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Popular Article

The Crucial Role of Probiotics: Enhancing Growth and Disease Resistance in Domesticated Silkworm in Animal Diets: Vital for Health and Productivity

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Abstract

The sericulture industry in India is a vital component of the agricultural sector, supporting millions of livelihoods and contributing significantly to foreign exchange earnings. However, challenges such as mulberry/silkworm diseases, climate change, resource depletion, and market uncertainties hinder silk production and expansion. The use of probiotics, which contain beneficial living microbes, has been shown to improve the behavior, health, disease resistance, pesticide tolerance, and environmental adaptability of the host organism. Studies have highlighted the efficacy of specific probiotic strains in enhancing silk yield, improving larval growth, and combating pathogens. Moreover, the identification of potent probiotic candidates from the gut microbiota of silkworms underscores the potential for developing tailored probiotic supplements to optimize silk production. However, despite these advancements, there remains a significant gap in our understanding of the gut microbiome of silkworms, particularly in India. Systematic studies focusing on the gut microbiota of domesticated & wild silkworm species prevalent in India are essential for developing targeted probiotic interventions. In essence, by unraveling the intricacies of the silkworm gut microbiome and harnessing probiotic interventions, we can pave the way for sustainable silk production, mitigating losses due to diseases and environmental stresses, and ensuring the continued prosperity of the sericulture industry in India.

Keywords: Silkworm, Probiotics, Mulberry, Disease, Resistance, India

Introduction:

Sericulture is one of the principals allied agricultural enterprises supporting the livelihood of more than 88 lakh people in India, besides netting a valuable foreign exchange of Rs.1848.96 crores



in 2021-22 (CSB, 2022). The silkworm, *Bombyx mori* is an economically important insect domesticated for the commercial production of silk, around which the whole sericulture industry is built upon. Yield loss due to various mulberry/silkworm diseases (Pebrine, Flacherie, Grasserie and Muscardine diseases caused by protozoa, bacteria, viruses and fungus respectively) is estimated to be around 30 – 40 % in India as a major yield-limiting factor. Various emerging challenges in terms of climate change, natural resources degradation, biotic and abiotic stresses, rural-to-urban migration & market vagaries, affect silk production and expansion. Thereby still silk is a high-value but low-volume product accounting for only 0.2% of the world's total textile production. The need to increase area under sericulture and improve productivity in county it is need of the hour to avoid huge losses resulting from diseases and maintain the good health of silkworm. There is a need for evolving novel disease and nutritional management strategies for improving sericulture productivity.

A solution to mitigate some of these challenges has been the administration of probiotics (adequate amounts of living microbes conferring health benefits on the host) found to enhance the host's behavior, health, disease resistance, pesticide tolerance, and environmental adaption. The gut microbial community has a direct impact on insect growth and health by defending the pathogens, triggering the innate immune system, breaking down certain food components, neutralizing dietary toxins and biosynthesizing nutrients. Environmental factors, including diet and human manipulation, likely influence the silkworm gut composition.

Gut microbiota: A potential source of immunity?

A healthy insect harbors microbiota that account for 1–10% of the insect's biomass and hence a healthy insect is termed a “multi organismal entity” (Douglas, 2015). The resident microbial flora in insects benefits the host by producing essential compounds like vitamins, digesting and metabolizing food, nutrient absorption, detoxification of toxins and pheromone production and immunity (Dillon & Dillon, 2004). However, in spite of the unfavourable and harsh environment that the gut provides for the gut microflora, several gut microbiota have been shown to carry out essential physiological functions in the lepidopterans (Paniagua et al., 2018). Thus, like other lepidopterans, silkworm is also associated with a large consortium of symbiotic microbes in their gut.

The use of probiotics is based on the interaction between the host and their gut microbes; hence, understanding the nature of this crosstalk is fundamental to understanding the process. Several studies have shown that there is host-specific intra-species diversity among the species and substantial strain diversity within *Bombyx mori* gut. Taha and Kamel, (2017) found *Bifidobacterium bifidum* to be an immunomodulating agent (increase in the activity of protease, amylase and invertase) increasing



raw silk production with fewer cocoons. *Lactobacilli* stimulated growth factors leading to an increase in the silk yield (Suraporn et al., 2015). Probiotics containing *Lactobacillus casei* improved larval weight, cocooning ratio, pupation ratio, and economic characters when larvae were infected with microsporidium *Nosema bombycis* (Suraporn and Terenius, 2021). Saranya et. al. (2019) reported *Staphylococci* containing probiotics feed increased larval growth and cocoon characters. The yeast, *Saccharomyces cerevisiae* containing probiotics feed act as immunomodulating agent with increasing raw silk production and protein content (Taha and Kamel, 2017). Hence, the microbiome studies has emerged as a potential tool to understand the physical, biochemical and molecular behavior of the host insect. However, further research is required to completely comprehend the mechanism underlying in regulating the host behavior and neurological processes via specific gut flora.

