***Review Article***

**Silkworm–Microbiome Interactions: Emerging Strategies for Gut Health, Artificial Diet Adaptation, and Sustainable Sericulture**

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ABSTRACT

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| Silkworms are economically and biologically significant insects widely cultivated for silk production. While genetic and nutritional research has long shaped traditional sericulture, recent advances in microbiome science have uncovered the crucial roles that gut microbial communities play in the health, development, and productivity of silkworms. These microbial partners facilitate digestion, immune modulation, detoxification, and even influence silk production. As artificial diets and mass-rearing conditions grow in popularity, understanding how gut microbiota adapt—and how they can be manipulated—is vital for improving sustainability and efficiency in sericulture. This review synthesizes recent scientific findings on the diversity, functionality, and plasticity of the silkworm gut microbiome. We highlight how factors such as diet, environment, and host genetics influence microbial communities and discuss the potential of probiotics, prebiotics, and microbial engineering as tools to enhance silkworm performance. Through an analysis of more than 30 studies, this paper aims to provide a comprehensive framework for integrating microbiome science into modern sericulture practices. |

*Keywords: Silkworm microbiome, Gut microbiota, Artificial diet, Probiotics, Prebiotics, Synbiotics, Microbiome engineering*

1. INTRODUCTION

Sericulture, the cultivation of silkworms for silk, has a long history across Asia, Africa, and parts of Europe. Silkworms are not only of economic importance but also serve as model organisms in genetic, physiological, and immunological studies [1,2,3]. Traditionally, silkworm development and silk production have been optimized through selective breeding and controlled feeding with mulberry leaves. However, the increasing adoption of artificial diets, the demand for year-round silk production, and climate-related stressors have complicated rearing practices [4,5].

In parallel, microbiome research has emerged as a transformative field, revealing the symbiotic relationships between host organisms and their gut microbial communities. Insects, including silkworms, harbour diverse microbial consortia that affect nearly every aspect of their biology—from digestion and detoxification to immunity and development [6,7]. The insect gut microbiome is known to influence host physiology through nutrient provisioning, hormonal modulation, competitive exclusion of pathogens, and interaction with host gene expression [1,8,9].

In recent years, the gut microbiota of silkworms has been recognized as a crucial factor in larval health and silk yield, particularly under the stress of artificial feeding or disease exposure [4,10]. Efforts to analyze and manipulate the silkworm microbiome through sequencing, probiotics, and diet modifications have gained traction, aiming to stabilize beneficial microbial communities and improve silk output in sustainable ways [11,12].

This review highlights recent advances in understanding silkworm–microbiome interactions, examining microbial diversity, host-microbe dynamics, dietary and environmental influences, and emerging microbiome-based strategies such as probiotic supplementation and artificial diet enhancement, offering insights for researchers and sericulture industries to harness microbiota for sustainable, health-optimized silk production.

1. **COMPOSITION AND DIVERSITY OF THE SILKWORM GUT MICROBIOTA**
   1. **Overview of Gut Anatomy and Microbial Niches**

The gut of silkworms is composed of the foregut, midgut, and hindgut. Among these, the midgut plays the most critical role in digestion and is typically the most densely colonized region in terms of microbial load [13]. The microbial populations vary across these compartments due to differences in pH, oxygen levels, nutrient concentration, and host-secreted enzymes. The midgut is slightly alkaline and provides an optimal environment for cellulolytic and proteolytic bacteria, while the hindgut tends to support fermentation processes due to its more anaerobic conditions [13,14,15].

* 1. **Core Microbiota in Silkworms**

High-throughput 16S rRNA gene sequencing has revealed that silkworm guts are typically dominated by bacteria from the phyla Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes [2,10,16]. The silkworm gut microbiota commonly includes bacteria from the genera *Enterococcus*, *Lactobacillus*, *Weissella*, *Bacillus*, and *Acinetobacter*, although the proportions of each can shift significantly depending on factors such as diet, environmental conditions, and the insect's developmental stage [4,15,17].

Enterococcus species, particularly *Enterococcus faecalis* and *Enterococcus faecium*, are often considered core microbiota in silkworms. These bacteria contribute to protein degradation and lactic acid production and may also offer pathogen resistance through competitive exclusion and antimicrobial compound secretion [18,19].

