Review Article

Silkworm gut microflora as a source: Biotechnological Applications

---

ABSTRACT

The term "microflora" has gained popularity as researchers explore the symbiotic relationships between microorganisms and their hosts, from our skin to our gut. Insects, including the silkworm, have mutualistic ties with their gut microflora, making them a valuable resource for studying essential molecules. The silkworm, with its rapid generation time, abundant genetic resources, well-defined genetic background, and numerous homologous genes, is an ideal model organism for various life science studies. Silkworm rearing is simple, inexpensive, and does not require ethical clearance, allowing the gut microflora of *Bombyx mori* to be harnessed for biotechnological applications. Silkworm gut microbes can synthesize and produce extracellular enzymes, such as cellulases, proteases, and lipases, which are crucial for industries ranging from biofuel production to waste management and textile processing. Additionally, these microbes can produce vitamins, such as vitamin B12, and metabolites like short-chain fatty acids (SCFAs), which have potential applications in the food and pharmaceutical industries. Antimicrobial substances produced by the gut microflora, including bacteriocins and lactic acid, are of particular therapeutic importance, offering natural alternatives to traditional antibiotics and contributing to the development of novel antimicrobial therapies. Moreover, the antioxidants generated by these symbionts, such as glutathione and superoxide dismutase (SOD), have significant implications for health supplements and cosmetics, owing to their ability to combat oxidative stress and support immune function. Beyond their metabolic capacities, silkworm symbionts can be used to enhance health, target and manage agricultural pests, and control vectors of human diseases in an environmentally friendly manner. Recent research has also highlighted the potential of mutualistic symbiotic bacteria for plastic disposal, with enzymes like PETases showing promise in degrading synthetic polymers. Despite extensive knowledge on the biology and physiology of the silkworm *Bombyx mori*, few studies have focused on its gut microflora. Modern genetic techniques, such as the genetic transformation of silkworms with genes of interest, may help overcome these research gaps. Increasing research efforts on silkworm gut microflora will not only reveal new symbiotic relationships but also identify new sources of biotechnologically important molecules and enzymes for therapeutic and industrial applications.

KEYWORDS: Silkworm, micro flora, symbionts, applications.

1. INTRODUCTION

Insects, one of the most diverse and ancient groups of organisms, inhabit various terrestrial and freshwater environments, showcasing their adaptability to a range of environmental conditions (Misof *et al.,* 2014). This prolonged coexistence has led to the evolution of a remarkable array of symbiotic microorganisms, which engage in diverse partnerships with their insect hosts (Chen *et al.,* 2016, 2018a).

Insect gut microbes display a vast taxonomic diversity, including prokaryotes (bacteria and archaea) and eukaryotes (fungi and protozoa). These microbial communities vary in complexity, degree of adaptation to the host gut environment, and their roles in the host's ecology and functions. The insect gut provides a unique habitat for microbial colonization, and these microbes offer their hosts numerous benefits. The necessity of gut microflora for basic activities in insects varies significantly. The diversification and evolutionary success of insects have largely depended on their complex interactions with beneficial microorganisms. These microorganisms enhance nutrient-deficient diets, aid in digestion, protect against predators, parasites, and pathogens, contribute to communication, influence disease vector effectiveness, and regulate mating and reproductive systems. Compared to mammalian guts, insect guts typically host fewer microbial species, though some insects possess vast populations of specialized bacteria (Engel and Moran, 2013).

A healthy insect is considered a "multi-organismal entity" due to its microflora, which can constitute 1 to 10 per cent of its biomass. These extensive gut microflora communities, especially in the digestive tract, play a significant role in mediating the diverse lifestyles of insect hosts (Barretto *et al.,* 2021). Microbial symbionts associated with insects offer immense resources for biotechnological applications due to their metabolic versatility and utility (Duplouy, 2018). These symbionts are promising sources for medicine, bioremediation, industrial processes, and agriculture (Berasategui *et al.,* 2016).

Insects, with their vast diversity and ecological significance, have long been a subject of scientific curiosity. Among the various aspects of insect biology that have captivated researchers, the study of insect gut microflora stands out for its complexity and relevance. The gut microflora, comprising a diverse array of bacteria, fungi, protozoa, and viruses, plays a crucial role in the life of insects. These microorganisms are not mere inhabitants of the gut but are active participants in numerous physiological processes that are vital for the insect’s survival, development, and evolution.

One of the primary roles of gut microflora in insects is the facilitation of digestion and nutrient acquisition. Many insects, particularly herbivores, consume diets that are rich in complex polysaccharides like cellulose, which they are unable to digest on their own. As highlighted by Dillon and Dillon (2004), gut bacteria assist in breaking down these complex carbohydrates into simpler sugars that can be absorbed and utilized by the insect. This symbiotic relationship is particularly evident in insects such as termites and certain beetles, which rely heavily on their gut microflora to extract energy from woody plant material. Without these microbial partners, the ability of these insects to thrive on such challenging diets would be severely compromised.

In addition to aiding digestion, gut microflora plays a vital role in the detoxification of harmful compounds. Many plants produce toxic secondary metabolites as a defence mechanism against herbivory. Insects that feed on these plants are often exposed to these toxins, which could be lethal if not properly managed. Douglas (2015) notes that gut microbes can detoxify these compounds, rendering them harmless and allowing the insect to continue feeding without adverse effects. This detoxification capability not only protects the insect but also expands its dietary options, enabling it to exploit a wider range of ecological niches. This relationship between gut microbes and their insect hosts illustrates the adaptive advantages conferred by a well-maintained gut microbiota.

The relationship between insects and their gut microflora extends beyond digestion and detoxification; it also plays a crucial role in modulating the insect’s immune system. Broderick and Lemaitre (2012) emphasize that the gut microbiota can influence the immune responses of insects, providing a first line of defense against pathogenic invaders. Some gut bacteria produce antimicrobial compounds that inhibit the growth of harmful pathogens, thus maintaining the health and stability of the gut environment. This protective role of the gut microflora is particularly important for insects that live in environments with high microbial loads, where the risk of infection is significant.

Furthermore, the gut microflora is instrumental in the development and reproductive success of insects. According to Jin and Jeon (2021), the composition of the gut microbiota can change throughout the life cycle of an insect, with different microbes playing key roles at various stages. For example, certain bacteria are essential for the proper development of the gut in larval stages, ensuring that the insect can efficiently process food as it grows. In adult insects, other microbes may influence reproductive success by affecting hormone levels or nutrient availability. This dynamic relationship between the insect and its gut microflora underscores the importance of these microorganisms in the overall fitness of the insect.

The diversity of insect gut microflora is another area of significant interest. Engel and Moran (2013) discuss how the composition of gut microbiota varies widely among different insect species, influenced by factors such as diet, environment, and host genetics. Despite this diversity, certain functional roles, such as digestion, detoxification, and immune modulation, are conserved across different species. This suggests that while the specific microbial species present in the gut may vary, the overall functions they perform are critical to the survival and success of their insect hosts.

Lepidoptera, including butterflies and moths, being one of the largest insect orders and a primary group of phytophagous agricultural pests, the microbial communities associated with them remain poorly understood. Recently, with the advent of high-throughput sequencing techniques, several studies have uncovered abundant and diverse bacteria in the guts of lepidopterans, yet the functional significance of these gut microbiomes is still largely unknown (Chen *et al.,* 2018b). The silkworm, *Bombyx mori* (Lepidoptera: Bombycidae), a model organism and domesticated insect, holds significance not only for basic research but also as a source of raw materials for the textile and biotechnology industries.

2. INSECT-MICROBE SYMBIOSIS

Symbiosis refers to the close relationship between two different species. These relationships can be 'obligate,' where both species are interdependent and cannot survive without each other, or 'facultative,' where the partnership is beneficial but not essential for survival. Obligate symbioses often evolve over long periods, while facultative symbioses may be more recent adaptations and can evolve into obligate symbioses over time.