The quantity and quality of silk produced depend on breed/hybrid, agro-climatic conditions, and overall physiological function of the silkworm as well as mulberry leaf nutrient status. Even though several studies have demonstrated gut bacteria in silkworm, their precise role in silkworm growth, development, silk production, and disease/stress tolerance is not clearly understood (Anand et al., 2010). However, it has been assumed that the genetic machinery of the silkworm does not code for cellulase genes, and mulberry leaf cellulose is rather digested by the gut symbiotic bacteria, such as *Enterobacter*, *Proteus vulgaris*, *Klebsiella pneumonia*, and *Citrobacter freundii* (Watanabe and Tokuda, 2010). Further, cofactors, particularly cobalamin forms, are neither synthesized by the silkworm nor obtained from the mulberry leaf, but play an essential role in propionate metabolism (propionate is a precursor for the biosynthesis of juvenile hormone); hence, they must have been obtained from the gut microbiota (Halarnkar and Blomquist, 1989). Several reports suggest key roles for gut bacteria in silkworm metabolism, growth, and development; yet, symbiotic relationships between the gut bacteria are far from fully understood.

Gut microbiota: A way forward to healthy silkworm...

In order to avoid this huge loss, maintaining the health of silkworm is the need of the hour. Therefore, several attempts are being made to improve the health of silkworms. Yeruva et al. (2019) identified potent probiotic bacterial communities from the silkworm *B. mori* gut through a metagenomic approach. Enterococcus has emerged as one of the predominant gut commensal bacterial and plays a fundamental role in the host physiology and health of the major economic agricultural insect, *Bombyx mori* (Zhang et al., 2022). The investigation carried for finding the effects of artificial domestication and evolutionary pressure on the gut bacterial diversity of *B. mandarina* and *B. mori* revealed substantial difference between the intestinal bacteria of both the species (Kumar et al., 2019).



A study by Saranya et al. (2019) reported indigenous Lactic acid bacteria (LAB) from *B. mori* gut with probiotic potential.

Liu et al. (2018) showed the importance of intestinal microorganisms in the host defence system against viral pathogens. The study conducted revealed the presence of *Bacillus pumilus* SW41 with lipase enzyme producing gene bearing antiviral activity against BmNPV. The enzymes in the silkworm gut are also known to influence the growth, development and resistance to diseases in silkworm that subsequently enable the silkworm to produce good quality cocoon and silk. Unban et al. (2022) isolated 51 pentose utilizing lactic acid bacteria with highest prevalence of 35 enterococci isolates, which included 20 isolates of *Enterococcus mundtii* followed by *Enterococcus faecalis* (eight isolates), *Weissella cibaria* (four isolates), *Enterococcus hirae* (two isolates), *Enterococcus lactis* (one isolate), and *Enterococcus faecium* (one isolate) showing antimicrobial activity against Eri silkworm pathogens. Hou et al. (2021) studied the effect of pesticide Guadipyr containing neonecotenoid and semicarbazone functional groups in one molecule. Results showed structure and richness of the midgut microbiota of silkworms were altered after being treated with 5.25 mg/L (1/10 of LC₅₀) of guadipyr. The expression of key genes in the Toll, IMD, and JAK/STAT pathways, which ultimately led to the downregulation of antimicrobial peptide genes (AMPs), such as *CecA*, *Defensin1*, *Leb*, and *glv2*, was reduced upon guadipyr exposure, concluding necessity to control the application of guadipyr in or around the mulberry fields. Possible mechanisms by which gut bacteria play role in insecticide resistance are, direct acquisition of pesticide degrading microbes from the environment, difference in gut bacterial composition and diversity, difference in xenobiotic degrading enzymes and presence of microbial xenobiotic degradation pathways and cross-acclimatization to related insecticides (Chaitra and Kalia, 2022).

Bhuyan et al. (2014) identified dominant gut bacteria as *Bacillus sp.*, *Proteus sp.* and *E. coli* and has shown to produce digestive enzymes amylase, pectinase, cellulase, xylanase and lipase that help in digesting the major ingredients pectin, starch, cellulose, xylan, lipids and fatty acids from primary host plant Som (*Presea bombycina* Kost) leaves. The digestion of food ingredients and utilization of nutrients is beneficial for better productivity of muga silk. The increased protease activity was attributed to the increased silk protein concentration for silk production.

