

## CONTENTS

**Declaration**

**Certificate**

**Acknowledgement**

**List of Tables**

**List of Figures**

**List of Plates**

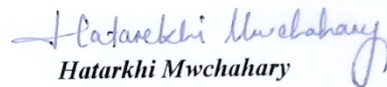
**List of Abbreviations and Symbols**

	<b>Page No.</b>
<b>CHAPTER-1: INTRODUCTION</b>	<b>1-10</b>
<b>CHAPTER-2: REVIEW OF LITERATURE</b>	<b>11-44</b>
<b>CHAPTER-3: MATERIALS AND METHODS</b>	<b>45-69</b>
<b>CHAPTER-4: RESULTS</b>	<b>70-114</b>
<b>CHAPTER-5: DISCUSSION</b>	<b>115-138</b>
<b>CONCLUSION</b>	<b>139</b>
<b>REFERENCES</b>	<b>140-176</b>
<b>APPENDIX</b>	<b>i-x</b>

## **DECLARATION**

*I, Hatarkhi Mwchahary, hereby declare that the Thesis entitled "Study on the Gut Bacterial Communities in Silkworm Samia ricini Fed on Different Food Plants" is the result of my own work, carried out at Department of Zoology, Bodoland University under the guidance of Dr. Dulur Brahma for the degree of Doctor of Philosophy and I have not submitted it earlier elsewhere for any other degree of any other University. Due acknowledgments have been made for the assistance and help taken during my work.*

*Date: 09/10/2024*  
*Place: Kokrajhar*

  
**Hatarkhi Mwchahary**  
*Department of Zoology*  
*Bodoland University*  
*Kokrajhar, Assam-783370*



**BODOLAND UNIVERSITY:DEPARTMENT OF ZOOLOGY**  
DEBARGAON, RANGALIKHATA, KOKRAJHAR-783370, BTR ASSAM

**DR. DULUR BRAHMA**  
ASSISTANT PROFESSOR  
Email:brahmadulur@gmail.com  
Contact No.+91 9101348701

Ref. No.:BU/ZOO-DEPTT./DB-PF/PhD Thesis/CF/01

Date: 04.10.2024

*I hereby certify that the present Thesis entitled "Study on the Gut Bacterial communities in Silkworm Samia ricini Fed on Different Food Plants" is an independent work of Ms. Hatarkhi Mwchahary submitted to Bodoland University for the award of the degree of Doctor of Philosophy (Zoology), has been carried out under my guidance at Department of Zoology, Bodoland University. Ms. Hatarkhi Mwchahary has fulfilled all the requirements laid by the university for the award of Doctor of Philosophy in Zoology under the faculty of Science and Technology.*

*To the best of my knowledge and belief, this work is original and has not been submitted so far in part or in full for the award of any degree or diploma of any University Institute.*

Date: 04.10.2024

Place: Kokrajhar

**Dr. Dulur Brahma**  
Assistant Professor  
Department of Zoology  
Bodoland University  
Kokrajhar, Assam-783370

## **ACKNOWLEDGEMENT**

*I express my heartfelt gratitude to the Almighty, the Merciful, and the Compassionate, whose grace and blessings have enabled me to successfully complete this thesis.*

*I am sincerely and wholeheartedly grateful to my guide Dr. Dulur Brahma, Assistant Professor, Department of Zoology, Bodoland University, for believing in me and providing me with the opportunity to pursue my Ph.D. under her guidance. Her kind guidance, suggestions and extended support throughout this endeavour have been invaluable.*

*My deepest appreciation goes to the Department of Zoology, Bodoland University, for providing me with the opportunity to conduct my research, sheltering me for five years as a Research Scholar, and furnishing all necessary infrastructure and technical support.*

*I extend my sincere thanks to Dr. Manjil Basumatary, Academic Registrar; Dr. Prahlad Basumatary, Former Deputy Registrar Academic; Administrative staff and Library staff, Bodoland University for their unwavering support during the course of my research work.*

*I also extend my deepest gratitude to the DRC members and all the teachers of Department of Zoology, Bodoland University Dr. Kushal Chowdhary, Head Department of Zoology; Prof. Hilloljyoti Singha; Dr. Ananta Swargiary, Dr. Khangembam Bronson Kumar and Mr. Bihung Basumatary, for their assistance and support during my Ph.D. journey.*

*I am sincerely thankful to Prof. Sujit Deka, Dean, Faculty of Science and Technology, Bodoland University for his active cooperation and guidance, which helped in shaping my research.*

*I am highly grateful to the experts Prof. Sandeep Das, Professor Department of Biotechnology; Dr. Arvind Kumar Goyal, Assistant Professor, Department of*