Endosymbiosis occurs when one symbiotic partner lives inside the body of the other, either within the host's cells (intracellular symbiosis) or outside the cells (extracellular symbiosis). Ectosymbiosis, on the other hand, involves one organism living on the surface of the host's body, including the digestive tract lining or exocrine glands.

Microbes participate in a wide array of symbiotic interactions with insects, spanning from mutualistic to parasitic relationships. Most of these symbiotic microorganisms positively impact their host, primarily by offering nutrition and/or defence (Xie *et al*., 2019). For instance, bacterial symbionts can broaden the dietary range of herbivorous insects, supply essential nutrients, and break down substances that are toxic to their hosts.

Symbiotic relationships in insect-microbe symbiosis can be categorized into three types:

- Mutualism

- Commensalism

- Parasitism

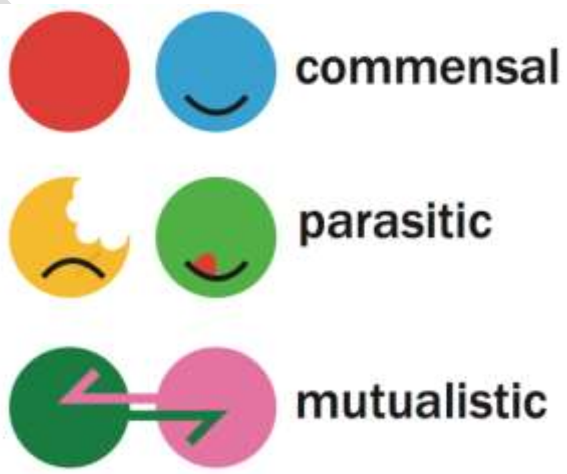


Fig 1. Symbiotic relationships in insect-microbe symbiosis

- **Mutualism**

Mutualism is a form of symbiosis where both partners benefit, often resulting in significant fitness gains for one or both parties. Mutualisms can involve resource-resource relationships, service-resource relationships, or service-service relationships.

- **Commensalism**

Commensalism is a symbiosis where one organism benefits from the other for food, shelter, or locomotion without affecting the host.

- **Parasitism**

Parasitism is a non-mutualistic symbiosis where one organism benefits at the expense of the other. Unlike predation, parasitism does not necessarily result in the host's death. In some cases, the parasitized host is killed, in which case the invader is known as a 'parasitoid'.

Interestingly, symbiotic relationships can sometimes shift from parasitism to mutualism, or vice versa, under certain conditions (Gerardo and Hurst, 2017). Although such transitions between different types of symbiotic relationships are frequently observed and have occurred numerous times throughout the history of microbial-eukaryote symbiosis, the mechanisms underlying these switches remain poorly understood. The rate of symbiont colonization, the impact of their abundance, modes of transmission, and host control are increasingly recognized as important factors influencing these changes in symbiont lifestyles. It is essential to determine whether these different lifestyles are interconnected through physiological, biochemical, or molecular pathways, and to identify the factors that trigger such shifts in symbiont behaviour (Skelton, 2016).

3. HOST-MICROFLORA SYMBIOTIC LIFESTYLE

The mechanisms behind switching among different symbiotic relationships are not fully understood, despite their frequent occurrence throughout the history of microbial-eukaryote symbiosis (Gerardo and Hurst, 2017; Rai and Agarkar, 2016). Understanding these interconnected lifestyles and identifying the triggers for changes in symbiont lifestyle is crucial.

The best-described mutualistic interactions revolve around the nutritional or protective benefits symbionts provide to their hosts. In defensive interactions, microorganisms protect their host against pathogens, parasites, parasitoids, or predators by producing antimicrobial compounds or toxins (Flórez *et al.,* 2015). In nutritional mutualisms, they produce nutrients such as amino acids and vitamins or digestive enzymes that help degrade dietary polymers or detoxify harmful secondary metabolites (Douglas, 2009).

In the insect world, the symbiotic relationship between the host and its gut microflora is crucial for survival. Dillon and Dillon (2004) discuss how herbivorous insects, which consume cellulose-rich plant material, rely on gut bacteria to break down these complex polysaccharides into simpler sugars. This relationship not only aids in digestion but also expands the dietary possibilities for the host, allowing it to exploit a broader range of ecological niches. This mutualistic interaction exemplifies the adaptive advantages conferred by a well-maintained gut microbiota.

Another critical aspect of the host-microflora symbiosis is the detoxification of harmful substances. Many plants produce toxic compounds to deter herbivores, but insects have evolved to harbor gut microbes that can neutralize these toxins, enabling them to feed on such plants. As Douglas (2015) notes, this detoxification process allows insects to broaden their diet and occupy diverse ecological roles, illustrating the evolutionary significance of symbiotic relationships.

In humans, the symbiotic relationship with gut microflora extends beyond digestion. The human gut microbiome, composed of trillions of microorganisms, plays a vital role in immune system development and function. According to Hooper *et al.* (2012), the gut microbiota helps train the immune system to distinguish between harmful and harmless agents, reducing the risk of autoimmune diseases.

Furthermore, the microbiota competes with pathogenic bacteria for resources and space, effectively protecting the host from infections.

The evolutionary perspective on symbiosis reveals that these relationships are not static but have co-evolved over millions of years, leading to highly specialized and interdependent systems. In some insects, for example, symbiotic bacteria have become so integral to the host's biology that they are passed from generation to generation through specialized structures known as bacteriomes. These bacteria provide essential nutrients that the host cannot synthesize on its own, making them indispensable to the insect's survival. Buchner (1965) emphasized the importance of these endosymbiotic bacteria in the evolution of insect nutrition.

However, the balance of these symbiotic relationships is delicate. Disruptions in the host-microflora balance, known as dysbiosis, can lead to significant health issues. For instance, in humans, dysbiosis has been linked to conditions such as inflammatory bowel disease, obesity, and even mental health disorders. As Turnbaugh *et al.* (2006) discuss, the composition of the gut microbiota is closely tied to the host's metabolic health, and alterations in this balance can have profound consequences.

4. Microflora of Domesticated Silkworms

The silkworm, *Bombyx mori*, is economically significant due to its role in silk production. It is also a valuable model organism for life science research, with rapid generation, abundant genetic resources, a fully sequenced genome, and numerous silkworm-specific genes (Meng *et al.,* 2017).
Despite the vast diversity of microbial flora in *B. mori*, few have been discovered and are poorly identified. More bacterial flora is found in the gut of fourth and fifth instar silkworm larvae, corresponding to their active feeding stage, aiding in growth and nutrient absorption (Pandiarajan & Krishnan, 2018).

Studies have also shown that *B. mori* gut microflora help combat infectious diseases. Eleven bacterial phylotypes isolated from the fifth instar *B. mori* gut can degrade various polysaccharides from the host diet. Cellulolytic bacteria in the midguts of *B. mori* aid in food digestion, nutrient absorption, and growth (Khyade and Marathe, 2012).

The domesticated silkworm, *Bombyx mori*, has been a critical organism in sericulture for thousands of years, providing the raw material for silk production. Alongside their economic importance, silkworms have also served as a model organism in scientific research. One area of particular interest is the study of their gut microflora, which plays a vital role in their digestion, immunity, and overall health. The microflora of domesticated silkworms consists of a complex community of bacteria, fungi, and other microorganisms that interact closely with the host, contributing to its physiological processes.

B. mori depends heavily on its gut microflora for the digestion of mulberry leaves, its primary food source. The leaves are rich in cellulose and other complex carbohydrates that the silkworm cannot digest on its own. As Bindu *et al.* (2014) explain, the gut bacteria help break down these complex molecules into simpler sugars that the silkworm can absorb and utilize as an energy source. This relationship is essential for the silkworm’s growth and development, as efficient digestion is directly linked to the production of high-quality silk.

The microflora of silkworms also plays a crucial role in modulating the immune system of the host. Cheng *et al.* (2017) note that certain gut bacteria in *B. mori* produce antimicrobial compounds that protect the silkworm from pathogenic infections. This protective effect is particularly important during the larval stage when the silkworm is most vulnerable to diseases. The presence of a healthy and balanced gut microbiome can enhance the silkworm's resistance to pathogens, reducing mortality rates and improving silk production.

