*Review Article*

The Role of Probiotic Supplementation in Silkworm (*Bombyx mori*) Biology: Current Insights and Future Perspectives

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ABSTRACT

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| Probiotic supplementation has emerged as a promising strategy to improve the health, growth, and silk productivity of *Bombyx mori*, the mulberry silkworm. Recent advancements in insect microbiome research have highlighted the functional significance of gut microbiota in nutrient assimilation, immune modulation, and physiological resilience. Studies indicate that incorporating beneficial microbes such as *Lactobacillus*, *Bacillus*, *Saccharomyces*, and *Bifidobacterium* into the silkworm diet enhances digestive enzyme activity, silk gland protein content, and disease resistance. These probiotics not only improve larval and cocoon traits but also suppress pathogenic infections by modulating gut microbial balance and immune responses. The effectiveness of probiotic supplementation, however, is often strain- and dosage-dependent, emphasizing the need for targeted formulations. In addition, integration with prebiotics or botanical additives has shown synergistic benefits, opening avenues for developing biofortified sericultural feeds. Despite promising outcomes, the absence of standardized protocols and limited understanding of long-term probiotic-host interactions remain major challenges. Recent molecular approaches such as metagenomics and transcriptomics offer new insights into the microbial dynamics within the silkworm gut, paving the way for precision probiotic applications. This review consolidates current knowledge on the functional roles of probiotics in silkworm biology and discusses their implications for sustainable, eco-friendly sericulture. The development of strain-specific, cost-effective probiotic interventions could significantly improve productivity while reducing dependence on chemical treatments, contributing to the resilience and profitability of the silk industry. |

*Keywords: Bombyx mori, Probiotic supplementation, Silkworm gut microbiota, Sericulture productivity, Digestive enzymes, Immune modulation, Cocoon and silk yield, Microbial symbiosis*

1. INTRODUCTION

Sericulture, the practice of rearing silkworms for silk production, represents a vital agro-based industry and source of livelihood, particularly in many Asian countries. Among the domesticated silkworm species, *Bombyx mori* L. (Lepidoptera: Bombycidae) holds the highest economic importance, contributing to over 90% of global raw silk production. This species is extensively utilized in commercial sericulture due to its remarkable ability to biosynthesize silk proteins through efficient nutrient conversion from mulberry leaves (*Morus* spp., Family: Moraceae) (Yeruva *et al.,* 2020; Hassan, 2020). Achieving optimal larval growth, enhanced disease resistance, and high silk yield is essential for improving both the economic viability and long-term sustainability of sericulture. Within this framework, nutrient assimilation efficiency and the maintenance of gut health emerge as critical determinants of silkworm performance.

*Bombyx mori* is a monophagous and host-specific insect, relying exclusively on mulberry leaves as its primary nutritional source. The quality of these leaves, which is significantly influenced by environmental factors, plays a central role in regulating larval growth, development, cocoon formation, and ultimately, silk quality (Gheorghe *et al.,* 2024). Beyond the inherent nutrients in mulberry, various dietary supplements such as vitamins, amino acids, proteins, and probiotics have demonstrated potential in improving nutrient utilization and enhancing the biological and economic traits of silkworms (Pachiappan *et al.,* 2021). Over the years, researchers have focused on enriching the nutritional profile of mulberry leaves through the incorporation of essential compounds like amino acids (e.g., glycine, asparagine, arginine, serine), vitamins (including B-complex and vitamin C), and minerals (such as calcium, phosphorus, zinc, magnesium, potassium, copper, and selenium), aiming to promote better larval health and productivity (Hassan *et al.,* 2020).

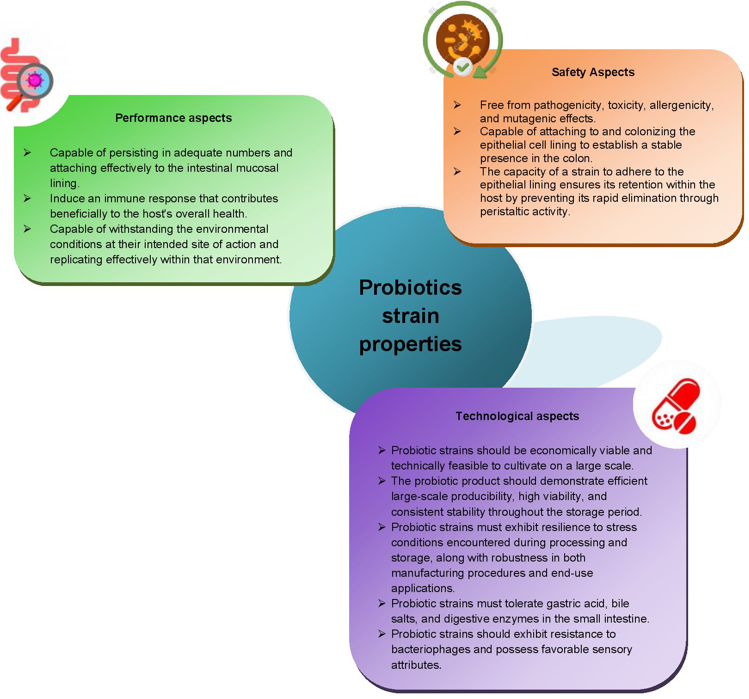
An increasingly recognized factor in silkworm health and productivity is the gut microbiota. The gut of *B. mori* harbors a diverse microbial community that plays vital roles in digestion, immune function, and overall physiological regulation. These symbiotic microorganisms act as important metabolic allies, aiding in the breakdown and absorption of complex plant-derived compounds, supplementing the host with essential nutrients and digestive enzymes, and thereby enhancing larval survival and fitness (Das *et al.,* 2024). Arasakumar *et al.,* (2023) emphasized that gut bacteria in silkworms contribute significantly to nutrient assimilation, defense against pathogens, and developmental processes. In light of these findings, manipulating gut microbiota through dietary supplementation, especially with probiotics, is emerging as a promising approach to improve silkworm health and silk production.

