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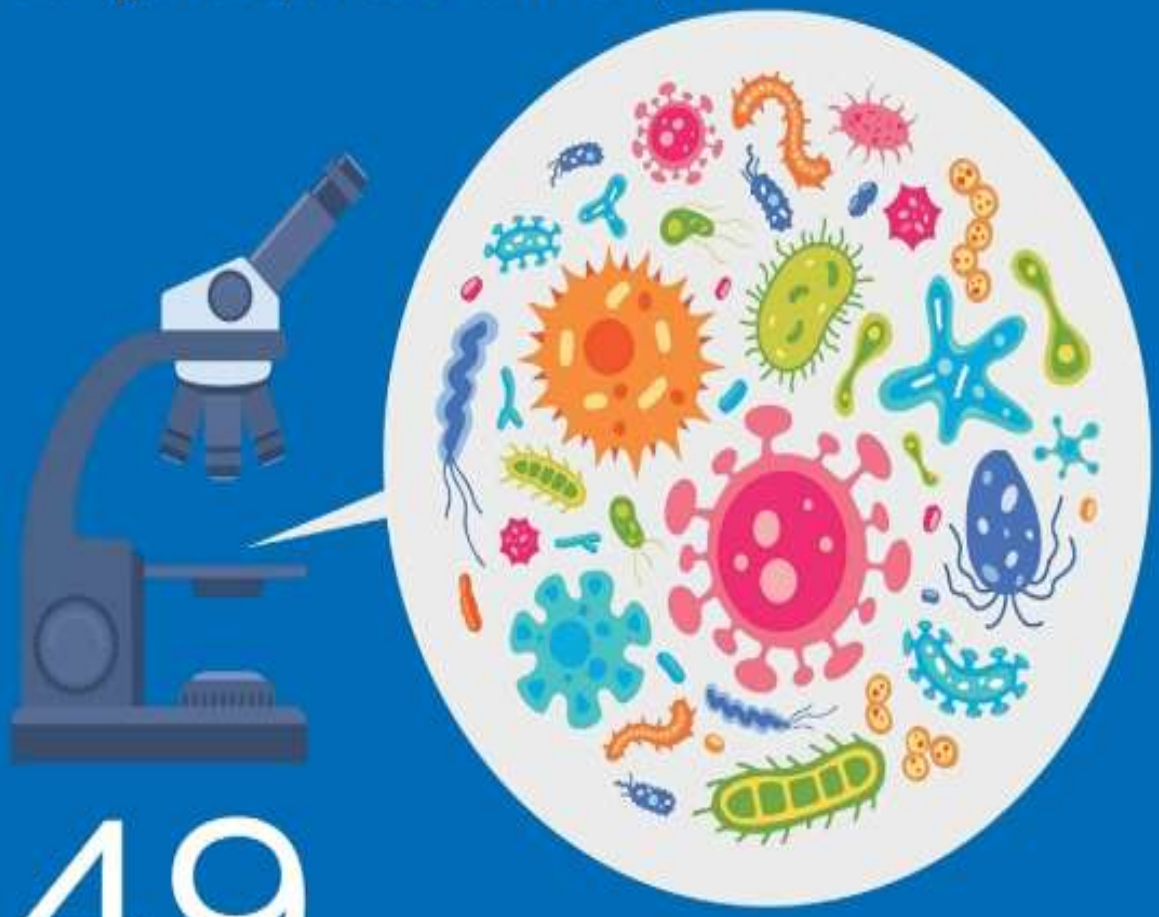

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Chapter 1 - The silkworm gut microbiota: A potential source for biotechnological applications

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Abstract

Abstract

Silkworm is a typical representative of the lepidopteran insects and has great importance in agriculture and economy. Like most lepidopterans, silkworm is also associated with a large consortium of symbiotic microbes. The commensal flora are found to play a crucial role in the survival of insects by

CHAPTER

1 The silkworm gut microbiota: A potential source for biotechnological applications

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1 Introduction

Insects are one of the oldest group of animals on our planet that are ubiquitous in nature and have been adapted to several environmental conditions represent the most diverse group of animals on earth. Since then a wide range of microbes are found associated with these insects (Chapman, 2007). The microbial inhabitants of insect gut comprise of a large taxonomic diversity ranging from prokaryotes (bacteria and archaea) to eukaryotes (fungi and protozoa). The colonized microbes may show either symbiotic and/or non-symbiotic relationship with the host insect (Bode, 2011).

A healthy insect harbours microbiota that account for 1–10% of the insect's biomass and hence a healthy insect is termed a "multi organismal entity" (Douglas, 2015). The resident microbial flora in insects benefits the host by producing essential compounds like vitamins, digesting and metabolizing food, nutrient absorption, detoxification of toxins and pheromone production and immunity (Dillon & Dillon, 2004; Rajagopal, 2009). The commensal flora are found to play a crucial role in the survival of the insects by protecting their insect hosts against the natural enemies and pathogens through varied mechanisms that include colonial resistance, production of toxins and insects' immune system activation to fight the invader organism (Douglas, 2015). Proportionately, this insect-microbe association throws light on a

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variety of secondary metabolites and other useful enzymes and molecules that can be derived from these microorganisms and used for a variety of purposes either for humans or for the insect itself.

2 Microbial diversity in the silkworm gut

The lepidopteran larvae harbour only a few or no bacteria as compared to the other insect orders owing to the rough environment like the alkaline pH, redox conditions, oxygen content, digestive enzymes, immune related compounds, type of food ingested and unstable habitats like moulting are the multiple challenges that the gut provides for microbial colonization (Hammer, Janzen, Hallwachs, Jaffe, & Fierer, 2017). However, in spite of the unfavourable and harsh environment that the gut provides for the gut microflora, several gut microbiota have been shown to carry out essential physiological functions in the lepidopterans (Paniagua Voirol, Frago, Kaltenpoth, Hilker, & Fatouros, 2018). Thus, like other lepidopterans, silkworm is also associated with a large consortia of symbiotic microbes in their gut.

2.1 Microbiota of domesticated silkworm

Silkworm *Bombyx mori* (Lepidoptera: Bombycidae) is a domesticated silk moth. It is a typical representative of the lepidopteran insects and has great importance in agriculture and the economy. Xia et al. (2004) termed the silkworm *B. mori* "economically important" due to its use in silk industry to maximize silk fibre productivity. The silkworm as a promising model organism in life science due to its short generation time, rich genetic resources, clearly sequenced genetic background and a considerable number of genes that are homologous to silkworm that makes it suitable for various life science studies (Meng, Li, Bao, & Sun, 2017). It is a powerful insect model in research owing to its relatively large size and easy maintenance and rearing (Xia, Li, & Feng, 2014).

Although the silkworm *B. mori* exhibits rich diversity of microbial flora, very few of them have been identified and poorly characterized (Pandiarajan & Krishnan, 2018). Kalpana, Hatha, and Lakshmanaperumalsamy (1994) have reported the gut microbial community throughout the life cycle of *B. mori*. Out of these, a greater number of bacterial flora were observed in the fourth and fifth instar larval gut that accounts for the active feeding stage of the larvae. These gut microbiota were observed to aid in host digestion and growth. Hui et al., (2010) reported 41 bacterial phylotypes in the midguts of the silkworm *B. mori* larvae by PCR/DGGE technique and 16S rDNA gene library analysis. Thangamalar, Ramesh, Subramanian, and Mahalingam (2009) revealed the existence of the bacteria belonging to Enterobacteriaceae in *B. mori* gut. Subramanian, Gadhave, Mohanraj, and Thangamalar (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. Prem Anand et al. (2010) found that the *B. mori* gut is colonized by a

variety of non-pathogenic microorganisms. The authors reported 11 bacterial phylotypes from the fifth instar *B. mori* gut that were capable of degrading various host-inaccessible polysaccharides from the host diet indicating the association of gut associated bacteria in nutrient absorption and the growth of the host insect. Khyade and Marathe (2012) indicated the presence of cellulolytic bacteria in the midguts of *B. mori* that helps in food digestion, absorption of nutrients and growth.