A lot study has been conducted to explore gut microbiota of domesticated mulberry silkworm, *B. mori* for understanding their role in silkworm health and potent to be used as probiotic feed supplement but yet not able to be the part of package of practices for silkworm rearing for commercial silk production. Shruti et al. (2019) studied the effect of probiotic feed supplements to mulberry silkworm



and found 2 per cent azolla was superior for larval growth and development. The mulberry leaves fortified with *Staphylococcus gallinarum* strain SWGB 7 and *Staphylococcus arlettae* strain SWGB 16 (10^8 cfu/ml) recorded maximum larval weight (4.12 g), effective rate rearing (96.36 %), cocoon weight (1.97 g), shell weight (0.37 g), pupal weight (1.60 g), shell ratio (18.78 %), silk productivity (4.81 g), filament length (1170.84 m), filament weight (0.31 g) and finer denier (2.38) besides reduced larval mortality (3.64 %) due to disease incidence compared to control (Saranya et al., 2019). Yeruva et al. (2019) through metagenomic approach identified predominance *Enterococcus* (30.30%) followed by *Bacillus* (16.96%) was observed in PM, whereas *Lactobacillus* (56.56%) followed by *Enterococcus* (10.58%) was seen only in CSR2 race of *Bombyx mori* silkworm. Interestingly, crossbreed midgut harbored diverse bacterial communities (36.21% *Lactobacillus*, 25.94% *Bacillus*, 8.1% *Enterococcus*, and 18.37% uncultured bacteria).

There are very few reports available on exploration of gut microbiota of wild silkworms across world. Gandotra et al. (2018) identified number of bacterial isolates identified a number of bacterial isolates viz., *Bacillus* spp, *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Pseudomonas stutzeri*, *Acinetobacter* sp. and *Alcaligenes* sp. inhabiting the gut of muga silkworm by using culture dependent techniques and generic identification using 16s rRNA probes. However, qualitative screening for enzymatic activities identified a number of gut bacterial isolates having significantly higher cellulase, amylase, lipase activities which may probably be contributing to the digestion and nutrition of their host insect, *A. assamensis*. Sixty culturable aerobic gut bacterial isolates comprising Firmicutes (54%) and Proteobacteria (46%); and twelve culturable facultative anaerobic bacteria comprising Proteobacteria (92%) and Firmicutes (8%) were identified inhabiting the gut of *S. ricini* (MsangoSoko et al., 2020). In addition, enzymatic assays indicated that 48 and 75% of culturable aerobic, and 75% of anaerobic gut bacterial isolates had cellulolytic, lipolytic and nitrate reductase activities, thus suggesting that they may be involved in food digestion and nutritional provision to the host. These bacterial isolates may be good sources for profiling novel genes and biomolecules for biotechnological application.

Unfortunately, there is lack of systematic study of gut microbiome of silkworm commonly found in India. As a dire need of time in order to enhance silkworm health and protecting them from diseases causing economic yield loss by providing nutrient rich diet with added microbes enhancing the health resulting higher silk production.

Conclusion:

The sericulture industry plays a crucial role in India's agricultural landscape, supporting



millions of livelihoods and contributing significantly to foreign exchange earnings. However, challenges such as mulberry/silkworm diseases, climate change, resource depletion, and market uncertainties hinder silk production and expansion. Despite its high value, silk remains a low-volume product globally. One promising solution to address these challenges lies in harnessing the potential of probiotics to enhance silkworm health and productivity. Probiotics, with their beneficial effects on behavior, disease resistance, and environmental adaptation, offer a novel approach to bolstering the sericulture industry. The intricate interplay between the silkworm host and its gut microbiota underscores the importance of understanding this relationship for effective probiotic application. Research indicates the diverse roles of gut bacteria in silkworm metabolism, growth, disease resistance, and silk production. Studies have highlighted the efficacy of specific probiotic strains in enhancing silk yield, improving larval growth, and combating pathogens. Moreover, the identification of potent probiotic candidates from the gut microbiota of silkworms underscores the potential for developing tailored probiotic supplements to optimize silk production.

However, despite these advancements, there remains a significant gap in our understanding of the gut microbiome of silkworms, particularly in India. Systematic studies focusing on the gut microbiota of wild silkworm species prevalent in India are essential for developing targeted probiotic interventions. In essence, by unraveling the intricacies of the silkworm gut microbiome and harnessing probiotic interventions, we can pave the way for sustainable silk production, mitigating losses due to diseases and environmental stresses, and ensuring the continued prosperity of the sericulture industry in India.

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