Gut bacterial community structure and functional analysis in the silkworm *Samia ricini* reared on the secondary food plant Tapioca (*Manihot esculenta* Crantz)

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Abstract
In the present study, the bacterial diversity in the gut of Eri silkworm (Samia ricini) fed with tapioca leaves was assessed using a culture-independent metagenomics approach. Additionally, the study aimed to predict the functional roles of gut bacterial communities by analysing gene involvement in various KEGG pathways. A total of 564 operational taxonomic units (OTUs) were identified, with Proteobacteria (69.15%), Firmicutes (28.37%) and Actinobacteria (1.77%) being the dominant phyla. Gammaproteobacteria was the most abundant class while Enterobacterales emerged as the dominant order, with Enterobacteriaceae being the most prevalent family. Co-occurrence analysis revealed nine bacterial genera: Lactobacillus, Enterococcus, Lactococcus, Enterobacter, Bacillus, Stenotrophomonas, Staphylococcus, Serratia and Klebsiella, suggesting similar habitat or nutritional niche preferences.

This study represents the report on gut bacterial populations in S. ricini larvae fed on tapioca leaves, a key secondary food widely adopted by rearers. PICRUSt2 analysis revealed diverse gene functions across KEGG pathways, particularly in membrane transport, carbohydrate metabolism, amino acid, lipid and nucleotide metabolism, cellular processing and signalling, gene expression and xenobiotic degradation, highlighting their roles in host physiology. These findings provide valuable insights into Eri silkworm gut microbiota, with dominant bacterial taxa having potential applications in probiotic development and biotechnological metabolite production.

Keywords: Metagenomics, 16S rRNA, gut bacteria, Eri silkworm, Tapioca.

Introduction
Insects represent the most varied group of living organisms on our planet and like all other animals, they also possess a complex ecosystem of microbes inside their gut, where they survive and multiply. These microbes perform significant functions in supporting the host's nutritional needs and contributing to the overall maintenance of health. The gut is a nutritionally dense ecosystem purported to harbour microorganisms approximately ten times as many as the total number of the animal cells and a hundred fold more microbial gene as there are animal genes[36]. Earlier works on gut bacteria of insects outlined the involvement of these bacteria in the processes of digesting and assimilating nutrients and imparting immunity to the insect host[1,12,13,17,19,48]. They are also known for their significant role in immunomodulation and providing host insect resistance against toxins and pesticides[39].

S. ricini is a domesticated silkworm reared extensively in the States in the north-eastern region of India. Eri silk is recognized for its distinct texture and is often used to make high-quality fabrics and textiles. Eri silkworm is unique in the sense that they feed on the foliage of a variety of food plants including Castor (*Ricinus communis*), Kesseru (*Heteropanax fragrans*), Borkesseru (*Ailanthus excelsa*), Barpat (*Ailanthus grandis*), Tapioca (*Manihot esculenta*), Gulancha (*Plumeria rubra*), Gamari (*Gmelina arborea*), Payam (*Evodia flaxinifolia*), Papaya (*Carica papaya*), Jatropha (*Jatropha curcas*), etc.[8,10,28,40,45] Secondary food plants play crucial role in the scarcity of the primary food plants and tapioca plant is widely adopted as a secondary food plant for *S. ricini* silkworm rearing due to its promising productivity, commercial benefits and economic viability, making it comparable to primary food plants.

The leaves of these food plants contain abundant nourishing elements that are crucial for developmental processes of the Eri silkworms. In general, gut microbiota of insects is diverse and can vary depending on the insect species, diet, developmental stage and environmental factors[23,29,30,41,51]. Some insects possess specific gut bacteria that produce essential vitamins and amino acids that are missing from their diet[3,38].

Like all insects, eri silkworms also have a complex ecosystem of microbes living inside their digestive tract. Recent researches have shown that the gut microbiota of Eri silkworm complements host digestion and assimilation by synthesizing important digestive enzymes and also provides resistance to pathogenic infections, which in turn are related to the host's physiological processes[32,33,46]. Studies have identified several bacterial species in the gut of Eri

silkworm, including *Bacillus*, *Enterococcus* and *Lactobacillus*. These bacteria are thought to help the Eri silkworm in breaking down complex plant material and obtaining nutrients that are difficult to digest.

The compositions of microbiota in midguts are reported to differ substantially among larvae feeding on varying diets[5]. However, the interrelation between the gut bacteria of *S.ricini* silkworm and their food plants is not yet fully understood. Therefore, investigating the importance of gut bacteria in Eri silkworms in relation to their host plants could hold significant implications for the silk industry. A more profound understanding of the dynamics between *S. ricini* silkworms and their associated gut bacteria could potentially pave the way to the development of new techniques for raising healthy and productive Eri silkworm breed, ultimately leading to higher-quality and high yield of Eri silk.