In addition to digestion and immunity, the gut microflora influences other aspects of silkworm physiology, including the absorption of nutrients and the regulation of metabolism. Takatsuka *et al.* (2005) report that the composition of the gut microbiota can affect the silkworm's ability to absorb essential nutrients from its diet, such as vitamins and amino acids. This interaction highlights the importance of maintaining a stable and diverse gut microbiome for the optimal health and productivity of domesticated silkworms.

Research into the gut microflora of *B. mori* has also revealed significant differences between wild and domesticated strains. Sun *et al.* (2016) found that domesticated silkworms have a more specialized gut microbiota compared to their wild counterparts, likely due to the controlled diet and environment in which they are raised. This specialization may contribute to the higher efficiency of silk production in domesticated strains, but it also makes them more dependent on their specific gut microflora for survival.

The study of the silkworm gut microflora is not only important for understanding the biology of *B. mori* but also has practical applications in sericulture. Researchers like Ishikawa *et al.* (2018) have explored the potential of manipulating the gut microbiome to improve silk yield and quality. By introducing beneficial microbes or optimizing the diet to support a healthy gut microbiome, it may be possible to enhance the productivity of silkworms and reduce the incidence of diseases in sericulture farms.

5. GUT MICROBIAL COMMUNITY IDENTIFICATION

Methods for the gut community identification have been divided into two approaches *i.e.*, **classical** and **molecular approach** which gets further classified into culture dependent and culture independent methods.

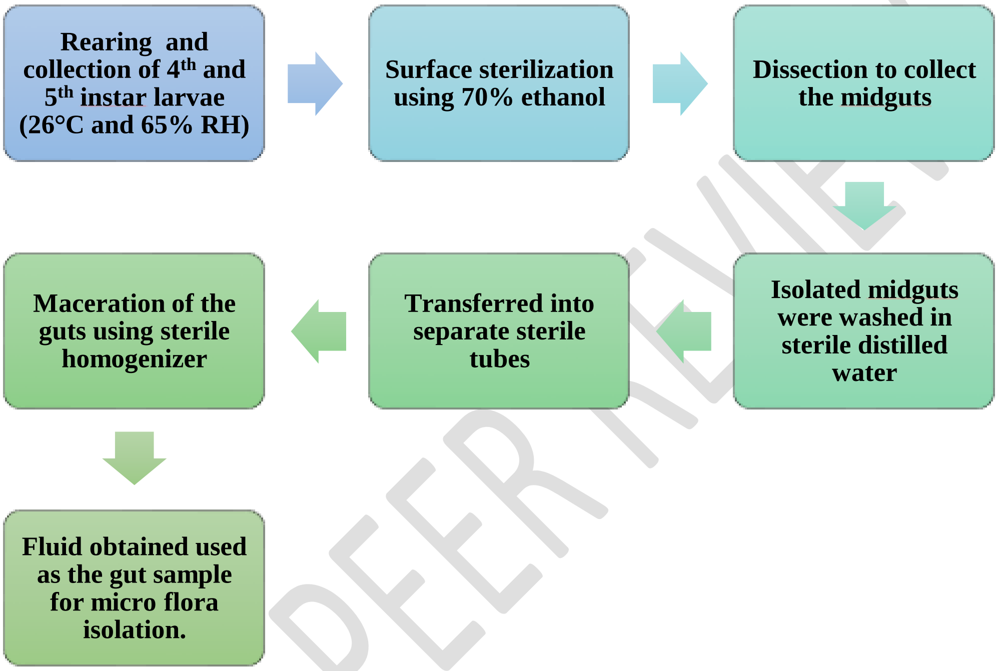
Table 1. Identification of Gut Microbial Community

Approach	Technique	Purpose	Reference
CLASSICAL APPROACH			
Cultural Dependent methods			
Plating/culturing technique	Culturing on various media	Isolating cultivable microorganism; studying the biochemical and physiological role of microorganism	Fraher, O'toole, and Quigley, 2012
Cultural Independent methods			
Direct microscopic analysis	Heat fixation and staining	Estimating bacterial load in the gut by analysing the microorganisms in the faeces of insects	Rautio, 2002
Monitoring specific enzymes and/or metabolites in faeces	Analytical techniques: MS etc.	Estimating microbial load in insect gut as metabolites in the faeces	O'Sullivan, 2000
MOLECULAR APPROACH			
Cultural Dependent methods			
Phenotypic fingerprinting	PAGE; bacteriophage typing	Identifying microorganisms using colony hybridization, monoclonal antibodies, etc.	Muller and Haridon, 1996
Genotypic fingerprinting	Colony hybridization with nucleic acid probes	Identification of gut symbiotic microbes.	Palmer *et al*., 2006
Cultural Independent methods			
PCR based	Gene targeting PCR; RAPD etc.	Identification of unculturable microorganisms.	Brauman *et al*., 2001

Metagenomics	cDNA library preparation	Detection and identification of microbial communities by analysing RNA which is then converted to cDNA for further analysis.	Cox-Foster *et al*., 2007

(Mahmod, 2014; Shi *et al.,* 2010)

6. Fig 2. COLLECTION OF GUT SAMPLE

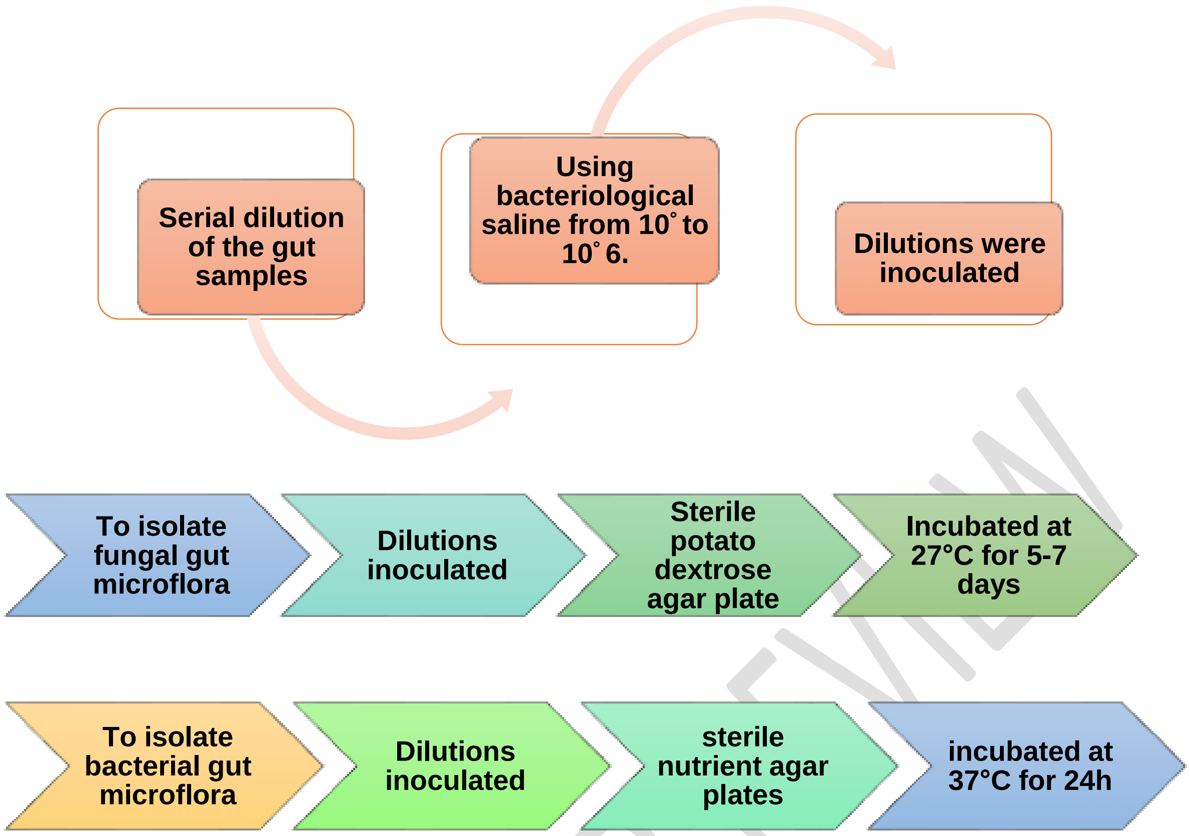


(Barretto *et al*., 2021)

The flowchart outlines the process of isolating gut microflora from the midguts of silkworm larvae. The process begins with the rearing and collection of 4th and 5th instar larvae at controlled environmental conditions (26°C and 65% relative humidity). The larvae undergo surface sterilization using 70% ethanol to remove external contaminants. Dissection is then performed to collect the midguts, which are subsequently washed in sterile distilled water to ensure sterility. The isolated midguts are transferred into separate sterile tubes and macerated using a sterile homogenizer to break down the gut tissues. The resulting fluid, which contains the gut sample, is used for the isolation of microflora. This systematic approach ensures that the gut sample remains uncontaminated and suitable for microbiological analysis.

