

Research Article

Potential of probiotic bacteria to improve silk production: Boosting the Sericulture Industry in Northeast India

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Abstract

Silkworms' Gut are home to various microorganisms, including viruses, fungi, and bacteria. Bacteria are the most prevalent and varied group, with more than 100 species known to exist. These bacteria are members of several phyla, such as Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes. A wide variety of beneficial bacteria are found in the gut of silkworms. Probiotics mainly influence some aspects of the immune system and gut health by interacting with the complex community of intestinal bacteria known as the gut microbiota. Certain bacteria produce essential vitamins, amino acids, and other nutrients, while others help break down dietary fibres. Silkworms are an invaluable model organism for gut microbiota research due to their significance in ecology and economy. The present review discusses the diversity and functions of bacteria in silkworm digestive tracts and explores potential applications of these microbes in other sectors of the economy. The findings of this study have highlighted the importance and potential of silkworm gut microbiota, as well as the prospective applications of these microbes in the future to enhance silkworm health and resistance to illness. Thus, comprehending the relationships between silkworms and the bacteria in their stomachs may offer fresh perspectives on the biology of these significant insects and open the door to the creation of innovative methods for increasing silk production.

Keywords: Lactobacillus, Probiotics, Sericulture, Silkworm

INTRODUCTION

An agro-based sector crucial to the transformation of the rural economy in India is sericulture. The states of the Northeast have a climate that is ideal for sericulture and a wealth of vegetation. Sericulture is achieving popularity in the Northeastern Indian states, especially in Assam, Manipur, and Nagaland. A significant primary-producing insect species that is raised and used commercially around the world to support the sericulture

sector is the silkworm. The global sericulture business has improved people's social and economic conditions by stimulating the textile sector, which has played a major role in declining unemployment in emerging nations (Ssemugenze *et al.*, 2021). The silkworm holds significant value in the sericulture industry since they yield precious silk fibres that are utilized in manufacturing fabrics, medicinal gadgets, and several other products. These holometabolous insects undergo the entire metamorphosis cycle, encompassing the egg, larva,

pupae, and adult stages. The importance of the silkworm gut microbiota in maintaining the health and productivity of these insects has led to an increase in interest in this area recently.

The gut bacteria of silkworms have a major role in the digestion and absorption of nutrients, the host's defence against infections, and the regulation of host immunity. Furthermore, these microbes may be used in a variety of fields, such as biotechnology, medicine, and agriculture. Insects are among the oldest species on Earth and represent the most diverse collection of organisms. Since then, these insects have been connected to various microbes (Ali *et al.*, 2023). By generating vital substances like vitamins, breaking down and metabolizing food, absorbing nutrients, detoxifying toxins, manufacturing pheromones, and boosting immunity, the resident microbial flora in insects helps the host (Zhao *et al.*, 2022). This insect-microbe relationship, in part, sheds light on a range of secondary metabolites and other beneficial chemicals and enzymes that may be extracted from these microbes and utilized for several reasons, either by humans or by the insect itself (Baretto *et al.*, 2021). The development of insects has been aided by their symbiotic relationships with helpful microorganisms, which improve diets deficient in nutrients, aid in the digestion of food components that are acid reflux, provide protection from pathogens, parasites, and predators, and facilitate communications between and within species. (Govindarajulu *et al.*, 2020).

The gut bacteria are important metabolic resources because they improve the digestion and absorption processes in the insect gut, enhance the digestion and absorption of complex plant metabolites, and supplement nutrients and digestive enzymes to increase the survival of insects (Khyade *et al.*, 2013; Das *et al.*, 2024). According to Arasakumar *et al.* (2023), insects like silkworms rely on gut bacteria for various benefits, including nutrient digestion, defence against pathogens, and growth enhancement. Certain microorganisms in the silkworm gut produce digestive enzymes, aiding in nutrient absorption. Studies have shown that supplementing silkworm diets with nutrients like cyanobacteria can improve larval growth and cocoon quality. The composition of gut microflora in silkworms includes both gram-positive (such as *Bacillus spp.* and *Enterococcus spp.*) and gram-negative bacteria (like *Escherichia coli* and *Klebsiella pneumonia*). Gut bacteria's functional roles in lepidopteran insects are only partially understood (Zhang *et al.*, 2022). Understanding the nutritional impact of gut bacteria in insects can help control pests and improve commercial insect rearing. Despite a wealth of information regarding the biology of silkworms, not much research has been done on their gut microbiome. As these microorganisms play crucial roles in silkworms' overall health and development, influencing economic traits like cocoon quality and yield, we will

examine the present understanding of silkworm gut bacteria in this review, as well as their roles, importance, and potential uses in the future (Gandotra *et al.*, 2018; MsangoSoko *et al.*, 2021; Liang *et al.*, 2018 and Liu *et al.*, 2018).

