A new research project proposal

Studies on synbiotics (combination of Probiotic and Prebiotic) induction for control of common diseases of silkworm, *Bombyx mori* L.

2014-16

Scientists

Dr.S.Chakrabarty, Scientist-B: Principal Investigator
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Silkworm Pathology Section
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Central Silk Board, Govt. of India, Ministry of Textiles
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PROFORMA – I (To be filled by applicant)

PART I: GENERAL INFORMATION

1. Name of the Institute / University / Organization submitting the Programme Proposal:

Central Sericultural Research & Training Institute, Berhampore - 742101

2. Status of the institute(s): Research & Development

3. Name(s) and designation(s) of the Executive Authority of the Institute /University forwarding the application:

Dr. S.Nirmal Kumar, Director, Central Sericultural Research & Training Institute, Berhampore-742101

4. Programme title:

Studies on synbiotics (combination of Probiotic and Prebiotic) induction for control of common diseases of silkworm, *Bombyx mori* L.

5. Category of the programme: Animal (A)

6. Specific area: Silkworm Protection

7. Duration: Two years

8. Total cost: Rs. 2.00 lakh

9. Is the programme single institutional or multi-institutional: Single institutional

10. If the Project is multi-institutional, please furnish the following: Name, Designation and Address of the Programme coordinator: Not applicable

11. Project summary:

Nutrition plays an important role in improving the growth and development of silkworm, *Bombyx mori* like other organisms. Silk production is dependent on larval nutrition. Nutritive value of mulberry leaves plays an important role in producing good quality of cocoon. As cellular defence mechanism are failed to control the disease and 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 immuno-stimulation could be achieved easily through feed supplement of Probiotics. Various researches have been carried out on the diet supplementation of mulberry leaves which is fed to silkworms. In recent years attempts have been made in sericulture with feed supplement of nutrients for production of good quality cocoons; thereby improve the quality of silk. Current research has found that probiotics contain live beneficial bacteria play a large role in reducing inflammation and disease symptoms of various diseases. The digestive system is home to different types of bacteria. They help keep the intestines healthy and assist in digesting food. They are also believed to help the immune system. These friendly organisms also help fight bacteria that cause diarrhoea. In probiotic therapy, live microbial feed supplements are improving the intestinal microbial balance of host. These non-pathogenic bacteria play a key role in enhancing resistance to colonization by exogenous potentially pathogenic organism. Probiotics inhibit microbial pathogen growth in the intestines. Probiotics can stabilize the structure in the intestinal barrier and maintain rigidity in the tight junctions between epithelial cells. Probiotics can also stimulate the body's innate defense mechanisms. As research continues to expand upon the use of probiotics with active disease, evidence continues to supports the use of oral probiotic supplements to help. Today, a mixture of live microorganism

(**probiotic**) and non-digestible oligosaccharides (**prebiotic**) have been demonstrated to modify the composition of the micro flora, restore the microbial balance and therefore have the potential to provide health benefits when normal intestinal flora is disturbed due to diarrhoea, food intoxification etc.

It is reported that humoral immune system has efficient self-defense mechanism against infection through induction of synbiotics (combination of prebiotic and prebiotic) one of the promising alternatives for passive immunization and simultaneously growth and development of the silkworm. Scientists of Silkworm Pathology Section has reported that feed supplement (*Oral immunization*) of probiotics contain, *Lactobacillus acidophillus* controlled bacterial disease ~ 88.62 % and other diseases ~ 55.25 % during unfavourable seasons (June – September) in multivoltine breeds, Nistari(M), M12(w) and M6DP(c).The combination of probiotics and prebiotics in a synbiotic preparation, has not been studied in silkworm earlier that might improve the survival of the beneficial bacteria enhancing their effects might be additive or even synergistic. Therefore, effort has been taken for making a **synbiotic preparation,** combination of **probiotic and prebiotic** to study the synergistic effect of the **live microorganisms** to control silkworm diseases, thereby increases the cocoon productivity.

PART II: PARTICULARS OF INVESTIGATORS

12.1.

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Name: Dr. A.K.Saha
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Institute / University:

PART III: TECHNICAL DETAILS OF THE PROGRAMME

15. Introduction
15.1. Definition of the problem

Crop loss due to diseases is a common factor but that annoys always when it surpasses the economic injury level. There is an average 40 % crop loss is attributed due to silkworm diseases in India. The disease incidence is prevalent during hot and humid seasons. Several

disease prevention options are available. These include chemical prevention measure based on direct antibiotic principle through innovation of different disinfectant. Due to the enormous use of disinfectants most of the pathogens are becoming resistant to those disinfectants and their efficacy in killing pathogens are decreasing even with higher doses of chemicals. The biological control measures and development of disease tolerant silkworm breeds are found to be insufficient to control the diseases. Presently studies are focused on resistance and immunity as alternative options to control the disease as innate immunity in multicellular organism is the first line of inducible host defense against invading bacterial, fungal, and viral pathogens. In insects, two different innate immune systems exist - cellular and humoral. Silkworms are easily and frequently attacked by pathogens due to failure of cellular defense system as Total Haemocyte Count (THC) remains almost same for a particular breed, so there is no way to increase further disease tolerance through cellular defence response of the silkworm breeds, whereas multiplication of pathogen is continue with high multiplication rate. As cellular defence mechanism are failed to control the disease and 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 immuno-stimulation could be achieved easily through feed supplement of Probiotics. Humoral immune system has efficient self-defense mechanism against infection through induction of synbiotics (combination of prebiotic and prebiotic) one of the promising alternatives for passive immunization and simultaneously growth and development of the silkworm.

15.2. Origin of the Proposal / Rationale of the Study

The digestive system is home to more than 500 different types of bacteria. They help keep the intestines healthy and assist in digesting food. They are also believed to help the immune system (Martin, 2001). These friendly organisms also help fight bacteria that cause diarrhea.

Probiotics: The term "probiotics" was first introduced in 1953 by Wener Kollath (Hamilton-Miller *et al*., 2003). Etymologically, the term appears to be a composite of the Latin preposition *pro* ("for") and the Greek adjective *βιωτικός* (biotic), the latter deriving from the noun *βίος* (bios, "life"). Various workers defined probiotics. Probiotics are live bacteria that may confer a health benefit on the host. The most promising health benefits are the prevention of diarrhea and enhancement of the immune system (Lilly and Stilwell, 1965). Probiotics are organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance (Parker in 1974). Probiotics are the live microbial food supplements beneficially affecting host by improving the microbial balance and enhanced the rapid cellular growth and development (Fuller *et al*., 1993). Probiotics are living ‘good’ bacteria intended to benefit colon health. These "good" bacteria have many important functions, such as helping to support your immune system and digestive tract. But good bacteria must be well-fed to perform at their best. Probiotics nourish the thousands of good bacterial species already living in the colon. Probiotics contain from one to a few species of bacteria which are added to the colon when they are ingested (eaten). Probiotcs occur naturally in fermented foods like curd. Probiotics may impact on bad bacteria by crowding them out. Probiotics are already widely used to prepare fermented dairy products that are becoming popular in India, Europe and Japan. These products favorably influence digestive functions and colonic flora.