*Biotechnology and Dr. Hemen Sarma, Associate Professor, Department of Botany Bodoland University for their guidance, and valuable suggestions during my Ph.D. journey for providing crucial suggestions that enhanced the quality of my research.*

*A heartfelt thanks goes again to Prof. Sandeep Das, Principal Investigator, Technology Incubation Centre (TIC), Bodoland University, for graciously allowing access to laboratory facilities crucial for my research progress. Without his assistance, completing certain aspects of my research on time would not have been possible.*

*Additionally, I extend my gratitude to the Department of Botany, Bodoland University, for their kind assistance and provision of instrumental facilities during my research work.*

*Special acknowledgment is due to Dr. Sanjib Baruah, Assistant Professor, Department of Botany and Dr. Sanswring Basumatary (Former Ph.D. Scholar), Department of Botany, Bodoland University for their assistance in identifying food plants used in my study and in the preparation and deposition of herbarium specimens.*

*I am highly thankful to the Ministry of Tribal Affairs, Govt. of India, for providing financial assistance throughout my research work under the scheme National Fellowship and Scholarship for Higher Education of ST Students.*

*I am also grateful to AgriGenome Labs Pvt Ltd., Kochi, Kerala, India, for their assistance in performing metagenomic sequencing, and to Eurofins Scientific for providing a platform for DNA sequencing.*

*Furthermore, I express my gratitude to Dr. Amaraja Joshi, Scientist C, National Centre for Cell Sciences (NCCS), Pune, Maharashtra, for her assistance during microbial identification work.*

*I am also thankful to Ms. Debajani Das, Ph.D. scholar Department of Biotechnology, Bodoland University and other staff members of TIC for their invaluable help and support during my work in the.*

*I also extend my heartfelt gratitude to our Lab assistant, Mr. Amit Mushahary, and all the dedicated staff members of the Department of Zoology for their invaluable assistance during my research work.*

*I gratefully acknowledge the timely help and moral support extended by my friends and colleagues, Mr. Swmdwn Brahma, Mr. Rajib Ratan Kashyap, Mrs. Fangleng Narzary and Mr. Paris Basumatary.*

*Lastly, my heartfelt appreciation and dedication goes to my family members for their unwavering support and encouragement throughout my Ph.D. journey. Their presence has been the cornerstone of my success.*

***Sincerely,  
Hatarkhi Mwchahary***

## LIST OF TABLES

<u>TABLE NO</u>	<u>TITLE</u>	<u>PAGE NO</u>
3.1.	Reading Table for API 20E strip analysis	60
3.2.	Genomic DNA concentrations of isolates	62
4.1.	Growth parameters of <i>S. ricini</i> larvae reared on different food plants	70
4.2.	Proximate analysis of food plants	71
4.3.	DNA Quantification result	74
4.4.	Sequencing data raw read summary	76
4.5.	Raw read summary with Phred quality score distribution (%)	76
4.6.	Base composition distribution of the samples (%)	77
4.7.	Trimmed and consensus read summary	79
4.8.	Pre-processing reads statistics	79
4.9.	Summary of OTUs	79
4.10.	Qualitative screening of digestive enzyme activities of bacterial isolates	98
4.11.	Table showing morphological characteristics of isolates	100
4.12.	Table showing Physiological characteristics of gut bacterial isolates	101-103
4.13.	Quantitative $\alpha$ - amylase enzyme activity of isolates	105
4.14.	Quantitative cellulase enzyme activity of isolates	106

4.15.	Quantitative proteinase enzyme activity of isolates	107
4.16.	Quantitative lipase enzyme activity of isolates	107
4.17.	Molecular identification NCBI Blast result of bacterial isolates	108



## LIST OF FIGURES

<u>FIGURE NO</u>	<u>TITLE</u>	<u>PAGE NO</u>
1.1.	Eri Silk moth ( <i>Samia ricini</i> )	2
3.1.	Metagenomic flowchart for the wet lab and bioinformatics protocols used	51
3.2.	Agarose gel electrophoresis of crude DNA isolated from bacterial isolates: A1-C1; A2-C2; A3-C3; A4-C4; A5-C5; A6-C6; A7-T1; A8-T2; A9-T3; A10-P1; A11-P2; A12-P3; A13-P4; B1-T4	61
3.3.	Agarose gel electrophoresis of 16S rRNA PCR products from bacterial isolates using universal primers: A1-C1; A2- C2; A3-C3; A4-C4; A5-C5; A6-C6; A7 T1; A8-T2; A9-T3; A10-P1; A11-P2; A12-P3; A13-P4; B1- T4	63
4.1.	Standard curves for larval gut digestive enzyme assay: A. Maltose standard curve; B. D-glucose standard curve; C. L-Tyrosine standard curve; D. p-Nitrophenol standard curve	73
4.2.	Gut digestive enzyme activities of <i>S. ricini</i> larvae across three sampled food Plants	73
4.3.	Gel electrophoresis profile of PCR amplification: L= Ladder; 1= Sample C; 2= Sample T; 3= Sample P	74
4.4.	Library preparation: TapeStation profile of A. sample C showing the expected size (601 bp) of the final library with a lower (25 bp) and upper (1500 bp) marker; B. TapeStation profile of sample-T showing the expected size (618bp) of the final library with a lower (25 bp) and upper (1500 bp) marker; C. TapeStation profile of sample P showing the expected size (605 bp) of the final library with a lower (25 bp) and upper (1500 bp) marker	75
4.5.1.	GC distribution plot of gut bacterial sequence reads from sample C	77
4.5.2.	GC distribution plot of gut bacterial sequence reads from Sample T	78