METHODOLOGIES FOR UNDERSTANDING THE DIVERSITY OF GUT BACTERIA

Understanding the microbial communities within silkworms is crucial for their development and ability to produce silk, as demonstrated by the studies on their gut microbiota. The approaches are as follows:

Culture-dependent methods

Isolation and Culturing: This isolates bacteria from a gut sample and grows them in a lab setting on certain media. Morphology, biochemical as well as functional characteristics can be ascertained from the cultured bacteria (Prashanthi and Kuntamalla, 2020)

Biochemical Assays: The functional roles of cultured bacteria in the gut ecosystem can be ascertained by testing them for particular enzymatic activity, such as lipase, cellulase, amylase, and protease, among others (Zhu *et al.*, 2022).

Culture-independent methods

Metagenomic sequencing: Examination of the complete microbial community, including non-cultivable bacteria, is possible through direct DNA analysis from the silkworm intestines (Yuan *et al.*, 2023)

Meta-transcriptomic sequencing: This method examines the RNA transcripts of the gut microbiota and offers information on the metabolic processes and active roles of these microorganisms in identifying the metabolic pathways and active genes in a given situation. It sheds light on the functional activities of microbial communities and the expression of genes (Zhu *et al.*, 2022).

16S rRNA gene sequencing: This technique provides information on microbial diversity by identifying and categorizing bacteria based on the 16S ribosomal RNA gene (Paoli *et al.*, 2022)

By identifying both cultivable and non-cultivable bacteria, these methods provide a more comprehensive understanding of the makeup and function of the gut microbiota. For example, investigations by Li *et al.* (2020) and Yeruva *et al.* (2020) explored gut bacterial diversity and identified putative probiotic strains using 16S rRNA gene sequencing and metagenomic sequencing, respectively.

Functional assays

Enzyme assays: To evaluate the functional potential of the microbiota, particular enzyme activities can be directly measured from gut samples in addition to biochemical assays. (Scardaci *et al.*, 2021)

Metabolomics: This method entails identifying and measuring the metabolites that intestinal bacteria create to provide light on their metabolic processes and interactions with the host. Gong and Yang (2012).

Combined approaches

A more thorough understanding of the dynamics of the gut microbiota can be obtained by combining culture-dependent and culture-independent techniques. Chen *et al.* (2020) used a combination of 16S rRNA gene sequencing and cultured gut bacteria to examine the role of gut microbiota in host insect resistance to pesticides. Similarly, by combining the two approaches, MsangoSoko *et al.* (2020) enhanced the examination of the gut microbiota of silkworms and discovered a diverse range of culturable and non-culturable bacteria.

By utilizing these techniques, researchers can promote sustainable sericulture methods and thoroughly understand gut microbiota's diversity, functional capacities, and functions in host physiology, health, and illness by using these techniques alone or in combination (Li *et al.*, 2022).

How do probiotics work to their advantageous effects?

According to Priyadharshini *et al.*, 2021 probiotics primarily affect several facets of gut health and immune function by interacting with the intricate ecology of microorganisms found in the gut, also referred to as the gut microbiota (Priyadharshini *et al.*, 2021). One important way that the native (autochthonous) gut bacteria maintain their presence and provide niche protection against newly ingested microorganisms, including pathogens, is through the advantageous use of intestinal microflora, also known as "colonization resistance" or the "barrier effect" (Lewis *et al.*, 2015; Pérez-Cobas *et al.*, 2015). Different advantageous effects of probiotics are mentioned in Fig. 1.

Real-world applications of gut bacteria in silkworms

According to Mwchahary *et al.* (2023), it is intriguing to observe the symbiotic relationship between intestinal bacteria and silkworms, which has many consequences for the general development and health of the silkworms. Fig. 2 represents some important real-world applications of gut microbiota in silkworms.

FUTURE POTENTIAL OF GUT BACTERIA IN SILKWORMS

Enhancing the general well-being and output of silkworms through manipulating gut microbes like *Lactobacillus spp.* and *Bacillus subtilis* will improve the quality and amount of silk produced. Creating therapeutic mol-

ecules like cytokines and enzymes from gut bacteria to generate antimicrobial chemicals that can work as natural antibiotics and cure a range of illnesses investigating host-microbe interactions in silkworms to learn more about immunity, metabolism, digestion, and behaviour, all of which will help design novel probiotics and prebiotics. breaking down complex polysaccharides in food plant leaves into simpler nutrients by using gut bacteria, particularly cellulose-degrading species, to improve digestion and lessen environmental pollution. Establishing the relationship between the gut microbiota of silkworms could help better understand the biology of these intriguing insects and pave the way towards advancing novel silk-producing techniques (Mwchahary *et al.*, 2023). The gut flora is a useful barrier to heavy metal uptake and influences heavy metal bioavailability and toxicity (Ding *et al.*, 2019; Richardson *et al.*, 2018). Overexposure to heavy metals has seriously harmed the biological system and the silkworm's gut microbiota's regular activities. However, the resistant microbes that aid in detoxification will be strengthened by the gut microbiota, lowering the toxicity of heavy metals (Chen *et al.*, 2023).

TECHNIQUES FOR IDENTIFYING THE DIFFERENT KINDS OF GUT FLORA AND THEIR FUNCTIONS

The gut microbiome of silkworms, which are essential to the textile industry, is necessary for their development, well-being, and ability to produce silk (Unban *et al.*, 2022). The gut microbiota of lepidopteran larvae has been studied using a variety of techniques; however, because of their difficult gut environment, which includes things like an alkaline pH, digestive enzymes, and unstable habitats, the larvae usually contain fewer or no microorganisms. Despite these difficulties, some intestinal microorganisms such as silkworms are essential to the physiology of lepidopterans (Zhang *et al.*, 2022). Thus, the intestines of silkworms, like those of other lepidopterans, are home to a complex variety of symbiotic microbes.

