ABSTRACT

The Eri silkworm (*Samia ricini*) is a unique and economically valuable insect known for its silk production. Unlike other silkworm species, Eri silkworms feed on a diverse array of food plants, showcasing their remarkable adaptability. Among their preferred food sources *Ricinus communis* (Castor) is always consider as a primary food plants for Eri silkworms. The choice of food plants used for rearing not only influences the nutritional composition of the silkworms but also plays a crucial role in shaping the dynamics of their gut bacterial communities. This intricate relationship between Eri silkworm and their food plants contributes not only to the quality of the silk produced but also underscores the ecological interconnectedness that defines their existence. Study of such symbiotic interaction provides valuable insights into the intricate balance between insect, plant and microbial communities, highlighting the importance of these relationships in the context of economic parameters of Eri silkworm.

Given the prevalence of Eri silkworm cultivation in the north-eastern states as a commercial venture, this study was meticulously designed to investigate the effects of three different food plants on the overall gut community structure of Eri silkworm. Considering primary castor (*Ricinus communis*), secondary tapioca (*Manihot esculenta*), and tertiary papaya (*Carica papaya*) food plants, the research aimed to unravel their effects on the growth patterns of Eri silkworm, with a particular emphasis on gut bacterial community structures and their functional significance. The selection of these food plants was based on their local availability and widespread usage among the community of local rearers, ensuring the practical relevance and applicability of the study. This research aims to deepen our understanding of the intricate gut microbial dynamics of Eri silkworm and their pivotal role in growth and development and production of quality silk within the context of the north-eastern states of India.

In this comprehensive study, the multifaceted influence of distinct food plants - *R. communis* (Sample C), *M. esculenta* (Sample T), and *C. papaya* (Sample P) on the intricate processes governing the growth and development of Eri silkworm larvae were investigated. The findings unravel a rich tapestry of developmental dynamics, where

Sample C exhibited not only a significantly short larval duration (19.87±0.26 cm), but also a substantial augmentation in both length (7.57±0.18 cm) and weight (6.92±0.21 g) in contrast to Sample T (6.97±0.18 cm; 5.71±0.26g respectively) and Sample P (6.02±0.15 cm; 4.33±0.21g respectively). Beyond the morphological dimensions, a comprehensive analysis of nutritional constituents highlights the supremacy of *R. communis*, elucidating the levels of crude protein (33.80±5.09g), and carbohydrates (40.37±2.59g), thus establishing a pivotal link between food source and larval nourishment.

The role of digestive enzymes is crucial for nutrient acquisition and assimilation, particularly in herbivores like *S. ricini* silkworms, which depend entirely on nutrients from their food plants for growth and development. This study also aimed at investigate the influence of gut digestive enzymes specifically α-amylase, cellulase, proteinase, and lipase activity- on *S. rici*ni larvae. By exploring the landscape of gut digestive enzyme activity, the study revealed significant variations ($p<0.05$) in the gut digestive enzyme activity among all three groups *i.e.* Sample C, Sample T and sample P. Among all studied enzyme activities, the highest gut enzyme activity was recorded in α-amylase and proteinase activity with 4.57 ± 0.33 U/ml and 2.41 ± 0.65 U/ml respectively in Sample C followed by Sample T (3.53± 0.05 U/ml and 2.46 ± 0.60 U/ml respectively) and Sample P (3.20 ± 0.07 U/ml and 5.80± 1.46 U/ml) respectively. These findings underscore how different food plants impact nutrient acquisition and assimilation, highlighting the importance of larval diet in shaping metabolic processes. Furthermore, they emphasizes the intricate relationship between food composition and physiological outcomes, potentially contributing to differential feeding habits.

Moreover, this exploration extends into the intricate world of bacterial communities residing within the gut of Eri silkworms using a culture-independent metagenomic approach. The analysis unveiled a complex interplay of composition, diversity, and functionality. Sample C exhibit a remarkable resemblance to Sample T in terms of gut bacterial composition compared to Sample P. The dominance of Proteobacteria and Firmicutes is observed in all three samples, in Sample C (69.84%; 25.64% respectively), followed by Sample T (69.15%; 28.37% respectively) and in

Sample P (65.12%; 21.48% respectively). At the genus level, Sample C was dominated by the genera *Enterococcus* (19.06%), *Klebsiella* (18.19%), and *Enterobacter* (9.18%). In Sample T, the dominant genera recorded were *Enterobacter* (16.49%), *Enterococcus* (12.74%), and *Klebsiella* (11.17%). Sample P was populated by the genera *Enterococcus* (13.26%), *Enterobacter* (12.29%), and *Klebsiella* (9.67%), highlighting the crucial role of these symbiotic bacterial communities in supporting silkworm health, as influenced by their food plants. Furthermore, the present study recorded a large proportion of unknown gut bacterial populations at lower taxonomic levels, *i.e.,* at the species level, with the highest occurrence in Sample P (90.94%), followed by Sample C (90.81% and Sample T (89.18%). This may be attributed to limitations in target gene length, limited reference databases, or the presence of novel bacterial species unidentified in the bacterial information database.

Widening the study perspective, this study navigates through the enzyme activities of gut bacterial isolates using culture dependent method, revealing a diverse array of roles in nutrient digestion. The samples were successfully isolated and fourteen bacterial species were identified, with three species consistently present across all three samples *i.e.* Sample C, Sample T and Sample P, which include *Mammaliicoccus sciuri* (GenBank OR016518), *Mammaliicoccus sp.* (GenBank OR82823), *Bacillus licheniformis* (GenBank OR739576), *Winslowiella sp.* (GenBank OR963258), *Brevundimonas diminuta* (GenBank: OR964999), *Staphylococcus sp.* (GenBank OR211560), *Mammaliicoccus sp.* (GenBank OR921980), *Bacillus sp.* (GenBank OR976063), *Bacillus subtilis* (GenBank OR923392), *Mammaliicoccus sciuri* (GenBank OR924301), *Winslowiella iniecta* (GenBank OR945735), *Klebsiella oxytoca* (GenBank OR958639), *Citrobacter sp.* (GenBank OR976270), and *Mammaliicoccus sp.* (GenBank OR958729), having role in the four digestive enzyme assay viz. α-amylase, cellulase, proteinase and lipase. Furthermore, this study also elucidates the significant physiological roles of these bacterial organisms. This multifaceted approach substantiates the pivotal role played by these endosymbiotic gut bacterial groups in influencing the overall health of Eri silkworm larvae, particularly within the context of their dietary preferences. The intricate interplay between larval diet, gut bacterial community structure and functional roles becomes increasingly apparent, adding depth to our understanding of the relationship between the Eri silkworm and its gut microbiota.

As the study concludes, it sets the stage for future research endeavours, advocating for advanced methodologies such as Meta-transcriptomics and proteomics to unveil the deeper functional implications of gut bacteria in response to plant-induced changes. This forward-thinking approach not only seeks to unravel the molecular intricacies but also holds the promise of transforming our understanding of the dynamic relationship between larval diet, gut bacteria, and the overarching physiological development of the economically pivotal Eri silkworm. In summation, this comprehensive investigation not only contributes nuanced insights into the enzymatic activities and molecular characteristics of bacterial isolates from Eri silkworms but also presents a holistic narrative that underscores the intricate and interdependent nature of larval diet, gut bacteria, and the overarching physiological development of this economically significant insect which will provide a base to future researchers based on metagenomic studies of Eri silkworm gut bacteria.