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Comparison of Gut Microbiota between Midgut of Healthy and Tiger Band Disease Infected Oak Tasar Silkworm, *Antheraea proylei* J.

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ABSTRACT

Antheraea proylei J., an economically significant silkworm in the Northeastern region of India, is exclusively domesticated for Tasar silk production. This silkworm is susceptible to various diseases caused by bacteria, viruses, and fungi, including the recently dreadful viral disease dubbed tiger band disease. This viral infection causes damage to silkworm larvae affecting cocoon production, which causes significant losses to the economy of the silk industry. The gut microbiota plays a crucial role in host nutrition and immunity against various pathogens. However, less information is available on the diversity and ecology of the gut microbiota of this tasar silkworm. In the present study, we analyze molecular characterization and histopathological examination of gut-associated bacteria of healthy and diseased silkworms. We observed a loss of turbidity, lumen distortion, and insignificant secretory layer in diseased silkworms compared to healthy silkworms. Also, body fat becomes vacuolated and soft when compared to healthy silkworms. Results of 16S rRNA gene sequencing reveal *Bacillus toyonensis* and *Bacillus thuringiensis* as abundant bacterial genera in healthy larvae, whereas *Bacillus aryabhattai* and *Bacillus megaterium* were found in diseased larvae. To the best of our knowledge, this is the first attempt to study *A. proylei* midgut microbiota from a biodiversity hotspot in North-Eastern India. The current study might provide valuable insights into understanding the disease prognosis of tasar silkworms and potential disease management strategies for these economic silkworms.

Keywords: *Antheraea proylei,* Gut microflora, 16S rRNA, Histopathology, Tasar silkworm.

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INTRODUCTION

The Northeastern region of India is home to a number of wild sericigenous insects. It is the center of wild silk culture, including muga, eri, oak tasar, and mulberry silk [1]. The tasar silk industry has had many socio-cultural and traditional linkages in India since immemorial. It plays a vital role in the rural economy. Hence it depicts its impact on the country's economy and agriculture [2]. The oak tasar silkworm, *Antheraea proylei* J, is an economically significant silkworm of Manipur reared for the production of tasar silk. It is interbred between the male Indian species of *A. proylei* with the female Chinese species of *A. pernyii* [3]. The yield and quality of tasar silk cocoons depend on climatic conditions, silkworm health, and nutrient absorption. Physiology and pathology of the silkworm's digestion, growth and nutrition, and immunity of

this silkworm are associated with the microbiota of the larvae's midgut. To date, no silkworm races are deemed ally resistant to diseases or pests. At the end of the larval stage, silk production is aided by silk glands whose infection affects silkworm growth and production, which causes severe losses to the linked economic activities [4]. As disease-infected silkworms fail to spin cocoons, analysis of cytological damages in the silk gland and gut is essential. Some histopathological studies showed Bombyx mori nucleopolyhedrovirus (BmNPV) associated with damaged internal tissue and silk glands following viral infection [5, 6]. Hence, knowledge of the microfloral changes of the gut in diseased conditions will help in understanding the health and nutrition of the silkworm [7] and give us ideas of management to improve the diseased condition during infection as such.

The intestinal tract microflora plays a vital role in the host's health by maintaining a normal ecological balance and regulating absorption, digestion, and assimilation [8]. Gut microflora is required in pheromone production, pesticide degradation and survivability, vitamin synthesis, and immunity against pathogens [9]. In addition, these bacteria also resist and compete with the invading microbes and their propagation and strengthen the immune system [10]. Microbial pathogens infect all animal species leading to disease and death. But their immune defense system helps in protection [11]. Insects, especially their larval forms, are more susceptible to pathogenic bacterial diseases and their virulence factors than vertebrates which further leads to alteration in the host defense mechanism [12]. Culture studies in laboratory conditions of insect gut bacterial communities do not reflect the entire microbial types and strains [13]. Culture-dependent methods screen only a few predominant bacteria genera and cannot detect bacterial genera with low abundance. The 16S rRNA gene is often used as a marker for identifying the diversity of bacterial species in insect gut microbiota [14]. Notably, the gut microbiota is involved in the health and nutrition of these important silkworms.