Material and Methods

Collection and Rearing of *S. ricini*: Healthy *S. ricini* layings, devoid of any diseases, contaminations, were collected from Directorate of Sericulture., Kokrajhar, Assam, India. The DFLs were allowed to hatch at room temperature. The larvae emerged out after 15 days of hatching, which were then carefully nurtured on tender leaves of tapioca (*M. esculenta*) food plant until 2nd instar stage. Then the mature instars i.e. from 3rd instar stage was provided with tapioca leaves as their diet until they reached the last pupal stage and the same process was repeated for three generations to maintain the homogeneity of the silkworm. After rearing three successful generations on tapioca leaves, mature 5th instar larvae were picked randomly and subjected to a 24 hours period of starvation before the extraction of the guts.

Extraction and harvesting of Gut: For the extraction of the guts, the process was initiated by rinsing the larvae in sterile water followed by surface sterilization using 75% ethanol (Molecular grade). After a final rinse in sterile water, the larval digestive tracts were carefully excised starting from mouth to anus under aseptic conditions. These extracted guts were then subsequently deposited into sterile Eppendorf tubes (1.5 ml) and promptly stored at -20ºC inside deep freezer for future processing.

Isolation of DNA and PCR amplification: Gut bacterial DNA was isolated using SDS DNA isolation method following the steps described by Han et al[24] and the extracted DNA was quantified in Qubit Fluorimeter (V.3.0). Bacterial DNA was then amplified using a universal primer under Master cycler nexus (Eppendorf India). The 16S rRNA V3-V4 region was specifically targeted for amplification, employing the V3 forward primer CCTACGGGNBG CASCAG and the V4 reverse primer GACTACNVGGGTA TCTAATCC. After amplification, the resulting product underwent examination on 2% agarose gel followed by gel purification to eliminate undesired amplifications.

Library Preparation and Gene Sequencing: Five nanograms (5ng) of the purified amplicons were employed for library construction with the NEBNext Ultra DNA library preparation kit. The quantification and assessment of library quality were conducted using the Agilent 2200 TapeStation (AgriGenome Labs Pvt. Ltd.).

Finally, the constructed library underwent sequencing on the Illumina HiSeq 2500 platform using 250x2 pair end cycle (AgriGenome Labs Pvt. Ltd.)

Data quality checking and Bioinformatics Processing: The initial (raw) reads acquired through Illumina sequencing were evaluated by using FastQC program (version 0.11.8) to assess their quality, utilizing default parameters. Prior to engaging in the bioinformatics analysis, a comprehensive examination encompassed quality and composition of the bases, content of the GC bases, uncertain bases and the presence of adapter dimers. The primer sequences were trimmed using PERL script. Trimmed good quality (Phred score, Q>20) sequences were subjected to pairwise merging using FLASH program (version 1.2.11) to generate consensus sequences for the V3-V4 amplicon allowing an overlapping range between 10 to 240 base pairs with zero mismatches. To maintain data integrity, chimeric sequences were meticulously removed using the UCHIME program (version 11), integrated into the VSEARCH tool.

Operational Taxonomic Units (OTU) clustering of consensus sequences was achieved based on their similarities using the Uclust program (97% similarity cut-off) within the QIIME software (Version: 1.9.1)[7]. From each OTU cluster, the representative sequences were carefully chosen and aligned against database in SILVA using PyNAST program. Additionally, each representative sequence was mapped to the SILVA OTUs database using RDP classifier for taxonomy classification.

The analysis included generating relative abundance plots at different taxa levels from phylum to species based on the identified OTUs. Within the sample dataset, both rare and abundant bacteria were identified, as previously described by Galand et al[18] and Aravindraja et al[2]. Other taxa beyond the top ten were collectively categorized as "Others", while sequences that lacking alignment with the taxonomic database were classified as "Unknown".

Co-occurrence analysis: SCNIC (Version: 0.6.2) was used for co-occurrence analysis of bacterial genera using genus level biom formatted file applying sparcc correlation method (distance metric). At last, a network plot was generated using a custom Python script developed in-house.

Alpha diversity: The assessment of microbial diversity within the sample involved the calculation of alpha diversity indices, namely the Shannon, Chao1 and observed species metrics, which were calculated using the pipelines in QIIME.

Phylogenetic Tree Construction: The FASTA sequences of identified species were subjected to alignment by ClustalW within MEGA11[44]. Neighbor-joining method was adopted for constructing phylogenetic tree as[37] using a bootstrap analysis of 1000 replicates for clustering taxa[16].

Functional annotation of gut bacteria: The assessment of functional potential within gut bacterial communities was carried out by assessing the KEGG pathway utilizing the data generated from metagenomic sequencing of gut samples, using the bioinformatics tool PICRUSt2[14].

