**Gut Microbiome Profiling of Eri Silkworm (Samia ricini) through 16S rRNA and Its Impact on Silk Yield in Dimapur, Nagaland**

**Abstract**

Eri silkworms, or Samia ricini, have gut microbiota that is essential to host physiology, especially for digestion, immunity, and silk production. The diversity of bacteria in S. ricini's midgut was examined in this work using 16S rRNA gene sequencing. Using BLASTn comparison with the NCBI GenBank database, four sample sequences (PPAI01, PPAI02, PETAI01, and PETAI02) were examined. *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Lysinibacillus* *fusiformis* are among the bacterial species in the Bacillaceae family with which the sequences showed a high degree of similarity (96.53%–98.29%). The production of digestive enzymes, antibacterial substances, and phytohormones by these bacteria suggests a possible symbiotic interaction with the host. According to the results, these microbial species improve gut health, aid in the manufacture of silk, and improve nutrient absorption. Future probiotic tactics aiming at enhancing larval health and sustainable sericulture can build on the findings of this work.

Key words: Samia ricini, Gut microbiota, Bacillus species, Probiotics, 16S rRNA sequencing, gut microbiota.

**Introduction**

The gut microbiota is a dynamic and varied population of bacteria found in the digestive tracts of insects. According to Douglas (2015), these microbial communities are crucial for host physiology, including immunological regulation, digestion, nutrition absorption, and pathogen defense. The gut microbiota plays an important role in host development and response to nutritional and environmental changes in lepidopterans, including the Eri silkworm (Samia ricini). The multivoltine, polyphagous silkworm species Samia ricini is mostly raised in Northeast India for its coarse, resilient silk, also referred to as endi or eri. S. ricini is raised on castor (Ricinus communis) and does well in semi-domesticated environments, in contrast to the domesticated Bombyx mori. As a result, its gut microbiome might be an indication of a more varied microbial community that is influenced by it and its host plant. Relatively little is known about the microbial ecology of the Eri silkworm stomach, despite its economic importance in rural sericulture, particularly when compared to the well-researched B. mori. Insect microbiology research has demonstrated that gut-associated bacteria can affect host growth, silk yield, illness resistance, and even stress tolerance (Engel & Moran, 2013). The core microbiota, many of which include members of the genera Bacillus, Enterococcus, Lactobacillus, and Pseudomonas, facilitate these interactions, which are frequently species-specific. It has been documented that bacteria such as Bacillus subtilis and Bacillus licheniformis in silkworms create compounds that inhibit the growth of pathogenic organisms and enzymes that facilitate the digestion of plant material (Ranjan et al., 2009).

Accurate identification and phylogenetic classification of gut microbiota have been made possible by developments in molecular biology, including the amplification and sequencing of the 16S ribosomal RNA (rRNA) gene. Because the 16S rRNA gene has both variable and conserved sections, it is a perfect target for determining evolutionary links and describing bacterial diversity. Sanger sequencing and other sequence-based techniques are still strong and trustworthy methods for taxonomic identification of culturable and unculturable microbial species. In this work, we amplified and analysed partial 16S rRNA gene sequences in order to examine the bacterial composition of the gut microbiota in Samia ricini. We found dominant bacterial taxa linked to the silkworm intestine by using phylogenetic analysis and sequence alignment with reference strains in the NCBI database. This work provides a basis for investigating probiotic and microbial improvement tactics in sustainable sericulture and adds to the expanding field of insect microbiome research.

**Methodology**

**Sample Collection and Bacterial DNA Extraction**

Larvae of the Eri silkworm (Samia ricini) in their fifth instar were chosen for gut microbial analysis because they were healthy. Larvae were surface sterilised with 70% ethanol and dissected to separate the midgut area under aseptic conditions. After being carefully collected, the stomach contents were suspended in sterile PBS (phosphate-buffered saline). After being homogenised, the samples were centrifuged to separate the bacterial cells.

The Cetyltrimethylammonium bromide (CTAB) technique, with modifications appropriate for bacterial isolates, was used to extract genomic DNA from the bacterial pellet. After being vortexed and resuspended in 500 µL of CTAB extraction solution, the pellet was incubated for 30 minutes at 60°C. After incubation, the lysate was combined with an equal volume of chloroform:isoamyl alcohol (24:1), centrifuged for five minutes at 14,000 × g, and then mixed again. To precipitate DNA, the aqueous phase was gathered and combined with 0.7 litre of cool isopropanol. After being cleaned with 70% ethanol, the resultant pellet was dried and reconstituted in 30 µL of TE buffer. A spectrophotometer was used to evaluate the purity and concentration of the DNA after RNase A was introduced to eliminate RNA contamination.