However, information on the gut bacterial communities of many silkworms in a diseased state, including *A. proylei,* is limited. The molecular characterization of gut microbiota regarding this economically significant silk moth remains less explored, and only a handful of DNA sequences are available [14, 15]. Additionally, very little is known about the effects of pathogens, their nutrient utilization, and the disease emergence of the silkworm in its gut microbiota. Therefore, research on the gut microbiota is of great importance in exploring the microbial diversity associated with silkworm production. To date, there are meager reports on profiling and histopathological studies of gut microflora in silkworms. Hence, in this present study, we aimed to compare the intestinal microflora of *A. proylei* of normal and Tiger band disease infected fifth instar larva reared under the same conditions using the 16*S* rRNA-based sequencing method. Furthermore, we also assessed the histopathological changes in the midgut and silk glands after infection to assess the tissue damage. Therefore, our study might provide insights for improving and managing the disease as a step toward the conservation of wild seri-biodiversity for ecological balance and sustainable economic viability.

MATERIALS AND METHODS

Sample collection

Healthy and infected fifth instar larvae of *A. proylei* were collected from the Regional Sericulture Research Station, Mantripukhri, Manipur, during the summer of 2019. The details of the location are furnished **(Table 1)**. Healthy and diseased tasar silkworms showing the signs of Tiger band disease were collected from the abovementioned area **(Figure 1**). The infected larvae suffering from Tiger band disease were recognized from their black band pattern similar to tiger stripes on the larva [14]. Properly sterilized equipment such as forceps, scissors, and hand gloves were used while collecting the insect sample. The collected samples were stored in an ice-cold storage box. Samples were brought from the field, and surface sterilization was done with 70% ethanol by submersion and rinsing three times using sterile distilled water before dissection. The larvae and midgut gut tissue was dissected in a sterile environment using dissection scissors. The collected intestinal contents will be immediately stored at 50% glycerol at -80^{0}C for future use [16]. Three-fifth of individual larvae were used for gut extraction and further processed for isolation of gut

bacteria. Following gut bacterial isolation, the larvae were maintained in sterile conditions at 26 ±1º C and 70 % RH at Animal Resources Division Laboratory, IBSD, Takyelpat, Manipur, India.

Table 1. Details of geographical conditions and locations of *A. proylei* from Regional Sericulture Research Station, Manipur.

Collection description of Oak Tasar Silkworm								
Locality	**District**	**Instars**	**GPS coordinates**	**Altitude**	**Season**	**Temp.(°C)**	**RH(%)**	**Host Plant**
Mantripukhri	Imphal West	4th	N24º50'19.20" E093º56'34.78"	773	April 2019	18-30	70-90	*Quercus serrata*
Mantripukhri	Imphal West	4th	N24º50'19.00'' E093º56'34.70''	772	June 2019	25-32	75-90	*Quercus serrata*
Mantripukhri	Imphal West	4th	N24º50'19.20'' E093º56'34.77''	772	September 2019	25-34	75-90	*Quercus serrata*
Mantripukhri	Imphal West	4th	N24º50'19.19'' E093º56'34.78''	773	June 2019	25-32	75-90	*Quercus serrata*
Mantripukhri	Imphal West	4th	N24º50'19.21'' E093º56'34.68''	773	August 2019	25-34	75-90	*Quercus serrata*
Mantripukhri	Imphal West	4th	N24º50'19.00'' E093º56'34.79''	773	June 2019	25-32	75-90	*Quercus serrata*

Table showing the details of the collection site of oak tasar silkworm from the state of Manipur during April – September 2019. 4th stage of worms was collected and brought to the laboratory for further investigation.

a) b)

Figure 1. Oak tasar silkworm larvae, *Antheraea proylei* J. a) healthy and, b) disease larva suffering from Tiger band disease.

Isolation and culture of the intestinal bacteria

Guts of healthy and diseased *A. proylei* larvae were homogenized in 0.86% NaCl solution [16]. The gut homogenates were serially diluted, inoculated in separate plate in triplicates, and incubated for 24-48 hrs at 37°C. The bacterial colonies were identified based on morphology, size, and color and purified following successive inoculation and streaking on corresponding agar plates as described [17]. The purified strains were cultured and maintained in glycerol stocks.

Histopathological investigation

For histopathological investigation, healthy and disease-infected fifth instar larvae were collected from Regional Sericulture Research Station, Mantripukhri, Manipur, India. Different organs like silk glands and guts were removed from normal, and nucleopolyhedrovirus (NPV) infected silkworms. The removed tissues were preserved in a 10% formaldehyde solution. The tissues were again fixed in Bouin's fluid. The water molecules were removed with the help of alcohol and embedded in paraffin wax for sectioning. 5-7µm thick tissues were stained with hematoxylin and eosin. Structural examination and histopathological changes were identified by visualization through Leica DM 3000 LED and photographed with Leica DFC450 C.

