	Year 3	Total (Rs)
1.	Chemicals (Media components, reagents, DNA isolation kits etc.)		2.00	2.00	2.00	6.00
Sub total						**6.00**

Other items	**Year 1**	**Year 2**	**Year 3**	**Total (Rs)**
B.3 Contingency:	1.00	1.00	1.00	3.00
Sub total B.3				**3.00**
B.4 Travel :	0.50	0.50	0.50	1.50
Sub total B.4				**1.50**
Sub-total of B (B.1+B.2+B.3+B.4)				**10.50**
Grand total (A+B)				**18.00**

Total cost of the project Rs: 18.00 lakhs

PART-V: EXISTING FACILITIES

19. Available equipment and accessories to be utilized for the project:

CSR&TI, Berhampore (SWPathology Section)

#	Name of the instruments	Make	Source	Year of Manufacture
1	Refrigerated Centrifuge	Remi	CSB	2007
2	Table-top Centrifuge	Remi	CSB	2006
3	Sartorius water purification system	Germany, 61316 RO and 611 DI	CSB	2007
4	Stereoscopic Binocular Compound Microscope	Wild Heerburgg M8, Make - Leitz	CSB	1993
5	Autoclave	Spac & services	CSB	-
6	Electronic balance	Anamed	CSB	-
7	Laminar air flow	Digitech	CSB	2016
8	BOD incubator	Indosati	NSSO	2016
9	Refrigerator	LG	CSB	-

PART VI: DECLARATION/CERTIFICATION

It is certified that

a) the research work proposed in the scheme/project does not in any way duplicate the work already done or being carried out elsewhere on the subject.
b) the same project proposal has not been submitted to any other agency for financial support.
c) the emoluments for the manpower proposed are those admissible to persons of corresponding status employed in the institute/university or as per the Ministry of Science & Technology guidelines (Annexure-III)
d) necessary provision for the scheme/project will be made in the Institute/University/State budget in anticipation of the sanction of the scheme/project.
e) if the project involves the utilisation of genetically engineered organisms, we agree to submit an application through our Institutional Biosafety Committee. We also declare that while conducting experiments, the Biosafety Guidelines of the Department of Biotechnology would be followed in toto.
f) if the project involves field trials/experiments/exchange of specimens, etc. we will ensure that ethical clearances would be taken from concerned ethical Committees/Competent authorities and the same would be conveyed to the Department of Biotechnology before implementing the project.
g) it is agreed that any research outcome or intellectual property right(s) on the invention(s) arising out of the project shall be taken in accordance with the instructions issued with the approval of the Ministry of Finance, Department of Expenditure, as contained in Annexure-V.
h) we agree to accept the terms and conditions as enclosed in Annexure-IV. The same is signed and enclosed.
i) the institute/university agrees that the equipment, other basic facilities and such other administrative facilities as per terms and conditions of the grant will be extended to investigator(s) throughout the duration of the project.
j) the Institute assumes to undertake the financial and other management responsibilities of the project.

Director
Chief Executive Authority
Date:…….

(K. Rahul)
Scientist-B
Principal Investigator
Date: 06-10-2016

(Zakir Hossain)
Scientist-D
Co Investigator
Date:07-10-2016