**PCR Amplification of 16S rRNA Gene**

The 16S rRNA gene was amplified using universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) as described by Weisburg et al. (1991). PCR reactions were carried out in a total volume of 25 µL containing: 2 µL of 10X PCR buffer, 0.5 µL of MgCl₂ (25 mM), 1 µL of dNTP mix (10 mM), 1 µL each of forward and reverse primers (10 pM), 0.5 µL of Taq DNA polymerase (5 U/µL), 1 µL of template DNA (25–40 ng), and the remaining volume adjusted with nuclease-free water.

A two-minute initial denaturation at 95°C was followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1 minute. These were the thermal cycling conditions for PCR amplification. The reactions were then stored or subjected to additional analysis at 4°C after a final extension at 72°C for 10 minutes. Ethidium bromide staining, UV visualisation, and 1.5% agarose gel electrophoresis were used to validate the PCR results.

**Purification and Sequencing**

As directed by the manufacturer, amplified PCR products exhibiting distinct bands were removed from the gel and purified using a gel extraction kit based on silica columns. Elution buffer (20 µL) was used to elute the DNA. An ABI 3500 Genetic Analyser was used to perform both forward and reverse sequencing on the purified amplicons after they had been subjected to Sanger sequencing using an Applied Biosystems BigDye Terminator v3.1 Cycle Sequencing Kit. The following thermal cycling conditions were used for the sequencing reactions: a 2-minute initial denaturation at 95°C, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and termination at 60°C for 4 minutes. The reactions were stored at 4°C until the cycles were finished and additional processing was done. EDTA and ethanol precipitation were used for post-sequencing purification in order to eliminate unincorporated dyes. This was followed by resuspension in Hi-DiTM formamide, denaturation at 95°C, and instant loading onto the sequencer.

**Sequence Analysis and Phylogenetic Identification**

High-quality consensus sequences were obtained by manually curating and assembling raw sequence data using BioEdit software. To find similar bacterial sequences, these sequences were BLASTn analysed against the NCBI GenBank database. The maximum % identity, alignment coverage, and E-value were used to choose the best hits. The closest phylogenetic matches were used to determine taxonomic categorisation, and sequences with ≥96% identity were regarded as belonging to the same or nearly similar species. The detected strains' taxonomic significance was interpreted in light of their established functions in digesting, host-microbe interactions, and health-promoting activities in insect guts. In order to create a neighbor-joining phylogenetic tree and validate the evolutionary relationships of the isolates, sequences were further aligned for phylogenetic comparison using ClustalW and MEGA software (v11).

**Results and Discussion**

The gut microbiota of Samia ricini (Eri silkworm) was thoroughly sequenced to demonstrate the taxonomic diversity and functional potential of the bacterial communities residing in the insect gut. Following 16S rRNA gene sequencing, BLASTn searches against the NCBI GenBank database were used to identify four representative sequences: PPAI01, PPAI02, PETAI01, and PETAI02. These genomes showed strong phylogenetic ties to known beneficial microorganisms, with 96.53% to 98.29 percent similarity to well-documented bacterial strains. The strains of Bacillus licheniformis, Bacillus subtilis, Bacillus fusiformis, and Bacillus amyloliquefaciens that have been found are well known for their metabolic adaptability and functions in immunological regulation, nutrition cycling, digestion, and antimicrobial defence. The possibility that these microorganisms are stable and functionally integrated components of the silkworm's gut ecology rather than sporadic ones is supported by their high sequence identity. The existence of these bacteria lends credence to the theory that S. ricini possesses a functionally varied and possibly symbiotic gut microbiota, which could have a major impact on the physiology of the larvae, their ability to absorb nutrients, their resistance to sickness, and eventually their ability to produce silk (Table 1). These results also support more general findings in entomological microbiome studies, indicating the importance of gut microbiota for insect adaption and health. Understanding these bacteria' precise functions and exploiting them for probiotic development and sustainable sericulture would require further research that focusses on their metabolic pathways and gene expression profiles.

**Table 1. Comparative 16S rRNA Sequence Analysis for Identification of Symbiotic Bacteria in Samia ricini**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sequence ID** | **Nucleotide Sequence (partial)** | **BLAST Hit Description** | **Percentage Identity** |
| PPAI01\_  query | CCAGTATCAGTATCAGACGTGATAGTCTGAGCGGACCGACTGGGAGCTTGCTCCCTTAGGTCAGCGGCGGACGGGTGAGTAACACGTGGGTAAGCCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGATTGAACCGCATGGTTCAATCATAAAAGGTGGCTTTTAGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGTGGCGAGCGTTGTCCGGAATTATCTGGCGCGTAAAGCGCGCGCAGCGGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACCTGTAGCAGTGGAAATGCGAGAGATGTGAGGAACACCAGTATCGTA | *Bacillus licheniformis* strain TB212 16S rRNA gene, partial sequence | 97.94% |
| PPAI02\_  query | TGTTTACTTTCGTATCATCGTGACAGTCGAGCGAACAGATCTGGGAGCTTGCTCCTTTGACGTTAGCGGCGCGACGGGTGAGTAACACGTGGGCAACCTACCCTATAGTTTGGGATAACTCCGGGAAACCGGGGCTAATACCGAATAATCTCTTTTGCTTCATGGTGAAAGACTGAAAGACGGTTTCGGCTGTCGCTATAGGATGGGCCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGAAAGCCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTAAGGGAAGAACAAGTACAGTAGTAACTGGCTGTACCTTGACGGTACCTTATTAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGGGGGGGGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCCTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGGACTTGAGTGCAAAGAGGAAAGTGGAATTCCAAGTGTAGAGCAT | *Lysinibacillus fusiformis* strain OSR6 16S rRNA gene, partial sequence | 98.29% |
| PETAI01\_  query | GGTTGTTGGTGCTACCGCTATCATCGTGATAGTCGAGCGGAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAAGAGGAGAGTGGAAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAAGGAGCGAAGCGTGGCGAAGCGAACAGGATCACATACCCGTGTTACTCCAACGCCGTCAACGATGATCTGCTACAGTCGTTAAGGAGGTTTCCGTCTCCTTAGTTGGTGACAGCTAGACGCACTGAAGTTCATCACGACTGGGGCGACGGACTGTAGCGGGAGTTGTTATACCTCTATAGGAAATTAGAGCAAAGCGCGCCTGGCAGACCCGTGAACCCCTAGTGAGTTTCTAGTACAAGGCATAGCACATAACGCATTACCGGCGTCTTGAACTGTCACTGTAGACTATCTAGGAATATCGGATCAGCATCGGCGAGCTACGACAAGTGCGCCTAGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAAGGGGGTTTCCGCCCCCTTAGTTGACTGCAGCTAACAGG | *Bacillus subtilis* strain MDR12 16S rRNA gene, partial sequence | 96.91% |
| PETAI02\_  query | TTACGTCTCTCTCTGCGCTACCTTGTTACGACTTACGTGACCTCGGGGACTCTCTGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCTGGGCTACACACGTGCTACAATGGGCAGAACAAAGGGCAGCGAAACCGCGAGGTTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACATCAGTATATGTCGGTGAGTGTAACTAAGTCGTAACAAGGTAGCCTATCTCTAGGATTGTCACAGGATGTCAAGACCCTGCTAAGGTTTCTTCGCGTGTACTTGGAATAAACCCCCCCCTCCCCTCCCA | *Bacillus amyloliquefaciens* strain BCd4 16S rRNA gene, partial sequence | 96.53% |

Table 2. BLASTn Identification of Gut Bacteria in Samia ricini Using Partial 16S rRNA Sequences

|  |  |  |
| --- | --- | --- |
| **Sequence ID** | **BLAST Hit Description** | **Percentage Identity** |
| PPAI01 | *Bacillus licheniformis* strain TB212 | 97.94% |
| PPAI02 | *Lysinibacillus fusiformis* strain OSR6 | 98.29% |
| PETAI01 | *Bacillus subtilis* strain MDR12 | 96.91% |
| PETAI02 | *Bacillus amyloliquefaciens* strain BCd4 | 96.53% |

All identified strains belonged to the phylum *Firmicutes*, family *Bacillaceae*, indicating a taxonomically coherent and functionally specialized gut bacterial community.

i. PPAI01\_query: Bacillus licheniformis strain TB212

The sequence PPAI01 showed 97.94% identity with the gram-positive, endospore-forming Bacillus licheniformis strain TB212, which is widely used in biotechnology and industry. This species is known for generating extracellular enzymes that catalyse the breakdown of proteins and polysaccharides, respectively, such as amylases and proteases (Schallmey et al., 2004; Hasan et al., 2021). These enzymatic processes are probably going to help Samia ricini's intestines extract nutrients from a fibrous, plant-based diet more effectively. In addition, B. licheniformis generates antibiotic substances such bacitracin, which are essential for suppressing harmful bacteria and preserving microbial homeostasis (Priest, 1977; Harwood, 2019). Its existence in S. ricini's gut microbiota points to a dual function in gut immunity and nutrition assimilation via metabolite-mediated pathogen suppression and competitive exclusion.

ii. PPAI02\_query: Lysinibacillus fusiformis strain OSR6

The bacteria Lysinibacillus fusiformis, which was formerly categorised under the genus Bacillus, shared 98.29% identity with sequence PPAI02. According to Ahmed et al. (2007) and Kämpfer et al. (2010), it is well-known for the breakdown of organic materials and its function in nitrogen transformation processes such ammonification. Additionally, the bacteria produces indole-3-acetic acid (IAA), solubilises phosphate, and releases siderophores, all of which are traits that promote plant growth (Shahid et al., 2020). The breakdown of complex plant-derived substrates and nitrogen provisioning—two essential processes for larval development and silk protein synthesis—may be aided by L. fusiformis in the stomach of S. ricini. Furthermore, its role in the synthesis of biocontrol agents emphasises how it probably contributes to the gut ecosystem's resistance to pathogens.

iii. PETAI01\_query: Bacillus subtilis strain MDR12

The sequence PETAI01 showed 96.91% similarity to the well-known model organism in microbial research, Bacillus subtilis strain MDR12. Proteases, cellulases, and other extracellular enzymes, as well as antimicrobial peptides like subtilin and the ability to build biofilms, are among the many noteworthy products of B. subtilis (Earl et al., 2008; Kowalska et al., 2022). It has a well-established probiotic potential in both insects and vertebrates, supporting intestinal health, enhancing immunological responses, and lowering pathogen colonisation (Hong et al., 2005; Wang et al., 2023). By improving immunological regulation and nutritional digestibility in the stomach of S. ricini, B. subtilis may have an indirect impact on silk output and larval viability. It may be a stable probiotic option for sericulture applications based on its capacity to produce hardy spores (Table1&2).

iv. PETAI02\_query: Bacillus amyloliquefaciens strain BCd4

The bacterial species Bacillus amyloliquefaciens strain BCd4, which is highly valued for producing secondary metabolites such as lipopeptides like iturin, fengycin, and surfactin—which are known to have strong antifungal and antibacterial properties—was 96.53% similar to the sequence PETAI02 (Chen et al., 2007; Yu et al., 2022). Because of its capacity to fix nitrogen, solubilise phosphate, and produce phytohormones, this bacterium is frequently used in sustainable agriculture as a biocontrol agent and plant growth stimulator (Idris et al., 2007). Through both enzymatic action and metabolite-mediated pathogen antagonism, B. amyloliquefaciens may help improve gut health, digestion, and immunological defence in S. ricini. By increasing nutrition absorption, its colonisation may result in improved metabolic activity and maybe higher-quality silk.

All four of the detected bacterial strains—Bacillus licheniformis, Bacillus subtilis, Bacillus fusiformis, and Bacillus amyloliquefaciens—belong to the family Bacillaceae, which is frequently linked to gut symbiosis in insects and is part of the phylum Firmicutes (Yun et al., 2014). In order to persist in the insect gut ecosystem, these bacterial species are distinguished by their metabolic versatility, ability to produce bioactive chemicals, and tolerance to a variety of environmental circumstances. The high sequence identity (96–98%) found for each strain demonstrates their possible functional significance in the Samia ricini gut and validates the accuracy of their identification. Together, these bacteria are probably responsible for a number of vital physiological functions in the host, such as effective digestion through the release of extracellular enzymes, preservation of gut homeostasis through the generation of antimicrobial metabolites, recycling of nutrients through processes like phosphate solubilisation and nitrogen transformation, and bolstering of host immunity through probiotic effects. These symbiotic functions support the idea that microbial communities have a major impact on host growth, development, and overall productivity and are in line with results from recent research on the gut microbiome of lepidopterans (Engel & Moran, 2013; Chen et al., 2021).

**Conclusion**

This study demonstrates that Samia ricini contains gut bacteria that are both metabolically active and crucial to symbiosis, especially those belonging to the genera Bacillus and Lysinibacillus. These microorganisms support vital physiological processes like immunological response, digestion, and possibly even increased silk synthesis. Their status as essential components of the gut microbiota is further reinforced by the significant sequence similarity of their identification. By boosting silk yield, decreasing disease incidence, and increasing larval health, the possible use of these bacterial strains as tailored probiotics in sericulture has the potential to completely transform sustainable silk farming. To clarify the metabolic processes and functional networks controlled by these symbionts, metagenomic and transcriptome investigations should be part of future research.

Author Declaration

The authors hereby declare that no generative AI technologies, including but not limited to Large Language Models (e.g., ChatGPT, GitHub Copilot) or text-to-image generators, were used in the writing, editing, or preparation of this manuscript.

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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 the writing or editing of this manuscript.

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