Several studies have also shown that the gut microflora of *B. mori* aids in fighting infectious diseases in the silkworm host. The microflora has been observed to change during infection especially during viral infection, e.g.; Sun et al. (2016) revealed that predominant bacteria *Delftia*, *Pelomonas*, *Ralstonia, Staphylococcus* and *Enterococcus* in healthy *B. mori* gut were altered during viral infection caused by BmCPV (*B. mori* cypovirus); Xingmeng and Fangwei (2002) 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; Li, Xia, Zhao, Sendegeya, and Zhu (2015) showed the presence of *Bacillus* species in the guts of silkworm that can be used as probiotics for silkworm disease management; Sun et al. (2013) reported the maximum horizontal gene transfer from bacteria and fungi to *B. mori* that improved the survival and fecundity of the silkworm.

The research on microflora of silkworm gut is biased towards the gut bacteriome and very little is known about the fungal gut communities. Chen et al. (2018) reported the presence of fungi belonging to the phyla Ascomycota and Basidiomycota along with bacteria belonging to the phyla Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. Yet there have been no reports on yeast species isolated from the *B. mori* larval gut until 2018. However, our study presented *Cryptococcus rajasthanensis* and *Blastobotrys bombycis* sp. nov. the yeast microflora of *B. mori* gut (Barretto et al., 2018; Barretto and Vootla, 2018b; Barretto & Vootla, 2020).

Furthermore, Chen et al., (2018) stated that "though the large amount of information on the biology and physiology of the silkworm *B. mori* is known, very few studies have been carried out on silkworm gut microbiota," indicating that the research on gut microflora of silkworm *B. mori* is still limited.

2.2 Microbiota of muga silkworm

The muga silkworm, *Antheraea assamensis* Helfer is a semi-domesticated silkworm reared exclusively in Northeast regions of India and known for its golden silk called "muga silk" (Chetia et al., 2017). Gandotra et al. (2018) studied the bacterial diversity in the gut of muga silkworm through culture-dependent techniques by using 16S rRNA sequencing. The diversity analysis indicated the presence of bacteria belonging to *Bacillus* spp., *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Pseudomonas stutzeri*, *Acinetobacter* sp. and *Alcaligenes* sp. among which *Bacillus* was the predominant genus contributing to 54% of total bacterial load in the gut of muga silkworm followed by *Serratia* (24%), *Pseudomonas* (10%) and *Alcaligenes* (6%). Another study by Haloi, Kalita, Nath, and Devi (2016) revealed the bacterial community in the gut of healthy muga silkworm. The 16S rRNA gene sequencing in

this study showed the presence of 11 bacterial isolates. *Pseudomonas aeruginosa* (DRK1), *Ornithinibacillus bavariensis* (DRK2), *Achromobacter xylosoxidans* (KH3) and *Staphylococcus aureus* (FLG1) were found to be dominant bacterial species in the healthy silkworm. These bacterial isolates were also observed in flacherie diseased muga silkworm. Bhuyan et al. (2018) identified gut bacteria of *A. assamensis* Helfer as *Bacillus pumilus* MGB05 with accession KP298708.2.

2.3 Microbiota of eri silkworm

The eri silkworm (*Philosamia ricini*) the producer of eri silk also popularly known as ahimsa silk, is a multi-voltine, non-mulberry silkworm reared in North-east parts of India especially in Assam, Nagaland and Meghalaya and some parts of China and Japan. It feeds primarily on castor (*Ricinus communis*) (Attathom, 2004). The gut bacterial communities of eri silkworm was studied by MsangoSoko et al. (2020) wherein the culture dependent 16S rRNA gene sequencing revealed the presence of 60 aerobic culturable bacteria comprising of Firmicutes (54%) and Proteobacteria (46%) and 12 culturable facultative anaerobes comprising of Proteobacteria (92%) and Firmicutes (8%). The metagenomic analysis revealed the presence of a diverse community of both culturable and un-culturable gut bacteria belonging to Proteobacteria (60%) and Firmicutes (20%) associated with seven orders.

2.4 Microbiota of tasar silkworm

Tropical tasar silkworm (*Antheraea mylitta* Drury) is a wild type, polyphagous, non-mulberry silkworm that feeds on food plants, viz. *Terminalia tomentosa*, *T. arjuna* and *Shorea robusta* and also on dozens of secondary food plants (Barsagade, 2017). The gut microbial community of tasar silkworm was reported by Rajan et al. (2020) from culture-dependent analyses, showing that the predominant bacterial community in the gut of *A. mylitta* Drury belonged to Proteobacteria. The gut bacteria were identified as *Pseudomonas*, *Erwinia*, *Enterococcus*, *Staphylococcus*, *Bacillus cereus*, *Lactobacillus* and *Micrococcus*. The non-culturable bacterial community in the gut of tasar silkworm was also evaluated by metagenomics approach depicting the abundance of Firmicutes in the gut. The abundant bacterial genera were found to be *Turicibacter* followed by *Ruminococcus*, *Rhodococcus*, *Prevotella*, *Delftia*, *Acinetobacter*, *Desulfomicrobium*, *Sphingomonas*, *Faecalibacterium*, *Staphylococcus*, *Ralstonia*, *Bacillus*, *Azospirillum*, *Candidatus* and *Kocuria*.

2.5 Microbiota of oak tasar silkworm

The Indian oak tasar silkworm (*Antheraea proylei* Jolly) is a domesticated silkworm in Northeast India especially in Manipur (Devi, Ponnuvel, Singh, Singh, & Dutta, 2012). Pimenta et al. (2005) reported novel species of ascogenous yeast belonging to genus *Geotrichum*. The novel species of yeast isolated from the gut of oak tasar silkworm (*Antheraea proylei* J.) is described as *Geotrichum silvicola* sp. nov.

3 Methods for gut microbial community identification

A number of procedures and techniques have been used to identify insect gut microflora. They basically involve classical approaches and molecular approaches which can be further divided into culture dependent and culture independent methods as shown in Table 1.

Sample preparation is an important step in evaluating the microbial load in the silkworm gut before isolating the gut microflora and identifying them. In our study we have isolated and identified microflora of silkworm *B. mori* gut (Fig. 1) and have standardized methods for obtaining high yields of bacterial colonies which are briefly described (Barretto et al., 2018; Barretto & Vootla, 2018a, 2018b).

3.1 Silkworm rearing and collection of gut sample

Fourth and fifth instar healthy silkworm larvae (*B. mori* race CSR2 X CSR4, Bivoltine hybrids) were collected and reared on fresh mulberry leaves in the laboratory at optimum environmental conditions of 26 °C and 65% RH (Relative Humidity). The silkworm larvae were surface sterilized using 70% ethanol and dissected separately to collect the midguts aseptically in the laminar air flow. The isolated midguts were washed in sterile distilled water to get rid of the food bolus and other contaminants. Isolated larval guts were transferred into separate sterile tubes under sterile conditions. Sterile bacteriological saline (0.85% NaCl) was pipetted into it followed by slight maceration of the guts using sterile homogenizer. The fluid obtained after maceration was used as the gut sample for microflora isolation.