DNA extraction and 16S rRNA sequencing

DNA was extracted from the gut intestinal contents using the Gsure Bacterial Genomic DNA isolation kit following manufacturer protocols.

The quality of the extracted DNA was checked on agarose gel and quantified using a Nanodrop spectrophotometer (Thermofisher Scientific) and then normalized to 200 ng/µL. 16S rRNA genes were amplified using universal primer, namely FD1(5'-AGAGTTTGATCCTGGCTCAG-3') and RD1(5'-AAGGAGGTGATCCAGCCGCA-3'), followed by PCR in a volume of 25µl containing 200 ng DNA, 5X Phusion HF buffer, 10mM each dNTP, 2.5 U of Phusion DNA Polymerase, 0.5µM of forward and reverse primers as listed before [18].

The PCR conditions were as follows: 94^{0}C for 5 min; 35 cycles at 94 ^{0}C for 1 min, 50 ^{0}C for 30 sec, 72 ^{0}C for 2 min, and a final extension at 72 ^{0}C for 10 min. The amplified PCR products were analyzed by 1% agarose electrophoresis and visualized under Gel Documentation System (Bio-Rad). The PCR product was further purified using a Gene JET purification column and sequenced in a BigDye® terminator kit following the manufacturer's conditions (Applied Biosystems Inc. ABI, Foster City, CA). Products were analyzed on ABI 3500xL Genetic Analyser (Eurofins Pvt Ltd, Bangalore, India). The purified PCR products were sequenced and aligned with sequences in the GenBank database using the Blast search algorithm [19]. These sequences were submitted in the GenBank and assigned accession numbers.

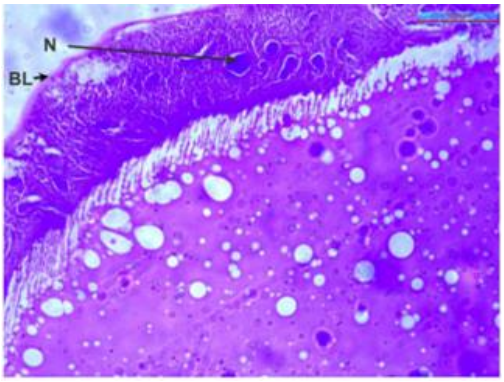
Sequence analysis

Online similarity searches were performed using the BLAST algorithm of GenBank. Sequence alignment was carried out with the CLUSTALW program by MEGA7 software [20]. Phylogenetic analyses of proteolytic bacteria based on the 16S rDNA gene were further used to establish evolutionary relationships. The 16S rDNA sequences obtained were used for reference nucleotide sequences search in the NCBI GenBank database using the BlastN algorithm tool [19]. The phylogenetic tree and evolutionary relationships of bacterial isolates were constructed by the Neighbor-Joining method [21] with the Kimura 2-parameter model [22]. Bootstrap analyses with 1000 replications were performed for each clade to provide statistical significance [23].

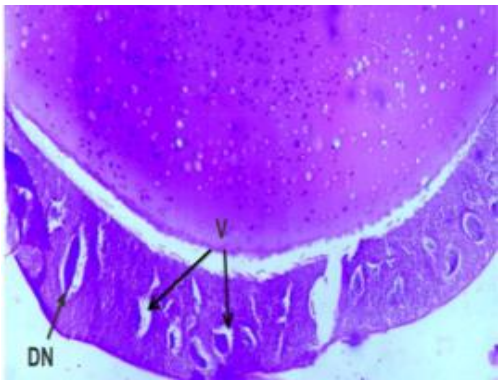
RESULTS AND DISCUSSION

In the fifth instar, healthy larvae are aggressive feeders; they feed most of the day and sleep little. They tend to find fresh leaves before finishing the previous ones. They are light green. The infected larvae show signs like the slowness of feeding and movement, fading of integument colors, inter-segmental region swelling, and oozing milky fluid from the mouth, anus, and body pores, depicting damage to internal organs. The integuments are thin and fragile skin and appear to be covered with a yellowish amorphous material with the formation of pores at the advanced stage of infection. The heavily infected larvae do not feed after molting; their body shrinks and later body becomes black. Many died during spinning. Few survivals spun thin and unreliable cocoons.