In silkworm artificial diets, some studies have reported a decline in diversity and a higher prevalence of *Enterococcus* and *Weissella*, suggesting a narrowing of microbial composition under nutrient-modified conditions [15,17]. Conversely, Lactobacillus tends to be more prevalent in silkworms fed natural mulberry leaves, which may support a more balanced and robust gut ecosystem [17,20].

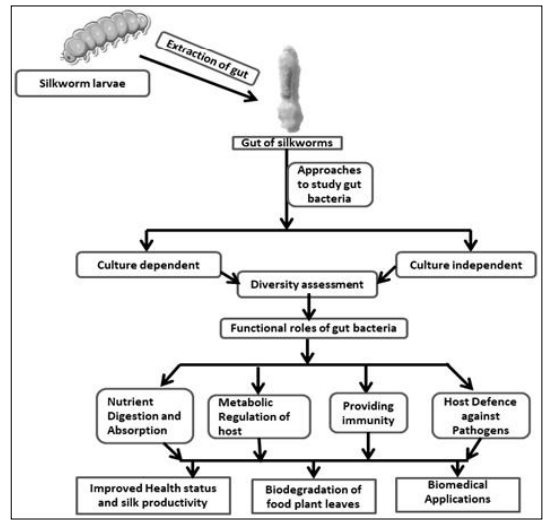
* 1. **Developmental and Temporal Dynamics**

The gut microbiome of silkworms is dynamic and undergoes significant shifts across the larval stages. For instance, microbial load increases as larvae grow, peaking around the 4th and 5th instars when feeding rates are highest [9,15,21]. Changes in moulting behaviour and gut peristalsis also affect microbial retention and turnover. Studies suggest that earlier instars have a simpler microbial community that becomes more complex as larvae age, possibly due to prolonged exposure to environmental microbes and the accumulation of partially digested substrates [2,13,22].

* 1. **Factors Affecting Microbial Diversity**

Microbial diversity in silkworms is influenced by both intrinsic and extrinsic factors. Intrinsic factors include larval age, genetic background, and immune status, while extrinsic factors include diet composition, ambient temperature, humidity, and microbial exposure in the rearing environment [15,23].

Interestingly, the same silkworm strain raised on different types of mulberry leaves (e.g., *Morus alba* vs. *Morus nigra*) shows variation in gut microbiota composition, presumably due to differences in leaf secondary metabolites and nutrient profiles [24,25]. Moreover, rearing season can also impact gut diversity, with larvae reared in autumn often displaying higher microbial richness than those reared in summer [26]. A summarized representation of how gut microbiota are studied, their functional roles, and future applications in silkworm biology is shown in Figure 1.



*Figure 1: Research approaches, functional roles, and future prospects of silkworm gut bacteria. This diagram illustrates the use of culture-dependent and culture-independent methods to assess microbial diversity, followed by functional outcomes such as nutrient digestion, metabolic regulation, immune support, and pathogen defence, which ultimately contribute to improved silk productivity and potential biomedical applications [27].*

1. **FUNCTIONAL ROLES OF THE SILKWORM GUT MICROBIOME**

Understanding the functional capabilities of gut microbiota in silkworms is essential for leveraging these communities to improve host health and silk productivity [12,15]. Gut microbes play several critical roles, including digestion of macronutrients, biosynthesis of essential compounds, modulation of host immune responses, and protection against pathogens. These microbes also contribute to detoxification processes and, potentially, the regulation of silk protein synthesis through metabolic cross-talk [28].

* 1. **Nutrient Digestion and Assimilation**

A key function of the silkworm gut microbiota is the enzymatic degradation of recalcitrant dietary macromolecules such as cellulose, hemicellulose, and glycoproteins [29]. These compounds, abundant in both mulberry leaves and formulated diets, often escape hydrolysis by host-derived enzymes, but are effectively metabolized by microbial communities harbouring cellulases, proteases, and detoxifying enzymes [8,29]. Symbiotic bacteria, such as species of *Bacillus*, *Enterococcus*, and *Lactobacillus*, produce enzymes like cellulases, amylases, proteases, and lipases that facilitate the breakdown of cellulose, starch, and proteins into absorbable monomers [30,31].