Microbial community inhabiting the digestive system of the silkworm feeding on mulberry leaves

Bombyx mori, a domesticated silk moth belonging to the Lepidoptera family Bombycidae, holds significant agricultural and economic importance (Zhou *et al.*, 2020). Xia *et al.* (2004) characterized *B. mori* as "economically significant" for its pivotal role in silk production. This species serves as a valuable model organism in life sciences due to its short reproductive cycle, abundant genetic resources, well-mapped genetic makeup, and a substantial number of genes homologous to other silkworm species, making it conducive for diverse life science investigations (Meng *et al.*, 2017).

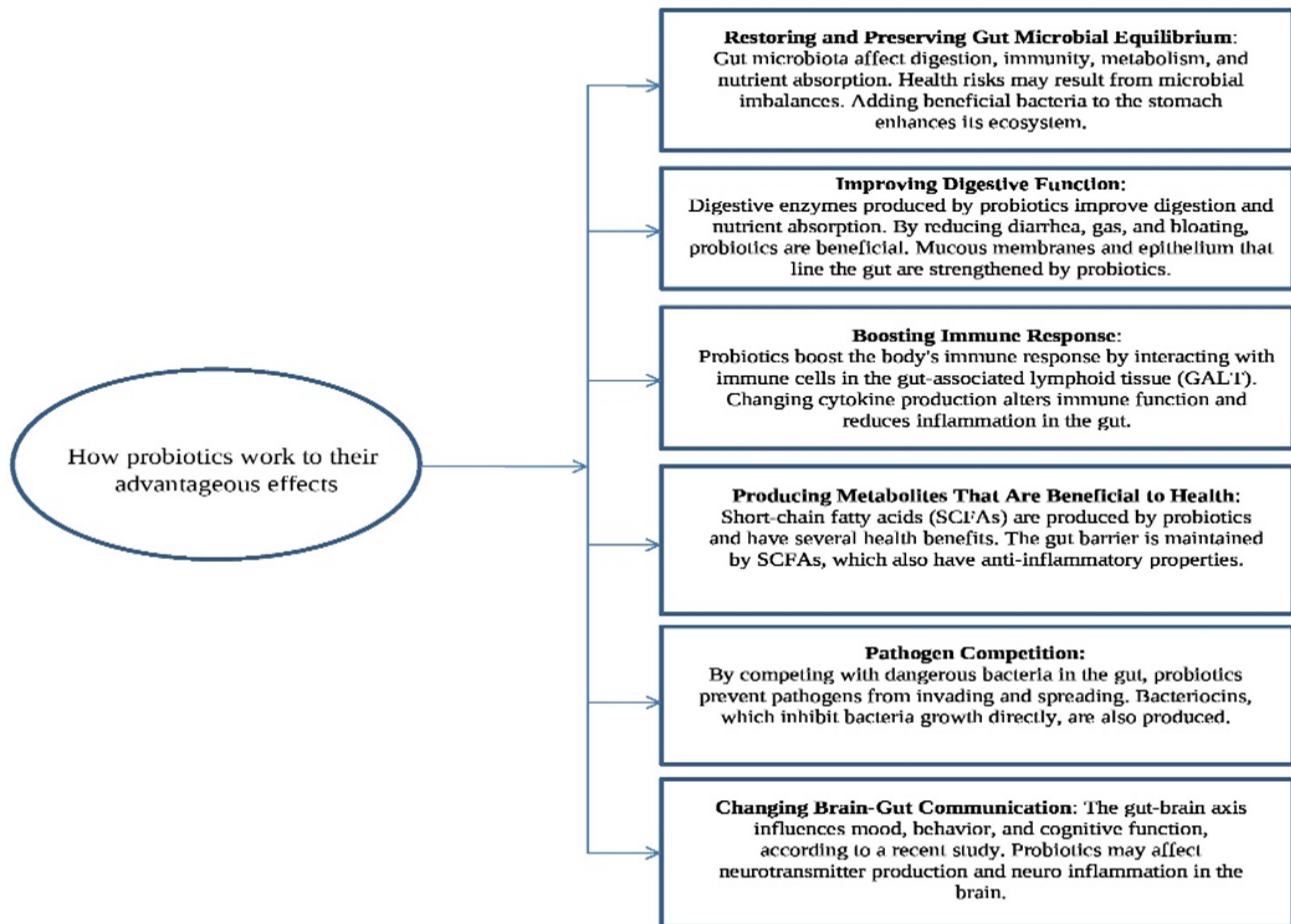


Fig. 1. Advantageous effects of probiotics (Li et al., 2024; Pachiappan et al., 2021; Suraporn et al., 2015).