Prebiotics: Prebiotics are specific kinds of fibers that nourish the friendly, "good" bacteria that live in your digestive system. These ‘good’ bacteria, called probiotics, have many important functions. The prebiotic fiber help the ‘good’ bacteria in digestive system grow and thrive, supporting overall health. Prebiotics are non-digestible substances that provide a beneficial physiological effect for the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria. Prebiotics are special form of dietary fiber. Prebiotics fiber is not affected by heat, cold, acid or time. Prebiotic Fiber is a naturally occurring, substance, found in thousands of plant species, though mostly in very small amounts. Prebiotics foster an environment in the colon which is hostile to bad bacteria. The benefits of prebiotics are supported by extensive research. Prebiotics pass though the stomach and small intestine unchanged. Thus far only two fructooligiosaccharides: oligofructose and inulin, fully meet the complete medical definition of ‘prebiotic’. The compound created from merging these two

prebiotics together is called Oligofructose-Enriched-Inulin and is considered a 'full-spectrum' prebiotics. Some foods presented as 'prebiotics' in and of themselves simply contain prebiotics. For example we often see honey presented as 'a prebiotic', while it is more accurate to simply say that honey contains a small amount of prebiotics, as do many other foods. Prebiotics enter colon where they nourish beneficial bacteria. The beneficial bacteria, typically within the hundreds of strains under the *Lactobacillus* and *Bifidobactor* families, create many health benefits through their action in the colon.

Synbiotics: Products that contain both probiotics and prebiotics. It is a mixture of live microorganisms (probiotic) and non-digestible oligosaccharides (prebiotic). Both prebiotics and probiotics must be ingested in sufficient quantity to have an impact and should not carry an excessive 'load' of sugar, calories, carbs etc. out of proportion to their benefit. One area where both prebiotics and probiotics are the same is that they must not bring excess calories, carbs, sugar, fat or other undesirables to the dietary mix. Probiotics often come in heavily-sugared yogurts and similarly, prebiotics sometimes arrive via a 'fiber bar' with chocolate icing, lots of sugar, etc.

15.3. Relevance to the current Issues and expected outcome

There is suggestive evidence that several probiotic strains with the prebiotic, oligofructose are useful in boosting the immune response. Indirect evidence has been obtained in studies aimed at preventing acute infectious disease (nosocomial diarrhoea in children, influenza episodes in winter) and studies that tested antibody responses to vaccines. Nowadays, the microbes *Lactobacilli* and *Bifido* bacteria are widely used in probiotic therapy. These bacteria are producing lactic acid that constitutes a major part of the normal intestinal microflora in all animals. Hence, the present attempt is a very good approach on *B.mori* to strengthen the host immunity to resist the microbial pathogenic attack and to promote good cocoon yield.

15.4. Objectives

1. Application of synbiotics (combination of probiotics and prebiotics) for eco-friendly silkworm disease management
2. Strengthen the immunity of silkworm to resist the microbial pathogenic attack
3. To promote good cocoon yield

16. Review of status of research and development on the subject:

16.1 International status:

The digestive system is home to more than 500 different types of bacteria. They help keep the intestines healthy and assist in digesting food. They are also believed to help the immune system (Martin, 2001). These friendly organisms also help fight bacteria that cause diarrhea. The original observation of the positive role played by certain bacteria was first introduced by Russian scientist and Nobel laureate Elie Metchnikoff, who in the beginning of the 20th century suggested that it would be possible to modify the gut flora and to replace harmful microbes with useful microbes. Metchnikoff had also observed that certain rural populations in Europe, for example in Bulgaria and the Russian steppes who lived largely on milk fermented by lactic-acid bacteria were exceptionally long lived. Based on these facts, Metchnikoff proposed that consumption of fermented milk would "seed" the intestine with harmless lactic-acid bacteria and decrease the intestinal pH and that this would suppress the growth of proteolytic bacteria. Metchnikoff himself introduced in his diet sour milk fermented with the bacteria he called "Bulgarian Bacillus" and found his health benefited. Friends in Paris soon followed his example and physicians began prescribing the sour milk diet for their patients.

Bifidobacteria were first isolated from a breast-fed infant by Henry Tissier . Tissier found that Bifidobacteria are dominant in the gut flora of breast feed babies and he observed clinical benefits from treating diarrhoea in infants with *Bifidobacteria*. The claimed effect was Bifidobacterial displacement of proteolytic bacteria causing the disease.

Silkworms are attacked frequently by pathogens due to failure of cellular defense system. Humoral immune system has efficient self-defense mechanism against infection through induction of defense molecules. These defence molecules include phenoloxidases, clotting factors, complement factors, lectins, protease inhibitors, antimicrobial proteins, Toll receptors, and other humoral factors found mainly in hemolymph plasma and hemocytes. These components, which together compose the innate immune system, defend invertebrate from invading bacterial, fungal and viral pathogens. 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 immuno-stimulation could be achieved easily through Probiotics.

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 good quality of cocoons (Sannappa, 2002).

The digestive system is home to more than 500 different types of bacteria. They help keep the intestines healthy and assist in digesting food. They are also believed to help the immune system. These friendly organisms also help fight bacteria that cause diarrhoea. In probiotic therapy, live microbial feed supplements are improving the intestinal microbial balance of host. These non-pathogenic bacteria play a key role in enhancing resistance to colonization by exogenous potentially pathogenic organism. Many bacterial strains have been evaluated for ability to normalize the properties of abnormal native microflora and reinforce various aspects of intestinal defense. Probiotics inhibit microbial pathogen growth in the intestines. Probiotics can stabilize the structure in the intestinal barrier and maintain rigidity in the tight junctions between epithelial cells. Probiotics can also stimulate the body's innate defense mechanisms, as with the increased production of the antimicrobial peptide 'defensins' in the intestines. As research continues to expand upon the use of probiotics with active disease, evidence continues to supports the use of oral probiotic supplements to help.

Current research has found that probiotics, or live beneficial bacteria, can also play a large role in reducing inflammation and disease symptoms of various diseases. In probiotic therapy, live microbial feed supplements are improving the intestinal microbial balance of host. These non-pathogenic bacteria play a key role in enhancing resistance to colonization by exogenous potentially pathogenic organism (Orhange and Nord, 2000).

Many bacterial strains have been evaluated for ability to normalize the properties of abnormal native microflora and reinforce various aspects of intestinal defense. Probiotics inhibit microbial pathogen growth in the intestines by inhibiting their ability to attach to the gut and colonize. *Lactobacillus plantarum in vitro* model demonstrated its ability to prevent adherence of a pathogenic strain, as well as increased the expression of protective proteins called mucins. Colonized probiotics ferment dietary fiber, and in doing so can induce pH and other chemical changes in the intestinal lumen (cavity) that also affect the inhibition of pathogen growth. Additionally, short-chain fatty acids are released as a byproduct of bacterial fermentation display anti-inflammatory properties in the epithelial (intestinal lining) cells. Probiotics can stabilize the structure in the intestinal barrier and maintain rigidity in the tight junctions between epithelial cells. Probiotics can also stimulate the body's innate defense mechanisms, as with the increased production of the antimicrobial peptide 'defensins' in the intestines. As research continues to expand upon the use of probiotics with active disease, evidence continues to supports the use of oral probiotic supplements to help.