Supplementation with gut-beneficial microbes, including *Lactobacillus* species, has been shown to enhance digestive enzyme activity and nutrient metabolism in silkworms [32]. Additionally, microbial fermentation of dietary fibers generates short-chain fatty acids (SCFAs) such as acetate and butyrate, which provide energy for gut epithelial cells and support intestinal homeostasis [29].

* 1. **Immune System Modulation**

Silkworms rely on innate immunity to defend against pathogens, as they lack adaptive immune systems. The gut microbiota contributes to immune defence in several ways. Commensal microbes stimulate the production of antimicrobial peptides (AMPs) and other immune effectors by interacting with pattern recognition receptors (PRRs) such as peptidoglycan recognition proteins (PGRPs) in the gut epithelium [33,34].

Certain bacteria like *Enterococcus faecalis* have been shown to activate the Toll and Imd signalling pathways, which are central to insect innate immunity [2]. In experiments where probiotic strains were introduced into silkworms infected with *Bacillus thuringiensis*, larvae displayed significantly lower mortality, elevated AMP expression, and higher levels of haemocyte proliferation compared to untreated controls [12].

Gut microbes also play a role in maintaining gut barrier integrity by regulating mucus production and tight junction proteins, thereby reducing pathogen entry [6,13]. These protective effects become especially important under artificial diet regimens, where immune suppression and dysbiosis are more likely to occur.

* 1. **Detoxification of Plant Metabolites and Environmental Toxins**

Silkworms are exposed to a variety of natural and anthropogenic toxins, including plant-derived secondary metabolites such as tannins and alkaloids, as well as pesticide residues and heavy metals. Microbes in the gut help mitigate these risks through enzymatic detoxification mechanisms [35].

Certain *Bacillus* and *Pseudomonas* strains have been identified as producers of enzymes like glutathione S-transferase, catalase, and laccase, which degrade toxic compounds and reactive oxygen species [36,37]. Silkworms fed on leaves treated with chlorpyrifos or other pesticides showed reduced growth and silk yield unless their diet was supplemented with probiotic strains capable of pesticide degradation [18].

Additionally, some gut microbes may bind to toxins or sequester them in biofilms, preventing their absorption across the gut epithelium [13,35]. This detoxifying role is especially important when silkworms are fed artificial diets made from plants and agricultural by-products, which often contain natural substances that can interfere with digestion or health [12,15].

* 1. **Contribution to Silk Production**

The relationship between gut microbiota and silk synthesis is still an emerging area of research. However, recent findings suggest that the gut microbiome can influence silk production indirectly through nutrient availability and directly via metabolic signalling pathways [15,35].

Silk is primarily composed of fibroin and sericin proteins, whose synthesis depends on the availability of amino acids, particularly glycine, alanine, and serine. Gut bacteria that enhance protein digestion and amino acid production thereby contribute to silk biosynthesis [15,38].

Furthermore, microbial metabolites such as SCFAs, polyamines, and vitamins may modulate host gene expression. In silkworms fed with *Lactobacillus plantarum*, fibroin gene expression in the silk gland was significantly upregulated, correlating with increased silk output and cocoon weight [11,20].

* 1. **Behaviour and Developmental Timing**

Although research in silkworms remains limited, emerging evidence suggests that the gut microbiome may influence behavioural traits and developmental timing [2,15]. Insights from other insect models indicate that gut bacteria can modulate processes such as moulting, pupation, and stress responses by interacting with host hormonal pathways and producing neuroactive metabolites [9].

Preliminary studies in silkworms suggest that gut microbial disruption through antibiotics can delay moulting, reduce feeding activity, and result in uneven cocooning [39]. More detailed investigations are needed, but the behavioural and endocrine effects of microbiota offer a novel frontier in silkworm biology that may eventually be exploited for precision management in industrial sericulture [15].