Microbial community residing in the gut of non-mulberry silkworm

Muga silkworm (*Antheraea assamensis* Helfer): The unique golden silk known as "Muga silk" is produced by the Muga silkworm, *Antheraea assamensis* Helfer, which is mostly grown in Northeastern India (Chetia et al., 2017). They feed on primary plants like Som (*Persea bombycina*) and Soalu (*Litsea polyantha*), secondary, plants include dighloti (*Litsea salcifolia*) and mejankari (*Litsea citrata*) and tertiary host plants, like *Cinnamomum obtusifolium* (Kumar et al., 2022). Using 16S rRNA sequencing and culture-dependent methods, Gandotra et al. (2018) investigated the diversity of bacteria in the muga silkworm's intestine. Their research identified numerous bacteria, including *Bacillus species*, *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Pseudomonas stutzeri*, *Acinetobacter species*, and *Alcaligenes species*. Of these, the most common genus was *Bacillus*, accounting for 54% of all the bacteria in the intestine of the Muga silkworm. Other common genera were *Serratia* (24%), *Pseudomonas* (10%), and *Alcaligenes* (6%) (Tan and Bautista, 2022).

Eri Silkworm (*Philosamia ricini*): The polyvoltine Eri silkworm, which is well-known for making eri silk, sometimes referred to as ahimsa silk, is mostly cultivated in

Northeastern India, specifically in the states of Assam, Nagaland, and Meghalaya, as well as in some areas of China and Japan. Castor (*Ricinus communis*) plants are its main source of nutrition (Attathom, 2004). MsangoSoko et al. (2020) examined the bacterial populations in the eri silkworms' guts. By using culture-dependent 16S rRNA gene sequencing, they identified 60 aerobic culturable bacteria, of which 54% were Firmicutes and 46% were Proteobacteria. They also discovered 12 culturable facultative anaerobes, of which 92% were Proteobacteria and 8% were Firmicutes. A diversified community of culturable and unculturable gut bacteria was also discovered by metagenomic analysis, with Proteobacteria (60%) and Firmicutes (20%) emerging as the two most common phyla, comprising seven orders (Lugli et al., 2023).

Tasar silkworm

Tropical tasar silkworm: The tropical tasar silkworm, scientifically known as *Antheraea mylitta* Drury, is a wild variety of silkworm that is polyphagous, meaning it feeds on a variety of plants. Its diet includes primary food sources like *Terminalia tomentosa*, *T. arjuna*, *Shorea robusta*, and numerous secondary food plants. Research by Barsagade (2017) indicates that the gut