In recent years, numerous nutritional interventions have been investigated in sericulture, including the supplementation of proteins, carbohydrates, amino acids, vitamins, sterols, hormones, and antibiotics, all aimed at improving cocoon yield in terms of both quantity and quality (Sannappa *et al.,* 2002). Among these, probiotics have attracted considerable interest due to their eco-friendly nature and multifaceted benefits. Certain probiotic strains have demonstrated the ability to inhibit the growth of harmful microbes within the silkworm body, thereby reducing disease incidence. For instance, *Streptomyces noursei*, known for its antibacterial properties, has shown potential as a sustainable biocontrol agent in silkworm disease management (Subramanian *et al.,* 2010a). Furthermore, oral supplementation of silkworm diets including both mulberry and *Samia ricini* foliage with cyanobacteria has been reported to improve larval and shell weight, ultimately enhancing the commercial characteristics of the cocoon (Ramesha *et al.,* 2010a; Masthan *et al.,* 2011). These findings collectively underscore the potential of probiotic supplementation as a strategy to improve gut health and optimize economic traits in silkworms.

Despite a growing body of experimental evidence supporting the positive effects of probiotics in insect systems, a consolidated understanding of their mechanisms, efficacy, and potential limitations in *Bombyx mori* remains limited. Therefore, this review aims to critically synthesize current research on probiotic supplementation in silkworm biology, focusing on its effects on gut microbiota modulation, nutrient utilization, immunity enhancement, disease resistance, and silk production. Additionally, emerging molecular insights, recent advances, and future prospects for integrating probiotics into sustainable sericulture practices are discussed to provide a comprehensive perspective for researchers and practitioners in the field.

2. An Overview of Probiotics: Definitions, Functional Characteristics, and Mechanisms of Action

All organisms possess a microbial ecosystem that can positively influence their health, and the discovery of probiotics has opened new avenues for exploring the impact of introducing beneficial microbes into insect systems (Gheorghe *et al.,* 2024). Specifically, the influence of these microorganisms is assessed for their ability to promote growth, improve reproductive performance, and reduce disease incidence under the stressful conditions encountered during rearing (Savio *et al.,* 2022). The term probiotics originates from the Latin word pro, meaning “for,” and the Greek word biotic, meaning “life,” collectively signifying “for life” (Kalokhe *et al.,* 2024). In other terms, probiotics can be defined as live microbial supplements that support host health by enhancing and maintaining a stable and balanced gut microbiota (Austin *et al.,* 1995; Mohanraj *et al.,* 2016). Numerous studies have highlighted the beneficial effects of probiotics in both humans (Chan *et al.,* 1985; Douillet & Langton, 1994; Gildberg *et al.,* 1997; Brigidi *et al.,* 2000; Brigidi *et al.,* 2001) and insects (Dillon & Dillon, 2004), demonstrating their broad applicability across biological systems. As a result, probiotic-based products are gaining widespread attention, reflecting their growing significance and demand across various applications. The presence of diverse bacterial populations in the gut of the mulberry silkworm (*Bombyx mori*) has been well documented by many researchers. Among these, several species from the genus *Streptococcus* have been identified as pathogenic to silkworm larvae, whereas *Lactobacillus* species have not exhibited any harmful effects. Despite extensive studies, the exact mechanisms underlying the beneficial interactions between the host and its gut microflora, or among different bacterial strains, remain unclear (Steinhaus, 1949; Kodama, 2001). Various species of lactic acid bacteria have been investigated in detail and recognized for their probiotic potential (Bruno *et al.,* 1993; Bernet-Camard *et al.,* 1997; Fuller, 1991; Sakamoto *et al.,* 2001). Experimental evidence indicates that probiotic supplementation in silkworms enhances larval growth, cocoon weight, shell weight, and pupation rates when fed with mulberry leaves treated with commercial probiotic formulations. In particular, beneficial effects have been observed from strains such as *Pediococcus*, members of the *Leuconostoc* genus, and *Lactobacillus plantarum* (Singh *et al.,* 2005). Choosing microorganisms for use as probiotics necessitates distinct traits or properties concerning their safety, functionality, and technological viability, as illustrated in Figure 1.