Today, a mixture of live microorganism (probiotic) and non-digestible oligosaccharides (prebiotic) have been demonstrated to modify the composition of the micro flora, restore the microbial balance and therefore have the potential to provide health benefits when normal intestinal flora is disturbed due to diarrhea, food intoxification etc. Probiotics prevent infections due to competition for binding sites and available substrates, lowering luminal PH, production of 'bactericins' and production of other antibacterial substances enhancement of intestinal motility

and up gradation of genes mediating innate immunity. Prebiotic promote the bifidobacterial growth. As innate immunity is an important defense system in *B.mori* , the non-pathogenic bacteria enhanced the immunity factors and reduced the susceptibility to bacterial pathogenic infections in *B.mori*.

As insect belong the same family (Rel family) with the mammals, the synergistic combination of probiotics may be used as more effective immunogenic in the study for development of antimicrobial property i.e., detergent properties of antibacterial proteins disrupt the cell membranes of the invading pathogen and lowering microbial lipoprotein as well as resisting it to penetrate vascular wall of the silkworm, thereby control silkworm diseases and increase cocoon productivity. Over the past several years, proteins belonging to the Rel family have been demonstrated to work in intracellular signalling for the activation of acute-phase protein genes in both mammals and insects. In the extracellular signalling of the recognition of microbes as foreign, insects and mammals have also been shown to employ homologues. Toll, a receptor on the fat body (an organ equivalent to the vertebrate liver) of *Drosophila* (3), and Toll-like receptor 2 on peripheral blood leukocytes of humans (4) have been indicated to work in the transduction of extracellular signals. The peptidoglycan recognition protein (PGRP) 1 is another example where homologues have been demonstrated or suggested to take part in the extracellular recognition of foreignness in the innate immunity of both mammals and insects. These common employments of homologues in the intracellular and extracellular signalling mechanisms indicate close links between innate immunity of mammals and insects [Lemaitre *et.al*. (1996) *Cell* 86, 973 – 983; Medzhitov *et.al*., (1997) *Nature* 388, 394 - 397; Kang, *et. al,* (1998). *Proc. Natl. Acad. Sci. U. S. A.* 95, 10078 -10082; Ochiai and Ashida (1999) *J. Biol. Chem.* 274, 11854 –11858].

As innate immunity is an important defense system in *B.mori* (Ponnuvel and Yamakawa, 2002) the non-pathogenic bacteria enhanced the immunity factors and reduced the susceptibility to bacterial pathogenic infections in *B.mori*. Scientists of Silkworm Pathology Section has already studied control of silkworm diseases through induction of different immunogens and it has been found that individual induction of Proline, Nicotinic acid and Ascorbic acid are very much effective for immunization to control the silkworm diseases to the tune of 67.33 ~ 84.22% in general and bacterial disease, 88.80 ~ 90.25 %, thereby increases the cocoon productivity. Oral immunization with *Lactobacillus acidophillus* has effected satisfactory result when mortality% was recorded significantly (P< 0.1) lower (27.0 %) compare to micronutrient supplement. The combination of probiotics and prebiotics in a synbiotic has not been studied. This combination might improve the survival of the bacteria crossing the upper part of the gastrointestinal tract, thereby enhancing their effects in the large bowel. In addition, their effects might be additive or even synergistic (*Am J Clin Nutr.*, 2000). Therefore, effort has been taken for making a synbiotic preparation, combination of probiotic and prebiotic to study the synergistic effect of the live microorganisms to control silkworm diseases, thereby increases the cocoon productivity. *Bifidobacterium bifidum*, known as *B. bifidum*, is a strain of bacteria commonly used as a probiotic. Probiotics are living microorganisms that simulate the beneficial bacteria found in the gastrointestinal tract of humans. *B. bifidum* is one of many bacterial strains that occur naturally in the gut flora, living in the colon. The inclusion of *B. bifidum* in a dietary regimen provides the host with a variety of following benefits (Erica Wickham, 2003).

Vitamin Synthesis: *B.bifidum* aids in the synthesis of B-complex vitamins and vitamin K in the intestines. This synthesis protects the body from deficiencies of these vitally important nutrients. The primary function of vitamin K is to regulate the blood clotting process. Vitamin K is also necessary to improve bone health, prevent bone fractures and reduce the risk of bleeding associated with long-term antibiotic use. B-complex vitamins are also essential to good health as they aid in energy production, promote normal growth and development, metabolize protein and carbohydrates, maintain nervous system function and aid in the creation of red blood cells (Erica Wickham, 2003).

Improved Immunity and Digestion: Supplementing the diet with B. bifidum helps improve digestion and enhance the immune phagocytic activity of the human body. First, B. bifidum promotes bacterial balance and optimal digestion, thereby discouraging the production of histamine, a chemical responsible for triggering an allergic reaction. Second, B. bifidum

enhances the body's natural antibody immune response. Therefore, regular B. bifidum in the diet fights against intestinal pathogens, digestive irregularities and histamine production, ultimately improving the body's immunity and avoiding the onset of allergic reactions (Erica Wickham, 2003).

Diarrhoea Treatment: Probiotics such as *B. bifidum* are especially effective in the treatment of diarrhea, including infectious diarrhea and diarrhea associated with antibiotic use. The probiotics are even safe for children with diarrhea. The restoration of bacterial symbiosis within the gastrointestinal tract as a result of probiotic supplementation results in a decrease in stool frequency, fecal weight and abdominal cramps (Erica Wickham, 2003).

Cancer Prevention: The article "Bifidobacterium as Probiotic Agents -- Physiological Effects and Clinical Benefits" states that bacterial irregularity of the intestinal flora influences the creation of cancer cells by producing enzymes that transform normal cells into active carcinogens. Harmful bacteria in the colon promote tumor production and tumor transformation in the gut. Evidence suggests that probiotics such as *B. bifidum* protect the host from activities within the body that cause the growth and transformation of healthy cells into cancer cells (Erica Wickham, 2003).

Lactobacillus acidophilus (Latin meaning *acid-loving milk-bacterium*) is a species in the genus *Lactobacillus*. *L. acidophilus* is a homofermentative species, fermenting sugars into lactic acid, and grows readily at rather low pH values (below pH 5.0) and has an optimum growth temperature of around 37 °C (99 °F). (Bâati *et.al*., 2000). *L. acidophilus* occurs naturally in the human and animal gastrointestinal tract, mouth, and vagina. Some strains of *L. acidophilus* may be considered to have probiotic characteristics (Ljungh *et.al*., 2006). These strains are commercially used in many dairy products, sometimes together with *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* in the production of acidophilus-type yogurt.

Health effects: Some strains of *L. acidophilus* have been studied extensively for health effects. Some *L. acidophilus* strains may be able to survive gastrointenstinal transit, being resistant to bile, low pH, and digestive enzymes. They may then be able to adhere to human epithelial cell lines and human intestinal mucus (Yuan-Kun Lee, 2009). A blend of bacterial strains including *L. acidophilus NCFM* decreased the incidence of pediatric diarrhea. *L. acidophilus* led to a significant decrease in levels of toxic amines in the blood of dialysis patients with small bowel bacterial overgrowth. At adequate daily feeding levels, *L. acidophilus* may facilitate lactose digestion in lactose-intolerant subjects (Sanders and Klaenhammer, 2001). A University of Nebraska study found that the *L acidohilus* feed on human skin from the inside out.supplemented with *L. acidophilus L1* and fed to cattle resulted in a 61% reduction of *Escherichia coli* O157:H7 (http://ard.unl.edu/rn/0902/ecoli.html). Research has indicated *L. acidophilus* may be helpful reducing serum cholesterol levels (Anderson J, Gilliland S, 1999).