1. **IMPACT OF DIET ON SILKWORM GUT MICROBIOTA**

Diet is among the most influential factors shaping the composition, diversity, and functional output of gut microbiota in silkworms [4]. As these insects are increasingly reared under controlled indoor environments and artificial feeding systems, understanding the consequences of dietary change on their gut ecosystems is essential for maintaining optimal health, development, and silk yield [9,15].

* 1. **Mulberry Leaves: The Traditional and Optimal Diet**

Natural plant-based diets rich in nutrients such as proteins, fiber, vitamins, and trace minerals have been shown to support a balanced gut microbiota in silkworms. These diets often contain bioactive compounds that influence microbial colonization and community structure [2,15].

Silkworms raised on such diets tend to harbour diverse and stable microbial communities dominated by beneficial genera like *Lactobacillus*, *Bacillus*, and *Enterococcus* [10,12]. These bacteria assist in cellulose digestion, production of short-chain fatty acids, and immune modulation, contributing to overall gut homeostasis. The presence of plant-derived prebiotics such as pectin and hemicellulose in mulberry leaves may support the growth of commensals, creating a mutually beneficial host–microbe relationship [4,15,25].

* 1. **Artificial Diets and Their Microbial Consequences**

To ensure year-round silk production and reduce dependence on seasonal mulberry crops, artificial diets have been developed. These diets generally include a mixture of mulberry leaf powder, wheat bran, soybean meal, vitamins, minerals, agar, and preservatives. While convenient and scalable, artificial diets often fail to replicate the complex chemical and microbial environment of fresh leaves [40].

Studies comparing the gut microbiota of silkworms fed artificial diets versus mulberry leaves show consistent patterns of reduced microbial diversity and increased dominance of lactic acid bacteria such as *Enterococcus* and *Weissella* [15,21]. This microbial simplification is associated with symptoms of dysbiosis, including gut inflammation, weakened immunity, slower growth, and lower cocoon weight [12,41].

Artificial diets may also lack critical prebiotic components or harbor heat-labile antimicrobial residues (from sterilization) that inhibit beneficial microbes. Consequently, larvae fed artificial diets often display altered fermentation profiles, reduced enzymatic activity, and thinner gut epithelia—markers of impaired digestive performance and nutrient absorption [15,42].

* 1. **Diet-Induced Dysbiosis and Its Consequences**

Dysbiosis in silkworms refers to an imbalance in the gut microbial community, where the proliferation of harmful or opportunistic bacteria disrupts the stability and functionality of beneficial microbiota [15,35,41]. This imbalance can significantly impair digestive efficiency, particularly through the depletion of proteolytic bacteria essential for protein assimilation [28]. As a result, nutrient uptake is compromised, leading to suboptimal growth and developmental delays. Additionally, dysbiosis has been associated with a weakened immune response, evidenced by the downregulation of antimicrobial peptide expression, making silkworms more vulnerable to infections by entomopathogens such as *Beauveria bassiana* and *Serratia marcescens* [43].

Beyond immune suppression and metabolic disruption, dysbiosis can also deteriorate silk quality. Studies have reported that microbial imbalances are linked to shorter filament length and reduced tensile strength of silk fibers [44]. Moreover, dysbiosis contributes to increased gut permeability—a condition often described as "leaky gut"—which facilitates systemic inflammation and oxidative stress throughout the organism [45]. These pathological changes further impair larval development and hinder the function of silk glands, ultimately affecting both the quantity and quality of silk production. Thus, maintaining a balanced gut microbiome is critical not only for silkworm health but also for optimizing sericultural outcomes [12,15,39].

* 1. **Adaptation of Gut Microbiota to Diet Transitions**

Despite the challenges associated with artificial diets, silkworms and their microbiota exhibit some degree of plasticity. When transitioned from artificial to mulberry leaf-based diets (or vice versa), gut microbial communities undergo substantial reorganization within 48–72 hours [9,15]. This microbial turnover is facilitated by changes in pH, gut motility, and substrate availability.

Interestingly, diet-induced microbial shifts are not always reversible. Larvae fed artificial diets for extended periods often retain a simplified microbiota, even after transitioning back to mulberry leaves [4,9]. This suggests a form of microbial “memory” or resistance to recolonization, which may reflect altered gut epithelial conditions or immune responses [24].