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**Fig. 1 Key properties of probiotic strains based on performance, safety, and technological aspects.** (Source: Pachiappan *et al.,* 2021)

Probiotics influence host health through a range of well-documented mechanisms that contribute to gastrointestinal integrity and immunological stability. These mechanisms primarily include strengthening of the intestinal epithelial barrier, enhanced adhesion to the mucosal lining, and inhibition of pathogenic colonization. By competing for receptor sites and nutrients, probiotics effectively exclude harmful microbes and create an unfavorable environment for their survival. Additionally, probiotic strains produce antimicrobial substances such as short-chain fatty acids and bacteriocins that further suppress pathogen growth. Importantly, probiotics also modulate immune responses by interacting with various immune cells, thereby contributing to the host's defense and overall physiological resilience. The major mechanisms of probiotic action are summarized in Table 1.

**Table 1. Mechanisms of action of probiotics**

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| **Mechanisms** | **Description** |
| Enhancement of Epithelial Barrier Function | The intestinal barrier relies on a combination of defense components, including the mucous layer, antimicrobial peptides, secretory immunoglobulin A (IgA), and epithelial junctional complexes, all of which are essential for maintaining epithelial integrity and safeguarding the host. |
| Enhanced adhesion to the intestinal mucosa | The successful colonization of probiotic bacteria within the host gut largely relies on their ability to adhere to the intestinal mucosal surface. Surface-associated adhesins play a crucial role in facilitating interactions between lactic acid bacteria, intestinal epithelial cells, and the surrounding mucus layer. |
| Suppression of Pathogenic Microbial Attachment to the Intestinal Mucosa | Mucin, a complex glycoprotein secreted by intestinal epithelial cells and constituting the primary component of mucus, plays a crucial role in hindering the adherence of pathogenic microorganisms to the gut lining. |
| Exclusion of Pathogens through Competitive Microbial Interactions | Probiotic bacteria adhere effectively to intestinal receptor sites, thereby inhibiting the attachment of pathogenic microorganisms. By creating an unfavorable microenvironment, occupying binding sites, secreting antimicrobial agents and beneficial metabolites, and outcompeting pathogens for essential nutrients, they suppress the growth and colonization of harmful microbes. |
| Biosynthesis of antimicrobial compounds | Probiotic microorganisms synthesize short-chain fatty acids that reduce intracellular pH, thereby creating unfavorable conditions for pathogenic microbes. Additionally, many strains release antimicrobial peptides and bacteriocins such as lactacin, plantaricin, and nisin which exhibit selective activity against specific pathogens. |
| Modulation of host immunological activity | Probiotic microorganisms are capable of modulating immune functions by engaging with epithelial cells, dendritic cells, macrophages/monocytes, and lymphocytes, thereby influencing host immune responses. |

Source: Bermudez-Brito *et al.,* (2012); Gheorghe *et al.,* (2024)

In addition to their primary functions, probiotic microbial communities within the host gastrointestinal tract can enhance nutrient assimilation and digestive efficiency, ultimately contributing to improved protein synthesis and utilization by the host (Rowland *et al.,* 2018). Probiotics also facilitate the breakdown of easily absorbable compounds and are capable of synthesizing certain vitamins that support enhanced nutrient uptake. In line with the concept that a beneficial gut microbial ecosystem is essential for eukaryotic metabolism, insects depend on a diverse array of microbial enzymes within their gut to effectively digest feed components. These enzymes further contribute to the release of vital molecules such as fermentable sugars and amino acids, ultimately promoting the insect’s growth and development (Liang *et al.,* 2015).

3. Silkworm Gut Microbiota

To optimize the application of probiotics, it is essential to gain a comprehensive understanding of the microbial populations naturally associated with the gut of silkworm larvae. In addition to their critical role in maintaining innate immune defense, the gut microbiota also contributes significantly to protecting the host against a range of pathogens, including bacteria, fungi, viruses, and microsporidia. Although ingestion is the primary route for bacterial and viral infections, fungal pathogens can also invade through the insect cuticle (Hong *et al.,* 2022). Notably, ecto-microbiota shares the same environmental origin as gut microbiota (Hong *et al.,* 2024) and is often established through exposure to fecal bacteria (Zhao *et al.,* 2024).

The gastrointestinal tract of silkworms harbors a diverse array of microbial communities that play a crucial role in digestion, immunity, and overall physiological development. These gut-associated microorganisms can be broadly categorized into Gram-positive and Gram-negative bacteria, each contributing distinct metabolic and functional benefits to the host. Studies have consistently identified specific bacterial taxa that dominate the gut microbiota of *Bombyx mori*, reflecting their adaptability and potential probiotic value in silkworm rearing systems. Table 2 summarizes the predominant bacterial species commonly reported in the gut environment of silkworm larvae.

**Table 2. Predominant Gram-Positive and Gram-Negative Bacterial Species Identified in the Gut Microbiota of *Bombyx mori***

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| **Gram positive** | **Gram negative** |
| *Bacillus circulans* | *Proteus vulgaris* |
| *Bacillus subtilis* | *Klebsiella pneumoniae* |
| *Enterococcus sp.* | *Escherichia coli* |
| *Staphylococcus aureus* | *Enterobacter sp.* |
|  | *Serratia liquefaciens* |
|  | *Aeromonas sp.* |
|  | *Citrobacter freundii* |

Source: Arasakumar *et al.,* (2023)

Advancements in next-generation sequencing (NGS) have greatly enhanced our understanding of the presumed microbial communities within silkworm larvae, revealing shifts in their composition in response to dietary variations and environmental stressors. Among the molecular techniques available, sequencing of the 16S ribosomal DNA (rDNA) gene encoding the small ribosomal subunit in prokaryotes and measuring approximately 1.5 kilobases in length is most frequently employed for assessing microbial diversity (Ponnusamy *et al.,* 2019). However, interpretation of these sequencing results must be approached with caution, as DNA-based approaches are unable to distinguish between viable and non-viable microbes, nor can they differentiate between metabolically active and dormant cells. This limitation may be addressed through RNA-based sequencing techniques, which offer more accurate insights into the active microbial population (Li *et al.,* 2017). Despite this, RNA-based methods are seldom utilized in studies investigating the silkworm gut microbiota (Chen *et al.,* 2018), and as a result, DNA-based analyses may lead to an overestimation of microbial richness within the silkworm gastrointestinal tract.

Numerous bacterial species inhabit the midgut of *Bombyx mori*, playing vital roles in various metabolic processes. Despite their importance, current knowledge regarding the diversity and characterization of these bacterial communities remains limited (Pandiarajan & Krishnan, 2018), as does our understanding of their specific contributions to the growth, development, and overall health of the silkworm (Yeruva *et al.,* 2020). Kalpana *et al.,* (1994) investigated the gut microbiota across the life cycle of *Bombyx mori* and reported that the intestinal bacterial population was more abundant during the fourth and fifth larval instars, which correspond to the active feeding phases, thereby facilitating better nutrient digestion and growth. Hui *et al.,* (2010), utilizing PCR/DGGE and 16S rDNA gene library analysis, identified 41 bacterial phylotypes present in the midgut of *B. mori* larvae. In a similar approach, Subramanian *et al.,* (2010b) employed 16S rRNA-based techniques and confirmed the occurrence of *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Streptomyces noursei* as part of the silkworm’s intestinal microflora. Prem Anand *et al.,* (2010) observed that by the fifth instar stage, the silkworm gut becomes colonized with 11 bacterial phylotypes that participate in the degradation of polysaccharides, thereby influencing nutrient uptake, digestion, and insect development. Li *et al.,* (2020) further explored the gut bacterial composition of two silkworm strains using 16S rRNA gene sequencing and noted significant variation in their microbial profiles. Yeruva *et al.,* (2020) applied a metagenomic approach to identify potential probiotic bacteria in the gut of *B. mori*, with *Lactobacillus* emerging as the predominant genus particularly *L. plantarum*, *L. rhamnosus*, *L. paracasei*, and *L. acidophilus* followed by *Enterococcus* and *Bacillus*. They proposed that both *Lactobacillus* and *Bacillus* spp. could be effectively incorporated as feed supplements with mulberry leaves to improve the economic traits of the silkworm. Similarly, Saranya *et al.,* (2019) also reported the probiotic potential of indigenous lactic acid bacteria isolated from the intestinal tract of *B. mori*.

In addition to their metabolic roles, several studies have highlighted the protective effects of the silkworm gut microbiota against infectious agents (Sun *et al.,* 2013; Li *et al.,* 2015; Sun *et al.,* 2016). Recently, Dee Tan and Bautista (2022) explored the bacterial diversity across four silkworm strains native to the Philippines using 16S rRNA gene amplicon sequencing. Their findings revealed the presence of five dominant bacterial genera such as *Pseudomonas*, *Sphingomonas*, *Delftia*, *Methylobacterium*, and *Acinetobacter* which were consistent with those observed in other silkworm populations. Although much of the current research on silkworm microflora has focused on the bacterial communities within the gut, fungal components of the gut microbiome remain largely underexplored. However, reports by Chen *et al.,* (2018) and Mwchahary and Brahma (2023) have documented the coexistence of bacterial phyla such as *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* alongside fungal members from the phyla *Ascomycota* and *Basidiomycota*. Further investigations into the yeast microbiota of *B. mori* have led to the identification of species like *Cryptococcus rajasthanensis* and *Blastobotrys bombycis* sp. (Barretto *et al.,* 2018; Barretto & Vootla, 2020; Barretto *et al.,* 2021). Importantly, the composition and abundance of the gut microbial community in silkworms are not static; they are modulated by several intrinsic and extrinsic factors, including the host’s genotype, diet, developmental stage, and surrounding environmental conditions (Mwchahary and Brahma, 2023).

The gut microbiota of *Bombyx mori* exhibits considerable diversity and functional relevance, influencing host metabolism, immunity, and disease resistance. While bacterial communities have been extensively studied, fungal populations remain underexplored. Variations in microbial composition are shaped by factors such as genotype, diet, developmental stage, and environmental conditions, underscoring the need for deeper functional analyses to fully exploit their potential in sericultural practices.

4. Functional Dynamics of Silkworm Gut Microbiota

The gut microbiota of *Bombyx mori* represents a dynamic consortium of microorganisms that function synergistically with the host to maintain homeostasis and promote physiological performance. These microbial inhabitants exert profound effects on nutrient metabolism, detoxification of harmful compounds, biosynthesis of essential metabolites, and regulation of immune responses. The functional versatility of gut microbes also extends to influencing the host’s developmental signaling pathways and contributing to resilience under environmental stress. As such, elucidating the functional dynamics of these microbial communities is fundamental not only for understanding host-microbe interactions but also for developing targeted microbial interventions to enhance silkworm productivity and health.

