



## Microbial partnerships in sericulture: A review on the gut bacteria of silkworms

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### Abstract

The gut of silkworms harbours a diverse community of bacteria which includes several beneficial and pathogenic species. Some of these bacteria help in the breakdown of dietary fibres, while others produce essential amino acids, vitamins and other nutrients. The main aim of this study is to review the literature on the diversity and functions of bacteria present in the gut of silkworms, and to explore the potential applications of these bacteria in various fields. The result of present study suggested that the gut of silkworms is a complex ecosystem comprising a diverse range of bacteria including members of the genera *Bacillus*, *Lactobacillus*, *Enterococcus*, *Enterobacter*, *Acinobacter*, *Citrobacter*, *Staphylococcus* and *Bacteroids* that play important roles in the digestion of food plants nutrients which are the primary food source of silkworms. They also produce enzymes and metabolites that are essential for the growth and development of silkworms. Furthermore, some gut bacteria have been shown to have probiotics properties, improving immune response and protecting against pathogenic infection by secreting AMP molecules and anti-viral factors. The findings of this study have highlighted the importance and the potentials of gut bacteria in silkworms and their future application by manipulating the gut microbiota of silkworms for enhancing their productivity, health, and resistance to disease. Therefore, understanding the interactions between silkworms and their gut bacteria could provide new insights into the biology of these important insects and pave the way for the development of novel strategies for the productivity of silk.

**Keywords:** Silkworm, gut, gut bacteria, microbial

### Introduction

Silkworms are important insects in the silk industry, providing valuable silk fibres used in the production of textiles, medical devices, and other materials. They are holometabolous insects that undergo a complete metamorphosis, consisting of egg, larval, pupae, and adult stages. During the larval stage, which lasts for 4-6 weeks, the silkworm feeds voraciously on food plant leaves, increasing in weight by over 10,000 times. The gut microbiota of silkworms has recently become an area of increasing interest due to its importance in maintaining the health and productivity of these insects. The gut bacteria in silkworms play vital roles in nutrient digestion and absorption, host defences against pathogens, and regulation of host immunity [1, 2, 3, 4, 5, 6, 7, 8]. Furthermore, these bacteria have potential applications in various fields, such as agriculture, medicine, and biotechnology. In this essay, we will review the current knowledge on gut bacteria in silkworms, their functions, important roles played by them, and their future applications.

### Gut bacteria in silkworms

Silkworms are an important model organism for studying gut microbiota due to their economic and ecological significance. The gut of silkworms contains a diverse population of microorganisms, including bacteria, fungi, and viruses. Among them, bacteria are the most abundant and diverse group, with over 100 bacterial species identified so far. These bacteria belong to various phyla, including

Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes.

### Approaches to study gut bacteria diversity and application

Silkworms are important insects in the textile industry and their gut microbiota play a crucial role in their growth, health, and silk production. To gain a better understanding of the gut bacteria of silkworms, several techniques have been employed, each with its own advantages and limitations. Culture-dependent methods have been used to isolate bacteria from the gut of silkworms and identify them based on their morphology, biochemical properties and important functionality. For example, Prasanna *et al.* (2014) isolated and identified amylase producing *Bacillus megaterium* from the gut of Eri silkworms using culture-dependent methods [9]. Similarly Important enzymes such as- amylase, cellulase, lipase, gelatinase, Xylynase, pectinase and endoglucanase producing gut bacteria were isolated and studies using traditional culture dependent method [2, 5]. However, culture-dependent methods can be biased towards the detection of fast-growing and cultivable bacteria, and may not accurately reflect the diversity of the gut microbiota. Culture-independent methods have been used to analyse the DNA or RNA extracted directly from the gut of silkworms, including 16S rRNA gene sequencing, metagenomic sequencing, and metatranscriptomic sequencing.