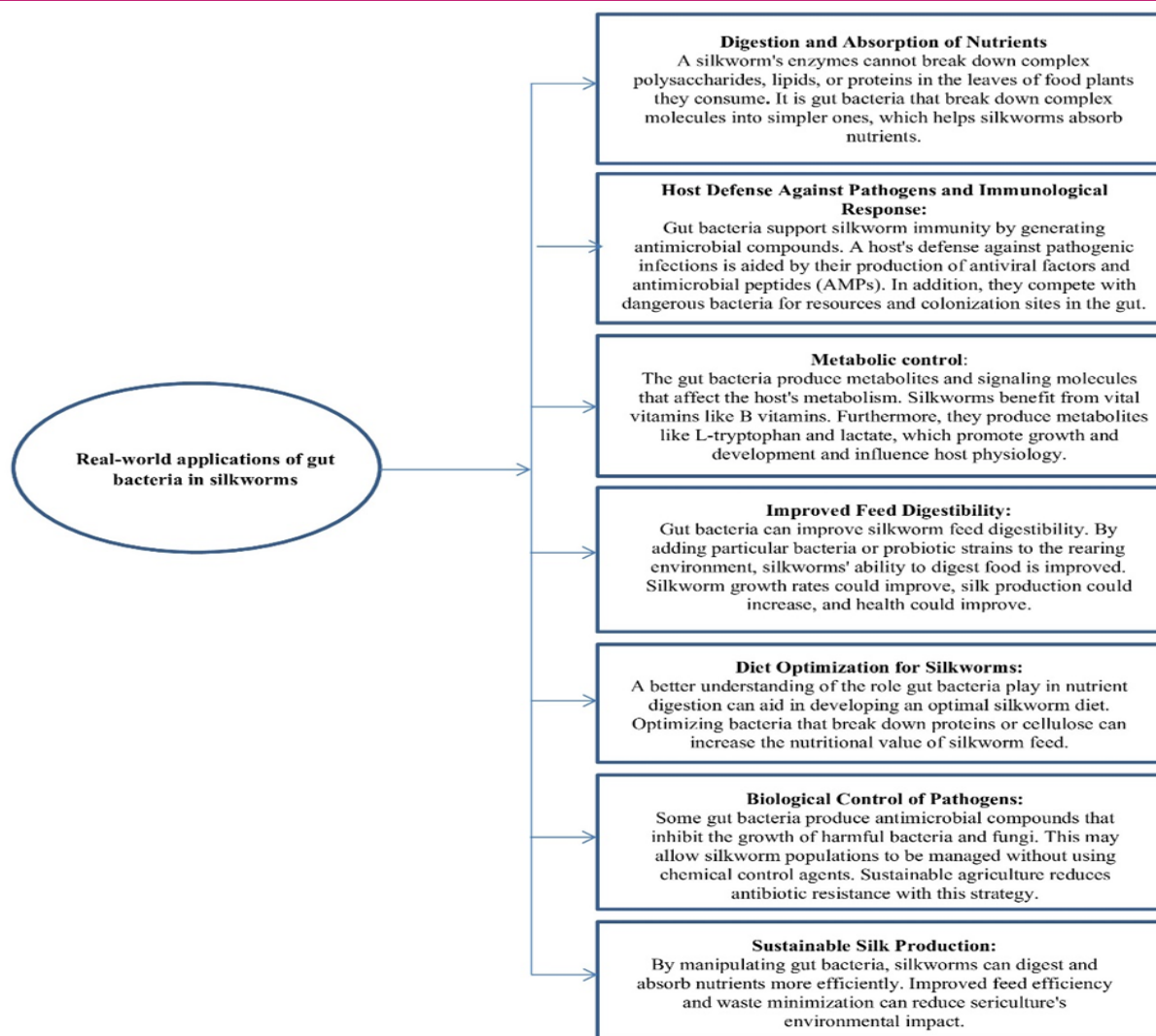


Fig. 2. Real-world applications of gut bacteria in silkworms (Mwchahary et al., 2023; Baddeley et al., 2021; Bandyopadhyay et al., 2019)

microbial community of this silkworm predominantly comprises bacteria belonging to the Proteobacteria phylum. Among the identified bacterial species in the gut are *Pseudomonas*, *Erwinia*, *Enterococcus*, *Staphylococcus*, *Bacillus cereus*, *Lactobacillus*, and *Micrococcus*. Moreover, Rajan et al. (2020) conducted a study using culture-dependent analyses and found that Firmicutes are abundant in the gut of the tasar silkworm. They identified several prevalent bacterial genera, including *Turicibacter*, *Ruminococcus*, *Rhodococcus*, *Prevotella*, *Delftia*, *Acinetobacter*, *Desulfomicrobium*, *Sphingomonas*, *Faecalibacterium*, *Staphylococcus*, *Ralstonia*, *Bacillus*, *Azospirillum*, *Candidatus*, and *Kocuria*.

Oak tasar silkworm: The Indian oak tasar silkworm, also known as *Antheraea proylei* Jolly, is a cultivated silkworm species primarily found in Northeast India, notably in Manipur (Devi et al., 2021). Pimenta et al. (2005) have identified a previously unknown species of ascogenous yeast from the genus *Geotrichum*. This

novel yeast species was discovered within the digestive system of the oak tasar silkworm and has been formally named *Geotrichum silvicola* sp. Nov (Pal et al., 2020). The composition of microbial populations in the gut of silkworms is influenced by various factors such as diet, developmental stage, and gender. Differences in gut microbiota have been observed between silkworms fed on mulberry leaves versus artificial diets, impacting nutrient metabolism and immune response. For instance, certain bacteria like *Advenella*, more prevalent in mulberry-fed silkworms, play a role in detoxifying harmful compounds in plants (Li et al., 2022). Additionally, silkworm gut microbes produce enzymes essential for digestion, such as cellulases, amylases, and proteases, with specific bacteria like *Bacillus* species being particularly effective. Probiotics, like *Streptomyces noursei* and *Lactobacillus* spp., are used to manage diseases in silkworms by enhancing immunity, inhibiting pathogens, and promoting beneficial gut microflora. Studies have shown that specific bacteria, including *Enterococcus* and *Lactobacillus*, contribute to silkworm

immunity and disease resistance (Unban *et al.*, 2022). Moreover, the silkworm's immune system responds to bacterial, fungal, and viral infections through pathways like IMD, Toll, JAK/STAT, and JNK/STAT, inducing the expression of antimicrobial peptides and enzymes. In summary, the gut microbiota of silkworms plays crucial roles in digestion, immunity, and disease management. Understanding these interactions can lead to developing probiotics and other strategies to improve silkworm health and production (Arasakumar *et al.*, 2023).

APPLICATIONS OF THE MICROBIOTA FOUND INSIDE SILKWORM GUT

Biotechnological applications

Silkworm stomach microbiota can be used for biotechnological purposes, such as controlling agricultural pests, preventing diseases, and improving insect well-being. Additionally, derived molecules or compounds can be used to create biomolecules with various applications. However, recent research has mainly focused on investigating the gut microbiota of healthy insects, with little research on bioactive substances found in insects' gut microflora. Less research has been done on the guts of lepidopteran insects, with enzymes essential to the industry being primarily identified from beetles and termites. (Barretto *et al.*, 2021). Furthermore, it is possible to manipulate the gut microbiota of silkworms to generate key metabolites, cytokines, and other therapeutic compounds that can be utilized to treat a variety of illnesses (Mwchahary *et al.*, 2023). Wood-feeding animals and symbiotic microorganisms successfully digest lignocellulose-rich biomass, making the gut a perfect model for advanced lignocellulose-degrading biorefinery. The gut's physicochemical properties and structure can guide process design and pro-

vide genetic resources (Ozbayram *et al.*, 2020).

Utilization in bioremediation and detoxification

The silkworm intestine is home to symbiotic gut microorganisms that can aid in detoxification and bioremediation, particularly in breaking down toxic substances and pesticides. Silkworms, being phytophagous insects, experience oxidative stress due to phenolic substances in their diet. Their gut microbiota produces enzymes that break down polysaccharides in mulberry leaves, a silkworm food source. However, the use of pesticides can lead to insect resistance, affecting agriculture. The gut microbiota of silkworms can also be used for fluorine detoxification, as fluoride-resistant breeds have different microbial communities (Barretto *et al.*, 2018). Heavy metals interact with the gut microbiome, causing growth retardation and altering its structure. The gut microbiome detoxifies heavy metals by altering physiological conditions and enzymes. Heavy metal-induced dysbiosis leads to diseases by advancements in developing recombinant probiotic strains, probiotics are used to address this issue (Arun *et al.*, 2021). Silkworms, lacking an acquired immune system, produce antimicrobial peptides (AMPs) as part of their innate immune response, attracting interest as potential medicinal agents amid antibiotic resistance (Miyashita *et al.*, 2015).

Applications on the well-being of silkworms

The probiotic potential bacterial species such as *Bacillus subtilis*, *Lactobacillus* spp., *Enterococcus hirae*, and *Bacillus* sp. can be used as probiotic to enhance and provide overall health status silkworms, which is directly related to economic traits of the silkworms. The gut bacteria in silkworms can be manipulated to improve the quality and quantity of silk production. The probiotic

Table 1. An overview of the microbial species found in *Bombyx mori*'s gut

Microbial Species	Location	Reference
<i>Bacillus subtilis</i> , <i>Streptomyces noursei</i> , <i>Pseudomonas fluorescens</i>	Gut	Subramanian <i>et al.</i> (2010)
<i>Enterobacteriaceae</i>	Gut	Thangamalar <i>et al.</i> (2009)
Various non-pathogenic microorganisms	Gut	Prem Anand <i>et al.</i> (2010)
Cellulolytic bacteria	Midgut	Khyade and Marathe (2013)
<i>Delftia</i> , <i>Pelomonas</i> , <i>Ralstonia</i> , <i>Staphylococcus</i> , <i>Enterococcus</i>	Gut during viral infection	Sun <i>et al.</i> (2016)
Bacteria (<i>Proteobacteria</i> , <i>Firmicutes</i> , <i>Actinobacteria</i> , <i>Bacteroidetes</i>)	Gut	Chen <i>et al.</i> (2018)
<i>Bacillus</i> species	Gut	Li <i>et al.</i> (2015)
Fungi (<i>Ascomycota</i> and <i>Basidiomycota</i>)	Gut	Chen <i>et al.</i> (2018)
<i>Blastobotrys bombycis</i> sp. Nov.	Gut	Barretto <i>et al.</i> (2018); Barretto & Vootla (2018)
<i>Cryptococcus rajasthanensis</i>	Gut	Barretto <i>et al.</i> (2018); Barretto & Vootla (2020)

Table 2. List of Gut bacterial species' functional role in the physiology of the Mulberry silkworm host

Silkworm gut bacteria	Host organism	Functions	References
<i>Proteus vulgaris</i> , <i>Klebsiella pneumoniae</i> , <i>Citrobacter freundii</i> , <i>Pseudomonas fluorescens</i> , <i>Erwinia sp.</i> , <i>Aeromonas sp.</i> , <i>Serratia liquefaciens</i>	<i>Bombyx mori</i>	Digestive enzymes production	Prem Anand et al. (2010)
<i>Enterococcus casseliflavus</i>	<i>Bombyx mori</i>	Growth and development by producing L-Tryptophan	Liang et al. (2022)
<i>Staphylococcus gallinarum</i>	<i>Bombyx mori</i>	Defence mechanism	Gibson et al. (2010)
<i>Enterococcus sp.</i> and <i>staphylococcus sp.</i>	<i>Bombyx mori</i>	Immune response against infection.	Sun et al. (2016)
<i>Lactobacillus</i> and <i>Bacillus</i>	<i>Bombyx mori</i>	Improvement of economic parameters	Yeruva et al. (2020)
<i>Bacillus aryabhatai</i> and <i>Bacillus sp.</i>	<i>Bombyx mori</i> L.	Cellulolytic activity	Pandiarajan et al. (2020)
<i>Streptomyces noursei</i>	<i>Bombyx mori</i>	Antimicrobial activity and disease management	Subramanian et al. (2010); Mohanraj et al. (2014)
<i>Enterobacter aerogenes</i> , <i>pneumoniae ssp. Pneumonia</i> , <i>Yersinia enterocolitica</i>	<i>Bombyx mori</i>	Growth and development, host defence, insecticide degradation and antagonism to entomopathogens.	Ramesh et al. (2010)
<i>Enterococcus mundtii</i>	<i>Bombyx mori</i>	Metabolism (Lactic acid and metabolites production) of host	Liang et al. (2018)
<i>Bacillus megaterium</i>	<i>Bombyx mori</i>	Liquefaction of starch	Prasanna et al. (2014)
<i>Enterococcus faecalis</i>	<i>Bombyx mori</i>	Protection against infection	Zhang et al. (2022)
<i>Bacillus pumilus</i>	<i>Bombyx mori</i>	Digestive and defence against antiviral factors BmNPV	Liu et al. (2018)
<i>Bacillus sp.</i> , <i>Brevibacterium sp.</i> , <i>Corynebacterium sp.</i> , <i>Staphylococcus sp.</i> , <i>Klebsiella sp.</i> , and <i>Stenotrophomonas sp.</i>	<i>Bombyx mori</i>	Digestive activity by producing lipase enzyme	Feng et al. (2011)