In this context, gut microbiota serves as a critical regulatory element for maintaining intestinal health (Qi *et al.,* 2022), owing to their beneficial symbiotic attributes and physiological contributions (Unban *et al.,* 2022). Their enzymatic repertoire is essential for catalyzing the degradation of complex substrates and liberating key nutrient molecules required by the host (Paniagua *et al.,* 2018). Specifically, the gut bacterial assemblages of the silkworm have been investigated for their ability to produce digestive enzymes that aid in breaking down leaf-derived nutrients (Taha & Kamel, 2017). Empirical evidence highlights multiple functional roles of these microbial communities, including participation in digestion, nutrient assimilation, detoxification, immune modulation, provision of growth-enhancing metabolites, antimicrobial compounds, and disease mitigation strategies (Barretto *et al.,* 2021; Sun *et al.,* 2017; Liu *et al.,* 2018; Liang *et al.,* 2022). Table 3 outlines the major functional contributions of representative gut bacterial species in *B. mori*.

**Table 3. Functional Roles of Gut Bacterial Species in Regulating the Physiological Processes of the Mulberry Silkworm (*Bombyx mori*)**

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| **Species** | **Functions** | **Reference** |
| *Proteus vulgaris*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Pseudomonas fluorescens*, *Erwinia sp.*, *Aeromonas sp*., *Serratia liquefaciens* | Synthesis of digestive enzymes | Prem Anand *et al.,* (2010) |
| *Bacillus sp.*, *Brevibacterium sp.*, *Cyanobacteria*, *Staphylococcus sp.*, *Klebsiella sp.*, *Stenotrophomonas sp.* | Lipid hydrolysis and lipase synthesis | Feng *et al.,* (2011) |
| *Enterococcus casseliflavus* | Promotion of growth and development through L-tryptophan biosynthesis | Liang *et al.,* (2022) |
| *Lactobacillus* and *Bacillus* | Optimization of traits contributing to economic yield | Yeruva *et al.,* (2020) |
| *Bacillus licheniformis* | Secretion of extracellular enzymatic compounds | Mala and Vijila, (2018) |
| *Streptomyces noursei* | Disease mitigation via antimicrobial efficacy | Subramanian *et al.,* (2010b); Mohanraj & Subramanian, (2014) |
| *Enterococcus faecalis* | Immunological protection from infectious agents | Zhang *et al.,* (2022) |
| *Enterobacter aerogenes, pneumoniae ssp. Pneumonia, Yersinia enterocolitica* | Promotion of physiological growth and development, enhancement of host immune responses, biodegradation of insecticidal compounds, and antagonistic action against entomopathogenic organisms. | Ramesh *et al.,* (2010b) |
| *Bacillus aryabhattai* and *Bacillus sp.* | Cellulolytic activity | Pandiarajan & Revathy, (2020) |
| *Enterococcus mundtii* | Regulation of host metabolic processes through the synthesis of bioactive metabolites and lactic acid | Liang *et al.,* (2018) |
| *Bacillus pumilus* | Facilitation of digestion and defense against antiviral agents, specifically BmNPV | Liu *et al.,* (2018) |
| *Bifidobacterium* | Stimulation of immune responses by enhancing the enzymatic activities of protease, amylase, and invertase, leading to increased raw silk production. | Taha *et al.,* (2017) |
| *Staphylococcus gallinarum* | Biological defense mechanism | Gibson & Hunter, (2010) |
| *Enterococcus sp.* and *Staphylococcus sp.* | Immunological defense against pathogenic invasion | Sun *et al.,* (2016) |
| *Staphylococcus gallinarum SWGB 7* and *Staphylococcus arlettae SWGB 16* | Enhanced growth performance, augmentation of economic traits, and strengthening of the immune system. | Saranya *et al.,* (2019) |

5. Effect of Probiotic supplementation in Silkworm

Probiotic supplementation has emerged as a promising strategy to enhance silkworm health, growth, and silk productivity. Recent studies have demonstrated that dietary inclusion of beneficial microbial strains such as *Lactobacillus*, *Bacillus*, and *Saccharomyces* can significantly improve digestion, immune response, and larval development in *Bombyx mori*. These probiotics exert their influence by modulating gut microbiota composition, enhancing enzymatic activity, reducing pathogenic load, and promoting nutrient assimilation. The application of strain-specific probiotics has also been linked to improvements in cocoon quality, silk yield, and disease resistance. The effects of probiotic supplementation in silkworms, including their functional roles and practical implications in sericulture, are discussed below based on key findings from various researchers.

Esaivani *et al.,* (2014) investigated the effects of fortifying mulberry leaves with the probiotic microorganism *Saccharomyces cerevisiae* on the enzymatic activity and economic traits of the silkworm *Bombyx mori*. Their findings revealed a significant elevation in the activity of digestive enzymes, particularly amylase and invertase, in probiotic-supplemented larvae compared to the control group. This enzymatic enhancement was associated with improved immunity and better silk yield, underscoring the probiotic's potential in promoting silkworm health and productivity. Building upon the positive influence of probiotic supplementation, Shruti *et al.,* (2019) evaluated the effects of various probiotic feed additives such as *Spirulina*, *Azolla*, yeast, and soy milk, administered at five different concentrations (1%, 2%, 3%, 4%, and 5%) to *Bombyx mori* hybrid PM × CSR-2 larvae from the fourth instar stage onward. These supplements were delivered once daily through mulberry leaves until the onset of spinning. Among the treatments, *Azolla* demonstrated superior performance, significantly enhancing the effective rate of rearing and cocoon weight at 50% spinning, followed by soy milk and yeast, compared to the unsupplemented control group. This study reinforces the potential of probiotic-enriched diets in improving key biological and economic traits in silkworm rearing.

The study by Kalokhe *et al.,* (2024) investigated the impact of probiotics, specifically Spirulina and Baker’s yeast, on the economic traits of mulberry silkworms (*Bombyx mori* L.). The results demonstrated significant improvements in key parameters when probiotics were administered. The highest cocoon weight (1.84 g) was observed with a combination of Spirulina and Baker’s yeast at 1.5% concentration, while Baker’s yeast at 1% yielded the highest shell weight (0.440 g) and shell ratio (27.09%). Additionally, Baker’s yeast at 1% concentration produced the maximum filament length (1216.67 m), and Baker’s yeast at 1.5% resulted in the highest filament weight (0.363 g). Spirulina at 2% concentration showed the lowest denier value (2.44), indicating finer silk quality. The study concluded that probiotics, particularly Baker’s yeast, significantly enhance silkworm productivity and silk quality, making them a promising supplement for sericulture.

Based on the findings of Starlin, (2022), probiotic and vitamin supplementation demonstrated a substantial influence on the physiological and economic parameters of *Bombyx mori*. Administration of probiotics such as Sporlac, in varying concentrations (2%, 4%, and 6%), significantly elevated the levels of total proteins, lipids, and carbohydrates across multiple larval tissues, including the silk gland, fat body, muscles, and haemolymph. Notably, larvae treated with 4% probiotic concentration exhibited superior outcomes in terms of larval weight, cocoon weight, shell weight, filament length, and protein content when compared to the control group. However, at higher concentrations (6%), a decline in several parameters was observed, indicating the importance of dosage optimization for probiotic and vitamin supplementation in silkworm rearing These enhancements indicate that specific concentrations of probiotics and vitamins can positively modulate metabolic activity and silk production, underlining their potential as functional dietary additives in sericulture.

Enhancing silk productivity through microbial intervention has emerged as a promising strategy in sericulture. A study conducted on the silkworm hybrid *Bombyx mori* (L × CSR2) revealed that dietary supplementation with select probiotic strains significantly improved larval biochemistry and cocoon quality. Six microbial treatments, including *Bacillus cereus*, *B. subtilis*, *B. amyloliquefaciens*, *Lactobacillus casei*, *L. plantarum*, and a mixed culture enriched with soy flour, were administered via mulberry leaf fortification from the third instar onward. Among these, *B. amyloliquefaciens*, *L. casei*, and the mixed probiotic diet notably increased silk gland protein concentration, alongside elevated carbohydrate and lipid levels in the larvae. The fibroin content was maximized in the *B. amyloliquefaciens* fed group, which recorded a yield of 0.310 g. Additionally, SDS-PAGE analysis demonstrated enhanced polypeptide expression at 30 kDa and 80 kDa, suggesting improved silk protein synthesis. These results highlight the functional benefits of probiotic-enriched diets in promoting larval health and enhancing silk yield in commercial silkworm rearing systems (Sekar *et al.,* 2016).

In an effort to improve the commercial traits of the silkworm *Bombyx mori*, Anisha *et al.,* (2022) evaluated the impact of probiotic-supplemented diets using Normagut, a commercial probiotic formulation containing *Saccharomyces boulardii*. The experiment involved foliar application of Normagut at 1%, 2%, and 3% concentrations on mulberry leaves, which were then fed to larvae from the third instar onward. Remarkably, the 2% treatment yielded the most significant results. Biochemical analysis revealed elevated levels of fat body protein (31.18 µg/mg), glycogen (20.21 µg/mg), and lipid (255.31 µg/mg). Similarly, haemolymph analysis showed enhanced protein (58.12 µg/ml), trehalose (365.06 µg/ml), and lipid (41.06 µg/ml) content. In the silk gland, free amino acids (34.32 µg/ml), protein (64.43 µg/mg), and lipid (15.27 µg/mg) were maximally expressed under the 2% probiotic regime. Corresponding economic traits such as larval weight (3869.23 mg), cocoon weight (2231.36 mg), shell weight (463.13 mg), shell ratio (21.72%), and filament length (912.24 m) also demonstrated notable improvement. These results clearly demonstrate that probiotic supplementation, particularly at 2% concentration, substantially enhances both physiological and commercial parameters in *B. mori*, indicating its potential application in sericulture productivity enhancement.

Fortification of mulberry leaves with probiotic strains significantly enhanced the biological performance and commercial traits of the bivoltine double hybrid silkworm (CSR6 × CSR26) × (CSR2 × CSR27). Among the treatments, supplementation with *Staphylococcus gallinarum* strain SWGB 7 at a concentration of 10⁸ cfu/ml resulted in the highest improvements across multiple parameters, including larval weight (4.12 g), effective rate of 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 a finer denier of 2.38. Moreover, larval mortality was reduced to 3.64%, indicating enhanced disease resistance. These findings demonstrate the beneficial role of probiotic supplementation in improving overall silkworm performance and silk yield. (Saranya *et al.,* 2019).