- **Fig 3. ISOLATION OF GUT MICROFLORA**

The gut samples are first subjected to serial dilution using bacteriological saline, ranging from a 10^0 to 10^{-6} dilution. These dilutions are then inoculated onto a suitable growth medium for culturing and isolating microorganisms. This method helps in determining the concentration of microorganisms within the gut samples by reducing the microbial load to a manageable level for analysis.



(Barretto *et al.*, 2021)

7. ENUMERATION AND IDENTIFICATION

The study of microbial populations in silkworms, particularly *Bombyx mori*, has garnered attention due to the significant role these microorganisms play in the health, development, and silk production of the host. Understanding the microbial load and identifying the various microbial species present in *Bombyx mori* can provide insights into their contributions to the host's physiology and potential impacts on sericulture practices. The process of enumeration and identification involves several steps, including viable count methods, morphological and biochemical characterization, and molecular techniques such as 16S rRNA and 18S rRNA gene sequencing.

The first step in quantifying the microbial load in *Bombyx mori* involves the use of the viable count method, which is widely recognized for its accuracy in determining the number of living cells in a culture. According to Lakshmi *et al.* (2020), the viable count method involves serial dilution of the microbial sample followed by plating on appropriate agar media. After incubation, the number of colony-forming units (CFUs) is counted, and the microbial load is calculated based on the dilution factor. This method provides a reliable estimate of the number of viable microorganisms present in the gut of *Bombyx mori*, including both bacteria and fungi.

Following enumeration, the next step is the morphological and biochemical characterization of the isolated colonies. Morphological characterization involves observing the shape, size, color, and other physical features of the colonies under a microscope. Biochemical characterization, on the other hand, involves performing various tests to determine the metabolic and enzymatic activities of the microbes. As described by Ramesh *et al.* (2018), common biochemical tests include the catalase test, oxidase test, and carbohydrate fermentation tests. These tests help differentiate between different microbial species and provide preliminary identification based on their physiological properties.

However, morphological and biochemical methods alone are often insufficient for precise identification, especially at the species level. To achieve accurate identification and classification, molecular techniques such as 16S rRNA and 18S rRNA gene sequencing are employed. The 16S rRNA gene is highly conserved among bacteria, making it an ideal target for sequencing to identify bacterial species. Similarly, the 18S rRNA gene is used for the identification of fungal species. According to Shankar *et al.* (2021), DNA is extracted from the microbial colonies, and the target rRNA genes are amplified using polymerase chain reaction (PCR). The amplified genes are then sequenced, and the sequences are compared against reference databases to identify the microbial species with high accuracy.

Gene sequencing provides several advantages over traditional methods, including the ability to identify unculturable microorganisms and detect species that are present in low abundance. Moreover, 16S rRNA and 18S rRNA sequencing can reveal the phylogenetic relationships between different microbial species, contributing to a better understanding of the microbial ecology within the silkworm gut. By integrating these molecular techniques with viable count methods and biochemical tests, researchers can achieve a comprehensive profile of the microbial communities in *Bombyx mori.*

8. BENEFITS OF GUT MICROFLORA TO SILKWORM

The gut microflora of silkworms *Bombyx mori* plays a pivotal role in their overall health and development. These microorganisms contribute to a range of physiological processes that are essential for the silkworm's survival, productivity, and resistance to diseases.

- **Production of Essential Compounds**

The gut microflora in silkworms is involved in the synthesis of essential compounds, such as vitamins and amino acids, which are crucial for the insect's growth and metabolic functions. For instance, specific gut bacteria can synthesize B vitamins that the silkworm cannot produce on its own. According to Anand *et al.* (2010), this symbiotic relationship ensures that the silkworm meets its nutritional requirements even when these nutrients are deficient in its diet.

- **Digestion and Metabolism of Food**

The gut microbiota aids in the digestion and metabolism of food, particularly in breaking down complex carbohydrates found in mulberry leaves, the primary diet of silkworms. As highlighted by Xiang *et al.* (2007), the enzymes produced by gut bacteria help in the efficient breakdown of cellulose and other polysaccharides, allowing the silkworm to extract maximum energy from its food.

- **Nutrient Absorption**

Gut microflora enhances the absorption of nutrients by maintaining a healthy gut environment and promoting the integrity of the gut lining. Chen *et al.* (2015) report that a well-balanced gut microbiome facilitates the efficient uptake of essential nutrients, such as glucose and amino acids, thereby supporting the silkworm's rapid growth during its larval stages.

- **Detoxification of Toxins**

Silkworms often encounter toxic compounds in their diet, which can hinder their development. The gut microflora plays a critical role in detoxifying these harmful substances. According to Zheng *et al*. (2014), certain gut bacteria possess detoxifying enzymes that neutralize these toxins, allowing the silkworm to safely process its food and avoid potential poisoning.

- **Pheromone Production and Immunity**

Gut microbes are also involved in the production of pheromones, which are vital for communication and reproductive behaviour in silkworms. Furthermore, the gut microbiota contributes to the host’s immune system, enhancing its ability to fight off infections. Li *et al.* (2017) explain that gut bacteria can stimulate the production of antimicrobial peptides, which protect the silkworm against pathogenic invaders.

- **Protection Against Natural Enemies and Pathogens**

The gut microbiota offers protection against natural enemies and pathogens by outcompeting harmful microorganisms for resources and producing substances that inhibit their growth. According to Sun *et al.* (2018), a healthy gut microbiome acts as a barrier against infections, reducing the likelihood of disease outbreaks in silkworm populations.

- **Improved Survival and Fecundity**

The presence of beneficial gut microflora has been linked to improved survival rates and fecundity in silkworms. A study by Wang *et al.* (2013) showed that silkworms with a well-established gut microbiome exhibited higher reproductive success and greater resilience to environmental stresses compared to those with disrupted microbiota.

- **Enhanced Growth, Development, and Disease Resistance**

Finally, gut microflora plays a vital role in promoting the overall growth and development of silkworms. Zhang *et al.* (2020) found that the presence of specific beneficial bacteria in the gut correlates with enhanced growth rates, better disease resistance, and improved silk production, making gut microbiota a key factor in the success of sericulture.

9. TARGETING INSECT-MICROBE SYMBIOSES FOR BIOTECHNOLOGICAL APPLICATIONS

Host-microbe interactions have been exploited for various biotechnological applications (Berasategui *et al.,* 2016):

1. Controlling agricultural pests and vector-borne diseases by targeting or utilizing symbiotic interactions.
2. Improving the health of economically important insects.
3. Utilizing symbiont-derived compounds, such as enzymes or bioactive molecules, for pharmaceutical or industrial processes.

Biotechnological applications of microbial symbionts in insects can include:

- Specialized compartments (e.g., bacteriomes)
- In the insect gut
- In insect tissues outside the gut (e.g., fat body)
- On the insect’s cuticle

These applications can help control insect pest populations, increase the survival of beneficial insects, and utilize symbionts for industrially important processes. Targeting insect-microbe symbiosis can be effective for biological control of agricultural pests by preventing their growth and survival, often using antibiotics or obstructing symbiont transmission pathways (Baumann, 2005; Salem *et al.,* 2015). However, due to antibiotic resistance concerns, anti-microbial peptides are a more cost-effective approach for pest control.