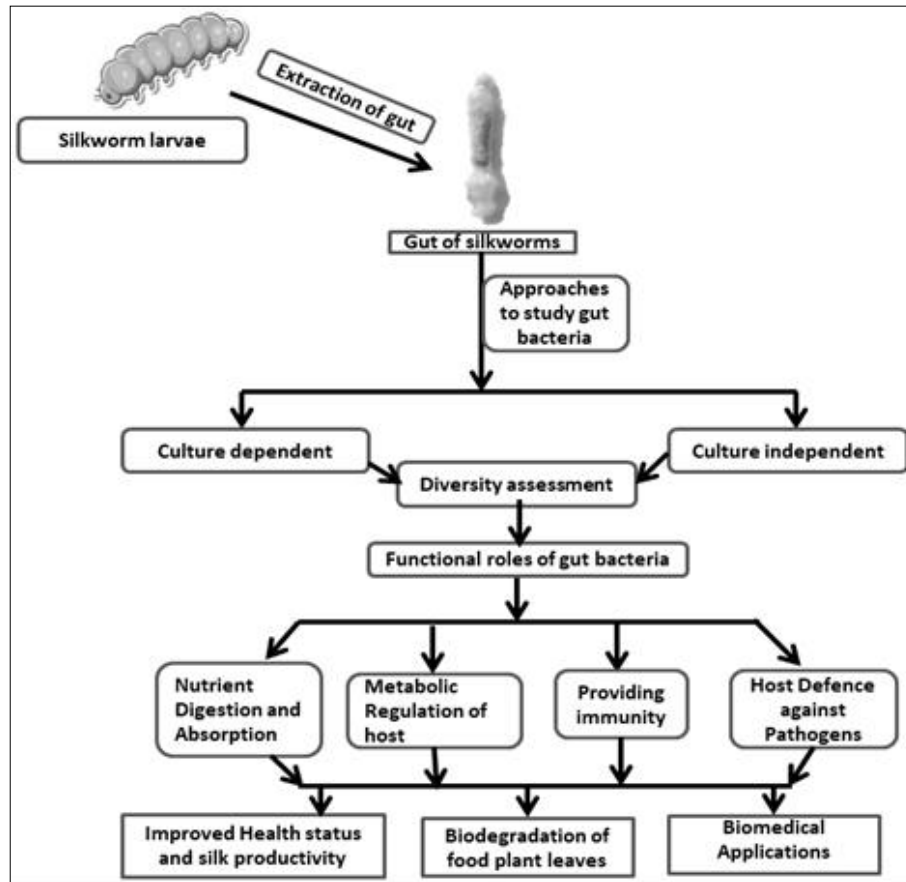


Fig 1: Gut bacteria of silkworm, Process roles and future prospects

These methods allow for a more comprehensive analysis of the gut microbiota, including the detection of non-cultivable and low-abundance bacteria. For example, Li *et al.* (2020) used 16S rRNA gene sequencing to analyse the gut bacterial diversity of two strains of silkworms and found significant differences in their gut bacterial profiles [10]. Similarly, Yeruva *et al.* (2019) used metagenomic sequencing to investigate and identify the probiotic potential gut bacteria in silkworm *Bombyx mori* [11]. Chen *et al.* (2018) used DNA and RNA based sequencing method to study and compare the microbiota of domesticated and wild mulberry silkworms [12]. The result revealed host specific variation in the gut bacterial population in the insect species. A study used high-throughput sequencing to characterize the gut microbiota of *Bombyx mori* silkworms under different feeding conditions. The researchers found that the composition and diversity of gut microbiota of silkworms were influenced by host developmental stage and feeding conditions, [13]. However combinations of both methods have been found to provide better insight on gut bacterial dynamics of silkworms. Chen *et al.* (2020) used both 16S rRNA gene sequencing method in combination of culturing of gut bacteria to investigate the effect of gut microbiota in providing resistance to host insect against organophosphate insecticides [14]. Similarly in a recent study by [15] who used combination of both culture-dependent and culture-independent techniques to analyse the gut microbiota of silkworm larvae in better ways. Their result revealed the presence of vast diversity of both culturable and unculturable gut bacteria, with presence of dominant phyla Proteobacteria and Firmicutes.

Overall, these techniques have provided valuable insights into the gut bacteria of silkworms and their role in silk

production and host health. By using a combination of these techniques, researchers can gain a more comprehensive understanding of the gut microbiota of silkworms and develop strategies to optimize silkworm growth, health, and silk production, as well as promoting sustainable sericulture practices.

#### Factor affecting diversity and composition of gut bacteria

Studies have shown that the gut microbiota of silkworms is dominated by bacteria from the phyla Firmicutes, Proteobacteria, and Actinobacteria. However, the composition and abundance of gut bacteria in silkworms can vary significantly depending on several factors, such as the host genotype, diet, age, and environment.

Host genotype and Phylogeny of the host has been shown to play a significant role in shaping the gut microbiota of silkworms, Studies have found that different individual silkworm strains from species have distinct gut bacterial profile, which may influence their susceptibility to diseases and overall health [5]. Similarly, Diet has also been shown to affect gut bacterial diversity in silkworms, feeding on certain artificial and antibiotics treated diet different from their natural diet can alter the gut composition and function, leading to changes in the host's physiology and silk production [16, 17, 18]. Age is another important factor that can affect the diversity and evenness of gut bacteria in silkworms. Studies have shown that the gut bacterial communities of silkworms undergo significant changes during their growth and development stages. For instances, the gut bacterial diversity tends to increase as larvae attend maturity [19, 12]. Gender of host silkworms also plays a role in shaping the gut microbiota of silkworms. Studies have

shown that different gender *viz.* male and female can have different gut microbiota compositions [20]. A change in the composition of the bacterial diversity of gut was also caused by Use of certain insecticides and disinfectant that certainly influencing host's physiology [10, 21]. The use of insect growth regulator can also be found to alter the diversity of intestinal bacteria [22]. Finally the seasonal factor and environmental factors, such as temperature, humidity and infection, can also influence the diversity of gut bacteria in silkworms [23, 24, 25, 26, 27]. Poor environmental conditions can lead to the proliferation of harmful bacteria and the suppression of beneficial ones, resulting in infection or disease that in turn affect the overall health and reduced silk production. Overall, understanding the factors that affect the gut bacterial diversity in silkworms is crucial for optimizing their growth, health and silk production, and for developing sustainable sericulture practices.

### Functions of gut bacteria in silkworms nutrient digestion and absorption

The gut bacteria in silkworms play a crucial role in the digestion and absorption of nutrients. The silkworm larvae feed on food plants leaves, which are rich in complex polysaccharides, such as cellulose, hemicellulose, lignocellulose, xylene, pectin, and complex lipids and proteins that cannot be digested by the host's own enzymes alone. Silkworms rely on the microbes in their gut to help them break down and digest food plant leaves that they eat. Carbohydrates, proteins and fats in the food into simpler compounds, which the silkworm can absorb and use for energy [23, 28, 2, 5, 29, 4].

### Host defence against pathogens and immune response

The gut bacteria in silkworms also play an important role in protecting the host against pathogenic infections. Gut bacteria also play a role in the host's immune response by producing antimicrobial substances and activating the immune system. For instance, *Enterococcus sp.* and

*staphylococcus sp.* found in *Bombyx mori* helps in the immune response against infections [20]. *Bacillus pumilus* and *Bacillus subtilis* isolated from the gut of silkworms are shown to have inhibitory activity, against several pathogenic bacteria by producing AMP and antiviral factors [30, 8]. *Enterococcus faecalis* isolated from *Bombyx mori* gut was also found in providing protection against *Nosema bombysis* the main microbe responsible for disease pebrine [31]. Furthermore, the gut bacteria can also compete with harmful bacteria for nutrients and colonization sites, thus preventing their growth and establishment in the gut. For example, the probiotic potential bacteria *Enterococcus hirae* and *Enterococcus faecalis* have been shown to reduce the colonization of pathogenic bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, and *Proteus vulgaris*, in the gut of silkworms by producing antibacterial activity against them and improve their survival rate and immunity under pathogenic challenge [32].

### Metabolic regulation of host

The gut bacteria in silkworms can also influence the host metabolism and hormone regulation through the production of various metabolites and signalling molecules. For example, the gut bacteria *Bacillus subtilis* in silkworms produces secondary primary metabolites, B vitamins, which enhance health status of silkworms [30]. *Turicibacter sp.* is also involved in lactate production [33]. In addition, the gut bacterium such as *Enterococcus casseliflavus* is involved in metabolic process of silkworms by producing L-Tryptophan that can modulate the host physiology [34]. Bacterial *Enterococcus mundtii* isolated from the gut of *Bombyx mori* is also found in associated with the metabolism of host by producing essential metabolites required for growth and development [7].

Overall, gut bacteria play a significant role in the overall health and wellbeing of the host. They help in digestion, nutrient production, disease resistance, defence mechanism, and various other functions of Silkworms.

**Table 1:** Functional role played by gut bacterial species in host silkworm host physiology

Sl. No.	Host organism	Silk worm gut bacteria	Functions	Source
1	<i>Bombyx mori</i>	<i>Enterococcus sp. and staphylococcus sp.</i>	Immune response against infection.	[20]
2	<i>Silkworm</i>	<i>Bacillus subtilis</i>	Producing secondary primary metabolites, B vitamin, AMP compound, enhance resistance	[30]
3	<i>Antheraea proylei</i> J.	<i>Bacillus toyonensis</i> and <i>Bacillus thuringiensis</i>	Promoting healthy health	[35]
4	<i>Antheraea mylitta</i>	<i>Turicibacter sp.</i>	Lactate production	[33]
5	<i>Antheraea assamensis</i>	<i>Bacillus spp, S. marcescens, S. maltophilia, P. stutzeri, Acinetobacter sp. and Alcaligenes sp.</i>	Digestive (cellulolytic, amylolytic and lipolytic) activities	[5]
6	<i>Samia ricini</i>	<i>Bacillus sp. and pseudomonas sp.</i>	Digestion and nutrition by producing enzyme cellulase	[6]
7	<i>Bombyx mori</i>	<i>Enterococcus casseliflavus</i>	Growth and development by producing L-Tryptophan	[34]
8	<i>Samia ricini</i>	<i>Acinetobacter sp., Enterobacter sp. Bacillus, spp., Citrobacter sp., Enterobacter sp., Pseudomonas spp.</i>	Digestion and nutrition	[29, 15]
9	<i>Bombyx mori</i>	<i>Proteus vulgaris, Klebsiella pneumoniae, Citrobacter freundii, Pseudomonas fluorescens, Erwinia sp., Aeromonas sp., Serratia liquefaciens</i>	Production of digestive enzymes	[4]
10	<i>Samia ricini</i>	<i>Acinobacter sp., Pseudomonas sp., Lysinobacillus contaminans, klebsiella pneumonia, Bacillus pumilus, Bacillus sp., Paenibacillus sp., Staphylococcus sp., Brachybacteriumr hamnosum, Pantoea sp.</i>	Lignocellulose degradation	[23]
11	<i>Bombyx mori</i>	<i>Staphylococcus gallinarum</i>	Defence mechanism	[36]
12	<i>Antheraea assamensis</i> Helfer	<i>Bacillus pumilus</i>	Disease resistance.	[28]

13	<i>Anthaea assamensis</i> Helfer	<i>Bacillus sp. Escherichia coli, Proteus sp.</i>	Digestion process	[37]
14	<i>Bombyx mori</i>	<i>Lactobacillus and Bacillus</i>	Improvement of economic parameters	[11]
15	<i>Bombyx mori</i> L.	<i>Bacillus aryabhatai and Bacillus sp.</i>	Cellulolytic activity	[38]
16	<i>Bombyx mori</i>	<i>Bacillus sp., Brevibacterium sp., Corynebacterium sp., Staphylococcus sp., Klebsiella sp., and Stenotrophomonas sp.</i>	Digestive activity by producing lipase enzyme	[2]
17	<i>Bombyx mori</i>	<i>Bacillus pumilus</i>	Digestive and defence against antiviral factors BmNPV	[8]
18	<i>Bombyx mori</i>	<i>Enterococcus mundtii</i>	Metabolism (Lactic acid and metabolites production) of host	[7]
19	<i>Bombyx mori</i>	<i>Bacillus megaterium</i>	Liquefaction of starch	[9]
20	<i>Bombyx mori</i>	<i>Enterococcus faecalis</i>	Protection against infection	[31]
21	<i>Bombyx mori</i>	<i>Enterobacter aerogenes, pneumoniae ssp. Pneumonia, Yersinia enterocolitica</i>	Growth and development, host defence, insecticide degradation and antagonism to entomopathogens.	[39]
22	<i>Samia ricini</i>	<i>Enterococcus hirae</i>	Tannin tolerance, production of cellulolytic enzymes, and antimicrobial activity against insect pathogenic	[32]
23	<i>Samia ricini</i>	<i>Bacillus spp., Citrobacter sp., Enterobacter sp., Pseudomonas spp</i>	Digestive process	[15]
24	<i>Bombyx mori</i>	<i>Streptomyces noursei</i>	Antimicrobial activity and disease management	[40, 41]
24	<i>Antheraea assamensis</i> Helfer	<i>Bacillus pumilus</i>	Cellulase activity	[28]

### Future applications of gut bacteria in silkworms

The gut bacteria in silkworms have several potential applications in various fields, such as sericulture, biotechnology, and medicine. Some of the future applications are discussed below.

### Improved health status and silk productivity

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

### Biodegradation of food plant leaves

The gut bacteria in silkworms can also be utilized for the biodegradation of food plant leaves, which are the main food source for silkworms. The food plants leaves are rich in complex polysaccharides, lignin, and other plant cell wall components that are difficult to digest and utilize. The gut bacteria in silkworms, especially the cellulose-degrading bacteria, can break down these complex polysaccharides into simpler sugars and other nutrients that can be more easily utilized by the host. This can not only improve the nutrient utilization efficiency of silkworms but also reduce the environmental pollution caused by the excessive use of food plant leaves.

### Biomedical applications

The gut bacteria in silkworms have several potential biomedical applications, such as the production of antimicrobial compounds, the modulation of host immunity, and the regulation of host metabolism and behaviour. For example, the *Bacillus pumilus*, *Enterococcus hirae* and *Enterococcus faecalis* can be utilized for the production of antimicrobial peptides that can be used as natural antibiotics against various pathogens<sup>[31]</sup>. In addition, the gut bacteria in silkworms can be engineered to produce therapeutic

molecules, such as enzymes, and important metabolites and cytokines, which can be used for the treatment of various diseases. Moreover, the gut bacteria in silkworms can be utilized as a model system for the study of host-microbe interactions and the development of new probiotics and prebiotics.

### Conclusion

In conclusion, the gut bacteria in silkworms play important roles in the host digestion, immunity, metabolism, and behaviour. The composition and diversity of the gut microbiota can be influenced by various factors, such as diet, developmental stage, and environmental conditions. The gut bacteria in silkworms can interact with the host through various mechanisms, such as nutrient digestion and absorption, immune regulation, and hormone modulation. The gut bacteria in silkworms have several potential applications in various fields, such as sericulture, biotechnology, and medicine. However, further studies are needed to elucidate the precise mechanisms underlying the host-microbe interactions in silkworms and to develop new strategies for the manipulation and utilization of the gut microbiota.

### Conflict of interest

Authors declared no conflict of interest.

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