Results

Bacterial diversity: Illumina sequencing of total gut bacterial DNA has generated 79716 total paired-end reads, from which 56754 pre-processed consensus sequences were produced after trimming of primer and chimeric sequences (Raw metagenomic sequencing data with accession ID SRR24874047 is available at NCBI SRA database). Out of 56754 pre-processed consensus sequences, a total of 2158 OTUs were identified. OTUs having reads more than 2 were only selected which resulted in a total of 564 OTUs. These OTUs were further processed and were taxonomically annotated into 5 phyla: *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Firmicutes* and *Margulisbacteria* [Fig. 1a]. The result showed that the gut bacterial population of larval gut of Eri silkworm fed on the leaves of tapioca was predominantly occupied by the phylum *Proteobacteria*, accounting for 69.15% of the total phyla followed by the phylum *Firmicutes* (28.37%) and phylum *Actinobacteria* (1.77%).

All other phyla, including *Margulisbacteria*, *Verrucomicrobia* had an average abundance of less than 1% and 0.17% of OTUs remained unidentified /unknown. The identified classes with the highest abundance were *Gammaproteobacteria* with 67.73% abundance followed by *Bacilli* (28.37%). Other classes identified were *Actinobacteria* (1.78%), *Alphaproteobacteria* (1.42%) and *Verrucomicrobiae* (0.35%) [Fig. 1b]. Order *Enterobacteriales* constitutes the most dominant in the gut sample (64.18%) in the order level analysis followed by order *Lactobacillales* (26.95%).

In addition, *Bacillales*, *Clostridiales*, *Xanthomonadales*, *Betaproteobacteriales*, *Frankiales* and *Pseudomonadales* were the less dominant orders observed in the gut sample (Fig. 1c). *Enterobacteriaceae (*64.18%), *Enterococcaceae* (13.47%) and *Streptococcaceae* (10.81%) were found to be the most dominant families [Fig. 1d].

On the other hand, less dominant families include *Sporichthyaceae*, *Brevibacteriaceae*, *Pseudomonadaceae*, *Xanthomonadaceae*, which were represented by less than 1% abundance, whereas *Burkholderiaceae* showed an abundance of 1.5% in the gut. The most abundant genera identified were *Enterobacter* (16.49%), *Enterococcus* (12.76%) and *Klebsiella* (11.17%) [Fig. 1e]. However, 40.07% of the OTUs at the genus level were unassigned/unknown.

Subsequently, in present investigation, a small fraction (10.82%) of total OTUs was identified at species level including *uncultured bacterium*, *Klebsiella variicola*, *Lactococcus lactis*, *Enterobacter asburiae*, *Staphylococcus sciuri*, *Delftia tsuruhatensis*, *Lactobacillus dextrinicus*, *Achromobacter xylosoxidans* subsp. *xylosoxidans*, *Lactobacillus senioris*, *Klebsiella oxytoca*, *Agrobacterium radiobacter*, *Corynebacterium variabile*, *Kocuria rhizophila*, *Acinetobacter baylyi*, *Micrococcus luteus*, *uncultured actinobacterium*, *bacterium LBS1* and *Serratia marcescens* [Fig. 1f], while 89.18% of the bacteria were reported as unknown at the species level.

Among these, *uncultured bacterium* (2.66%) showed highest percent of abundance followed by *Klebsiella variicola* (2.48%) and *bacterium LBS1* (1.06%). *Klebsiella oxytoca* and *Lactococcus lactis* both were observed to have an abundance of 0.53% while all the remaining species showed less than 0.2% abundance each. The phylogenetic clustering of the identified species has been shown in fig. 4.

Co-occurrence analysis: From the result of co-occurrence analysis of gut sample, nine bacterial genera were seen mostly connected to one another as *Lactobacillus*, *Enterococcus*, *Lactococcus*, *Enterobacter*, *Bacillus*, *Stenotrophomonas*, *Staphylococcus*, *Serratia* and *Klebsiella.* [Fig. 2].

Alpha diversity: Shannon metric quantifies the observed abundances of OTUs, encompassing both diversity and evenness of species whereas chao1 metric serves as an estimator for species richness. The observed species metric quantifies the unique OTUs recorded within the sample. In present study, the rarefaction curves for alpha diversity revealed the presence of high species diversity and high species richness of gut bacteria in Eri silkworm reared on tapioca leaves [Fig. 3].

Functional annotation of gut bacteria: Functional analysis of gut bacterial community reared on *M. esculenta* food plant identified multiple roles of gut bacteria involved in various KEGG pathways with diverse levels of gene involvement [Fig. 5]. The most prominent pathways recorded were related to membrane transport and metabolism including carbohydrate, amino acid, lipid, nucleotide, cofactor, vitamin and energy metabolism, representing a high number of genes involved. Genetic information processing pathways like replication, repair, transcription and translation demonstrated substantial gene counts. KEGG pathway also exhibited different degree of bacterial gene involvement in various organismal systems, such as the digestive, nervous and endocrine systems.

Small gene participation was exhibited by pathways associated with diseases such as cancers and cardiovascular

diseases, immune system diseases, metabolic diseases etc. Other pathways related to immune system, signal transduction and environmental adaptation also exhibited gene participation. These findings highlight the complex interplay of gut bacterial genes in various biological processes and provide insights on the possible contributions of these gut bacterial communities on host *S. ricini.*

Discussion

Studies on gut bacteria of insects show lot of attention with their increasing important role in various fields. Insects form mutually beneficial relationship with microorganisms either through inheritance or dietary acquisitions, these symbiotic partners may play a role in breaking down food at the cellular level and providing nutritional support to the host organism[35].

This study investigated the diversity of the gut bacterial population in *S. ricini* fed on the leaves of *M. esculenta* food plant and revealed the occurrence of diverse bacterial populations, with 2158 Operational Taxonomic Units (OTUs) identified from the 56754 reads. The dominant phylum observed within the gut sample was *Proteobacteria* with *Firmicutes* and *Actinobacteria* following closely as the subsequent dominant groups.

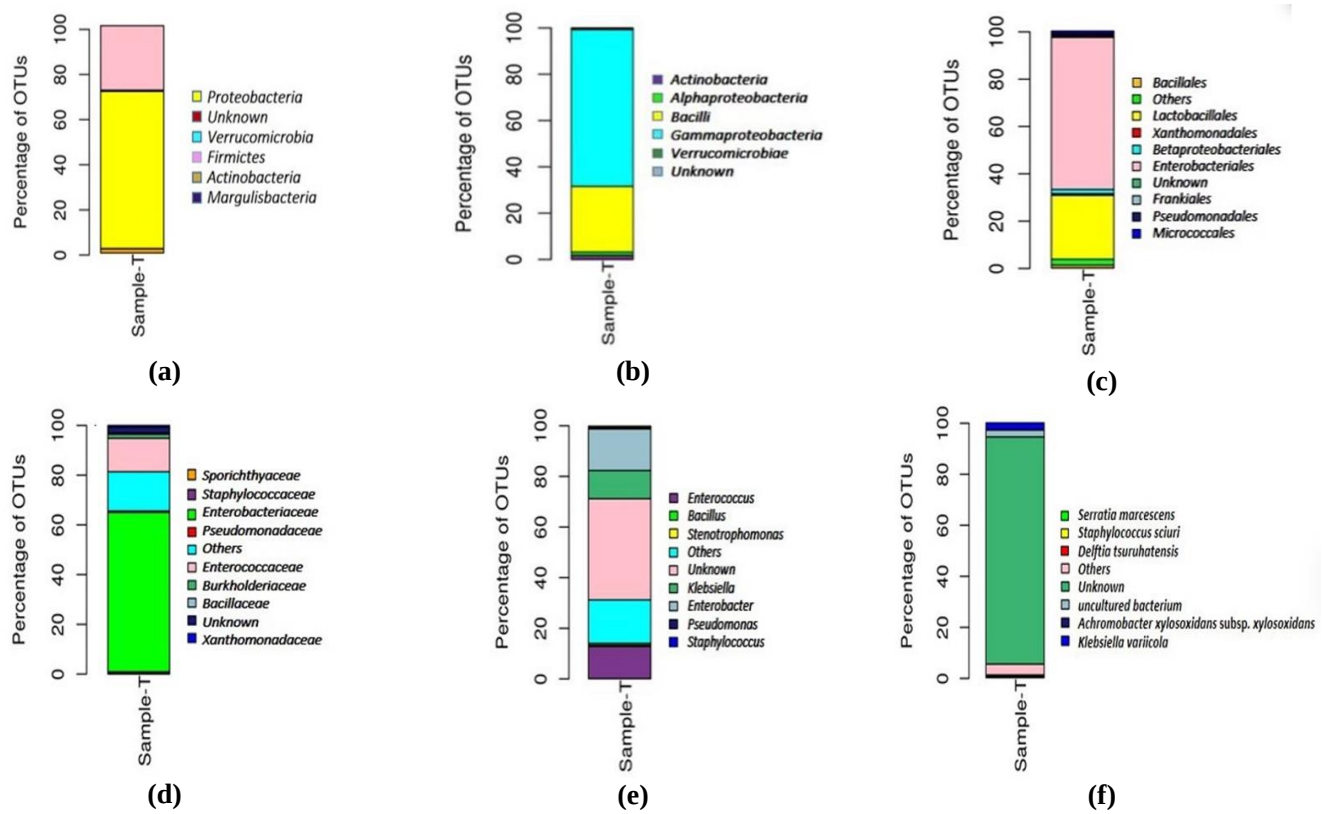

Figure 1: Relative abundance of gut bacteria across various taxonomic level (OTUs) (a) Phylum, (b) Class, (c) Order, (d) Family, (e) Genus, (f) Species