Microbial symbionts also show promise in reducing the frequency and effectiveness of insect vectors of human diseases like malaria, dengue, yellow fever, and Chagas disease. Techniques such as Para transgenesis (genetic modification of bacterial symbionts) and the manipulation of insect vectors to shorten their lifespan or reduce fertility are being explored (Ben Beard *et al.,* 2002).

The incompatible insect technique (IIT) is another strategy, similar to the sterile insect technique (SIT), where sexually incompatible males are released to mate with females, leading to non-viable eggs (Knipling, 1955).

Insect symbionts as probiotics can enhance efforts to control agricultural pests and vectors. For example, lactic acid bacteria (LAB) from honey bees could be used as probiotics to boost bee immunity (Olofsson and Vásquez, 2008).

Insects and their symbiotic bacteria produce a variety of digestive enzymes, making them adaptable to diverse nutritional resources. These enzymes, such as pectinases, cellulases, and ligninases, are used in industries like detergents, food production, and biofuel generation (Phitsuwan *et al.,* 2013).

10. BIOTECHNOLOGICAL APPLICATIONS OF SILKWORM GUT MICROFLORA

The biotechnological applications of silkworm gut microflora can be categorized into:

a) Industrial Applications

- **Production of important enzymes:**

- Enzymes such as amylases, cellulases, pectinases, and laccases are utilized in various sectors, including pharmaceuticals, food, bioremediation, fuel, detergents, paper, and textiles (Vermelho et al., 2013).
- Facilitating the digestion of lignocellulosic components.
- Providing nutrients and aiding in food digestion.
- Expanding the cellulolytic toolbox for efficient biomass deconstruction.

Findings	Authors

Silkworm gut micro flora produce enzymes that facilitate the digestion of lignocellulosic components which they feed on that is otherwise difficult to digest.	Vermelho *et al.,* 2013
Amylase production by *Bacillus megaterium* isolated from *B. mori* gut is suitable for liquefaction of starch in detergent, textile, food and other industrial applications.	Prasanna *et al.,* (2014)
Cellulolytic and amylase microorganisms in the gut of silkworm larvae and identified them as genus *Enterococcus, Erwinia* and *Pantoea* can be exploited for further biotechnological applications.	Liang *et al.,* (2015)
An alkaline-tolerant alpha amylase and xylose isomerase characterized in the silkworm *B. mori* gut bacterium EMB156 are highly potent for lactate fermentation.	Liang *et al.,* 2018; Yan and Wu, 2016

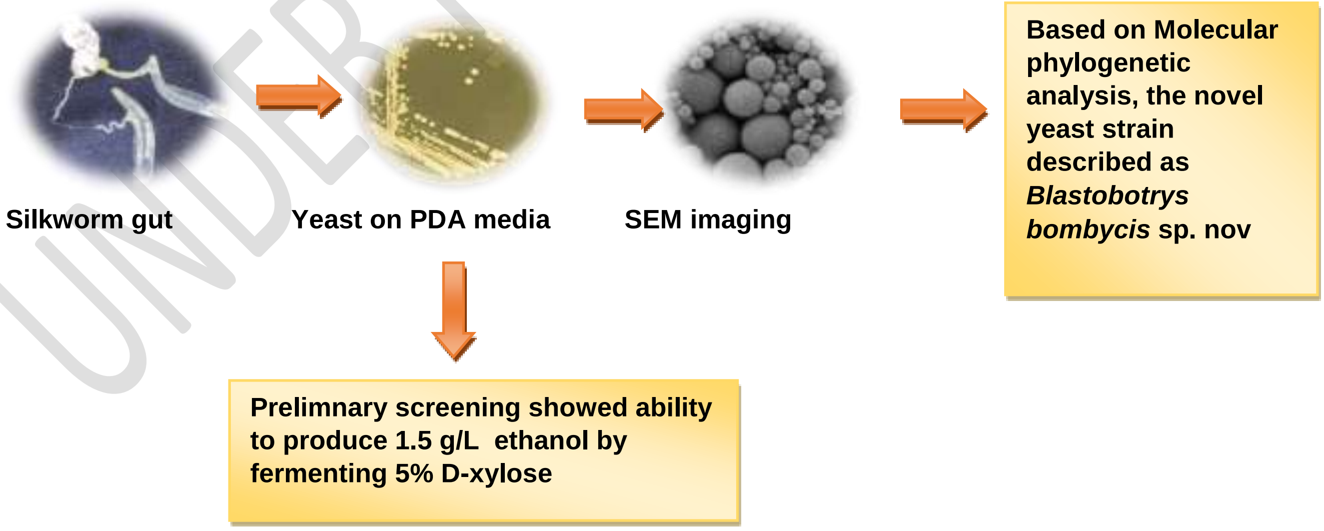
- **Production of vitamins**

The provision of vitamins for silkworm growth and benefit is one of the advantageous functions of silkworm gut micro flora. Due to the numerous advantages that naturally produced vitamins have over those that are chemically created, the usage of vitamins derived from microbial forms is becoming more and more important.

Findings	**Authors**
Lactic acid bacteria isolated from silkworm gut belongs to the genus *Lactobacillus* produce various B-group vitamins including folates, riboflavin and vitamin B_{12} amongst others that produce beneficial effects in humans.	LeBlanc *et al.,* 2011
Vitamin B_{12} producing *actinomycetes* from gut lumen of silkworm larvae shown to increase the synthesis of nucleic acids and proteins in the silk gland of the insect.	Halarnkar and Blomquist (1989)
Showed the presence of vitamin B_{12} in silkworms produced by the intestinal microorganisms of host.	Sridhara and Bhat (1966)

- **Ethanol production**

One of the most potential green substitutes for fossil fuels or petroleum-based products is the use of microbes to convert waste mulberry lignocellulosic material into bioethanol (Thirupathaiah *et al*., 2018).



Based on Molecular phylogenetic analysis, the novel yeast strain described as *Blastobotrys bombycis* sp. nov

Prelimnary screening showed ability to produce 1.5 g/L ethanol by fermenting 5% D-xylose

Blastobotrys bombycis **sp. nov. obtained from silkworm** ***B. mori*** **gut micro flora with potency for ethanol production (Barretto** ***et al.,*** **2018).**

b) Human Therapeutic Applications

The gut microflora of insects, particularly silkworms, offers numerous health benefits for humans:

- **Therapeutic Properties:** Insect gut microflora is a promising source for discovering therapeutic properties such as anti-cancer, antimicrobial, and anti-inflammatory effects (Bode, 2011).
- Probiotics: Probiotic strains of the genus Lactobacillus, isolated from the gut of B. mori, offer health benefits like cholesterol-lowering, antimicrobial, and immunomodulatory properties (Bhalchandra and Pathade, 2011).
- **Antimicrobial Peptides (AMPs):** AMPs are low molecular weight proteins with potential as future drugs due to their antagonistic effects on bacteria, fungi, and viruses. Six AMP families from B. mori include cecropin, defensin, moricin, gloverin, attacin, and lebocin.
- **Astronaut Food:** Due to the presence of cellulase and amylase-producing bacteria (Enterococcus, Erwinia, and Pantoea), silkworms could be an ideal protein source for astronauts in Biogenerative Life Support Systems during long-term space missions (Tong *et al.,* 2011).
- **Serratiopeptidase:** This proteolytic enzyme, derived from Serratia marcescens isolated from the silkworm intestine, has anti-inflammatory, anti-epidemic, and analgesic properties. It's used in various medical fields such as surgery, otorhinolaryngology, gynecology, and dentistry (Bhagat *et al.,* 2013; Miyata *et al.,* 1970).

c) Bioremediation and Detoxification

Bioremediation utilizes biological processes to remove or neutralize pollutants. Silkworms and their gut symbionts produce detoxifying enzymes that help resist toxins from both human activities and natural sources (Douglas, 2013).

- Detoxifying Enzymes: Laccase and linamarase (-D-glucosidase) have biotechnological applications. Linamarase from gut bacteria (*Acinetobacter, Bacillus, Klebsiella*, and *Alcaligenes*) acts on cyanogenic glucosides in food plants (Idowu *et al.,* 2009). Laccases are used in chemical synthesis, bioremediation, textiles, wine stabilization, and biosensors (Kunamneni *et al.,* 2008).

- Alpha Amylase and Xylose Isomerase: The *B. mori* gut bacterium EMB156 produces these enzymes, which are effective for lactate fermentation and have potential future biotechnological applications.

d) Disease and Pest Control

Silkworm gut microflora produce extracellular enzymes, vitamins, and antimicrobial peptides (AMPs) that enhance growth, development, and disease resistance.

- Antimicrobial Peptides (AMPs): AMPs from silkworm gut microflora have antagonistic effects on fungi, bacteria, and viruses. They activate the host immune system without harming non-target cells (Nesa *et al.,* 2020).

- Horizontal Gene Transfer: Transferring genes from bacteria and fungi to *B. mori* can enhance survival and fecundity. Bacillus species in the gut can be used as probiotics to treat silkworm diseases. *Bacillus pumilus* SW41 has antiviral properties against BmNPV, reducing viral infectivity and abundance (Liu *et al.,* 2018).

e) Environmental Applications

Plastic pollution is a significant environmental issue, with many plastics being slow to degrade due to their chemical structure. However, silkworm gut bacteria show promise in biodegrading plastics.

- **Biodegradation of Plastics:** Silkworm gut bacteria can biodegrade plastics like polyethylene (PE), which has been traditionally considered nonbiodegradable.

-**Bio-plastics Production**: Bio-plastics, such as poly lactic acid (PLA), are both bio-based and biodegradable. *Enterococcus mundtii* EMB156, isolated from the B. mori gut, is a productive lactic acid-producing strain that can convert various carbon sources into lactic acid, useful in disposable packaging (Krishnamurthy & Amritkumar, 2019; Liang *et al.,* 2018; Jem & Tan, 2020).

11. SIGNIFICANT STUDIES PERTAINING TO SILKWORM GUT MICRO FLORA

Reported outcomes	**Authors**
Revealed the existence of the bacteria belonging to *Enterobacteriaceae* in *B. mori* gut.	Thangamalar *et al.,* (2009)
Reported 41 bacterial phylotypes in the midguts of the silkworm *B. mori* larvae by PCR/DGGE technique and 16S rDNA gene library analysis.	Hui *et al.,* (2010)

Showed the presence of beneficially important bacteria in the *B. mori* gut including *Bacillus subtilis*, *Pseudomonas fluorescens* and *Streptomyces noursei* by using 16S rRNA probes.	Subramanian *et al.,* (2010)
Found that the *B. mori* gut is colonized by a variety of non-pathogenic microorganisms.	Anand *et al.,* (2010)
Indicated the presence of cellulolytic bacteria in the midguts of *B. mori* that helps in food digestion, absorption of nutrients and growth.	Khyade and Marathe, (2012)
Revealed that predominant bacteria *Delftia, Pelomonas, Ralstonia, Staphylococcus* and *Enterococcus* in healthy *B. mori* gut were altered during viral infection caused by *Bm*CPV (*B. mori* cypovirus).	Sun *et al.,* (2016)
Evaluated the relationship between *Nosema bombycis* and *Enterococci* and found that the Enterococcal load increased in the guts of *B. mori* post *Nosema bombycis* infection.	Xingmeng and Fangwei, (2002)
Showed the presence of *Bacillus* species in the guts of silkworm that can be used as probiotics for silkworm disease management.	Li *et al.,* (2015)
Reported the maximum horizontal gene transfer from bacteria and fungi to B. mori that improved the survival and fecundity of the silkworm.	Sun *et al.,* (2013)

12. CONCLUSION

Rearing *Bombyx mori* is a simple, cost-effective process that does not require ethical approval, making its gut microflora a valuable resource for biotechnological applications. Silkworm symbionts offer potential for improving health, managing agricultural pests, and controlling vectors of human diseases in an environmentally friendly manner. Their metabolic capabilities are particularly useful in the development of bioplastics, widely used in disposable packaging.

Despite extensive knowledge about the biology and physiology of silkworms, research on their gut microflora remains sparse. This gap can be bridged through improved culture methods, advanced genomic and genetic tools for gene identification and expression, and genetic transformation techniques. Understanding host-symbiont ecology can guide targeted searches for substances of practical use, such as antibiotics, detoxifying enzymes, cellulases, and lipases.

Increasing research efforts in silkworm gut microflora will uncover intriguing new symbiotic relationships and potentially lead to the discovery of novel molecules and enzymes with therapeutic and industrial applications, advancing the field of biotechnology.

13. LIMITATIONS

Research on silkworm gut microflora has been limited due to several challenges:

- Difficulties in cultivating and isolating microbial targets.
- Certain bioactive compounds are produced only in vivo within the symbiotic relationship.
- Bioactive compounds cannot be produced in axenic cultures.

14. OVERCOMING THESE OBSTACLES

To address these challenges, the following approaches can be employed:

- Advances in culturing techniques, such as broth culture, agar-based dipstick culture, and agar plate culture.
- Utilization of genomic and genetic tools like allozymes, mitochondrial DNA, and microsatellites for gene identification and heterologous expression.
- Application of modern genetic techniques, including genetic transformation of organisms with encoding genes.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

15. REFERENCES

Anand AAP, Vennison SJ, Sankar SG, Prabhu DI, Vasan PT, Raghuraman T, et al. Isolation and characterization of bacteria from the gut of *Bombyx mori* that degrade cellulose, xylan, pectin, and starch and their impact on digestion. Journal of Insect Science. 2010; 10(107): 1-13.

Barretto DA, Avchar R, Carvalho C, Sampaio JP, Vootla SK, Baghela A. *Blastobotrys bombycis* sp. nov., a D-xylose-fermenting yeast isolated from the gut of the silkworm larva *Bombyx mori*. International Journal of Systematic and Evolutionary Microbiology. 2018; 68(8): 2638–2643.

Barretto DA, Gadwala M, Vootla SK. The silkworm gut microbiota: A potential source for biotechnological applications. Methods in Silkworm Microbiology. 2021; 1-26.
Baumann P. Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. The Annual Review of Microbiology. 2005; 59:155–189. doi:10. 1146/annurev.micro.59.030804.121041

Ben Beard C, Cordon-Rosales C, Durvasula RV. Bacterial symbionts of the Triatominae and their potential use in control of Chagas disease transmission. The Annual Review of Entomology. 2002; 47: 123–141.

Berasategui A, Shukla S, Salem H, Kaltenpoth M. Potential applications of insect symbionts in biotechnology. Applied Microbiology and Biotechnology. 2016; 100:1567–1577.

Bhagat S, Agarwal M, Roy V. Serratiopeptidase: A systematic review of the existing evidence. International Journal of Surgery. 2013; 11(3): 209–217.

Bhalchandra PM, Pathade GR. Antibacterial and cholesterol reducing lactic acid bacteria from silkworm (*Bombyx mori*) gut environment-A review. Nature, Environment and Pollution Technology. 2011; 10(2): 319–326.

Bindu S, Arun KV, Nataraju B. Role of gut microflora in domesticated silkworm, *Bombyx mori* L. Journal of Sericulture Technology. 2014; 45(2): 128-133.

Bode HB. Insect-associated microorganisms as a source for novel secondary metabolites with therapeutic potential. In A. Vilcinskas (Ed.), Insect biotechnology. 2011; pp. 77–93.

Brauman A, Dore J, Eggleton P, Bignell D, Breznak JA, Kane MD. Molecular phylogenetic profiling of prokaryotic communities in guts of termites with different feeding habits. FEMS Microbiology Ecology. 2001; 35(1): 27–36.

Broderick NA, Lemaitre B. Gut-associated Microbes of *Drosophila melanogaster*. Gut Microbes. 2012; 3(4): 307-321.

Buchner P. Endosymbiosis of Animals with Plant Microorganisms. Interscience. 1965; Publishers.

Chen B, Du K, Sun C, et al. Gut bacterial and fungal communities of the domesticated silkworm (*Bombyx mori*) and wild mulberry-feeding relatives. The ISME Journal: Multidisciplinary Journal of Microbial Ecology. 2018; 12:2252–2262.

Chen B, Teh BS, Sun C, Hu S, Lu X, Boland W, Shao Y. Biodiversity and activity of the gut microbiota across the life history of the insect herbivore *Plutella xylostella*. Scientific Reports. 2015; 5(1): 1-12.

Chen B, Teh BS, Sun C, Hu S, Lu X, Boland W, Shao Y. Biodiversity and activity of the gut microbiota across the life history of the insect herbivore *Spodoptera littoralis*. Scientific Reports. 2016; 6: 29505.

Chen B, Yu T, Xie S, Du K, Liang X, Lan Y. Comparative shotgun metagenomic data of the silkworm *Bombyx mori* gut microbiome. Scientific Data. 2018a; 5(1): 1–10. https://doi.org/10.1038/sdata.2018.285.

Cheng Y, Ling E, Li X. The impact of gut microbiota on the health and immune responses of silkworm, *Bombyx mori*. Insect Biochemistry and Molecular Biology. 2017; 89: 25-31.

Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, Moran NA, et al. A metagenomic survey of microbes in honey bee colony collapse disorder. Science. 2007; 318(5848): 283–287.

Dillon RJ, Dillon VM. The Gut Bacteria of Insects: Nonpathogenic Interactions. Annual Review of Entomology. 2004; 49(1): 71-92.

Dillon RJ, Dillon VM. The Gut Bacteria of Insects: Nonpathogenic Interactions. Annual Review of Entomology. 2004; 49(1)7: 1-92.

Douglas AE. Microbial brokers of insect-plant interactions revisited. Journal of Chemical Ecology. 2013; 39: 952–961. doi:10.1007/s10886-013- 0308-x

Douglas AE. Multiorganismal Insects: Diversity and Function of Resident Microorganisms. Annual Review of Entomology. 2015. 60: 17-34.

Douglas AE. The microbial dimension in insect nutritional ecology. Functional Ecology. 2009; 23:38–47. doi:10.1111/j.1365-2

Duplouy A. Uncovering the hidden players in Lepidoptera biology: the heritable microbial endosymbionts. 2018; Peer J 6: e4629

Engel P, Moran NA. The Gut Microbiota of Insects – Diversity in Structure and Function. FEMS Microbiology Reviews. 2013; 37(5): 699-735.

Flórez LV, Biedermann PHW, Engl T, Kaltenpoth M. Defensive symbioses of animals with prokaryotic and eukaryotic microorganisms. **Natural Product Reports**. 2015; 32: 904–936. doi:10.1039/C5NP00010F

Fraher MH, O'toole PW, Quigley EM. Techniques used to characterize the gut microbiota: A guide for the clinician. Nature Reviews Gastroenterology and Hepatology. 2012; 9(6): 312.

Gerardo N, Hurst G. Q&A: Friends (but sometimes foes) within: the complex evolutionary ecology of symbioses between host and microbes. BMC Biology. 2017; 15:126.

Halarnkar PP, Blomquist GJ. Comparative aspects of propionate metabolism. Comparative Biochemistry and Physiology. B. Comparative Biochemistry. 1989; 92(2): 227.

Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science. 2012; 336(6086): 1268-1273.

Hui X, Muwang L, Yong Z, Liping Z, Yuehua Z, Yongping H. Bacterial community in midguts of the silkworm larvae estimated by PCR/DGGE and 16S rDNA gene library analysis. Acta Entomologica Sinica. 2010; 50(3): 222–233.

Idowu AB, Edema MO, Oyedepo MT. Extracellular enzyme production by microflora from the gut region of the variegated grasshopper *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae). International Journal of Tropical Insect Science. 2009; 29:229–235. doi:10.1017/S1742758409990312

Ishikawa Y, Tamura T, Sezutsu H. Potential applications of gut microbiota in sericulture: A review of current research. Journal of Insect Biotechnology and Sericulture. 2018; 87(3): 190-199.

Jem KJ, Tan B. The development and challenges of poly (lactic acid) and poly (glycolic acid). Advanced Industrial and Engineering Polymer Research. 2020; 3(2): 60–70.

Jin L, Jeon HH. The Role of Gut Microbiota in Insect Development and Adaptation. Current Opinion in Insect Science. 2021; 45: 55-61.

Khyade VB, Marathe RJ. Diversity of bacterial flora in the mid gut of fifth instar larvae of silkworm, *Bombyx mori* (L) (RACE: PM X CSR2). International Journal of Science and Nature. 2012; 3(4): 905–914.

Knipling EF. Possibilities of insect control or eradication through the use of sexually sterile males. Journal of Economic Entomology. 1955. 48: 459–462.

Krishnamurthy A, Amritkumar P. Synthesis and characterization of eco-friendly bioplastic from low-cost plant resources. SN Applied Sciences. 2019; 1(11): 1432.

Kunamneni A, Plou FJ, Ballesteros A, Alcalde M. Laccases and their applications: a patent review. Recent Patents on Biotechnology. 2008; 2:10–24

Lakshmi G, Ramesh B, Shankar M. Enumeration of Gut Microflora in *Bombyx mori* Using Viable Count Method. Journal of Sericulture and Entomology. 2020; 25(2): 143-150.

LeBlanc JG, Lain˜o JE, del Valle MJ, Vannini VV, Van Sinderen D, Taranto MP, et al. B-Group vitamin production by lactic acid bacteria–current knowledge and potential applications. Journal of Applied Microbiology. 2011; 111(6): 1297–1309.

Li GN, Xia XJ, Zhao HH, Sendegeya P, Zhu Y. Identification and characterization of Bacillus cereus SW7-1 in *Bombyx mori* (Lepidoptera: Bombycidae). Journal of Insect Science. 2015; 15(1): 136.

Li H, Qi Y, Jasper H. *Drosophila* intestinal response to bacterial infection. Developmental and Comparative Immunology. 2017; 70: 67-70.

Liang X, Fu Y, Liu H. Isolation and characterization of enzyme-producing bacteria of the silkworm larval gut in bioregenerative life support system. Acta Astronautica. 2015; 116: 247–253.

Liang X, Sun C, Chen B, et al. Insect symbionts as valuable grist for the biotechnological mill: an alkaliphilic silkworm gut bacterium for efficient lactic acid production. Applied Microbiology and Biotechnology. 2018; 102: 4951–4962.

Liu R, Wang W, Liu X, Lu Y, Xiang T, Zhou W, et al. Characterization of a lipase from the silkworm intestinal bacterium Bacillus pumilus with antiviral activity against *Bombyx mori* (Lepidoptera: Bombycidae) nucleopolyhedrovirus in vitro. Journal of Insect Science. 2018; 18(6): 3.

Mahmod F. Novel methods to study intestinal microbiota (Master’s thesis). Norwegian University of Life Sciences, A˚s. 2014.

Meng L, Li H, Bao M, Sun P. Metabolic pathway for a new strain *Pseudomonas synxantha* LSH-70 : From chemotaxis to uptake of n-hexadecane. Scientific Reports. 2017; 7: 39068.

Misof B, Liu SL, Meusemann K, et al. Phylogenomics resolves the timing and pattern of insect evolution. Science. 2014; 346:763–767.

Miyata K, Maejima K, Tomoda K, Isono M. Serratia protease part I. purification and general properties of the enzyme. Agricultural and Biological Chemistry. 1970; 34: 310–318.

Muller, C, GMC, L’Haridon R. Selective enumeration of *Bacteroides vulgatus* and *B. distasonis* organisms in the predominant human fecal flora by using monoclonal antibodies. Applied and Environmental Microbiology. 1996; 62(2): 735–73.

Nesa J, Sadat A, Buccini DF, Kati A, Mandal AK, Franco OL. Antimicrobial peptides from Bombyx mori: A splendid immune defense response in silkworms. RSC Advances. 2020; 10(1): 512–523.

O'Sullivan DJ. Methods for analysis of the intestinal microflora. Current Issues in Intestinal Microbiology. 2000; 1(2): 39–50.

Olofsson TC, Vásquez A. Detection and identification of a novel lactic acid bacterial flora within the honey stomach of the honeybee *Apis mellifera*. Current Microbiology. 2008; 57: 356–36.

Palmer C, Bik EM, Eisen MB, Eckburg PB, Sana TR, Wolber PK, et al. Rapid quantitative profiling of complex microbial populations. Nucleic Acids Research. 2006; 34(1): 5.

Pandiarajan J, Krishnan M. Comparative bacterial survey in the gut of lepidopteran insects with different bionetwork. Microbiology. 2018; 87(1): 103–115.

Phitsuwan P, Laohakunjit N, Kerdchoechuen O, Kyu K, Ratanakhanokchai K. Present and potential applications of cellulases in agriculture, biotechnology, and bioenergy. Folia Microbiologica, 2013; 58:163–176. doi:10.1007/s12223-012-0184-8

Prasanna VA, Kayalvizhi N, Rameshkumar N. Characterization of amylase producing Bacillus megaterium from the gut microbiota of silkworm *Bombyx mori*. Research Journal of Chemistry and Environment. 2014; 18(7): 38–45.

Prem Anand AA, Vennison SJ, Sankar SG, Gilwax Prabhu DI, Vasan PT, Raghuraman T, et al. Isolation and characterization of bacteria from the gut of *Bombyx mori* that degrade cellulose, xylan, pectin and starch and their impact on digestion. Journal of Insect Science. 2010; 10(1): 107.

Rai M, Agarkar G. Plant–fungal interactions: what triggers the fungi to switch among lifestyles? Critical Reviews in Microbiology. 2016; 42: 428–438.

Ramesh B, Lakshmi G, Kumar A. Morphological and Biochemical Characterization of Bacteria from Silkworm Gut. International Journal of Sericulture Research. 2018; 32(1): 55-63.

Rautio M. Indigenous intestinal microbiota and disease. Doctoral dissertation Faculty of Sciences. 2002; Department of Biosciences, University of Helsinki.

Salem H, Florez L, Gerardo N, Kaltenpoth, M. An out-of-body experience: the extracellular dimension for the transmission of mutualistic bacteria in insects. Proceedings of the Royal Society B: Biological Sciences. 2015; 282. doi:10.1098/ rspb.2014.2957

Shankar M, Kumar A, Lakshmi G. Molecular Identification of Gut Microflora in Silkworm *Bombyx mori* by 16S rRNA and 18S rRNA Gene Sequencing. Journal of Insect Biotechnology and Sericulture. 2021; 40(3): 215-224.

Shi W, Syrenne R, Sun JZ, Yuan JS. Molecular approaches to study the insect gut symbiotic microbiota at the 'omics' age. Insect Science. 2010; 17(3): 199–219.

Skelton J, Doak S, Leonard M, Creed RP, Brown BL. The rules for symbiont community assembly change along a mutualism parasitism continuum. Journal of Animal Ecology. 2016; 85:843–853.

Sridhara S, Bhat JV. Trace element nutrition of the silkworm *Bombyx mori* L. II. Relationship between cobalt, vitamin B12, benzimidazole and purines. Proceedings of the Indian Academy of Sciences—Section B. 1996; 63: 17–25.

Subramanian S, Gadhave KR, Mohanraj P, Thangamalar A. Use of 16S rRNA probes for characterization of gut microflora of silkworm (*Bombyx mori* L.) breeds. Karnataka Journal of Agricultural Sciences. 2010; 22(3): 476–478.

Sun BF, Xiao JH, He SM, Liu L, Murphy, RW, Huang, DW. Multiple ancient horizontal gene transfers and duplications in lepidopteran species. Insect Molecular Biology. 2013; 22(1): 72–87.

Sun L, Lu M, Lin H, Vincent M, Bi C, Guo Y. Characterization of the gut bacterial community of larval and adult silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) by high-throughput sequencing. AMB Express. 2018; 8(1): 1-11.

Sun L, Zhou W, Chen Y. 2016. Comparative analysis of gut microbiota between wild and domesticated silkworms, *Bombyx mori.* Microbial Ecology. 2016; 72(1): 254-262.

Sun Z, Lu Y, Zhang H, Kumar D, Liu B, Gong Y., et al. Effects of BmCPV infection on silkworm *Bombyx mori* intestinal bacteria. PLoS One. 2016; 11(1): e0146313.

Takatsuka J, Kunimi Y, Shiotsuki T. Influence of gut microbiota on the absorption and metabolism of dietary nutrients in silkworm, *Bombyx mori.* Applied Entomology and Zoology. 2005; 40(2): 211-219.

Thangamalar, A., Ramesh, G. K., Subramanian, S., and Mahalingam, C. A. 2009. Use of biochemical kits for characterization of Enterobacteriaceae from the gut of silkworm, Bombyx mori L. Karnataka Journal of Agricultural Sciences, 22(3): 514–516.

Thirupathaiah Y, Chandel AK, Sivaprasad V. Potential Applications of Enzymes in Sericulture. In: Singh O, Chandel A. (eds) Sustainable Biotechnology- Enzymatic Resources of Renewable Energy. 2018; Springer, Cham. https://doi.org/10.1007/978-3-319-95480-6_18

Tong, L., Yu, X. and Liu, H. 2011. Insect food for astronauts: Gas exchange in silkworms fed on mulberry and lettuce and the nutritional value of these insects for human consumption during deep space flights. Bulletin of Entomological Research, 101(5): 613.

Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444(7122): 1027-1031.

Vermelho, A. B., Noronha, E. F., Filho, E. D. X. F., Ferrara, M. A., Bon, E. P. S., Rosenberg, et al. 2013. Prokaryotic enzymes: diversity and biotechnological applications. In E. Rosenberg, E. F. DeLong, E. Stackebrandt, S. Lory, & F. Thompson (Eds.), The prokaryotes-applied bacteriology and biotechnology, pp. 2123–2240. Springer.

Wang Y, Gilbreath III TM, Kukutla P, Yan G, Xu J. Dynamic gut microbiome across life history of the malaria mosquito *Anopheles gambiae* in Kenya. PLoS ONE. 2013; 6(9): e24767.

Xiang H, Wei GF, Jia S, Huang J, Miao XX, Zhou Z, et al. Microbial communities in the larval midgut of laboratory and field populations of cotton bollworm (*Helicoverpa armigera*). Journal of Insect Science. 2007; 7(40): 1-9.

Xie S, Lan Y, Sun C, Shao Y. Insect microbial symbionts as a novel source for biotechnology. World Journal of Microbiology and Biotechnology. 2019; 35 (2): 25. doi:10.1007/s11274-019-2599-8.

Xingmeng L, Fangwei W. Inhibition of cultured supernatant of enterococci strains on germination of *Nosema bombycis* spores in vitro. Can ye kexue = CanyeKexue =Acta Sericologica Sinica. 2002.; 28(2): 126–128.

Yan S, Wu G. Analysis on evolutionary relationship of amylases from archaea, bacteria and eukaryota. World Journal of Microbiology and Biotechnology. 2016; 32: 24.

Zhang X, Pang X, Wang J, Wang L, Zhao L. The influence of gut microbiota on sericulture sustainability. Journal of Applied Microbiology. 2020; 128(4): 989-998.

Zheng H, Nishida A, Sekiya H, Fujisaki T, Matsuura K, Uchida K. Gut bacterial flora and chemical defenses in larvae of the Chinese oak silk moth, *Antheraea pernyi*. Insect Biochemistry and Molecular Biology. 2014; 49: 1-9.