4.5.3.	GC distribution plot of gut bacterial sequence reads from Sample P	78
4.6.	Bar plot representing the relative reads and OTU proportion	80
4.7.1.	Relative abundance of bacterial OTUs at Phylum level	81
4.7.2.	Relative abundance of bacterial OTUs at Class level	81
4.7.3.	Relative abundance of bacterial OTUs at Order level	81
4.7.4.	Relative abundance of bacterial OTUs at Family level	82
4.7.5.	Relative abundance of bacterial OTUs at Genus level	82
4.7.6.	Relative abundance of bacterial OTUs at Species level	82
4.8.	Combined Phylogenetic tree of gut bacterial communities in all samples	84
4.9.	Krona plot of bacterial population of two dominant phylum: A. Sample C; B. Sample T; C. Sample P	86
4.10.	Alpha diversity index curves for all three samples: A. Shannon rarefaction curve; B. Observed species rarefaction curve; C. Chao1 rarefaction curve	87-88
4.11.	Comparative analysis of gut bacterial Operational Taxonomic Units (OTUs) in Venn diagram: showing shared and unique OTUs across samples C, T, and P	88
4.12.	Co-occurrence analysis of bacterial genera in gut samples: A. Sample C; B. Sample T; C. Sample P	89-90
4.13.	Principal Coordinate Analysis (PCoA) between Samples: C, T and P	91
4.14.1.	Comparison of KEGG functional predictions in the gut bacterial communities of <i>S. ricini</i> fed on different host plants Level 1- Overall functional pathways	92

4.14.2	Comparison of KEGG functional predictions in the gut bacterial communities of <i>S. ricini</i> fed on different host plants Level 2-analysis of metabolic pathways	93
4.14.3.	Heatmap showing comparison of KEGG functional predictions in the gut bacterial communities of <i>S. ricini</i> fed on different host plants Level 3- analysis of functional proteins	95
4.14.4.	Comparative analysis of KEGG pathway predicted functional proteins using STAMP Bar Plots with Extended Error Bars Illustrating Level 3 comparisons between samples: (A) Sample C and T; (B) Sample C and P; (C) Sample P and T	96-97
4.15.	Standard curves: A. Maltose standard curve for $\alpha$ -amylase; B. D-glucose standard curve for cellulose; C. L-Tyrosine standard curve for protease; D. p-Nitrophenol standard curve for lipase	104
4.16.	Phylogenetic tree of bacterial isolates and closet relatives constructed based on Neighbor-Joining method with bootstrap test of 1000 replicates	110
5.1.	Phylogenetic relationships of gut bacterial population of three samples	126
5.2.	Digestive enzyme activities of gut bacterial isolates from three samples	134

## LIST OF PLATES

<u>PLATE NO</u>	<u>TITLE</u>	<u>PAGE NO</u>
<b>PLATE 1:</b>	Photograph of food plants used for rearing of <i>S. ricini</i> : A. Castor ( <i>Ricinus communis</i> ); B. Tapioca ( <i>Manihot esculenta</i> ); C. Papaya ( <i>Carica papaya</i> )	65
<b>PLATE 2:</b>	Photograph of submitted herbarium sheets of sampled food plants: A. <i>R. communis</i> (BUBH0000827); B. <i>M. esculenta</i> (BUBH0000828); C. <i>C. papaya</i> (BUBH0000829)	66
<b>PLATE 3:</b>	Photograph of Rearing of <i>S. ricini</i> on: A. <i>R. communis</i> leaves; B. <i>M. esculenta</i> leaves; C. <i>C. Papaya</i> leaves	67
<b>PLATE 4:</b>	A-I: Photos of experimental procedures of gut bacterial community studies	68
<b>PLATE 5:</b>	Mixed culture of gut bacterial isolates: A. Mixed culture isolated from <i>R. communis</i> fed larvae (Isolate C); B. Isolated from <i>M. esculenta</i> (Isolate T); C. Isolated from <i>C. papaya</i> fed larvae (Isolate P)	69
<b>PLATE 6:</b>	Positive result for qualitative $\alpha$ -amylase and Cellulase activity of bacterial isolates (A-N)	111
<b>PLATE 7:</b>	Positive result for qualitative Proteinase and Lipase activity of bacterial isolates (A-I)	112
<b>PLATE 8:</b>	Culture plate (A to N): Gut bacterial isolates isolated from <i>S. ricini</i> gut reared on <i>R. Communis</i> (C1-C6); <i>M. esculenta</i> (T1-T4); <i>C. papaya</i> (P1-P4) leaves	113
<b>PLATE 9:</b>	Gram staining of gut bacterial Isolates from <i>S. ricini</i> gut reared on <i>R. Communis</i> (C1-C6); <i>M. esculenta</i> (T1-T4); <i>C. papaya</i> (P1-P4) leaves	114