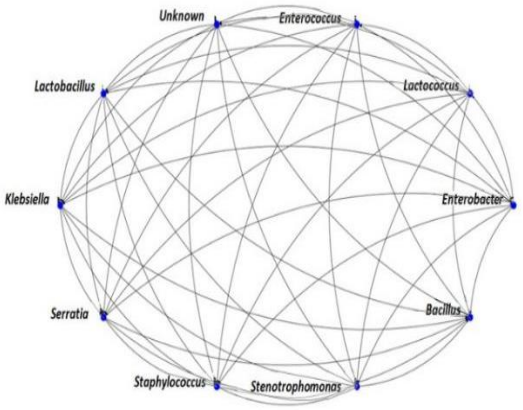

Figure 2: Gut bacteria network plot illustrating their co-occurrence at genus level

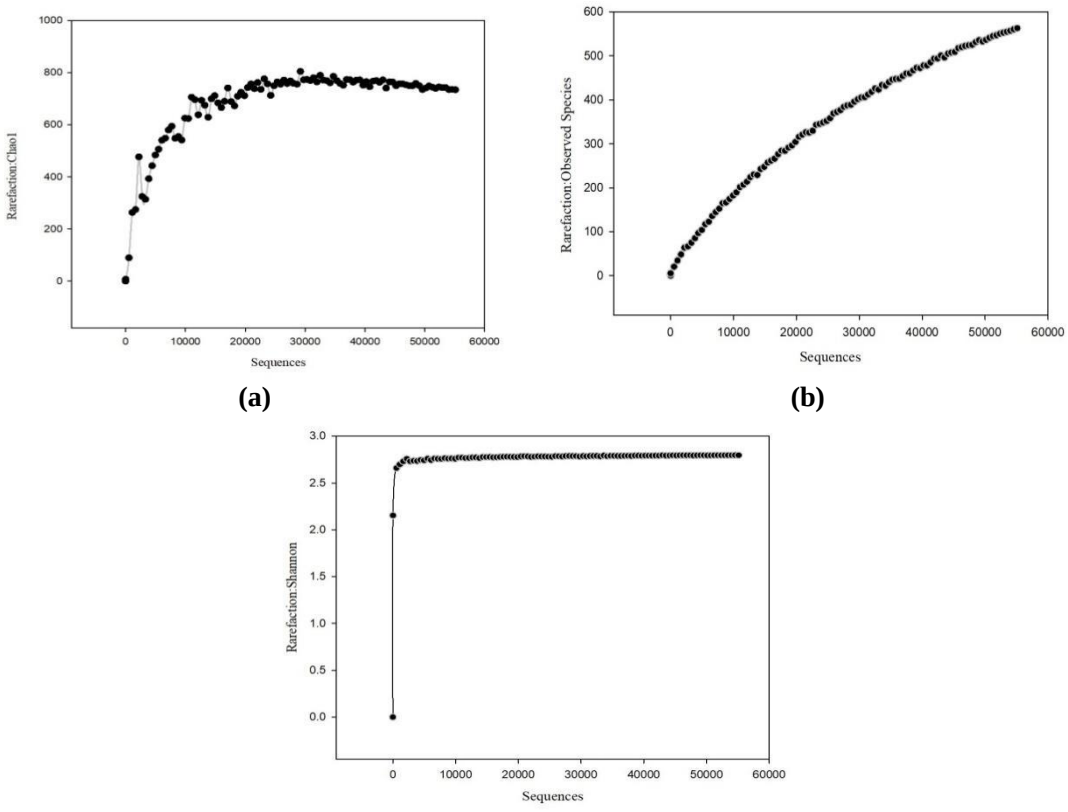

Figure 3: Alpha diversity indexes (rarefaction curves) (a) Chao1, (b) Observed species, (c) Shannon curve

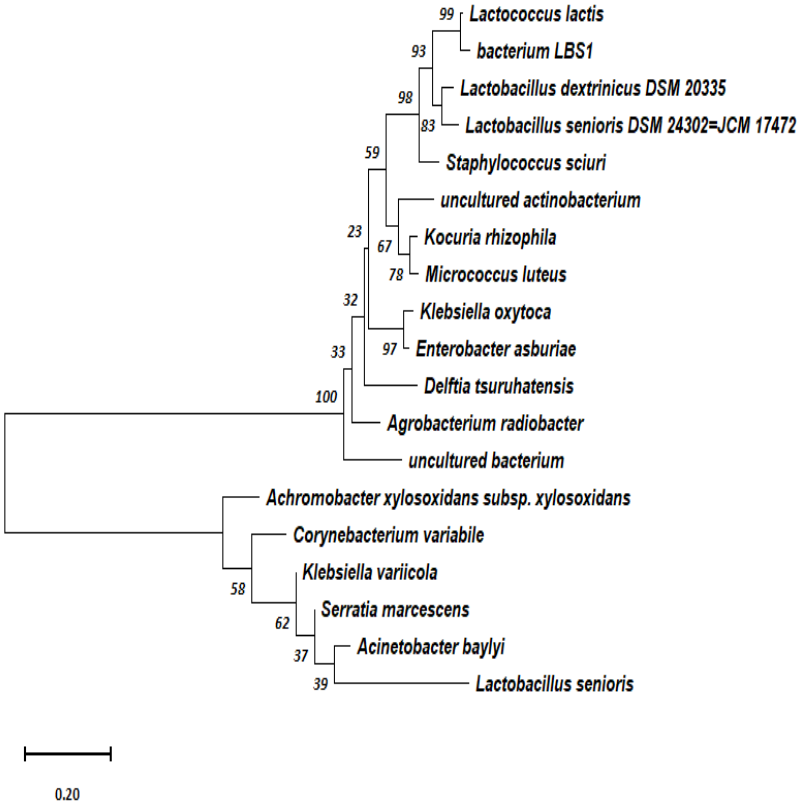

Figure 4: A phylogenetic tree constructed based on neighbour-joining method in MEGA11. Taxonomic grouping was assessed through a bootstrap test involving 1000 replicates and the resulting percentage of replicate trees was indicated alongside the branches. The evolutionary distances were calculated using the Maximum Composite Likelihood method and are presented as the number of base substitutions per site

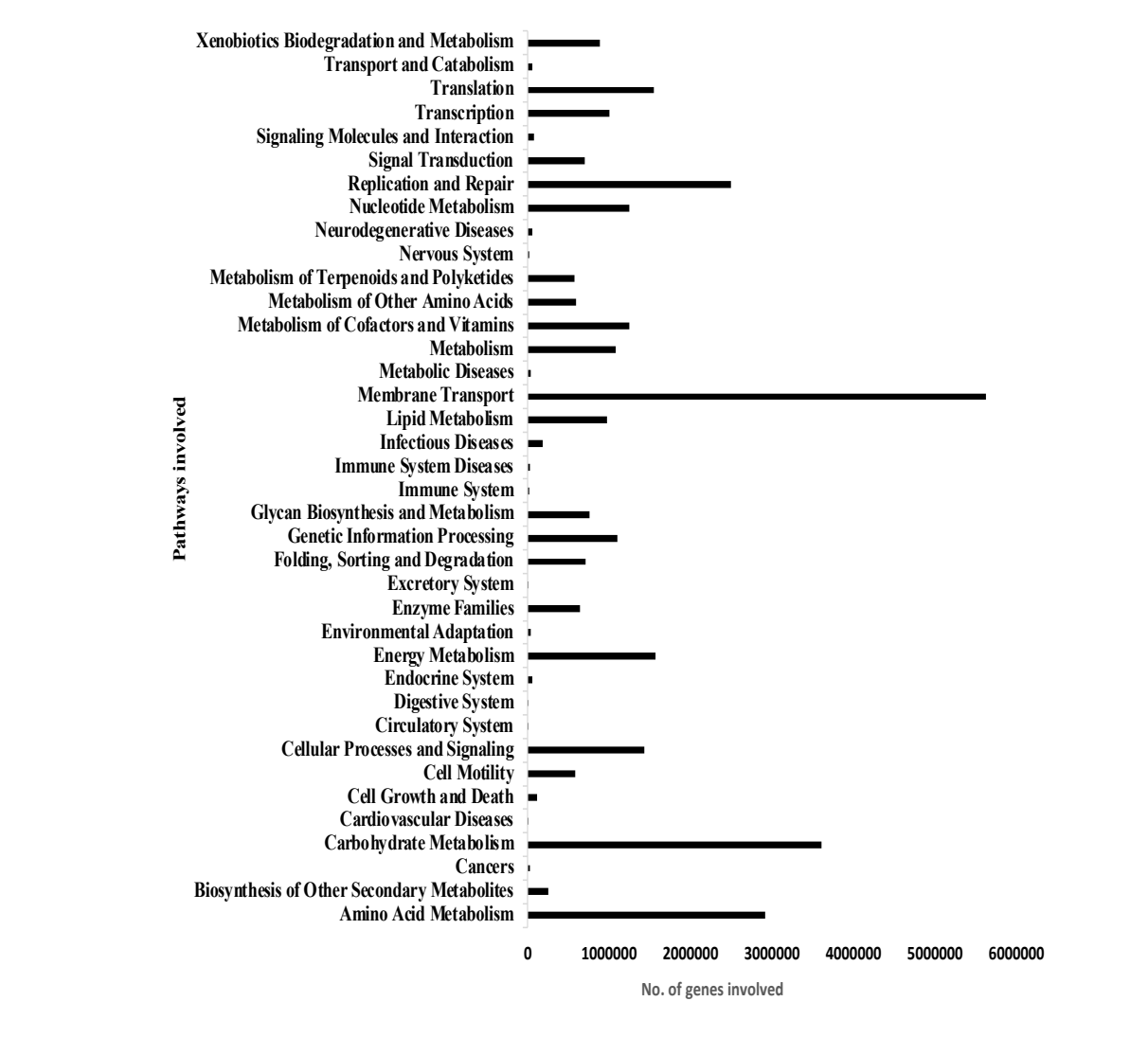

Figure 5: KEGG pathway functional annotation of *S. ricini* gut bacterial genome

Similar observations were reported by MsangoSoko et al[34], who reported *Proteobacteria* and *Firmicutes* as dominant phyla in castor fed eri silkworm represented with 60% and 20% of total sequences respectively.

However, present result showed higher abundance percentages of dominant phyla, occurrence of additional phyla and the lesser proportion of unknown phyla in the gut sample of Eri silkworm when reared on tapioca food plant. Such results may be indicative of the influences of food plant and location variability in the Eri silkworm. Similar findings were also reported in other insects[11,51]. Chen et al[9] described the relative association of diversity and composition of gut flora with host plants, as well as host's silkworms growth phases and the physiological conditions.

Colman et al[11] also confirmed the influences of diet and host's taxonomy on gut microbial composition. The crucial roles played by the members of phylum *Proteobacteria* and *Firmicutes* have been demonstrated by different researchers in terms of nutrients digestion and absorption, metabolism and protection of the host against diseases in insects[1,4,17,46]. This indicates the correlative roles of *Proteobacteria* and *Firmicutes* in the Eri silkworm larvae reared on tapioca leaves which might be complementing the overall growth and development, survivability and providing resistance to the larvae. The most abundant class identified was *Gammaproteobacteria*, with *Enterobacteriales* being the most dominant order and *Enterobacteriaceae*, *Enterococcaceae* and *Streptococcaceae* as the most dominant families.

In the lower taxa, at the genera level, the most abundant genera identified were *Enterobacter, Enterococcus*, *Klebsiella* and *Lactobacillus*, whereas 40% of the OTUs were unknown in terms of genus. Similarly, the occurrence of *Enterobacter* and *Enterococcus* as dominant genera has been previously recorded in the gut of silkworms[9,30,32]. Broderick et al[5] reported that *Enterobacter* and *Enterococcus* occur most prominently within the gut of the gypsy moth larval stage reared on varying diets. Previous studies have revealed the gut bacteria such as *Enterococcus* and *Enterobacter* obtained from the lepidopteron insect, contributing positively to the well-being of host by

regulating the functions such as host defence against pathogens, nutrition, metabolism (producing amino acids, lactic acid, metabolites), production of digestive enzymes, regulation of gut pH and tannin tolerance[1,25,30,42,46]. This suggests a symbiotic partnership between the host and these steadfast microbial partners, providing a stable and consistence source for the host insect's metabolic processes.

Interestingly, co-occurrence analysis of the gut sample revealed that nine bacterial genera including *Lactobacillus*, *Enterococcus*, *Lactococcus*, *Enterobacter*, *Bacillus*, *Stenotrophomonas*, *Staphylococcus*, *Serratia* and *Klebsiella*, were found to co-occur together in the Eri silkworm larval gut. This indicates that these gut bacteria might have co-existed in the gut tissue either due to common habitat preference or shared resources or mutual interactions within the gut of *S. ricini* fed with the leaves of tapioca host plant. However, the result of present investigation also showed that 40.07% of the OTUs were unknown among genera. A huge fraction (89.18%) of total OTUs was unknown at the level of species and only 10.82% OTUs have been identified including uncultured and some other known bacterial species [Fig. 1f].

The presence of these unclassified taxa in the gut sample could be possible due to the limitations in the reference databases, as diversity studies rely on the existing databases to classify the bacteria. Limitations in methodology for accurate identification, evolutionary novelty or some of the bacteria found in insect gut may be new that are not closely related to known bacterial taxa[51]. This report is in accordance with the observations of earlier researchers[26,34], who reported the occurrence of unassigned taxa. It was concluded that such observations could be due to the appearance of novel microbes or failure of resolution as targeting only small segment encompassing the V3-V4 area of 16S rRNA gene would not be able to resolve the proper taxonomic annotation.

Overall, the information provided and the earlier findings elucidated the diverse nature of insect's gut microbial community and played pivotal role in facilitating the host's growth and development including immune function. However, additional high throughput investigation is needed to gain a more comprehensive understanding of the precise roles carried out by the bacterial taxa identified in the Eri silkworm gut and their interactions with one another. Additionally, this study highlights the dominance of the *Proteobacteria*, *Firmicutes* and *Actinobacteria* phyla in the larval gut of the Eri silkworm. The prevalence of *Proteobacteria*, *Firmicutes* and *Actinobacteria* has additionally been documented by other workers in the digestive tract of many insects inclusive of bees, bumblebees, termites and ants[6,22.27,31,50].

The prevalence of *Proteobacteria* within the gut of insect in significant numbers could be linked to their capability to adjust to shifting environmental circumstances. Furthermore, the co-occurrence of several genera in the gut sample suggests that these bacteria may have functional interactions that promote to the general well-being and vitality of the Eri silkworm.

For instance, the species of *Enterobacter*, *Erwinia* and *Klebsiella* were reported to render help in digestion and nutrition through production of carbohydrate degrading enzymes[1]. Habineza et al[21] further reported participation of *Lactococcus* in digestion and nutrient provisioning.

Although tapioca is generally regarded as a secondary food source for *S. ricini*[45], the Eri larvae reared on its leaves is associated with less mortality. This could be due to evolutionary adaptation of Eri insect to tapioca leaf feeding or coordinated functioning of host defense and gut microbiota. This is supported by prior studies that have discussed the potential impact of *Lactobacillus* on the survivability of *Bombyx mor*i during parasite infection[43]. Similarly, Wang et al[47] reported the involvement of *Enterobacter* species in detoxification and manipulation of defence responses in host.

It is also interesting to note that several of these genera, including *Lactobacillus*, *Bacillus* and *Enterococcus* are known to have probiotic properties and may fulfil a beneficial role associated with digestive health of Eri silkworm[46,50]. In general, the existence of such wide diversity of bacterial taxa in Eri silkworm gut could possibly help in regulating the physiological processes and thus influencing the host insect's development and growth.

The current investigation also predicted the roles of gut bacterial community in Eri silkworm and revealed their various roles in various processes of metabolism, physiological and immunological pathways. One of the key observations was the significant gene involvement in the membrane transport and metabolism; particularly carbohydrate metabolism pathway. These outcomes indicate the vital function of the gut bacteria in metabolism and utilization of carbohydrate food components of plant leaf used for rearing, highlighting their potential impact on the energy metabolism of Eri silkworm.

In addition, the amino acid metabolism and lipid metabolism pathway displayed substantial gene involvement. This suggests the importance of gut bacteria in metabolism of proteins and lipids and their potential contribution to host nutrition and development. This aligned well with the report of Zhang et al[52] who emphasized the significance of gut microbial inhabitants in maintaining the health and nutrition of the silkworm.

Moreover, the involvement of sufficient amount of genes associated with energy metabolism further emphasizes the significance of gut microbes in energy homeostasis and nutrient processing within the host. This conclusion is substantiated by the results of Habineza et al[21] who

documented the contribution of gut bacteria in fostering the growth and advancement of Red palm weevil (RPW) by regulating its nutrient metabolism. Similarly, participation of gut bacteria in various processes within the circulatory, nervous, digestive and immune systems implies that the gut bacteria potentially play active role in maintaining the host's overall physiology and enhancing its immunity.

Insects' growth and development are intricately linked to their resident gut microorganisms, which assist in digesting dietary components, upgrade diets deficient in nutrients, support intra- and interspecies communication and safeguard against parasites, predators and diseases[20,42]. In this investigation, substantial gene participation in genetic information processing, replication, repair and gene expression processes was also predicted suggesting the conceivable effect of the gut bacterial community on host's gene expression and protein synthesis mechanisms. In addition, pathways linked to the immune system, signal transduction, environmental adaptation, as well as other biological processes exhibited differing levels of gene participation. This showcases the intricate functional potential of the gut microbiome in the host silkworm.

The finding on the role in xenobiotic degradation suggests the contribution of gut bacteria in degradation of toxic substances consumed by the host. This is consistent with the findings of Xia et al[49] who reported the detoxifying role of abundant bacteria in the Diamondback moth (*Plutella xylostella*). Furthermore, involvement of a very small proportion of genes involved in infectious diseases and certain human disease indicating only small gut bacteria may be harmful in certain condition.

Overall, the findings of this PICRUSt2 analysis illuminate the diverse functional activities exhibited by the gut bacterial community in the Eri silkworm. These discoveries offer valuable perspectives into the functional roles of gut bacteria in silkworm *S. ricini*, laying the foundation for future investigations into host-microbe interactions and their ramifications for silk production and disease susceptibility in silkworm populations.

Conclusion

This study provided report on the structural and functional potentials of the gut bacterial community in the larval gut of Eri silkworm reared on secondary food plant, Tapioca (*M. esculenta*). The dominance of *Proteobacteria* and *Firmicutes* and the co-occurrence of several key genera with beneficial roles hold considerable significance for the overall health and well-being of the silkworm.

Moreover, these gut microbes can be biotechnologically exploited for exploring novel enzymes and potential biomolecules. It also helps in developing strategies for improving the health and productivity of the Eri silkworm through manipulation of its gut microbiota. However, further investigation is required to unravel the functional roles of these gut bacteria and delve into their possible applications in insect rearing and disease management.

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