Antibiotics taken orally will also kill beneficial bacteria, including *L. acidophilus*. After a therapy that includes antibiotics, patients are occasionally instructed to take an *L. acidophilus* treatment in order to recolonize the gastrointestinal tract (Yuan-Kun Lee, 2009). To that effect, *L. acidophilus* is often sold in health stores in pill or powder form as a nutritional supplement, as well as being available in many yogurts. A part of the claims in favor of such treatment refer to attaining a better digestion thanks to a recovered normal intestinal flora. *L. acidophilus* LA-5 produces 'bacteriocin CH5' that is both antibacterial and inhibitory against certain yeasts and molds and is effective against both *Salmonella typhimurium* and *Campylobacter jejuni* (Yuan-Kun Lee, 2009). It has been shown to improve bowel regularity and has been shown to have a preventative effect against traveller's diarrhea, as well as antibiotic related bowel issues (Yuan-Kun Lee, 2009).

Because of its relation to gut-associated lymphoid tissue (GALT), *L. acidophilus* LA-5 has been associated with positive effects on the immune system such as increased cytokine, phagocytic activity and antibody production, as well as phagocytosis of *Salmonella* and *L. acidophilus* NCFM has even been shown to reduce incidence of symptoms of fever, cough and runny nose (Yuan-Kun Lee, 2009). Anti-inflammatory effects have also been observed in people consuming *L. acidophilus* NCFM. Additionally *L. acidophilus* LA-5 has shown to inhibit growth of

breast cancer cells, and positive effects on chemotherapy patients (Yuan-Kun Lee, 2009). An improvement of lipid metabolism has also been linked to *L. acidophilus* LA - 5 (Yuan-Kun Lee, 2009). Animal studies of NCFM have indicated that it reduces intestinal pain by inducing u-opioid and canabinoid receptors in the intestines (of animals), but this effect has not been sufficiently shown in humans yet (Yuan-Kun Lee, 2009). Other benefits of *L. acidophilus* include the production of vitamin K and lactase, and some strains may produce bacteriocins such as acidolin, acidophilin and lactocidin. A study published in the Journal of the American College of Nutrition reported that yogurt containing *L. acidophilus* L1 has the potential to reduce risk for coronary heart disease by 6 - 10% by reducing serum cholesterol concentration.

Matthias Rath M.D. (1992) presented new therapeutic approaches of **nutritional supplements** to achieve this therapeutic aim.Dietary supplementation of L-proline could prevent the binding of LDL to lipoprotein (a) already deposited in the vascular wall and, release already deposited LDL from the atherosclerotic lesions.

Uses for lactose intolerance: There are many fermented dairy products that use *L. acidophilus* including yogurt and some types of cheese. Sweet acidophilus milk is consumed by individuals who suffer from lactose maldigestion and intolerance, which occurs when enzymes (lactase) cannot break down lactose (milk sugar) in the intestine. Failure to digest lactose results in discomfort, cramps and diarrhea (Roos and Katan, 2000). Some bacteria have been shown to improve lactose digestion by providing *β-galactosidase*, while some *L. acidophilus* strains have been linked to improvement in symptoms and indicators of lactose indigestion (Yuan-Kun Lee, 2009).

Strains with described health effects:

Strain	**Producer**
Lactobacillus acidophilus DDS-1	Nebraska Cultures[12]
Lactobacillus acidophilus LA-5	Chr. Hansen
Lactobacillus acidophilus NCFM	Danisco

Vaginal microbiota (flora): While *Lactobacillus* species are part of the vaginal flora, the most common species are *Lactobacillus crispatus*, *L. gasseri*, *L. jensenii*, and *L. iners* -- modern identification methods have not found *L. acidophilus* to be common in the vagina (Forsum, *et al.,*2005; Holmes, King, *et al*., 2007). In lab experiments, *L. acidophilus* seemed to decrease *Candida albicans*' ability to adhere to vaginal epithelial cells; however, *L. acidophilus*' role in preventing yeast infections is unclear because this species of *Lactobacilli* has also been found not to have a very strong ability to adhere to (and thereby colonize) the vaginal cells. Certain spermicides and contraceptive creams can kill *Lactobacillus* species in the vagina, clearing the path to possible yeast infections.

Research into the potential health effects of supplemental probiotics has included the molecular biology and genomics of *Lactobacillus* in immune function, cancer, antibiotic-associated diarrhoea, travellers' diarrhoea, pediatric diarrhoea, Lactose intolerance, Blood cholesterol, Blood pressure, Loss of vitamin, Eczema etc.Preliminary research is evaluating the potential physiological effects of **multiple probiotic strains**, as opposed to a single strain. As the human gut may contain several hundred microbe species, one theory indicates that this diverse environment may benefit from consuming multiple probiotic strains, an effect that remains scientifically unconfirmed. Members of the *Bacillus cereus sensu lato* species group are frequently found in invertebrates. *B. cereus* has been identified in the gut of numerous insects, including aphids, mosquito larvae and cockroaches and in certain arthropods this organism exists in a special filamentous or 'Arthromitus' stage within the intestine. *B. cereus* as well as *B. mycoides* in the vegetative form has also been found in abundance in the gut of the earthworm. *B.anthracis* has been found in the faeces of tabaniid flies (various horse and deer flies) and this is believed to help disseminate and transmit anthrax. *B. thuringiensis* is considered an insect pathogen due to its unique ability to produce large crystal protein inclusions during sporulation. These inclusions have bio-pesticide activity and are active against larvae from different insect

orders including Lepidopetera, Diptera and Coleoptera. *B. thuringiensis* does not grow in the soil, yet its presence there is believed to arise from insect deposition and it has been shown to proliferate in the earthworm gut. It has been suggested that members of the *B. cereus sensu lato* species group possess two life cycles, one where the bacteria live in a symbiotic relationship with their invertebrate host and a second life cycle where they can proliferate in a second invertebrate or vertebrate host. Other *Bacillus* species found in the gut of insects include *B. licheniformis*, *B. cereus*, *B. sphaericus*, *B. circulans*, *B. megaterium*, *B. alvei*and *B. pumilus*. As well as *B. thuringiensis* a number of other spore formers are insect pathogens that gain entry to the host via the GIT, these include *Paenibacillus larvae* (formerly *Bacillus larvae*) that infects domestic honeybees and two species that produce parasporal crystals and are pathogenic to larvae of various *Coleoptera*, *Paenibacillus popillae* (formerly *Bacillus popillae*) and *Paenibacillus lentimorbus* (formerly *Bacillus lentimorbus*).