3.2 Isolation of gut microflora

Serial dilution of the gut samples were carried out using bacteriological saline from 10^0 to 10^{-6}. 100 µL of the dilutions 10^0, 10^{-4}, 10^{-5} and 10^{-6} were inoculated onto the respectively labelled sterile Nutrient agar plates. The plates were incubated at 37 °C for 24 h in order to isolate the bacterial gut microflora. Similarly 100 µL of the dilution 10^0, 10^{-1}, 10^{-2} and 10^{-3} were inoculated onto the sterile Potato Dextrose agar plate followed by incubation at 27 °C for 5–7 days in order to isolate the fungal gut microflora.

3.3 Enumeration of gut microflora and identification

The microbial load was counted by viable count method. Morphologically distinct microbial colonies were sub-cultured and maintained for further studies. Morphological and biochemical characterization was carried out to identify the bacterial colonies up to the genus level followed by identification by 16S rRNA gene sequencing method to identify the bacteria at its species level. Similarly morphological characterization was carried out of to identify the genera of fungi followed by molecular characterization using 18S rRNA gene sequencing method.

Table 1 Different methods to study gut microbial community of insects (Mahmod, 2014; Shi, Syrenne, Sun, & Yuan, 2010).

Sl. no	Approach	Technique	Purpose	References
I	*Classical approaches*			
1.	Culture dependent method			
(i)	Plating/culturing technique	Culturing on various media	Isolating cultivable microorganism; studying the biochemical and physiological role of microorganisms	Fraher, O'toole, & Quigley, 2012
2.	Culture independent methods			
(i)	Direct microscopic analysis	Heat fixation and staining	Estimating bacterial load in the gut by analysing the microorganisms in the faeces of insects	Rautio, 2002
(ii)	Monitoring specific enzymes and/or metabolites in faeces	Analytical techniques: NMR; MS; FT-IR; FT-Raman spectroscopy, etc.	Estimating microbial load in insect gut as metabolites in the faeces can be correlated with metabolism of specific microbes	O'Sullivan, 2000
II	*Molecular approaches*			
1.	Culture dependent method			
(i)	Phenotypic fingerprinting	PAGE; bacteriophage typing; serotyping	Identifying microorganisms using colony hybridization, monoclonal antibodies, etc. specific for a genus, species or strain without sub-culturing	Corthier, Muller, & L'Haridon, 1996
(ii)	Genotypic fingerprinting	Colony hybridization with nucleic acid probes; pulse field gel electrophoresis; ribotyping	Identification of gut symbiotic microbes	Palmer et al., 2006
2.	Culture-independent methods			
(i)	PCR based	Gene targeting PCR: 16S rRNA/ 18s rRNA gene sequencing	Identification of unculturable microorganisms	Brauman et al., 2001
		Molecular fingerprinting techniques: DGGE; TGGE; SSCP; RAPD	Identification of microbial diversity through analysis of the 16S/18s rRNA from different microbial species and then generating the molecular fingerprinting and phylogenetic affiliation of the gut microflora	Smalla et al., 2007
(iii)	Oligonucleotide probe based hybridization	FISH technique	Identification of symbiotic microbiota of the insect gut by using fluorescently labelled probes that target the 16S/18s rRNA sequence specific for a bacterial/fungal genus or species	Turroni, Ribbera, Foroni, van Sinderen, & Ventura, 2008
III	*Meta-omics*			
1.	Metagenomics	Metagenome sequencing and downstream analysis; cDNA library screening	Detection and identification of microbiota	Harmon, Moran, & Ives, 2009
2.	Metatranscriptomics	cDNA library preparation	Detection of microbial communities by analysing RNA which is then converted to cDNA for further analysis	Cox-Foster et al., 2007
3.	Metaproteomics	2D gel electrophoresis, MALDI; ESI; MS/MS; LC-MS, etc.	Understanding the significance of insect gut symbiotic microbe to the proteome level	Wilmes & Bond, 2004
4.	Metabolomics	Analytical methods: NMR; MS	Studying the metabolic profiles	Gong and Yang (2012)

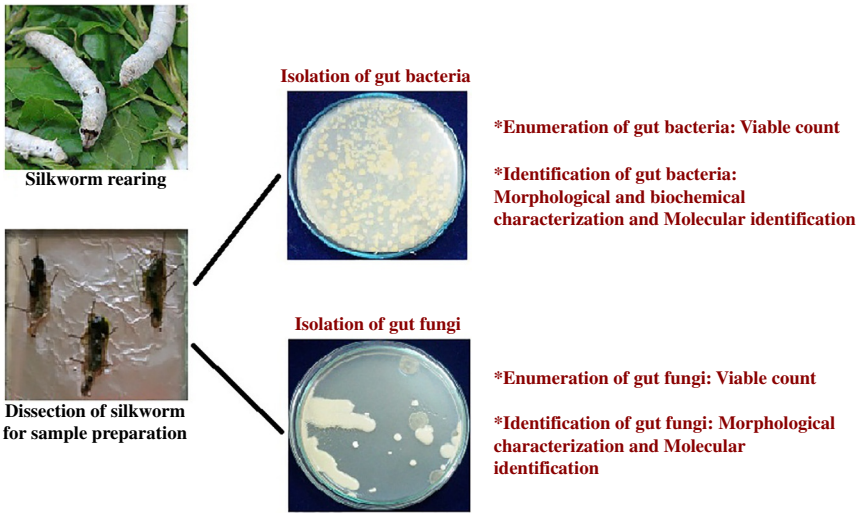


FIG. 1

Silkworm rearing, sample preparation, isolation and identification of silkworm *B. mori* gut microflora. The domesticated silkworm was dissected under sterile conditions and the sample was prepared by macerating the gut in sterile saline. The sample was then diluted and inoculated on nutrient agar and potato dextrose agar. Upon incubation the microbial flora of the silkworm gut was enumerated and identified.

4 Biotechnological applications of silkworm gut microbiota

The insect gut microbiota application in biotechnology can be achieved in two ways: (1) by targeting or using the mutualistic relationships in controlling agricultural pests and vector-borne diseases or to improve the health of economically important insects, (2) by utilizing the symbiont-derived compounds or molecules such as enzymes or bioactive molecules. In order to achieve this, the recent research has focused on exploration of gut microbiota of healthy insects for the screening and production of biomolecules with various biotechnological applications.

Although numerous secondary metabolites with therapeutic properties (antimicrobials, anticancer agents and antioxidants) have been identified isolated and studied from insect-associated microbes from the exoskeleton and/or external environment that protect the insect from harmful invaders, only a few studies have been reported on bioactive compounds from the insect gut microflora. Also the industrially important enzymes like cellulases, pectinases, ligninases, proteases and other detoxifying enzymes have been isolated from beetles and termite guts but very few from the lepidopteran insect gut (Berasategui, Shukla, Salem, & Kaltenpoth, 2016). The study on microbes with the potency for ethanol production is again biased towards beetle and termite gut microflora as compared to that of the lepidopterans. This may be attributed to the fact that the study of the microbial community in the

lepidopteran gut is inadequate as compared to other insect orders owing to the inimical environment the insect gut presents to the colonizing microbes. Although the gut microbiota of the lepidopteran insect (in this case the silkworm) with biomolecules producing ability have been reported, the research on utilization of these secondary metabolites with biotechnological application especially in therapeutics is yet to be explored.

Recent studies have demonstrated that silkworm gut microbes could synthesize and produce extracellular enzymes, vitamins, metabolites, antimicrobial substances and antioxidants which help in digestion, absorption of nutrients, inhibiting colonization of pathogenic microorganisms and stimulating host immune response (Thirupathaiah, Chandel, & Sivaprasad, 2018).