* 1. **Strategies to Improve Artificial Diet Outcomes via Microbiome Modulation**

To mitigate the drawbacks of artificial diets, several microbiome-based interventions have been explored. Probiotic supplementation with strains like *Bacillus subtilis*, *Lactobacillus plantarum*, and *Weissella cibaria* has been shown to improve gut microbial balance, enhance immunity, and increase silk yield by supporting digestion and suppressing harmful bacteria [12,40].

Advanced strategies such as synbiotic formulations—combinations of probiotics and prebiotics—offer synergistic effects that improve diet palatability and microbial colonization [40]. These formulations not only support the growth of beneficial bacteria but also help suppress potential pathogens by enhancing gut competitive exclusion. The inclusion of functional ingredients like oligosaccharides or herbal extracts can further modulate the microbial ecosystem and improve nutrient utilization. Fermenting artificial diets prior to feeding has also shown potential in enhancing probiotic viability and gut adaptation [12,15,35].

1. **PROBIOTICS, SYNBIOTICS, AND MICROBIOME ENGINEERING IN SILKWORM REARING**

Microbiome-targeted interventions such as probiotics, synbiotics, and engineered microbial consortia are gaining attention as promising tools to enhance gut health, nutrient absorption, and silk productivity in silkworms. These strategies aim not only to restore microbial balance, particularly under artificial diets, but also to manipulate the gut ecosystem for improved physiological outcomes [7,11].

*Figure 2: Microbiome modulation strategies in sericulture. Probiotic, prebiotic, synbiotic, and microbiota transplantation approaches aim to enhance gut health, immune activation, silk yield, and disease resistance. (Source: Author’s own illustration).*

Probiotics—live beneficial microorganisms—have demonstrated positive effects on silkworm health. Species like *Lactobacillus plantarum* and *Bacillus subtilis* enhance larval weight, cocoon quality, immune function, and stress resistance, particularly in nutrient-limited rearing systems [12,20,46]. These microbes support digestion, suppress pathogens, and improve gut stability by producing antimicrobial compounds and antioxidants.

Prebiotics, such as mulberry leaf polysaccharides and inulin, selectively stimulate beneficial bacteria like *Lactobacillus* and *Bifidobacterium* [46,47]. When combined with probiotics, they form synbiotics, which synergistically improve microbial diversity and larval performance. Some studies have also used plant-based compounds like green tea polyphenols or garlic extracts alongside microbial inoculants to enhance immunity and offer antifungal or antiviral effects [12,15,48].

Fermenting artificial diets with beneficial microbes before feeding has emerged as a bio-enhancement strategy. This process partially digests macromolecules, increases metabolite availability, and detoxifies harmful compounds. Fermented feeds are better accepted by larvae, improve gut colonization, and can boost silk yield significantly while reducing the need for antibiotics [12,15,39].

Microbiota transplantation has also been tested, with gut contents from healthy larvae transferred into dysbiotic or antibiotic-treated individuals to restore microbial balance and improve performance [39]. Precision engineering approaches are beginning to design synthetic microbial consortia with specific functions such as fiber degradation or short-chain fatty acid production [15]. The future may see genetically engineered probiotics tailored to produce silk-enhancing or immunomodulatory compounds, though their environmental safety and regulatory approval remain major considerations.

Despite their potential, these interventions face challenges. Probiotic colonization success varies with host genotype and environmental conditions, and strain effectiveness is often inconsistent [12]. Technical issues such as storage stability, formulation, and delivery methods also limit field-scale application. Regulatory frameworks, especially for genetically modified strains, are still evolving. Nonetheless, with expanding knowledge of silkworm–microbe interactions, microbiome engineering offers a promising direction for sustainable and precision-driven sericulture [16,17].

1. **FACTORS INFLUENCING THE SILKWORM GUT MICROBIOME**

The composition and functionality of the silkworm gut microbiome are shaped by a combination of environmental conditions, host genetic background, and research methodologies. Understanding these variables is essential for designing reproducible experiments and developing microbiome-informed sericulture strategies [2,10,26].