Nutrition plays an important role in improving the growth and development of silkworm *Bombyx mori* like other organisms (Legay, 1958). As the 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 nutrient such as proteins, carbohydrates, amino acids, vitamins, hormones, and antibiotics etc. for better performance of good quality of cocoons (Sannappa, 2002). Various researches have been carried out on the diet supplementation of mulberry leaves which is fed to silkworms. 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 immuno-stimulation could be achieved easily through Probiotics. The *lactobacillus plantarum* is a probiotic which improves the cocoon production of mulberry silkworm *Bombyx mori* (Singh *et al*., 2005). Certain probiotic bacteria inhibit the growth of microbes. *Streptomyces noursei* are probiotic microbes which prove the antibacterial activity and good eco-friendly management of silkworm diseases. *Streptomyces noursei* isolated from silkworm breeds revealed their antibiotic potential against a range of Gram positive and Gram negative bacteria and it was found to inhibit the germination of conidia of entomopathogens *B. bassiana and M. anisopliae in vitro* (Mohanraj, 2007). Probiotic applications of *S. noursei* have resulted in increase of endogenous actinomycetes population by 123.08 and 141.86 %, respectively in PM and CSR2. Application of probiotics has paved way for eco-friendly silkworm disease management (Subramanian *et al*., 2009).Recently efficacy of yeast as a nutrient supplement had been tried in sericulture. Probiotic supplementations improved commercial characteristics, disease resistance and protein in the silkworm, *Bombyx mori L.* Feed supplementation not only enhanced economic and nutritional parameters but also prevent bacterial infection in *B.mori* (Amala Rani *et. al.,*2011). wjbr.interscholar.org.

Mode of action: Prebiotics affect intestinal bacteria by increasing the numbers of beneficial anaerobic bacteria and decreasing the population of potentially pathogenic microorganisms. Probiotics affect the intestinal ecosystem by stimulating mucosal immune mechanisms and by stimulating non-immune mechanisms through antagonism / competition with potential pathogens. These phenomena are thought to mediate most beneficial effects, including reduction of the incidence and severity of diarrhea, which is one of the most widely recognized uses for probiotics. Probiotics reduce the risk of colon cancer in animal models, probably due to their role in suppressing the activity of certain bacterial enzymes that may increase the levels of pro-carcinogens. Probiotics prevent infections due to competition for binding sites and available substrates, lowering luminal PH, production of 'bactericins' and production of other antibacterial substances enhancement of intestinal motility and up gradation of genes mediating innate immunity. Prebiotic promote the bifidobacterial growth. As innate immunity is an important defense system in *B.mori* , the non-pathogenic bacteria enhanced the immunity factors and reduced the susceptibility to bacterial pathogenic infections in *B.mori*.

Probiotic and host interaction: When some digestive disorders happen due to infection or after taking antibiotics, the balance of friendly bacteria in the intestines becomes disturbed. Intestinal problems can also arise when the lining of the intestines is damaged. Taking probiotics help to repair. "Probiotics can improve intestinal function and maintain the integrity of the lining of the intestines," (Stefano Guandalini, 2001). Symbiosis between microbiota and the host can be optimized by pharmacological or nutritional interventions in the gut microbial ecosystem using probiotics or prebiotics. Probiotic as Immunologic action: There's also evidence

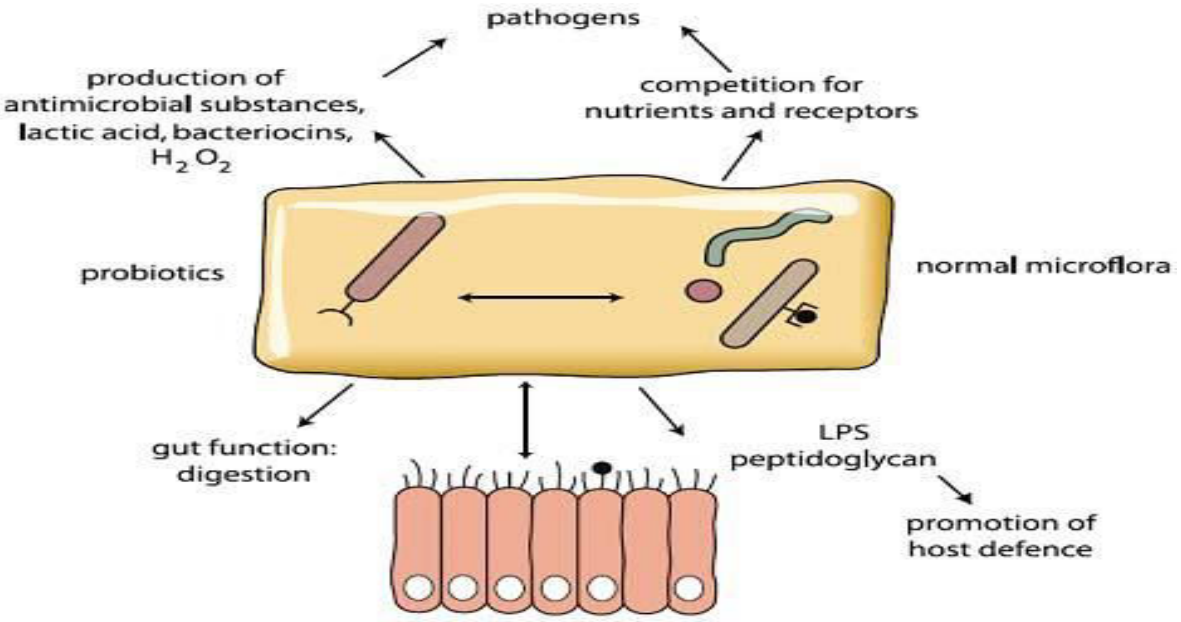
that probiotics help and maintain a strong immune system. “In societies with very good hygiene, a sharp increase in autoimmune and allergic diseases,” (Guandalini,2000).“That may be because the immune system isn’t being properly challenged by pathogenic organisms. Introducing friendly bacteria in the form of probiotics is evidenced to challenge the immune system in healthy ways.”Activate local macrophages to increase antigen presentation to B lymphocytes and increase secretory immunoglobulin A (IgA) production both locally and systemically Modulate cytokine profiles Induce hypo responsiveness to food antigens.

Probiotics as Non-immunologic action:

- Digest food and compete for nutrients with pathogens
- Alter local pH to create an unfavorable local environment for pathogens
- Produce bacteriocins to inhibit pathogens
- Scavenge superoxide radicals
- Stimulate epithelial ‘mucin’ production
- Enhance intestinal barrier function
- Compete for adhesion with pathogens
- Modify pathogen-derived toxins

Probiotics Mode of action:

- Metabolic effects: production of short-chain fatty acids, fat metabolism, absorption of ions (Ca, Fe, Mg)
- Enhancing host immunity (IgA production, cytokine modulation, etc.)



The normal microbiota and probiotics interact with the host in metabolic activities and immune function and prevent colonization of opportunistic and pathogenic microorganisms (Sullivan and Nord, 2005).