The influence of *Lactobacillus acidophilus* on the growth performance of two Thai silkworm strains, Nang Lai and Nang Lai × 108, demonstrated notable enhancements in biological and economic traits when administered through probiotic-enriched mulberry leaves. In the study, *L. acidophilus* was applied at a concentration of 10⁸ cells/mL to leaves fed to larvae from the second to fifth instars. For Nang Lai, probiotic treatment resulted in a higher survival rate (92.66 ± 1.52%) compared to the control (84 ± 1.00%), along with improved larval weight in the fifth instar (1.26 ± 0.05 g vs. 1.18 ± 0.05 g), pupation ratio (91 ± 1.00% vs. 82.33 ± 1.52%), cocooning ratio (91.33 ± 1.52% vs. 85 ± 1.00%), cocoon weight (1.08 ± 0.09 g vs. 0.94 ± 0.07 g), and cocoon shell ratio (14.95 ± 0.06% vs. 12.78 ± 0.15%). Similarly, Nang Lai × 108 exhibited a survival rate of 91 ± 1.73% versus 80.33 ± 0.58% in the control, with significant improvements in larval weight (1.70 ± 0.09 g vs. 1.53 ± 0.05 g), pupation (90.33 ± 1.52% vs. 83 ± 1.00%), cocooning ratio (90.33 ± 0.57% vs. 81 ± 1.00%), cocoon weight (1.18 ± 0.01 g vs. 1.15 ± 0.00 g), and shell ratio (16.67 ± 0.51% vs. 16.01 ± 0.62%). These findings affirm the beneficial role of *L. acidophilus* in promoting silkworm health, growth, and silk productivity through improved physiological efficiency and nutrient assimilation (Suraporn *et al.,* 2015).

A substantial enhancement in the enzymatic activity and economic performance of double hybrid silkworms was observed following dietary supplementation with selected probiotic strains. Among the tested probiotics *Lactobacillus rhamnosus*, *Saccharomyces boulardii*, and *Bifidobacterium longum* feeding silkworm larvae with 3% *B. longum* significantly elevated amylase (67.89 mg/g) and invertase (42.73 mg/g) activities compared to control values (23.63 mg/g and 22.34 mg/g, respectively). Additionally, supplementation with 3% *B. longum* led to the highest improvements in key commercial traits: cocoon weight (1.88 g), pupal weight (1.25 g), shell weight (0.33 g), and shell ratio (26.40%), exceeding the control group's respective values of 1.46 g, 1.12 g, 0.26 g, and 23.21%. Similar enhancements were observed for *L. rhamnosus* and *S. boulardii*, although to a lesser extent. These findings suggest that probiotic-enriched mulberry leaves can significantly boost silk yield and larval vigor by modulating gut enzymatic profiles and nutrient utilization efficiency, thus providing a promising biological tool in sericulture enhancement strategies (Pachiappan *et al.,* 2021).

In addition to enhancing growth and silk productivity, probiotics have also shown promise in bolstering the immune defense mechanisms of silkworms. Notably, *Francisella tularensis* established a symbiotic relationship with silkworms, where its presence in the hemolymph influenced host immunity. Following colonization, typical immune responses such as melanization and nodulation were suppressed. However, pre-inoculation with *F. tularensis* significantly upregulated the expression of antimicrobial peptides, thereby improving resistance against subsequent infections by pathogenic bacteria. These findings suggest that probiotic symbionts not only contribute to silkworm development but can also play a pivotal role in disease resistance, offering potential advantages for sericultural health management (Suzuki *et al.,* 2016).

Suraporn and Terenius (2021) studied the protective effects of *Lactobacillus casei* supplementation against *Nosema bombycis* infection in Thai polyvoltine silkworm strains. Their findings revealed that administering *L. casei* at a concentration of 10⁸ cells/mL significantly improved disease resistance, with larval survival increasing to 91% compared to 68% in the infected control group. Additionally, they observed enhanced larval weight during the fifth instar (2.70 ± 0.96 g), higher pupation ratio (95%), cocoon weight (1.33 ± 0.33 g), and shell weight (0.23 ± 0.33 g). These improvements were linked to enhanced gut health, strengthened immunity, and competitive inhibition of the pathogen, suggesting that *L. casei* could serve as an effective probiotic intervention for disease management in silkworms.

Attia *et al.,* (2025) demonstrated that supplementing *Bombyx mori* larval diets with *Lactobacillus acidophilus*, *L. casei*, or *L. delbrueckii* (10⁶–10⁸ CFU/ml) from the late 3rd instar significantly enhanced growth and disease resistance. Their findings revealed that *L. acidophilus* at 10⁸ CFU/ml optimized larval weight (2.28 g) and growth rate (312%) while reducing mortality (3.33–13.48%) compared to untreated groups. Scanning electron microscopy confirmed probiotic colonization in the foregut, with bacterial adhesion mediated by biofilaments. Histochemical analyses further showed elevated fibroin and sericin production in silk glands, particularly in *L. acidophilus*-treated larvae, alongside improved resistance to flacherie disease. The study underscores the potential of *Lactobacillus* probiotics as a sustainable approach to enhance sericulture productivity by modulating gut microbiota, nutrient absorption, and immune responses under climatic stressors.