Table 3. List of gut bacterial species' functional role in the physiology of the Muga silkworm host

Silkworm gut bacteria	Host organism	Functions	References
<i>Bacillus spp.</i> , <i>S. marcescens</i> , <i>S. maltophilia</i> , <i>P. stutzeri</i> , <i>Acinetobacter sp.</i> and <i>Alcal-</i>	<i>Antheraea assamensis</i>	Digestive (cellulolytic, amylolytic, and lipolyt-	Gandotra et al. (2018)
<i>Bacillus pumilus</i>	<i>Antheraea assamensis</i>	Cellulase activity, and	Bhuyan et al. (2018)
<i>Bacillus sp.</i> , <i>Escherichia coli</i> , <i>Proteus sp</i>	<i>Antheraea assamensi</i>	Disease resistance.	
	<i>Helper</i>	Digestion process	Bhuyan et al. (2014)
<i>Pseudomonas aeruginosa</i> , <i>Achromobacter xylosoxidans</i> , <i>Staphylococcus aureus</i>	<i>Antheraea assamensi</i>	Have antimicrobial activity	Haloi et al. (2016); Menetrey et al. (2021)
	<i>Helper</i>		

Table 4. List of Gut bacterial species' functional role in the physiology of the Eri silkworm host

Silkworm gut bacteria	Host organism	Functions	References
<i>Enterococcus hirae</i>	<i>Samia ricini</i>	Tannin tolerance and production of cellulolytic enzymes	Unban et al. (2022)
<i>Acinetobacter sp., Enterobacter sp. Bacillus, spp., Citrobacter sp., Enterobacter sp., Pseudomonas spp.</i>	<i>Samia ricini</i>	Digestion and nutrition	MsangoSoko et al. (2022) MsangoSoko et al. (2020)
<i>Acinobacter sp., Pseudomonas sp., Lysinobacillus contaminans, klebsiella pneumonia, Bacillus pumilus, Bacillus sp., Paenibacillus sp., Staphylococcus sp., Brachybacterium hamnosum, Pantoea sp.</i>	<i>Samia ricini</i>	Lignocellulose degradation	Gogoi et al. (2023)
<i>Bacillus spp., Citrobacter sp., Enterobacter sp., Pseudomonas spp</i>	<i>Samia ricini</i>	Digestive process	MsangoSoko et al. (2020)
<i>Bacillus sp. and pseudomonas sp.</i>	<i>Samia ricini</i>	Digestion and nutrition by producing cellulase	MsangoSoko et al. (2021)

potential bacterial species such as *Bacillus subtilis*, *Lactobacillus sp.*, *Enterococcus hirae*, and *Bacillus sp.* can be used as probiotic to enhance and provide overall health status silkworm, which is directly related to economic traits of the silkworms. The gut bacteria in silkworms can be manipulated to improve the quality and quantity of silk production (Mwchahary et al., 2023).

Manufacture of enzymes vital for industrial applications

Microorganisms present in the silkworm gut are involved in the different enzyme production for digestion. They secrete the cellulose, starch, protein, pectin, xylan, lipids and fatty acids degrading enzymes (Arasakumar et al., 2023). Enzymes like amylases, cellulases, pectinases, and laccases are essential in various sectors like medicines, food, bioremediation, fuel, detergents, paper, and textiles. Microbial en-

zymes, particularly those from insects' gut microbiota, are crucial for breaking down lignocellulosic materials, which are the main building block of plant cell walls. *Bacillus megaterium* and *B. mori's* intestines produce amylase, which is most active at 40°C and pH 8.0. The gut microflora of *B. mori* larvae produces various enzymes, including pectinase, xylanase, cellulase, and starch. *Bacillus sp., Proteus sp.,* and *E. coli* have been found to produce enzymes, with *B. pumilus* MGB05 showing antibacterial characteristics against *E. coli* and *P. aeruginosa*. (Vermelho et al., 2013; Prasanna et al., 2014; Prem Anand et al., 2010). According to Gandotra et al. (2018), there are a lot of bacterial isolates in the stomach of the Muga silkworm, *A. assamensis*, and they have a lot more lipase, cellulase, and amylase activity. *Bacillus pumilus* MGB05 was isolated by Bhuyan et al. (2018) from the gut larvae of *A. assamensis*. The isolate exhibited antibacterial characteristics

Table 5. List of Gut bacterial species' functional role in the physiology of the Tasar silkworm host

Silkworm gut bacteria	Host organism	Functions	References
<i>Bacillus toyonensis</i> and <i>Bacillus thuringiensis</i>	<i>Antheraea proylei J.</i>	Promoting healthy health	Devi et al. (2022)
<i>Turicibacter spp.</i>	<i>Antheraea mylitta</i>	Lactate production	Rajan et al. (2020)
<i>Bacillus pacificus, Bacillus mycoides, Bacillus mycoides, Bacillus toyonensis, Bacillus thuringiensis</i>	<i>Antheraea proylei</i>	Promoting healthy health	Devi et al. (2022)
<i>Bacillus aryabhatai, Bacillus zanthoxyli, Bacillus megaterium, Bacillus flexus</i> and <i>Bacillus simplex</i>	<i>Antheraea proylei</i>	Found in diseased condition, toxicity-ameliorating and have growth-promoting abilities	Devi et al. (2022); Pishchik et al. (2021)
<i>Pseudomonas, Erwinia, Enterococcus, Staphylococcus, Bacillus cereus, Lactobacillus</i> and <i>Micrococcus</i>	<i>Antheraea mylitta</i>	Have antimicrobials activity	Singh et al. (2021); Rajan et al. (2020); Amaning et al. (2022)

Table 6. Differences in silkworms raised on fresh mulberry leaves compared to those raised on artificial diets (Dong et al., 2018)

SI No.	Aspect	Silkworms Raised on Fresh Mulberry Leaves	Silkworms Raised on Artificial Diet
1.	Gut Microbiota Composition	Dominated by Cyanobacteria, Firmicutes, and Proteobacteria	Dominated by Firmicutes or Proteobacteria
2.	Functional Predictions of Intestinal Bacteria	Stable functional predictions	Varied functional predictions
3.	Immune Response	Enhanced immunity with specific bacteria abundance (e.g., <i>Enterococcus</i> , <i>Lactobacillus</i>)	Altered immune response; susceptibility to infections and environmental stresses
4.	Nutrient Metabolism	Efficient digestion aided by specific bacteria (e.g., <i>Bacillus circulans</i> , <i>Proteus vulgaris</i> , <i>E. coli</i> , <i>Citrobacter freundii</i>)	Altered nutrient metabolism; significant differences in lipase-producing bacteria abundance
5.	Co-evolution of Microorganisms	Stable microbiota composition observed across different silkworm strains	The shift in microbiota composition resembles that seen in other animals fed artificial diets

against *E. coli* and *P. aeruginosa*, as well as cellulase and β -glucosidase activities.

Production of vitamins and ethanol

The benefits of vitamin supplied by insect gut bacteria for insect growth and health are becoming more widely recognized. The discovery of vitamin B12 in silkworms by Sridhara and Bhat (1966) was ascribed to intestinal microbes (Serrato-Salas and Gendrim, 2023). Human-beneficial B-group vitamins like folate, riboflavin, and vitamin B12 are produced by lactic acid bacteria from the intestines of silkworms, notably those from the genus *Lactobacillus sp.* (LeBlanc et al., 2011). Actinomycetes that produced vitamin B12 were found in the intestinal lumen of silkworm larvae (Unban et al., 2022). These actinomycetes improved the synthesis of proteins and nucleic acids in the silk gland. In terms of producing ethanol, enzymatic processes that employ microbes to transform lignocellulosic waste from mulberries into bioethanol present a viable, environmentally beneficial substitute for fossil fuels. According to a study, the gut microbiome of *B. mori* (race CSR2xCSR4, Bivoltine hybrid) included the yeast species *Blastobotrys bombycis sp. Nov.*, which can produce ethanol. As the second most prevalent sugar in lignocellulosic biomass, 5% D-xylose may be fermented by this yeast strain to produce 1.5 g/L of ethanol (Barretto et al., 2018).

Conclusion

Much research has looked into the variables influencing the economic feasibility of silkworms and the amount of silk they produce to improve sericulture industrial sustainability. The gut microbiome is one such element affecting silkworm immunity, disease resistance, and nutritional absorption. The microbiome's main job in the silkworm's digestive system is to facilitate the process-

es of absorption and digestion. It generates the enzymes needed to break down starch, cellulose, Xylan, and pectin. The digestive juices' pH levels impact these enzymes' efficiency, allowing them to optimize their activity accordingly. These helpful bacteria mainly create enzymes that facilitate plant digestion and antimicrobial peptides that strengthen the host's defences against infections. Moreover, the host's immune system's response to a range of infectious illnesses is regulated by the gut microbiota of the silkworm. It produces antimicrobial peptides in response to certain infections and triggers immunological signalling pathways. High throughput 16S rRNA gene amplicon sequencing is one example of an advanced genetic sequencing approach that provides information about the digestive tract microbiomes of various local silkworm strains by revealing details about their bacterial populations. It is hypothesized that along the course of evolution, silkworms and the microbes in their stomachs have forged an advantageous alliance that makes life and reproduction easier. This is analogous to the wider acknowledgement of microorganisms' symbiotic roles in diverse settings, referred to as the "microbiome," which impacts immunity and lifestyle. Though much is known about the biology and physiology of silkworms, less is known about their gut microbiome. Thus, the silkworm gut microbiota presents tremendous potential because of its ease of use, cost, and ethical ease of rearing.

Conflict of interest

The authors declare that they have no conflict of interest.

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