Current research has found that probiotics, or live beneficial bacteria, can also play a large role in in reducing inflammation and symptoms of Crohn’s disease. Many bacterial strains have been evaluated for ability to normalize the properties of abnormal native microflora and reinforce various aspects of intestinal defense. Probiotics inhibit microbial pathogen growth in the intestines by inhibiting their ability to attach to the gut and colonize (Bernet *et al*., 1994). A study

looked at the beneficial activity of *Lactobacillus plantarum* in an *in vitro* model of colon cells and demonstrated its ability to prevent adherence of a pathogenic *E. coli* strain, as well as increased the expression of protective proteins called mucins (Mack et al., 1999). Colonized probiotics ferment dietary fiber, and in doing so can induce pH and other chemical changes in the intestinal lumen (cavity) that also affect the inhibition of pathogen growth (Le Blay et al.,1999 and Babakissa et. al.,2003). Additionally, short-chain fatty acids are released as a byproduct of bacterial fermentation display anti-inflammatory properties in the epithelial (intestinal lining) cells (Wilson et al,1997).

Probiotics can stabilize the structure in the intestinal barrier and maintain rigidity in the tight junctions between epithelial cells. *Lactobacillus* GG was shown in multiple studies to stabilize the mucosal barrier, as well as inhibit the activation of proinflammatory cytokines that have been shown to be responsible for much of the cell death in the intestines Probiotics can also stimulate the body's innate defense mechanisms, as with the increased production of the antimicrobial peptide defensins in the intestines (Wehkamp *et. al*.,2005).

16.2. National status:

This Institute had already conducted a research project (ARP 008) on cellular defense mechanism of silkworms and screened the available multivoltine and bivoltine breeds. Besides, a Pilot Study has been completed on immunization of silkworm. The outcome of all these studies indicates advantages of humoral immunity derived components as well as their application in the field to manage the silkworm disease. Krishnan *et al*. (2000) reported that the difference in protein concentration between healthy and diseased larvae becomes more pronounced as the disease progress, indicating that after NPV infection of fat body cells, normal protein synthesis and release is greatly reduced. The large reduction in protein concentration in haemolymph at 96 hours post inoculation occurred at a time when most fat body cell is infected. This reduction late in disease (i.e., appearance of external disease symptoms) may be due to sequestration of protein by infected cells for virus encoded protein synthesis, particularly that of occlusion body development or which large quantities would be needed.

The CSR&TI, Berhampore screened silkworm breeds based on cellular defense mechanism (Krishnan *et al*., 2000). Mitra *et al* (2011) worked on Characterization of haemocyte types, their counts in different breeds of silkworm, *Bombyx mori* L. and their progressive changes following bacterial inoculation. This Institute also isolated different strains of virulent bacteria, fungus and microsporidia from infected silkworm larvae. Chakrabarty *et al.* (2011) worked on immunological impact of some chemicals, botanicals, antibacterial proteins and live nonpathogenic bacteria in silkworm, *Bombyx mori* L. to control bacterial disease. The outcome of all 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 for immunization of silkworm for controlling disease of silkworm. In our study, we observed that mortality% was recorded lowest in all the treatments supplements with ascorbic acid, so oral immunization with *L.acidophillus* is giving satisfactory result than micronutrients. Total pupal protein per gram tissue of body weight were found are at par in the larvae immunized with micronutrient supplements and healthy control batches of larvae, it indicates that immunization is not interfering the protein level required for formation of silk in later stages. Protein profile of 'vaccinated, larvae (muga) challenged with live (pathogenic) bacteria was similar to that of the control suggesting unhindered metabolism (Choudhury *et al,* 2004). It has been known that insects inoculated with live non-pathogenic bacteria can acquire resistance to subsequent challenge by bacterial pathogen (Boman and Hultmark, 1987).

Scientists of Silkworm Pathology Section has already studied on feed supplement (Oral immunization) of *Lactobacillus acidophillus* etc and spraying the selective dose of immunogen on the mulberry leaf and fed (*Per oral*) to the silkworm larvae at the time of 2nd feeding after resuming from 2nd moult. The larvae were challenged with selective dose of pathogenic bacteria (log phase) after a stipulated gap of immunization. Probiotcs, *Lactobacillus acidophillus* etc. have potentiality to control bacterial disease to the tune of 88.62 % and other diseases, 55.25 % during unfavourable seasons (June – September) in multivoltine breeds, Nistari(M), M12(w) and

M6DP(c). Probiotcs, *Lactobacillus acidophillus* stood first (*Rank-1*) in controlling the bacterial diseases in M12 (w) considering the economic parameters among the above three breeds.

Recent publications of Scientists of this Institute on the subject are 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, Oct, 30 – Nov., 01, 2010 *'Advances in Parasitology: A novel approach towards a disease free world'*, Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal,India. Published by Prof. P.K.Banddyopadhyay, *Editor In Chief*, Printed at East India Photo composing Centre, 69, Sisir Bhaduri Sarani, Kolkata -700 006 , p-244 - 251. *Full paper published on 23.01.12.*

2) Mitra,P., Chakrabarty S., Bandopadhyay P.K and Haldar D.P (2012). Characterization of haemocyte types, their counts in different breeds of silkworm, *Bombyx mori* L. and their progressive changes following bacterial inoculation. Proceeding of the 22nd Indian Congress of Parasitology, Oct, 30 – Nov., 01, 2010 *'Advances in Parasitology: A novel approach towards a disease free world'*, Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal,India. Published by Prof. P.K.Banddyopadhyay, *Editor In Chief*, Printed at East India Photo composing Centre, 69, Sisir Bhaduri Sarani, Kolkata -700 006, p .196 - 205. *Full paper published on 23.01.12.*

16.3. Importance of the proposed programme in the context of the current status:

Economic benefit resulting from reduced mortality of silkworms due to silkworm disease by adopting the recommended formulation of synbiotics

16.4. Anticipated products processes/ technology packages, information or other outcome from the programme and their expected utility:

Technology for synbiotic therapy of silkworm to prevent the silkworm diseases and thereby increase the productivity may be patented for the benefit of industry.

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

For proper implementation of the programme, expertise available with proposed investigation group.

16.6. List of five experts in India in the proposed subject area:

#	Name	Designation	Address
1	Dr. Mitradas M.Panicker panic@ncbs.res.in 0802366001	Senior Scientist	Neurobiology, National Centre for Biological Sciences, TIFR, GKVK, Bellary Road, Bangalore-65, Karnataka
2	Dr. B. G. Unni bgunni@rrljorhat.res.in 0376 – 2372710	Scientist-G	North East Institute of Science and Technology, Formerly RRL, CSIR, Jorhat,Assam -785 006
3	Dr. Sailas Benchamin sailasben@yahoo.co.in 0494-2401144 Extn 406, 407	Associate Professor	Department of Biotechnology, Calicut University, Calicut, Kerala -673 635
4.	Dr.C.S.Patil, Director, kssrdi.conf@gmail.com	Director	KSSRDI, Thalaghattapur, Bangalore -560062
5.	Prof Veena Tandon tnadonveena@gmail.com	Professor	Dept. of Zoology, North Eastern Hill University,Shillong,Meghalaya