The three layers of silk glands, such as tunica propria, glandular layer, and tunica intima, were visible in normal silkworms. The secretory cells have rich secretory granules with a broader layer compared to tunica propria. The nuclei are more or less circular in shape and rest on basal lamina, separating the layer from hemocoel. Tunica propria is narrow, and the lumen consists of silk mass. The glandular zone has a large number of hemocytes containing branched nuclei **(Figure 2a)**. In the disease-infected worm, the silk gland shows a loss of tunica propria integrity, and the secretory layer is not easily distinguishable from tunica propria. The cells of the silk gland are ruptured and damaged in a diseased state. The nuclei are hypertrophied and spindle-shaped and filled with inclusion bodies. Vacuolisation is also prominent. Lumen is seen distorted with stranded oil droplets in the silk mass. The silk mass loses compactness and becomes less dense **(Figure 2b)**.



a)



b)

Figure 2. a) Silk gland of normal silkworm showing intact tunica propria (TP) and rich nuclei (N) (Bar=100µm). b) The diseased silk gland showing loss of tunica propria (TP) integrity. At the advanced stage of infection, larvae show small spherical nuclei (N) with the appearance of vacuoles (V) (Bar=100µm)

The midgut represents the longest segment of the alimentary canal and comprises the outer muscle layer and inner epithelial layer separated by a basement membrane. Histology of normal silkworms' midgut shows a layer of enteric epithelial cells resting upon a basement membrane. An outer layer of longitudinal muscle cells and an inner layer of circular muscle cells follow the basement membrane. The epithelium is folded into villi. The midgut epithelium cells are differentiated into three types, columnar cells, goblet or secretory cells, and regenerative cells. Columnar cells are tall, contain granular cytoplasm, and are closely associated with each other. The nuclei are singular, large, spherical, or elliptical and situated in the middle or apical half of the cells. Goblet and regenerative cells are interspersed apically and basally, respectively. The Goblet cells are flask-shaped and contain centrally located nuclei with the bulk of the cytoplasm in the basal region. The apical portion secretes mucus into the lumen. The regenerative cells are small and irregular in shape. They replace the destroyed epithelial cells during molting. The basal cells lie in between the columnar cells and the basement membrane **(Figure 3a).**

In the diseased silkworm, the epithelial layer of the midgut lacked continuity. The slides of the diseased larvae show deformed columnar cells with abnormal nuclei, mass vacuolization with scattered secretions, indistinct basement membrane, and villi borders. Both secretory goblet and absorptive columnar cells of *A. proylei* midgut are hypertrophied, and large vacuoles are formed. Absorptive cells are fully loaded, indicating loss of their absorption. The inter-cellular spaces were widened, and individual cells detached from the basement membrane. The nuclear changes range from hypertrophy to pyknotic, and even anuclear cells were noticed. Goblet, regenerative and basal cells were less appreciable in histology compared to normal. Necrotic cell debris is dispersed in the lumen and hemocoel. All these cells contained viral inclusion bodies in variable quantities. The midgut of the host larva is mainly destroyed due to infection. Thereby, typical cellular arrangement is disorganized. Also, bacterial aggregates were seen with dark masses inside the lumen, indicative of its infections intruding the epithelial layer **(Figure 3b)**. Enzymatic granules secreted by midgut columnar cells are involved in the digestion and absorption of products, while the secretary function is carried out by the goblet cells. The regenerative cells divide and replenish the old and worn-out apoptotic cells with a ready to function cells. A thorough morphometric and histologically informative picture can be drawn out of the silkworm gut morphology state of healthy and diseased as described in an earlier study on passalids (Bess beetles) [24]. We investigated the histopathology of healthy and diseased silkworm larvae to understand the in-depth pathogenic effects a disease imposes on the cells, tissues, and organs. As infected silkworms fail to spin cocoons, analysis of cytological damages in the silk gland is essential. The digestive system in silkworm larvae needs much attention as oral entries of pathogenic microbes are quite common. Apart from the dual function of digestive and absorptive, the midgut region also provides a barrier to invading parasites. Our results show that silk glands of infected larvae are ruptured and deformed, along with the formation of lump cells compared with healthy larvae. The cytotoxic effects result in the loss of silkworms' ability to maintain homeostasis.