4.1 Applications in agriculture and silkworm health

The sericulture industry involves processes like cultivation of mulberry trees, rearing of silkworm on mulberry leaves to produce cocoons, silk reeling and weaving. Silkworm rearing is the most crucial and sensitive process in the silk industry as the larvae are most susceptible to non-infectious and infectious diseases (Takeda, 2009). The non-infectious diseases include development of symptoms like flaccidity caused by environmental, nutritional and chemical factors. The silkworm *Bombyx mori* is susceptible to various infectious diseases which are caused by various microorganisms. The diseases caused by viruses in *B. mori* has been reported by Watanabe (1986) include *Bombyx mori* Cytoplasmic Polyhedrosis virus (BmCPV) by a Reovirus; *Bombyx mori* Infectious Flacherie virus (BmIFV) by a picornavirus, *Bombyx mori* Densonucleosis virus (BmDNV) by a densovirus and *Bombyx mori* nuclear polyhedrosis virus (BmNPV) by a Baculovirus. Bacteria such as *Streptococcus faecalis*, *S. faecium*, Staphylococcal species and *Serratia marcescens* cause bacterial flacherie individually or in combination with viruses such as BmIFV and BmDNV (Selvakumar & Datta, 2013). Another important infectious disease is Muscardine, a fungal disease caused primarily by *Beauveria bassiana* and *Metarhizium anisopliae* (Kumar, Singh, Babu, Ahsan, & Datta, 1999). Pebrine is yet another disease caused by a microsporidia, *Nosema bombycis*. Recently several other microsporidian species viz., *Vairimorpha*, *Pleistophora* and *Thelophania* can cause disease in silkworm, which are transovarially transmitted and were known to be responsible for eradication of silk industry in Europe (Bhat, Ifat, & Kamili, 2009). These diseases cause significant loss of cocoons at the farmer's level thereby affecting the economy relying on the silk industry.

In order to avoid this huge loss, maintaining the health of silkworm is the need of the hour. Therefore, several attempts are being made to improve the health of silkworms. Yeruva, Vankadara, Ramasamy, and Lingaiah (2019) identified potent probiotic bacterial communities from the silkworm *B. mori* gut through a metagenomic approach. The diversity of bacterial profile in the *B. mori* (Pure Mysore, PM: multivoltine; CSR2: bivoltine and PM × CSR2: crossbreed) gut showed an abundance of the genus *Lactobacillus* including *L. plantarum*, *L. rhamnosus*, *L. paracasei* and

L. acidophilus in the midgut followed by *Enterococcus* and *Bacillus*. The reports suggest that *Lactobacillus* and *Bacillus* species could further be supplemented to the silkworm through mulberry feed for the improvement of economic characteristics of the silkworm. A study by Saranya, Krishnamoorthy, Balachandar, and Tilak (2019) reported indigenous Lactic acid bacteria (LAB) from *B. mori* gut with probiotic potential. Subramanian, Mohanraj, and Muthuswamy (2009) has studied the probiotic application of *Streptomyces noursei* (Brown et al. ATCC® 1455™) an actinomycete isolated from silkworm. The study has demonstrated the increase in endogenous actinomycete population in the *B. mori* gut on the administration of the strain. The study also depicted antimicrobial potentiality of culture filtrate of *Streptomyces noursei* (Brown et al. ATCC® 1455™) against gram-positive and gram-negative bacteria including *Bacillus* sp., *Staphylococcus* sp., *E. coli*, *Xanthomonas* sp., *Pseudomonas* sp. and *Micrococcus* sp. These results provide a boon for application of *Streptomyces noursei* (Brown et al. ATCC® 1455™) for eco-friendly management of silkworm diseases. A study by Mohanraj and Subramanian (2014) also reported the antibacterial activity of the gut bacteria *S. noursei* against bacterial pathogens viz. *S. aureus*, *E. coli, Xanthomonas oryzae*, *Pseudomonas* sp., *Micrococcus* sp. and *B. subtilis*. A study reported by Wang et al. (2016) explored the distribution and probiotic function of a strain *B. subtilis* (strain no. 951NA) isolated from *B. mori* fourth instar larval gut. The isolated strain showed the presence of a gene encoding the neutral protease enzyme. A study by Yang et al. (2011) reported amylase producing probiotic bacteria *B. cereus* from the *B. mori* intestine.

Liu et al. (2018) showed the importance of intestinal microorganisms in the host defence system against viral pathogens. The study conducted revealed the presence of *Bacillus pumilus* SW41 with lipase enzyme producing gene bearing antiviral activity against BmNPV. The study showed the reduction of BmNPV infectivity in vitro resulted in decreased viral DNA abundance and viral occlusion bodies. The lipase produced by the *Bacillus pumilus* SW41 probably acts directly against the budded virions thereby acting as potential antiviral factor for silkworm against BmNPV.

The enzymes in the silkworm gut are also known to influence the growth, development and resistance to diseases in silkworm that subsequently enable the silkworm to produce good quality cocoon and silk. Esaivani, Vasanthi, Bharathi, and Chairman (2014) reported the adaptability of *B. mori* for nutrient feed supplement and their influence on enzymatic profiles and commercial characteristics. In this study the silkworm larvae fed with a probiotic strain *Saccharomyces cerevisiae* along with mulberry feed has shown significant variation in enzymatic profile in silkworm gut. The probiotic has been shown to increase the commercial characteristics of the silkworm such as cocoon characters and silk characters. Similarly, Gunasekhar and Somayaji (2019) demonstrated the effect of *Burkholderia cepacia*, an endophytic bacteria of mulberry leaves on silkworm growth and enzymatic activity. It is noted that administration of *Burkholderia cepacia* in *B. mori* increased the growth compared to normal which in turn increased the silk yield. The increase in weight of the larvae and cocoon was in correspondence with the increased digestive enzymes such as

amylase and protease. The increased protease activity was attributed to the increased silk protein concentration for silk production. Suraporn, Sangsuk, Chanhan, and Promma (2015) reported the effects of the probiotic *L. acidophilus* on silkworm *B. mori* races Nang Lai and Nang Lai X 108. The study indicated that *L. acidophilus* stimulated growth factors in silkworm leading to increased silk yield and improved silk harvest. Similarly, Nishida et al. (2017) reported a rice bran pickle isolate *Lactobacillus paraplantarum* 11–1 isolated from Rice bran pickles. This probiotic strain was reported to activate innate immunity and improve insect survival against bacterial infection. Taha and Kamel (2017) reported the effect of mulberry leaves supplemented with the probiotics *S. cerevisiae* and *Bifidobacterium bifidum* on *B. mori*. The administration of these probiotics strains has shown an increase in digestive enzymes protease, invertase and amylase that has shown to have beneficial effects on the cocoon production.

Bhuyan, Gogoi, Neog, and Subramanian (2014) suggested that the isolation, characterization and identification of muga silkworm (*Antheraea assamensis* Helfer) gut bacteria will provide insights to assess the beneficial gut bacteria in formulating consortia for the better growth of *A. assamensis* and production of muga silk. In this study, the dominant gut bacteria were identified as *Bacillus* sp., *Proteus* sp. and *E. coli* and has shown to produce digestive enzymes amylase, pectinase, cellulase, xylanase and lipase that help in digesting the major ingredients pectin, starch, cellulose, xylan, lipids and fatty acids from primary host plant Som (*Presea bombycina* Kost) leaves. The digestion of food ingredients and utilization of nutrients is beneficial for better productivity of muga silk. Bhuyan et al. (2018) also reported the cellulase activity of *Bacillus pumilus* MGB05 isolated from midgut of muga silkworm that suggested the beneficial role of the bacteria in digestion. The isolate has shown antimicrobial activity against *E. coli* and *P. aeruginosa* that plays an essential role in disease resistance. The results suggest the potential role of *B. pumilus* MGB05 to be used as probiotic in enhancing silk productivity. A study by Gandotra et al. (2018) has reported the enzymatic activities of cellulase, amylase and lipase from gut bacteria of *A. assamensis* contributing to digestion and nutrition of the host that would benefit muga silk productivity.