17. WORK PLAN

17.1 Materials: Following materials will be used in the study is depicted below:

#	Probiotics	Brand name	Producer
1	*Bifidobacterium animalis* DN 173 010	Activia	Danone/Dannon
2	*Bifidobacterium animalis* subsp. *L*	*actis* Bb-12	Chr. Hansen
3	*Bifidobacterium breve* Yakult	Bifiene	Yakult
4	*Bifidobacterium infantis* 35624	Align	Procter & Gamble
5	*Bifidobacterium lactis* HN019 (DR10)	Howaru Bifido	Danisco
6	*Bifidobacterium longum* BB536	Morinaga	Milk Industry
7	*Lactobacillus acidophilus* LA-5	Chr.	Hansen
8	*Lactobacillus acidophilus* NCFM	Danisco	DanActive
9	*Lactobacillus casei* DN-114 001	Actimel,	Danone/Dannon
10	*Lactobacillus casei* CRL431	Chr.	Hansen
11	*Lactobacillus casei F19*	Cultura	Arla Foods

PATHOGENICITY STUDY:

The surface sterilized silkworms will be homogenized aseptically. An aliquot of the homogenate will be streaked on the nutrient agar plates and incubated one day at 36 ± 2ºC. The bacterial colonies will be observed, and an individual colony will be separated. The individual colonies will be identified by selective media subjected to biochemical test. Likewise, viral and fungal strain will be identified following standard procedure. Silkworm larvae will be artificially infected with the pathogens to determine pathogenicity. The 3rd, 4th and 5th instar larvae will be fed with high concentration of 1x10^{9} cells / ml of live bacteria, polyhedra and conidia through microbial injection method. LD50 value will be determined at 4th instar larvae by the method of Reed and Munch (Woolf, 1968). The original pathogen suspension was serially diluted in serial physiological saline from (1.0 x10^{1} to 1.0x10^{9} cells / ml). The load of pathogen will be determined by serial dilution and plate counting techniques for bacterial strain. Haemocytometer will be used for counting of viral polyhedra and fungal conidia. Three dilutions (1.0x10^{7} to 1.0 x10^{9}) with three replications will be allowed to infect the worms. From each dilution, 0.2 ml of pathogen culture will be injected to the upper portion of the skin of silkworm. The lethal concentration for 50% mortality will be calculated as per the Reed and Muench using the formula (Savithri and Murali Mohan, 2003).

$$LD50 = \text{Lower limit} \frac{\text{50-\% of Lower limit}}{\text{Upper limit - Lower limit}} \times (\text{Log upper limit - Log lower limit})$$

PREPARATION OF SYNBIOTICS

In the present experiment, different strains of *Lactobacillus* and *Bifidobacterium* will be used as 'Probiotic' therapy to the silkworm through feed supplementation. Different concentrations of probiotic solution will be prepared (1 - 10 %) from stock solution for the treatment. Then different concentrations (1 - 10%) of 'Prebiotic' will be prepared. Synbiotics will be made with the different combinations of probiotics and prebiotics. Synbiotics will be tested on silkworm to feed the silkworm as feed supplement.

ADMINISTRATION OF SYNBIOTICS TO *B.MORI*

All silkworm larvae will be fed untreated leaves until the end of 2nd instar stage. The 3rd Instar larvae will be divided into groups for the treatment. Three sets of larvae will be treated with three short listed synbiotics. Then these three sets after treated with short listed synbiotics will be challenged with bacterial, viral and fungal pathogens separately. The synbiotic combinations in different concentrations will be sprayed on mulberry leaves separately and the treated leaves will be allowed to semi dry for 15 minutes. Leaves were given for four times in a day. Leaves of the

healthy control larvae will be sprayed with distill water and the water was dried before feeding. The leaves of the infected control larvae will be sprayed with pathogen. and to be semi-dried before feeding. Three replications were maintained for each treatment. The weights of the worms will be measured by the electronic balance. The fecal matter and unfed leaves will be removed from the bed daily. The observations on economic parameters such as mature larval weight, cocoon weight, pupal weight, shell weight, shell percentage, filament length, denier, sericin and fibroin content will be determined following standard procedure. Food consumption and growth parameters to measure Energy budget, will be measured on dry weight basis following Waldbauer (1968).

ECONOMIC CHARACTERS

LARVAL WEIGHT: Larval weight will be taken by using an electronic balance in gram

COCOON CHARACTERS: The mature fifth instar larvae will be picked up from rearing trays and will be released on Chandrika for spinning the cocoon. The cocoons will be harvested after 5 days of spinning. Assessments of various cocoon parameters will be made as follows.

COCOON WEIGHT: Ten randomly selected cocoons (five male and five female) were taken and weighed using an Electronic balance. The weight was expressed in grams.

PUPAL WEIGHT: After removing the floss, the cocoons will be cut open and the pupae will be taken out without causing any damage to them. Then the ten pupae (five male and five female) will be weighed using an electronic balance.

SHELL WEIGHT: Ten shell weight (five male and five female) of the cocoon, after removing the floss and pupa will be weighed using an electronic balance.

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

Shell % = Shell weight x 100 / Cocoon weight

SILK CHARACTERS

FILAMENT LENGTH: Cocoons from each replication will be stifled in boiling water and threads from individual cocoons will be reeled using an epprouvette and observed for their silk characters such as silk filament length and silk filament weight.

RENDITTA: CSTRI have given certain constants that can be used for estimating the renditta from the shell %. The constants suggested by them are given below:

- 165 for cocoon with shell % of 14-16%
- 150 for cocoon with shell % of 17-20%
- 133 for cocoon with shell % of 21-23%

$$\text{Renditta} = \frac{\text{Constant}}{\text{Shell Ratio}}$$

DENIER: Denier is the unit, used to denote the thickness of silk filament. It is the weight of 9,000m length of silk expressed in grams. The value of denier varies from 1.7 to 2.8. It is calculated by using the formula

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

Filament denier is used to estimate the number of cocoons required to reel the silk of a specific denier. Filament denier is measured using an epprouvette and a denier scale.

SERICIN AND FIBROIN CONTENTS OF THE COCOON: Individual cocoons will be taken in a weighing crucible to which 20ml of 0.5% percent KOH will be added and will be allowed to remain soaked for 6 hours. The protein sericin will be removed by washing in boiling distilled water twice, leaving behind the protein filament, fibroin. Then the crucible containing fibroin will be oven dried at 90ºC for 24 hours. The weight of fibroin and sericin will be determined by the following formulae.

Sericin Content (g) = Initial dry weight of the shell – Dry weight of the shell after alkali treatment

Fibroin Content (g) = Dry weight of the shell - Sericin content

The protein content of the haemolymph of V Instar larvae of control and synbiont treated will be estimated by adopting Lowry's method.

ENERGY BUDGET:

The gravimetric method described by Waldbaucer (1968) will be used to determine food consumption and growth parameters of all experiments. The initial mean dry matter of larvae will be estimated by weighing and then killing ten larvae from the group used in the experiment, over-drying them at 60ºC for 6 days, and re-weighing them. Thus, the initial dry weight of each larva was calculated from its fresh weight and the mean percent dry matter of an aliquot of similar larvae. The initial dry weights of diets(s) will be measured by taking ten aliquots of each diet and over drying them to constant weight to establish the average percent dry weight of the diet. The dry weights fed to the larvae will be determined by multiplying the fresh weight of feed diet (s) by this constant.