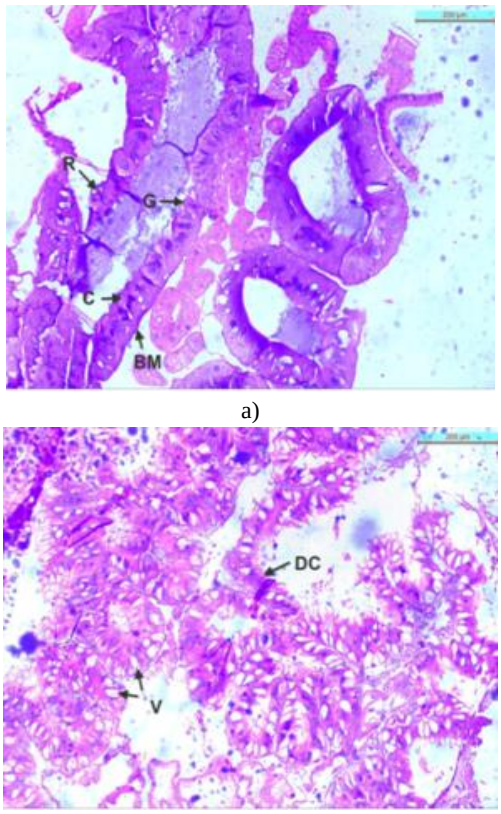


Figure 3. a) Cross section of the midgut of healthy fifth instar larvae of *A. proylei* showing columnar epithelial cells (CEC)and compact darkly stained nucleus (N) (Bar= 200µm). b) Cross section of the midgut of TBD fifth instar larvae of *A. proylei* showing hypertrophied columnar epithelial cells (CEC) and vacuoles (V) in the cytoplasm (Bar= 200µm).

Histopathological changes in the midgut by bacterial and viral infections have been documented in other silkworms [25-28]. It appears that the pathology concerning the Tiger band disease is mainly localized in the silk glands and midgut. And the most common pathological features observed include nuclei hypertrophy, the inclusion of bodies in cytoplasm, and vacuolation, ultimately leading to insect death. These changes in the midgut epithelium observed are in alignment with the findings of the study reported [29].

The infection process is believed to have started at the midgut and continued by penetration through the basement membrane of the midgut wall into the hemocoel disseminating into hemocytes, hypodermis, muscle, fat body, trachea, silk gland, etc. Highly alkaline gut juices with pH ranging from 9.5 to 11.5 and possibly enzymatic degradation are believed to play essential roles in the dissolution and disintegration of ingested viral pathogens in Lepidoptera insects [30].

Endocytosis of viral bodies present in silkworm diets initiated the process of infection, followed by intracellular replication and multiplication in large numbers and shedding after cells burst [31]. But the silk gland has an extracellular fibrous matrix, the basal lamina, and the virus needs to penetrate this lamina to pass on the infection to the gland. Recent studies showed that basal lamina thickness and matrix organization limit the passage of macromolecules by acting as a selective barrier [6]. The tracheal system plays an essential role in spreading the infection from the haemocoel to the silk gland [32]. The trachea penetrates basal lamina, and the virus gains access to glandular epithelium through this route to establish a new replication cycle, as evident by nuclear enlargement and viral inclusion bodies.

We analyzed the phylogenetic similarity and affiliation of 16S rRNA sequences in both identified and unidentified strains using the optimal criteria set for distance in the neighbour-joining analysis of MEGA 6 and similarity using the BLAST search program. Analysis of sequences revealed that the majority of the intestinal bacteria floras showed 99% similarity. The gut bacterial isolates' 16SrRNA sequences produced in the study were deposited into GenBank vide accession nos. MT416410.1 to MT416415.1and the details are presented **(Table 2)**.

Table 2. Details of 16S rRNA gene sequence of isolates of gut bacteria of ***A. proylei*** in our study.

Sl No.	Sample ID of Tasar Silkworm	Organism with the closest match from GenBank	Site of Collection	GenBank Accession No:	Similarity
1	N_H02	*Bacillus toyonensis*	Mantripukhri	MT416410.1	99.55%
2	D_C03	*Bacillus aryabhattai*	Mantripukhri	MT416411.1	99.89%
3	D_D03	*Bacillus aryabhattai*	Mantripukhri	MT416412.1	99.55%
4	D_E03	*Bacillus aryabhattai*	Mantripukhri	MT416413.1	99.66%

5	D_F03	*Bacillus aryabhattai*	Mantripukhri	MT416414.1	98.99%
6	D_G03	*Bacillus aryabhattai*	Mantripukhri	MT416415.1	99.77%

BLAST (Basic Logic Alignment Search Tool) was used to compare 16S ribosomal RNA gene sequences. Sample ID represents different gut bacteria isolated from oak tasar silkworm from the geographical locations with the highest similarity (98-100%) to their closest matches from the GenBank database. GenBank sequences submitted with assigned accession numbers MT416410.1 to MT416415.1.

16S rRNA gene sequence analysis of tasar silkworm A*. proylei* revealed that Firmicutes form the predominant group, with Bacillus comprising ten different species depicted as the dominant genus. Bacillaceae was abundantly represented in the gut of both healthy and diseased A. *proylei,* as shown **(Table 3)**. Bacillus was found to be the predominant genera in our study from laboratory culturing of gut bacterial isolates of *A. proylei.*

Table 3. Taxonomic profile of gut bacterial isolates *of A.proylei.* Identified by 16S rRNA gene sequences

Samples	Group	Family	Genus	Species
Healthy Silkworm	Firmicutes	Bacillaceae	Bacillus	*Bacillus toyonensis* *Bacillus thuringiensis* *Bacillus pacificus* *Bacillus mobilis* *Bacillus mycoides*
Disease Silkworm	Firmicutes	Bacillaceae	Bacillus	*Bacillus megaterium* *Bacillus aryabhattai* *Bacillus zanthoxyli* *Bacillus flexus* *Bacillus simplex*

Phylogenetic analysis of gut microflora of diseased and normal tasar silkworms showed 6 % differences between the groups. The evolutionary phylogenetic distances were determined using the Maximum Composite Likelihood method [33]. A phylogenetic tree was constructed **(Figure 4)** using the 16S rRNA sequences by the neighbour-joining method with 1000 bootstraps better to understand the evolutionary relationship of the intestinal bacteria.

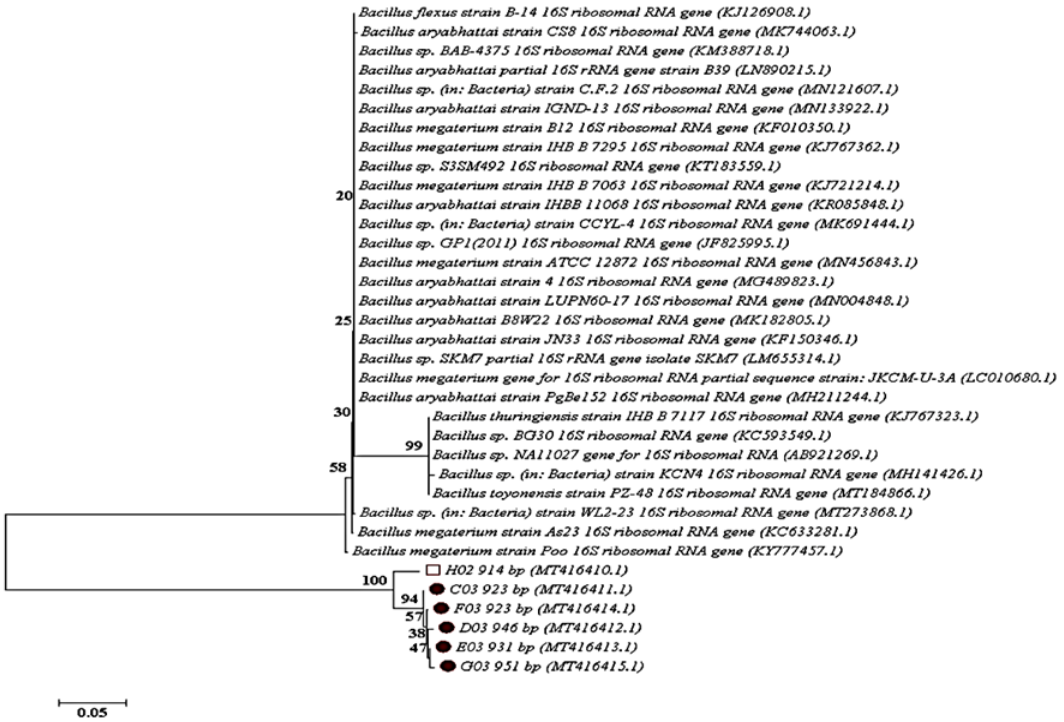


Figure 4. Phylogenetic tree of gut bacteria isolated from the gut of *Antheraea proylei*

In the diseased silkworms, *Bacillus aryabhattai* seems to be the most abundant genera found in all the diseased worms. In healthy silkworms, microflora, like *B. toyonensis* and *B. thuringiensis* are the predominant species and share high sequence similarity. This showed more than 99% identity with the sequences deposited in the GenBank database.