The Lepidoptera contains the most devastating agricultural pests worldwide and is the second most diverse order of insects (Sree & Varma, 2015). In recent decades, pesticides and insecticides have been widely used in controlling the agricultural loss due to these insects but the pesticide toxicity is also noticed against non-target insects and pests including silkworm. Li et al. (2020) demonstrated the adverse effect of organophosphate pesticide, phoxim on silkworm health. The study reported the alteration of gut microbiota in *B. mori* upon phoxim exposure leading to stressful conditions leaving the insect susceptible to diseases and compromised health status. The study showed the increased abundance of non-dominant bacteria *Staphylococcus* and reduction in abundance of the dominant genera *Methylobacterium* and *Aurantimonadaceae* in phoxim-treated silkworm. Phoxim inhibited the expression of antimicrobial peptides (AMPs) at mRNA level and enhanced pathogenesis of *Enterobacter cloacae* towards silkworm larvae thereby affecting the immune

system. The change in microbiota upon phoxim exposure affects the normal functioning of the intestinal tract of silkworm causing midgut injury. This study is useful for studying the toxicity of insecticides against target and non-target insects thereby avoiding the losses in the sericulture industry.

4.2 Applications in bioremediation and detoxification

Bioremediation of pesticides and detoxification of toxic natural products is another application of symbiotic gut microflora. Silkworms belong to the phytophagous insect group and hence the gut is not sterile and undergoes oxidative stress during oxidation of phenolic compounds that are present in the food bolus (Summers & Felton, 1994). In these cases, the microbiota is known for detoxifying the plant compounds. The domesticated silkworm (*B. mori*) feeds on mulberry leaves which contain polysaccharides pectin, xylan, cellulose and starch. A study by Prem Anand et al. (2010) showed that the gut bacterial community of *B. mori* including the Gram-positive *Bacillus circulans* and Gram-negative *Proteus vulgaris*, *Klebsiella pneumoniae*, *E. coli*, *Citrobacter freundii*, *Serratia liquefaciens*, *Enterobacter* sp., *Pseudomonas fluorescens*, *P. aeruginosa*, *Aeromonas* sp., and *Erwinia* sp. have been shown to produce a variety of enzymes including pectinases, xylanases, cellulases and amylases with polysaccharide degrading properties. The gut of these silkworms have alkaline pH and the enzymes of the bacterial isolates were shown to have highest activity at alkaline pH.

Insecticides are effective in combating insect attack on food crops causing huge economic loss to the agricultural industry. However, overuse of these insecticides is known to increase the insecticide resistance in many insect pests (Indiragandhi, Anandham, & Sa, 2011). It is well established that the insect-resistant hosts carry diverse gut-associated microbial load with insecticide degrading capabilities (Almeida, Moraes, Trigo, Omoto, & Consoli, 2017). Fluoride compounds mainly released during production of aluminium, steel, glass fibre, phosphate fertilizers, bricks, tiles and ceramics can lead to fluorosis in silkworms causing serious loss in sericulture. Li, Xia, Tang, and Zhu (2016) investigated the relationship between intestinal microflora and fluoride resistance of silkworm. It was noted that the gut microflora of fluoride-resistant and fluoride-susceptible strains showed a difference in microbial communities. The fluoride treated silkworm showed the abundance of two dominant bacterial groups (Firmicutes and Proteobacteria) followed by Bacteroidetes, Cyanobacteria, Fusobacteria, Chloroflexi, Euryarchaeota, Thaumarchaeota, and unclassified species. This study gives insights in to the fluoride degrading capacity of silkworm gut microbiota.

4.3 Human therapeutic applications

The insect gut microflora as a source of novel bioactive compounds is gaining interest since a number of bioactive compounds have been obtained such as antiviral, antimalarial and antimicrobial peptides (Chernysh et al., 2002), novel metabolites

and enzymes (Zhang & Brune, 2004). Bode (2011) investigated the insect-associated microorganisms as a source for the discovery of novel secondary metabolites with therapeutic properties like anti-cancer, antimicrobial, anti-inflammatory, etc.

Our previous research on activity of Staphyloxanthin obtained from *Staphylococcus gallinarum* KX912244 reported the antimicrobial, antioxidant and anticancer properties (Barretto & Vootla, 2018a). The bacterial isolate *S. gallinarum* was isolated from the midgut of *B. mori* (race CSR2X CSR4, bivoltine hybrid). The pigment of *S. gallinarum* showed antimicrobial activity against human pathogens *E. coli*, *S. aureus* and *C. albicans*. The pigment also showed anticancer activity against four cancerous cell lines such as Dalton's lymphoma ascites (DLA) with IC_{50} value $6.20 \pm 0.02\,\mu g/mL$, Ehrlich ascites carcinoma (EAC) possessing IC_{50} value $6.48 \pm 0.15\,\mu g/mL$, Adenocarcinomic human alveolar basal epithelial cells (A549 Lung carcinoma) with IC_{50} value $7.23 \pm 0.11\,\mu g/mL$ and Mus mucus skin melanoma (B16F10) indicating IC_{50} value $6.58 \pm 0.38\,\mu g/mL$ and less cytotoxicity towards non-cancerous human fibroblast cell lines (NIH3T3) with IC_{50} value of $52.24\,\mu g/mL$ and is thus biocompatible (Fig. 2). Another pigment isolated from the yeast *Cryptococcus rajasthanensis* KY627764 from *B. mori* gut was reported

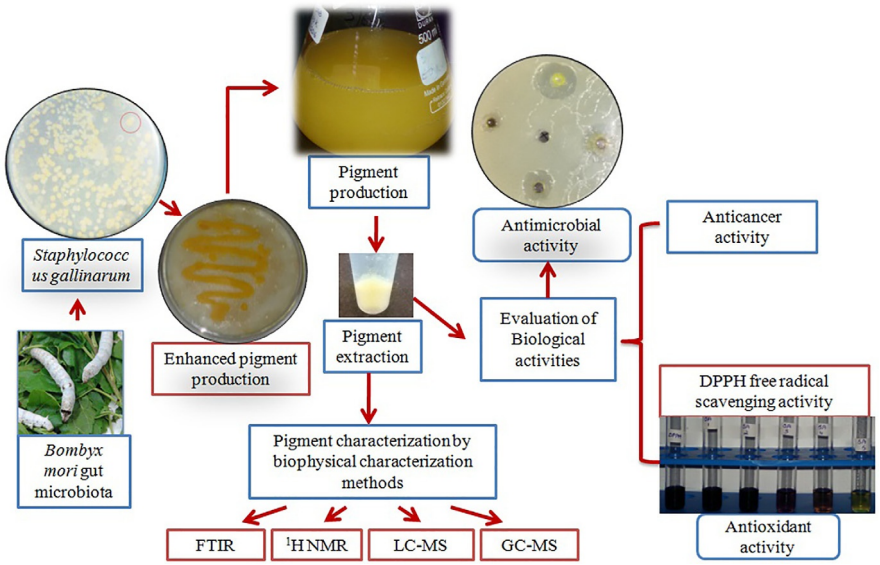


FIG. 2

Therapeutic applications of staphyloxanthin obtained from *S. gallinarum* isolated from silkworm *B. mori* gut microflora (Barretto & Vootla, 2018a). The bacteria producing yellow pigment was isolated and the pigment production was enhanced on milk salt agar. The pigment was extracted and characterized as Staphyloxanthin and the producer strain as *S. gallinarum*. Staphyloxanthin has shown biological activities including antimicrobial activity, antioxidant activity and anticancer activity *in vitro*.