* 1. **Environmental Factors**

External factors such as temperature, humidity, seasonal variation, and hygiene conditions strongly affect microbial diversity and stability in silkworms. Elevated temperatures can reduce microbial richness by inhibiting heat-sensitive bacteria like *Lactobacillus*, while high humidity promotes mold growth and microbial contamination [28,49]. Seasonal shifts also influence microbial communities—larvae reared in cooler months often show higher microbial richness, possibly due to differences in mulberry leaf chemistry and environmental exposure [2]. Hygiene plays a dual role; while poor sanitation increases pathogenic risks, excessive sterilization may suppress exposure to beneficial environmental microbes. Balancing cleanliness with natural microbial exposure is crucial for supporting a healthy gut ecosystem [15,39].

* 1. **Host Genetics and Immune Modulation**

Genetic variation among silkworm strains has a significant impact on gut microbial composition. Even under identical diets and rearing conditions, different strains exhibit distinct dominant genera such as *Enterococcus*, *Lactobacillus*, or *Acinetobacter* [15,21,46]. These differences may stem from host factors like gut physiology, metabolic activity, or immune signaling. The innate immune system, through mechanisms such as antimicrobial peptide production and oxidative regulation, plays a key role in maintaining microbial balance. Silkworms with genetically enhanced immune responses—such as those upregulating Toll or Imd pathways—tend to exhibit reduced microbial diversity but enhanced pathogen resistance [50,51]. In hybrid or genetically modified strains, shifts in microbial profiles may also result from altered host metabolism or physiological demands [15,35,39].

* 1. **Methodological and Experimental Considerations**

Reliable microbiome characterization depends heavily on methodological consistency. Sample collection methods, preservation protocols, and sequencing platforms all influence outcomes. Rapid gut dissection followed by freezing in liquid nitrogen is recommended to maintain microbial integrity, as delays can allow opportunistic taxa to overgrow [10,15]. DNA extraction protocols that lack mechanical disruption may underrepresent Gram-positive microbes, and primer selection during 16S rRNA amplification can introduce taxonomic bias [10,52]. While short-read sequencing remains popular, long-read technologies like Oxford Nanopore and PacBio offer higher taxonomic resolution. To ensure reproducibility, experiments should adopt standardized bioinformatics pipelines and validated reference databases [53]. Moreover, using germ-free or gnotobiotic larvae, mock communities, and complementary validation techniques such as qPCR or metabolomics can help improve data quality and enable more robust cross-study comparisons [54].

1. **FUTURE PERSPECTIVES**

Recent advances in silkworm gut microbiota research are transforming sericulture from a system focused solely on breeding and diet to one informed by microbiome science. Gut microbes are now seen as active partners in enhancing health, development, and silk yield [15,55]. Microbiome diagnostics may soon enable early detection of disease or stress, allowing farmers to adopt precision sericulture strategies through tailored diets and probiotic interventions [56]. Personalized microbial formulations adapted to specific silkworm strains or artificial diets could enhance larval health and productivity [12,15].

However, practical challenges must be addressed before widespread implementation. These include ecological risks from engineered microbes, strain-specific limitations, and the technical complexities of scaling up live microbial products [8,56]. Additionally, regulatory frameworks for microbial use in insect farming—especially for genetically modified strains—remain underdeveloped in many regions [57]. Despite these obstacles, integrating microbiome-targeted approaches holds significant promise for building a more sustainable, resilient, and high-efficiency future for global silk production.

1. **CONCLUSIONS**

Silkworms and their gut microbiota form an intricate, adaptable, and functionally significant partnership that underpins larval development, immune competence, and silk production. The gut microbiome is shaped by factors such as diet, environment, host genotype, and microbial exposure, and can be modulated using probiotics, prebiotics, and engineered microbial strategies.

As sericulture shifts toward controlled rearing and artificial diets, microbiome-targeted interventions offer a promising route to overcome the limitations of conventional practices. By integrating microbiome science into silkworm breeding, nutrition, and disease management, the industry can move toward more resilient, productive, and sustainable silk farming systems. In sum, the silkworm gut microbiome is not merely a bystander but a central player in modern sericulture, offering new tools and insights for the next generation of biological innovation.

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