Mohanraj *et al.,* (2016) evaluated how probiotic and botanical supplements influence the growth and cocoon quality of *Bombyx mori*, using different treatments on two silkworm races-Pure Mysore (PM) and CSR2. They compared the effects of *Lactobacillus* spp. (10⁶ CFU/mL), a combination of *Lactobacillus* with *Psoralea corylifolia* (2%), and a native mix of *Bacillus subtilis* and *B. tequilensis* (10⁶ CFU/mL). Among these, the combination of *Lactobacillus* with *P. corylifolia* produced the most significant improvements. In CSR2, larval weight increased by 18.94% (4.17 g vs. 3.38 g in control), and shell ratio improved by 19.23% (26.63%). Similarly, in PM, larval weight rose by 14.52% (3.1 g vs. 2.65 g), and shell ratio by 14.37% (24.36%). *Lactobacillus* alone also showed a positive effect, enhancing CSR2 cocoon weight by 10.23% and shell weight by 22.73%. The *Bacillus*-based treatment yielded moderate benefits, including a 13.11% increase in larval weight for CSR2. These improvements were linked to better gut microbial balance, higher nutrient assimilation, and enhanced digestive enzyme activity, indicating that combining probiotics with botanicals can effectively boost silkworm growth and silk production.

Probiotic supplementation has consistently demonstrated a multifaceted role in enhancing silkworm physiology, with marked improvements observed in growth, immunity, and silk production. Across various experimental setups, the incorporation of microbial strains such as *Lactobacillus*, *Saccharomyces*, *Bacillus*, and *Bifidobacterium* has led to significant biochemical and economic gains. These benefits include elevated digestive enzyme activity, increased silk gland protein content, improved nutrient assimilation, and enhanced expression of immune-related biomarkers. Notably, the effects were often strain- and dosage-specific, highlighting the importance of precise formulation. Moreover, studies have shown that probiotics can reduce disease incidence and improve resilience under pathogenic or environmental stress. Together, these findings establish microbial supplementation not just as a growth enhancer, but also as a potential tool for strengthening silkworm health and productivity in commercial sericulture.

6. Challenges and Future prospects

Despite the promising benefits of probiotic supplementation in enhancing silkworm growth, immunity, and silk yield, several critical challenges remain. One of the foremost issues is the lack of standardized protocolsfor probiotic strain selection, dosage, and mode of administration. Most studies vary widely in terms of microbial species used, concentrations applied, and silkworm races tested, making it difficult to draw universally applicable conclusions. Additionally, the mechanisms underlying host-microbiota interactions remain incompletely understood, especially at the molecular and genomic levels. There is also limited knowledge regarding the long-term impacts of probiotic administration on silkworm physiology, including potential microbial resistance, shifts in native gut flora, or unintended effects on silk protein synthesis. Furthermore, environmental and genetic variability such as differences in climate, mulberry leaf quality, and silkworm strain—can significantly influence probiotic efficacy, complicating the generalization of findings.

Looking ahead, future research must focus on metagenomic**,** transcriptomic, and metabolomic analyses to unravel the precise molecular pathways influenced by probiotics in Bombyx mori. There is also a need to develop tailored probiotic formulations that are compatible with specific silkworm races and rearing conditions. Moreover, the integration of RNA-based microbiome profiling could help identify viable and metabolically active gut microbes, offering deeper insight into their functional roles. Exploring synergistic effects of probiotics with prebiotics, botanicals, or immune-stimulating agents could pave the way for next-generation biofortified feeds. Importantly, field-level validation and cost-benefit analysis are essential to transition from experimental success to scalable, eco-friendly practices in commercial sericulture. Ultimately, addressing these challenges could unlock the full potential of probiotic biotechnology for sustainable and disease-resilient silkworm rearing systems.

7. Conclusion

The integration of probiotic supplementation in silkworm rearing has emerged as a promising biotechnological advancement, demonstrating significant improvements in larval growth, cocoon quality, disease resistance, and overall physiological performance. Empirical findings have consistently shown that specific probiotic strains particularly from the genera *Lactobacillus*, *Bacillus*, *Bifidobacterium*, and *Saccharomyces* modulate the gut microbiota of *Bombyx mori*, enhancing nutrient assimilation, enzymatic activity, and immune responses. These benefits collectively contribute to improved silk yield and product quality.

Furthermore, the synergistic use of probiotics with botanical additives or prebiotics has amplified their efficacy, highlighting the potential for tailored biofortified feed strategies. However, the strain-specific and dosage-dependent nature of these effects underscores the necessity for standardized protocols and targeted formulations. With advancements in molecular tools such as metagenomics, transcriptomics, and functional microbiome analysis, there is a growing opportunity to deepen our understanding of host-microbiota interactions and develop precision probiotic interventions for sericulture.

In this context, probiotic supplementation offers a sustainable, eco-friendly, and economically viable approach to strengthening silkworm health and optimizing silk production. Continued interdisciplinary research and field validation will be critical in translating these insights into scalable solutions that support the long-term resilience and profitability of the sericulture industry.

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