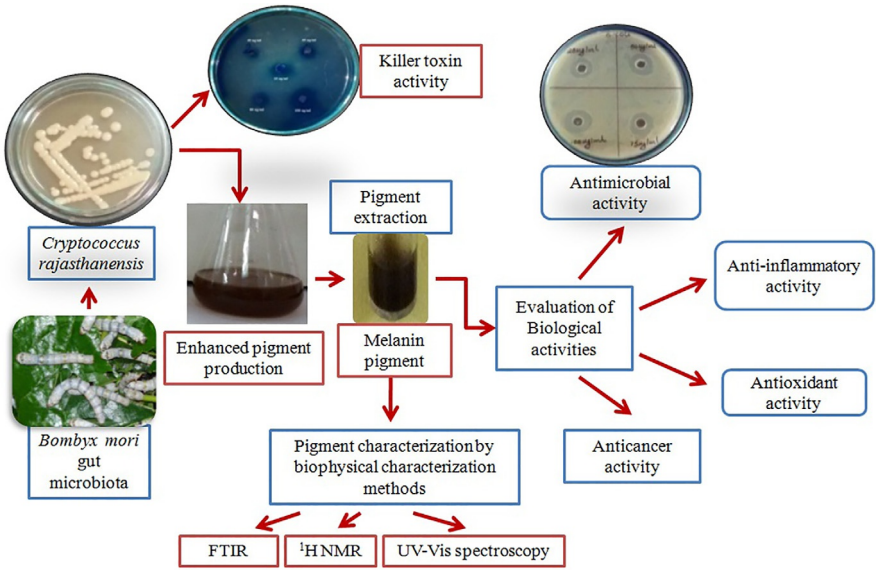
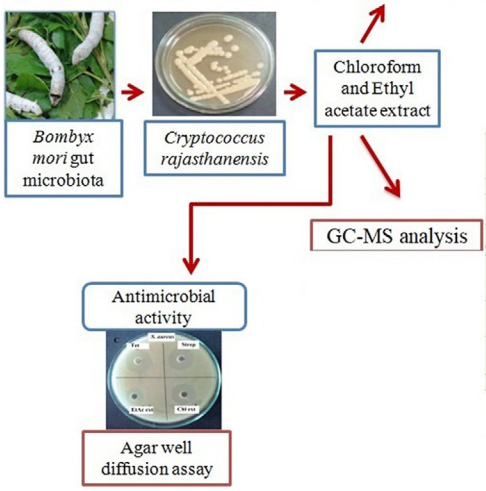


FIG. 3

Therapeutic applications of melanin obtained from *Cryptococcus rajasthanensis* isolated from silkworm *B. mori* gut microflora (Barretto & Vootla, 2020). The yeast producing brownish pigment was isolated and the pigment production was enhanced in minimal L-tyrosine glycine medium. The pigment was extracted and characterized as melanin and the producer strain as *C. rajasthanensis*. This pigment has shown biological activities including antimicrobial activity, antioxidant activity, anti-inflammatory and anticancer activity *in vitro*. The yeast strain has also exhibited killer toxin activity.

by us to produce a brownish-black pigment which on characterization was found to be melanin (Fig. 3). This pigment showed good antimicrobial, antioxidant, anti-inflammatory and anticancer activities studied *in vitro* (Barretto & Vootla, 2020).

The ethyl acetate and chloroform extracts of the yeasts isolate *Cryptococcus rajasthanensis* KY627764 from *B. mori* gut has also shown a wide range of bioactive compounds with biological activities (Barretto & Vootla, 2018b). The GC-MS chromatographic analysis of the chloroform and ethyl acetate extracts revealed the presence of various bioactive compounds like phenol 2,4-bis(1,1-dimethylethyl)—which is known for its antibacterial and antioxidant activity; Pentadecane previously reported to bear antibacterial activity; 1,2-benzenedicarboxylic acid dibutyl ester known to possess antibacterial activity, Nonadecane is antimicrobial and cytotoxic; 2,5-octadecanoic acid methyl ester known for its antiviral, antibacterial and antioxidant properties; tetratetracontane is antibacterial, tetracosane has shown cytotoxicity towards gastric cancer cells by inducing apoptosis; Celidoniol deoxy also known as nonacosane is antibacterial and anti-inflammatory, etc. (Fig. 4).



IR υ_{max} (cm^{-1})	(Vibration mode)	Functional groups
3425.37	H-bonded OH-stretching	Alcohols, phenols
2925.59	C-H stretching	Alkanes
1639.51	N-H bending	1°, 2° amines
1410.39	C–C stretch (in–ring)	aromatics
1075.60; 1032.16	C-O stretching; C-N stretching	Alcohols, carboxylic acids, esters, ethers; Aliphatic amines
558.12	C-Br stretching	Alkyl halides

Compound name	Retention time	Peak Area (%)	Molecular weight	Activity
Pentadecane	26.065	1.05	212 ($C_{15}H_{32}$)	Antibacterial
Phenol-2,4-Bis(1,1-Dimethylethyl)-	26.459	8.16	206 ($C_{14}H_{22}O$)	Antioxidant Antibacterial
Octadecane	29.384	1.99	254.494 ($C_{18}H_{38}$)	Antifungal agent for plant and human pathogens
1,2-Benzenedicarboxylic acid, dibutyl ester	32.749	3.10	278.344 ($C_{15}H_{22}O$)	Antibacterial Antifouling Pesticide
Nonadecane	39.218	15.64	268 ($C_{19}H_{40}$)	Antimicrobial and cytotoxic
Calidoniol, deoxy (Nonacosane)	42.142	15.06	408 ($C_{29}H_{60}$)	Antibacterial Antiinflammatory
Tetratetracontane	43.562	11.16	619.185 ($C_{44}H_{90}$)	Antibacterial

FIG. 4

C. rajasthanensis isolated from silkworm *B. mori* gut microflora as a source of bioactive compounds (Barretto & Vootla, 2018b). FTIR and GC-MS analysis of the ethyl acetate and chloroform extract of *C. rajasthanensis* indicated the production of bioactive molecules by this strain. The extracts also exhibited antimicrobial activity.

Bhalchandra and Pathade (2011) elucidated the probiotic potential of *Lactobacillus* sp. isolated from *B. mori* gut. The report highlighted various health benefits for humans from probiotic strains belonging to genus *Lactobacillus* isolated from the *B. mori* gut. These *Lactobacillus* sp. have shown cholesterol lowering property, antibacterial potential, immunomodulatory activity, etc. The beneficial role of bacteriocins produced by the probiotic *Lactobacillus* sp. has also been reviewed.

Silkworms are prone to a large number of infectious diseases that cause huge loss to the sericulture industry, thus production of disease-resistant varieties of silkworm is gaining interest. Silkworms depend solely on their innate immune response to combat invader pathogens as they lack acquired immune system (Miyashita, Takahashi, Ishii, Sekimizu, & Kaito, 2015). Antimicrobial peptides (AMPs) are one of the chief components of the innate immune system in silkworms apart from the production of melanin and the generation of reactive oxygen species (ROS) (Chen & Lu, 2018). According to World Health Organization (2014), a large number of bacterial pathogens are developing resistance to a wide range of commonly used antibiotics for human therapy. As the rate of multidrug and extreme drug resistance cases are predominant in the recent decade due to overuse of antibiotics, the use of

insect AMPs have become a major area of interest in disease treatment (Buhroo, Bhat, Bali, Kamili, & Ganai, 2018). The gut microbiota of silkworm has been reported to produce antimicrobial peptides (Garcia-Gutierrez, Mayer, Cotter, & Narbad, 2019). Nesa et al. (2020) reviewed the importance of AMPs from *B. mori*. AMPs are low molecular weight proteins exerting an antagonistic effect against fungi, bacteria and viruses. AMPs produced from *B. mori* so far identified, belong to six different families viz. cecropin, defensin, moricin, gloverin, attacin and lebocin, which are produced by the Toll and immune deficiency (IMD) pathways with diverse modes of action. The mechanisms of action depend on microbial pathogens and are still under investigation. A study by Islam, Bezbaruah, and Kalita (2016) has reviewed the application of *B. mori* AMPs in plants and animal disease management. The study also explained the worth of the *B. mori* AMPs in clinical sectors as follows: (i) broad spectrum antimicrobial activities, (ii) low probability of development of resistance and (iii) easy synthesis. These are potential antimicrobial drugs of the future for human disease therapy. Due to their small size AMPs diffuse easily through the plasma membrane of the target cell thereby activating the host immune system without affecting the non-target cells making them potent antimicrobial drugs for human health. They are non-toxic to the non-target cells or human cells and hence are biocompatible in nature.

Several ecological models have been investigated for Bioregenerative Life Support System (BLSS) on ground and shorter space flights to sustain astronauts on long-term missions, e.g., Mars mission wherein BLSS is envisioned with closed nutrient and gas loops in order to solve the problem of limited food and oxygen resources (Häder, Braun, & Hemmersbach, 2018). Silkworm (*B. mori* L.) larvae are being investigated as an animal protein source for astronauts in BLSS during future long-term deep space missions (Tong, Yu, & Liu, 2011). Liang, Fu, and Liu (2015) investigated the role of gut microbiota of *B. mori* larvae to be used as an ideal animal protein source as food for astronauts in BLSS. The study showed the presence of cellulase and amylase producing bacteria belonging to the genus *Enterococcus*, *Erwinia* and *Pantoea* as dominant populations in silkworm reared using BLSS. These intestinal bacteria with digestive enzymes have been shown to improve the digestibility of feed used in BLSS that enhances nutrient absorption. The microbiota have also been shown to prevent disease in BLSS. Thus, the study concludes that the gut microbiota of silkworm can be used as probiotics to improve the gut microecology of silkworm that enhances the yield and quality of animal proteins to be used in BLSS for the health benefit of the crew on long-term space missions.

Serratiopeptidase is a proteolytic enzyme used as an effective drug with anti-inflammatory, anti-edemic and analgesic effects that has a very long history in various specialties like surgery, otorhinolaryngology, gynaecology and dentistry (Bhagat, Agarwal, & Roy, 2013). This enzyme was basically used by *B. mori* pupae in allowing the emerging moth to dissolve its cocoon (Miyata, Maejima, Tomoda, & Isono, 1970). Serratiopeptidase (Serratia E15 protease) was originally obtained from *Serratia marcescens* that was isolated from the silkworm gut in the late 1960s.

4.4 Industrial applications

4.4.1 Production of industrially important enzymes

Several enzymes such as amylases, cellulases, pectinases, laccases, etc., have been widely used in the field of biotechnology in pharmaceutical, food, bioremediation, fuel, detergent, paper and textile industries etc. (Vermelho et al., 2013). Microorganisms provide a rich source of enzymes in biotechnological applications. Further these microbial enzymes present some advantages over the enzymes obtained from plants and animals. Insect gut microbiota produce enzymes that facilitate the digestion of lignocellulosic components which they feed on that is otherwise difficult to digest. Lignocellulosic biomass is the main component of the plant cell wall and contains a complex network of lignin, cellulose and hemicellulose. It also contains proteins and lipids. The enzymes like ligninases, amylases, cellulases and others utilize the lignocellulosic components as substrates.

Prasanna, Kayalvizhi, and Rameshkumar (2014) reported amylase production by *Bacillus megaterium* isolated from *B. mori* gut. The optimal temperature and pH for the enzyme activity were found to be 40 °C and 8.0 respectively. These unique characters suggested the suitability of the enzyme in liquefaction of starch in detergent, textile, food and other industrial applications where high temperature and alkaline conditions are necessary. Yang et al. (2011) revealed the gut bacterial isolates such as *B. cereus* DZ-a and *B. cereus* DZ-h with amylase activities as 20.5 and 24.2 U/mL respectively. The gene encoding for amylase production was cloned in *E. coli* demonstrating successful expression in the host.

Feng, Wang, Zhou, Liu, and Wan (2011) evaluated the lipase producing ability of gut microbiota from fourth and fifth instar *B. mori* larvae. The lipolytic ability examined on a Rhodamine B agar plate depicted 9 lipase-producing bacteria belonging to genera *Bacillus*, *Brevibacterium*, *Corynebacterium*, *Staphylococcus*, *Klebsiella* and *Stenotrophomonas*. Liu et al. (2018) investigated the lipase producing ability of *B. pumilus* SW41 isolated from *B. mori* L. gut microbiota. The strain was shown to encode the lipase gene which was further cloned and expressed in *E. coli* BL21 using the DE3 expression system. The enzyme expressed in *E. coli* was then purified and its activity was evaluated as 277.40 U/mg at the optimum temperature (25 °C) and pH (8.0). Wang et al. (2016) explored the probiotic function of enzyme-producing *B. subtilis* strain (No. 951NA) from fourth instar *B. mori* larval gut. The bacterial isolate produced neutral protease when cultured on nutrient agar casein medium. The enzyme depicted an activity of 24.2 U/mL. The gene encoding the enzyme was cloned and expressed in *E. coli*. The transformant showed efficient expression for neutral protease. Soma Prabha and Prabakaran (2017) reported lipase producing *Bacillus* sp. from the gut of *B. mori* with optimum temperature and pH for lipase production to be 30 °C and 9.0 respectively. The lipase production was observed to be maximum at late exponential phase.

Kalpana et al. (1994) evaluated the bacterial flora inhabiting the *B. mori* gut and found the bacteria is rich in amylase, caseinase, gelatinase, lipase and urease production. The abundance of protease producers was highest followed by lipid and

polysaccharide digesters. Prem Anand et al. (2010) reported the production of enzymes like pectin, xylan, cellulase and starch from the gut microflora of *B. mori* L. The gut bacteria producing these enzymes are identified as the Gram-positive bacteria *Bacillus circulans* and the Gram-negative bacteria *Proteus vulgaris*, *K. pneumoniae*, *E. coli*, *Citrobacter freundii*, *Serratia liquefaciens*, *Enterobacter* sp., *Pseudomonas fluorescens*, *P. aeruginosa*, *Aeromonas* sp. and *Erwinia* sp., out of which isolates *P. vulgaris*, *K. pneumoniae*, *Aeromonas* sp. and *C. freundii* showed cellulase production when cultured on CMC agar media. These four isolates also showed xylanolytic activity. *P. fluorescens* and *Erwinia* sp. showed pectinase production. *K. pneumoniae* indicated amylase production by degrading starch. *Serratia liquefaciens* showed cellulase, xylanase and pectinase production and *B. circulans* showed production of all the four enzymes. Liang et al. (2015) showed prevalence of cellulolytic and amylase microorganisms in the gut of silkworm larvae and identified them as genus *Enterococcus*, *Erwinia* and *Pantoea* with amylase and cellulase production that can be exploited for further biotechnological applications. Mala and Vijila (2017) reported cellulase, amylase and lipase production by bacterial isolate BMGB42 obtained from gut microbiota of *B. mori* larvae fed on mulberry leaves fortified with *Aloe vera* and *Tinospora cordifolia*. The enzyme assay for cellulase, amylase and lipase found activities of 0.428, 1.02 and 11.33 (U/mL/min) respectively.