Generally, *Bacillus* species are widely used as biopesticides and biofertilizers in agricultural practice [34], and as potential probiotics for both animals and humans [35-37]. Hence our results also might help in the potential application of *Bacillus* species against a variety of pathogenic gut microflora. Many Lepidopteran insects, including silkworms, are described as culturally and economically valuable insects from the remote eras till now. A wide variety of bacterial classes, from obligate endosymbionts to facultative ones, reside as gut microflora of most insects [38]. Further investigation and detailed analysis of bacterial diversity and correlation of existence within the gut and their interactions with the insect host from varied geographical regions will prompt us to better recognize and comprehend their essential roles in the overall health and disease process. Some meager information on insect gut flora correlation of tasar silkworm, *A. proylei* found in Northeastern regions of India is available, which is incomprehensible and non-conclusive. Laboratory culture-derived 16s rRNA gene sequence-based approaches in this study were used to assess the gut bacterial groups associated with tasar silkworm populations in Manipur, North-eastern India.

Our analysis shows that the tasar silkworm harbored unique bacterial flora in its gut, with Firmicutes as the major group and Bacillus being the predominant genera constituting almost 100 percent of total gut bacterial isolates from *A. proylei.* Earlier studies have also documented that the gut of the gypsy moth, *L. dispar* (Lepidoptera), lodge Pseudomonas and Bacillus species as the dominant bacterial communities [4]. The results of the present study align with that of *Bombyx mandarina* and *Bombyx mori* gut bacterial diversity study, where phylum Firmicutes is found to be the predominant bacteria through 16S rRNA gene sequencing [39]. Bacillus is also the most predominantly found bacterial genus, as confounded in an earlier studies, comprising nearly 18% of the total gut flora populace in 21 different insect species [40]. A limited diversity was revealed in the 16S rRNA gene analysis of *P. xylostella* larvae gut bacteria [41]. The phylogenetic analysis of gut bacterial isolates from tasar silkworm showed limited bacterial phylogenetic diversity.

Our analysis showed Firmicutes as the dominant family forming a significant clade with Bacillus as the dominant genus with ten different species **(Figure 4)**. Molecular phylogenetic analysis further reveals that Firmicutes and Bacillus are the predominant clade and genera in healthy and diseased silkworms. Detailed characterization and further investigation from different geographical locations in the Northeastern region of India would advance our understanding of the diversity and composition of gut bacteria of oak tasar silkworms and their disease management.

Diseased larvae are a potential focus of subsequent virus outbreaks in silkworms [42]. Due to high virulence, pathogenicity is retained in the deceased larvae and continues to spread in the silkworm rearing rooms hence prevention and control are very important in preventing viral transmission. They should be removed carefully with the skin intact and undisturbed as much as possible

Variation in the infection characteristics of nucleopolyhedrovirus virus infections is due to factors such as the isolate's virulence, amount of ingested virus particles, larval maturity, co-infection with other microbial pathogens, and suitable rearing conditions and parameters like temperature, population density, and food availability [32, 42]. Food reserve becomes insufficient for morphogenesis of silkworm organs as most of the infected fat body tissues were exhausted by replication of the virus. The adults emerging from these cocoons exhibited deformities and were less active. Devitalizing effects of viral diseases of Lepidoptera include developmental retardation, reduced pupal and adult weights, lower silk synthesis, and ultimately diminished silk production.

CONCLUSION

The present study is one of the foremost attempts toward a genetic and histopathological characterization of the midgut microbiota of *A.*

proylei found in Manipur, the North-eastern state of India. A more comprehensive and thorough analysis of midgut bacteria is required for a better understanding of the involvement of microbiota in the areas of immunity, nourishment, and breeding of this important silkworm. This present study not only offers insight into the process of gradual degradation of *A. proylei* larvae midgut but also elucidates the extent of damage that occurs therein after its infection, which also affects the silk glands. The probiotic candidature of the role of the potential gut microflora will also be a challenge ahead. Further, our findings might advance our understanding in the field of disease resistance traits and silkworm *A. proylei* management.

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