## LIST OF ABBREVIATIONS AND SYMBOLS

%	: Percentage
°C	: Degree Celsius
µg	: Microgram
µl	: Microliter
µM	: Micromolar
1492R	: 1492 Reverse
27F	: 27 Forward
2D	: Two Dimension
A	: Adenosine
ACE	: Abundance-based Coverage Estimator
ADS	: Arginine Dihydrolase
AMP	: Antimicrobial Peptide
AMY	: Amygdalin
API	: Analytical Profile Index
ARA	: Arabinose
BLAST	: Basic Local Alignment Search Tool
BmCPV	: <i>Bombyx mori</i> Cytoplasmic Polyhedrosis Virus
BOD	: Biological Oxygen Demand
bp	: Base pair
CIT	: Citrate Utilization
CMC	: Carboxymethylcellulose
CMER&TI	: Central Muga Eri Research and Training Institute
D	: Days
dH <sub>2</sub> O	: Distilled water
DMSO	: Dimethyl sulfoxide
DNA	: Deoxyribonucleic acid
DNSA	: 3,5, Dinitrosalicylic acid
EDTA	: Ethylenediamine tetra acetic acid
FASTA	: Fast adaptive Shrinkage Threshold Algorithm
FLASH	: Fast Length Adjustment of Short reads

g	: Gram
GC	: Guanosine Cytosine
GEL	: Gelatinase
GLU	: Glucose
h	: Hour
H <sub>2</sub> S	: H <sub>2</sub> S production
HCL	: Hydrochloric acid
IND	: Indole production
INO	: Inositol
IQR	: Interquartile Range
KEGG	: Kyoto Encyclopedia of Genes and genomes
L	: Liter
LAF	: Laminar Air Flow
LDC	: Lysine Decarboxylase
M	: Molar
Mb	: Megabyte
MEL	: Melibiose
mg	: Milligram
min	: Minute
ml	: Milliliter
mM	: Millimolar
MRSA	: Methicillin-Resistant <i>Staphylococcus aureus</i>
MSN	: Mannitol
n	: Number
NCBI	: National Center for Biotechnology Information
NJM	: Neighbor joining method
ng	: Nanogram
nm	: Nanometer
OD	: Optical Density
ODC	: Ornithine Decarboxylase
ONPG	: Ortho Nitro Phenyl-βD- Galactopyranosidase
OTU	: Operational taxonomic unit

OX	: Oxidase
PBS	: Phosphate Buffered Saline
PC	: Principal Coordinate
PCoA	: Principal Coordinate analysis
PCR	: Polymerase Chain Reaction
PERL	: Practical Extraction and Report Language
PICRUST	: Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
pmol	: Picomole
pNPP	: p-nitrophenylpalmitate
Q	: Phred Score
QIIME	: Quantitative Insights into Microbial Ecology
RHA	: Rhamnose
rpm	: Rotation per minute
RNA	: Ribosomal Ribonucleic acid
S	: Svedberg
SAC	: Saccharose
SD	: standard deviation
SDS	: Sodium Dodecyl Sulfate
sec	: Second
SOR	: Sorbitol
sp.	: Species
STAMP	: Statistical Analysis of Metagenomics Profile
T	: Thymine
TAE	: Tris Acetate Ethylenediaminetetraacetic acid
TCA	: Trichloroacetic acid
TDA	: Tryptophane Deaminase
TE	: Tris EDTA
U	: Unit
UCHIME	: Ultra-fast Chimera Identification and Removal
UPGMA	: Unweighted Pair Group Method with Arithmetic Mean
URE	: Urease

UV-VIS	: Ultraviolet-Visible
V	: Volt
VP	: Voges Proskauer
w/v	: Weight by volume