The enzyme producing capability of Muga silkworm, *Antheraea assamensis* gut microbiome has also been explored. Gandotra et al. (2018) revealed the abundance of bacterial isolates *Bacillus* sp., *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Pseudomonas stutzeri*, *Acinetobacter* sp. and *Alcaligenes* sp., inhabiting the gut of *A. assamensis*. The qualitative screening for digestive enzymes for these isolates showed significantly higher enzymatic activities for cellulase, amylase and lipase. In vitro screening for production of enzymes like amylase, cellulase, pectinase, xylanase and lipase demonstrated ability for production of digestive enzymes by bacterial isolates of *Bacillus* sp., *Proteus* sp. and *E. coli* isolated from the gut of *A. assamensis* (Bhuyan et al., 2014). Bhuyan et al. (2018) revealed that the extracellular cellulase enzyme producing strain *Bacillus pumilus* MGB05 isolated from the gut larvae of *A. assamensis* Helfer showed CMC hydrolysing zone utilizing CMC as the sole carbon source. The cellulase activity was evaluated to be 0.262 U/mL at 72 h of incubation under submerged conditions in a medium supplemented with CMC and Filter paperase (FPase) activity was found to be 0.022 U/mL in media supplemented with wheat bran. *B. pumilus* MGB05 also revealed β-glucosidase activity of 3.71 U/mL. The optimum conditions for β-glucosidase production were found to be pH 6.0 at 50 °C. This isolate also depicted antibacterial activity against *P. aeruginosa* and *E. coli*.

4.4.2 Production of vitamins

One of the beneficial roles of insect gut microflora is the provision of vitamins for the growth and benefits of insects. The use of vitamins produced from microbial forms is gaining importance due to the various benefits of naturally produced vitamins over

the chemically synthesized ones. Sridhara and Bhat (1966) showed the presence of vitamin B_{12} in silkworms that was reported to be produced by the intestinal microorganisms of the host. 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). A study by Halarnkar and Blomquist (1989) reported 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. Dong et al. (2018) revealed the presence of bacteria belonging to genus *Enterococcus*, *Clostridium*, *Pseudomonas*, *Escherichia* and *Lactococcus* in *B. mori* gut reared on artificial diet soybean meal in comparison to the ones reared on fresh mulberry leaves. The dominant microbial flora is heavily correlated with vitamin biosynthesis including thiamine and riboflavin.

4.4.3 Production of ethanol

Enzymatic approaches using microbes that leads to conversion of mulberry lignocellulosic biomass of waste to bioethanol production maybe one of the most promising eco-friendly alternatives to fossil fuels or petroleum-based products (Thirupathaiah et al., 2018). Our previous study has reported *Blastobotrys bombycis* sp. nov. a newly isolated species of yeast belonging to gut microbiota of *B. mori* (race CSR2xCSR4, Bivoltine hybrid) with ethanol producing properties (Fig. 5). The yeast showed the capability to produce 1.5 g/L ethanol by fermenting 5% D-xylose (Barretto et al., 2018). D-xylose is the second most abundant sugar in lignocellulosic biomass and the utilization of this sugar by yeasts in bioethanol production may be useful in producing second generation ethanol (Cadete & Rosa, 2018). Feng et al. (2011)

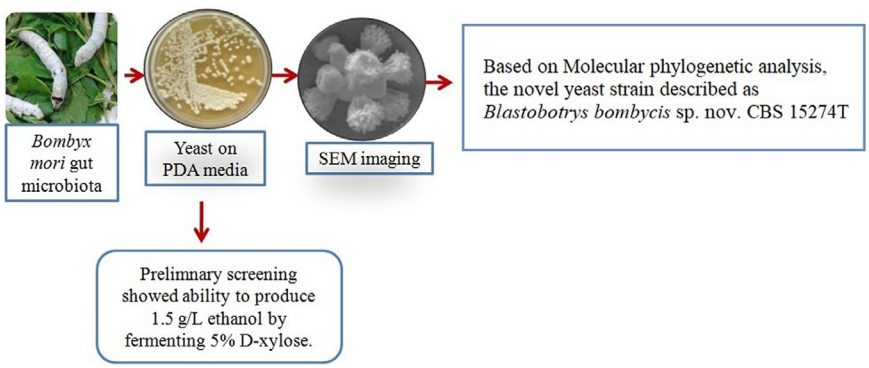


FIG. 5

Blastobotrys bombycis sp. nov. obtained from silkworm *B. mori* gut microflora with potency for ethanol production (Barretto et al., 2018). *Blastobotrys bombycis* sp. nov was identified as a novel species of *Blastobotrys* isolated from *B. mori* gut microflora. This strain has shown the potency for ethanol production by fermenting D-xylose.

demonstrated the ethanol producing property of recombinant *Proteus mirabilis* JV strain. The genes responsible for ethanol fermentation by microbes such as pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase II (*adh* II) were cloned from *Zymomonas mobilis* and transformed into the cellulolytic bacteria *P. mirabilis* JV that was i isolated from *B. mori* gut. The recombinant bacteria were able to produce 1% ethanol using the substrates CMC and 4% pretreated sugarcane bagasse (Sobana Piriya et al., 2012).

4.4.4 Other biotechnological applications

The alarming rate of environmental pollution caused by plastic wastes has gained tremendous attention that has lead to the development of Bio-plastics. Poly lactic acid (PLA) is one of the predominantly used bio-plastics which is both bio-based and bio-degradable and is widely used in many disposable packaging (Jem & Tan, 2020). Lactic acid is the prominent source for the synthesis of PLA (Krishnamurthy & Amritkumar, 2019). Liang, Sun, Chen, et al. (2018) isolated *Enterococcus mundtii* EMB156 from the gut of *B. mori* as an efficient lactic acid producing bacterial strain. The isolate is shown to grow at alkaline pH (10) and converts various carbon sources to lactic acid. The high-yield lactic production at alkaline pH by *E. mundtii* EMB156 offers advantages in downstream fermentative processes. The amylase enzyme activities were reported to be quite stable at high pH thereby facilitating lactic acid production. Yan & Wu (2016) and Liang et al. (2018) reported alkaline tolerant amylase and xylose isomerase enzymes from the *B. mori* gut bacterium *E. mundtii* EMB156.

5 Conclusion

Microbiome has become a buzz word in trying to correlate the symbiotic role of microorganisms everywhere around us ranging from our skin to the gut. Microbes have proven to dictate the way we live and they also affect our immunity. The mutualistic relationships between the insect host and the gut microbiota opens the way to exploit the insect gut microflora as a rich source for exploring essential molecules (Bode, 2011). Although the large amount of information on the biology and physiology of the silkworm is known, very few studies have been carried out on silkworm gut microbiota. Hence, the research on gut microflora of silkworm *B. mori* is still limited (Chen et al., 2018).

The gut microbiota of *B. mori* can be used as a potent source for biotechnological applications as the rearing of *B. mori* is an easy and cost effective task that does not require any ethical clearance Furthermore, the identification of novel microorganisms and novel molecules from the gut microbiota of silkworm will be useful in the field of biotechnology in future especially in order to discover novel molecules with therapeutic potency and also to develop some important enzymes that will be useful in industrial applications.

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Glossary

Domesticated silk moth The silkworm *Bombyx mori* which is stocky creamy-white in colour and is almost entirely under human care. It feeds on mulberry leaves.

Multi-voltine It refers to silkworm having more than two broods or generations per year.

Sericulture It is also called as silk farming that refers to raising silkworm for silk production.