Nutritional indices: Nutritional indices will be calculated according to Waldbauer (1968) and Scriber and Slansky (1981) [CR= Consumption rate; GR= Growth rate; AD= Approximate digestibility; ECD= Efficiency of conversion of digested food to biomass; ECI= efficiency of coversion of ingested food to biomass] These indices are calculated on a dry weight basis as follows

$$CR = \frac{\text{Wt of food eaten}}{\text{Duration of exp.(days)}}$$

$$GR = \frac{\text{Wt gain}}{\text{Duration of exp.(days)}}$$

$$ECI = \frac{\text{Wt gain}}{\text{Wt of food eaten}} \times 100$$

$$AD = \frac{\text{Wt of food eaten} - \text{wt of faeces}}{\text{Wt of food eaten}} \times 100$$

$$ECD = \frac{\text{Wt gain}}{\text{Duration of exp.(days)}} \times 100$$

Weight gain will be calculated by the subtracting the final dry weight of the pupa from the initial dry weight of the larva; mean weight of an insect during the feeding period was calculated

to be one half the sum of its initial and final weight. Data will be analyzed statistically to verify the result.

17.2. Methodology

E-01: *In vitro* and *in vivo* screening of synbiotic preparation against common silkworm pathogens

Selection, screening and culture of probitics will be studied. Different concentrations of synbiotic preparation with Ologosaccharides will be tested against common silkworm pathogens i.e., *Staphyloccus vitulinus*, *Nuclear Polyhedrosis virus* (*Bm*NPV) and *Beauveria bassiana* in *in vitro*. Synbiotic preparation will be sprayed with selective dose along with suitable medium to the larvae of *B.mori* (Nistari) after resuming from 3rd moult (1st day '0' hr.).Treatment will be done with selective dose of pathogen after a stipulated gap (48 hours). A batch of healthy control and infected control larvae will be maintained. Larval mortality % will be recorded from inoculations of pathogens to till the formation of cocoon. The observations on economic parameters such as mature larval weight, cocoon weight, pupal weight, shell weight, shell percentage, filament length, denier, sericin and fibroin content will be determined following standard procedure. Food consumption and **energy budget** will be measured on dry weight basis (Waldbauer, 1968). Data will be analyzed statistically to verify the result. Two rearings each in unfavourable and favourable seasons will be conducted.

E-02: Testing of effective synbiotic preparation and record disease incidence in various and silkworm breeds / hybrids

The most effective **synbiotic preparation** will be tested in other silkworm breeds like M12w, CB5, O, G, MCon1 and M_6DPc frequently used for hybrid seed preparation and hybrids, N x M 12w, N x MCon1, MCon1 x MCon4, N x (SK6 x SK7). After resuming from 3rd moult the silkworm will be treated with more effective Synbiotic preparation. Then the same procedures for rearing, inoculation, data recording etc., to be adopted as like E01.One rearing each in unfavourable and favourable season will be conducted.

E03: Assessment of cocoon / reeling performance and post cocoon disease incidence in breeds / hybrids

Rearing will be conducted with new effective synbiotic preparation to the suitable breed / hybrids in normal condition. Reeling performance like Filament Length, Non-breakable Filament Length and Denier will also be recorded. Pupation, Grainage and Post cocoon disease incidence performance will also be assessed for suitable breeds / hybrids and result will be analyzed statistically. EO3 will run simultaneously with EO2 to reduce time.

E04: Determination of shelf life of the effective Synbiotic preparation

Shelf life of newly developed effective formulation of immunogen will be assessed by freezing from - 25˚C to 25˚C from 01 month to 06 months. EO4 will run simultaneously with EO2 to reduce time.

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17.3. Proprietary / patented items, if any, expected to be used for this programme: No.

17.4. Suggested plan of action for utilization of the expected outcome from the programme:

Application of Synbiotic therapy to control silkworm diseases thereby increase cocoon productivity.

17.5. TIME SCHEDULE OF ACTIVITIES GIVING MILESTONES

Period of study	Achievable targets
1- 3 Months	E-01 : Selection of probiotics / prebiotics and Synbiotic preparation
4 - 9 months	E- 01.1: *In vitro* screening / testing of Synbiotic preparation
10 - 12 Months	E-01.2 : *In vivo* screening / testing of Synbiotic preparation
13 - 15 Months	E-02: 1st trial of testing of effective synbiotic preparation and record disease incidence in various and silkworm breeds / hybrids. E03: Assessment of cocoon / reeling performance and post cocoon disease incidence in breeds / hybrids
16 - 18 Months	E-02: 2nd trial of testing of effective synbiotic preparation and record disease incidence in various and silkworm breeds / hybrids. E03: Assessment of cocoon / reeling performance and post cocoon disease incidence in breeds / hybrids
19 - 21 Months	E-02: 3rd trial of testing of effective synbiotic preparation and record disease incidence in various and silkworm breeds / hybrids E03: Assessment of cocoon / reeling performance and post cocoon disease incidence in breeds / hybrids
22 - 24 Months	E04: Determination of shelf life of the effective Synbiotic preparation and submit final report

17.6. Programme Implementing Agency/ Agencies:

Name of the Agency	Address of the Agency	Proposed Research Aspects	Proposed Amount (Rs in lakh)	Cost Sharing %
Central Silk Board	BTM Layout, Madivala, Bangalore	Studies on Synbiotics (combination of Probiotic and Prebiotic) induction for control of common diseases of silkworm, *Bombyx mori* L.	2.00	100%
Total:			2.00	100%

PART IV: BUDGET PARTICULARS

18. BUDGET (Rs in lakh):

[In case of multi-institutional programmes, the budget details should be provided separately for each of the institute]

A. Non-Recurring (e.g. equipments, accessories, etc.):

#	Item	Year 1	Year 2	Total (Rs in lakh)
1.	Refrigerator 300 L double door	0.25	0	0.25
2.	Air Conditioner 1.0 ton Split type 3 star	0.30	0	0.30
3.	Laminar Air flow Vertical	1.00	0	1.00
	Sub total A			**1.55**

#	Item	Year 1	Year 2	Total
1.	Cost of materials	0.25	0	0.25
2.	Cost of medium / glass ware	0.20	0	0.20
	Sub-total B2	**0.45**	**0**	**0.45**
		G.Total 2.00 lakh		

Total : Rs 2.00 lakh

PART VIII: DECLARATION / CERTIFICATION

It is certified that

a. The research work proposed in the programme does not in any way duplicate the work already done or being carried out elsewhere on the subject.
b. The same programme has not been submitted to any other agencies 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 programme will be made in the Institute in anticipation of the sanction of the scheme.
e. If the programme involves the utilization of genetically engineered organism, it is agreed that we will ensure that an application will be submitted through our institutional bio-safety committee and we will declare that while conducting experiments, the bio-safety guidelines of the Department of Biotechnology would be followed *in toto.*
f. If the programme involves field trials / experiments/ exchange of specimens etc we will ensure that ethical clearances would be taken from the concerned ethical committees/ competent authorities and the same would be conveyed to the Department of Biotechnology before implementing the programme.
g. It is agreed by us that any research outcome or intellectual property right(s) on the invention(s) arising out of the Programme 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 agrees that the equipment, the basic facilities and such other administrative facilities as per terms and conditions of the grant will be extended to investigators through out the duration of the programme.
j. The institute assumes to undertake the financial and other management responsibilities of the programme.

Signature of Co-coordinator
Date:

Signature of Exe. Authority
Date:

Signature of Principal Investigator
Date:

Signature of Co- Investigators
Date: