CONTENTS

<table>
<thead>
<tr>
<th>TITLE</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgement</td>
<td>i – iv</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>v – ix</td>
</tr>
<tr>
<td>List of Tables</td>
<td>x – xiv</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xv – xxiii</td>
</tr>
</tbody>
</table>

CHAPTER 1 INTRODUCTION 1 – 7

CHAPTER 2 REVIEW OF LITERATURE 8 – 40

2.1 Introduction 8 – 9

2.2 Bamboo shoot as a food bioresource 9

2.2.1 Economy and gastronomy of edible bamboo shoots 9 – 17

2.2.2 Bamboo shoot as health food: Nutritive and health values of edible bamboo shoots 18 – 22

2.2.3 Toxic and anti-nutritive contents of bamboo shoots 22 – 24

2.3 Fermented bamboo shoot products: International scenario 24 – 25

2.4 Diversity of fermented bamboo shoot products in North East India 25 – 26

2.5 Microbiology of traditional fermented bamboo shoots 26 – 27

2.6 Methods used for studying microbial ecology of food fermentation 27

2.6.1 Culture-dependent methods used for studying the 27 – 29
2.6.2 Culture-independent molecular methods used for studying the microbial ecology of food fermentation

2.6.3 Culture-independent quantification of microbial community associated with fermented foods

CHAPTER 3 MATERIALS AND METHODS

3.1 Experimental design 41
3.2 Fermented bamboo shoot products 42
3.3 Bamboo shoot fermentations and sampling 42 – 46
3.4 pH, titratable acidity and temperature measurement 46
3.5 Isolation and enumeration of microorganisms 47 – 48
3.6 Genomic DNA extraction from isolated cultures 48 – 49
3.7 Molecular identification of culturable microorganisms 49 – 51
3.8 Metagenomic DNA extraction 51 – 53
3.9 PCR-DGGE analysis 53 – 54
3.10 Determination of DGGE band identity 54
3.11 Barcoded PCR and multiplexed Illumina amplicon sequencing 54 – 56
3.12 Illumina data analysis 56 – 57
3.13 Determination of microbial diversity estimates 57 – 58
3.14 Microbial community statistical analysis 58 – 59
3.15 Determination of core microbiota 59 – 60
3.16 qPCR to estimate microbial population 60 – 61
3.17 *Lactobacillus plantarum* isolates and reference strains for genetic and functional diversity 61

3.18 Preparation of chromosomal DNA plugs 61 – 62

3.19 *In situ* chromosomal DNA restriction digestion of *Lactobacillus plantarum* and REA-PFGE 62 – 63

3.20 REA-PFGE data analysis 64

3.21 Selection of MLST loci and designing of *Lactobacillus plantarum* specific MLST primers 64 – 65

3.22 Sequencing and MLST data analysis 65 – 68

3.23 Phenotypic and functional characterization of *Lactobacillus plantarum* 68

3.24 Phenotypic characterization and morphological observation of *Meyerozyma guilliermondii* and *Meyerozyma caribbica* 68 – 69

3.25 *In silico* analysis and restriction enzyme selection for differentiation of *Meyerozyma guilliermondii* and *Meyerozyma caribbica* 69 – 70

3.26 Yeasts mitochondrial DNA (mtDNA)-RFLP 70 – 71

3.27 Yeasts electrophoretic karyotyping 71

3.28 Accession numbers of nucleotide sequences of culturable isolates and Illumina amplicon data 71 – 87

**CHAPTER 4 RESULTS** 88 – 285

4.1 **DOCUMENTATION OF INDIGENOUS TRADITIONAL KNOWLEDGE OF** 88 – 98
FERMENTED BAMBOO SHOOTS OF NORTH
EAST INDIA

4.1.1 Traditional fermented bamboo shoot products of North East India 88 – 89

4.1.2 Traditional processes involved in bamboo shoot fermentation for production of Soidon, Soibum, Hikhu and Sele 89

4.1.2.1 Short-duration spontaneous bamboo shoot fermentation – Traditional preparation of Soidon 89 – 91

4.1.2.2 Long-duration spontaneous bamboo shoot fermentation – Traditional preparations of Soibum, Hikhu and Sele 92 – 98

4.2 PROTOCOL DEVELOPMENT AND OPTIMIZATION 99 – 128

4.2.1 Extraction of genomic DNA as cell-free DNA lysate 99

4.2.2 In silico restriction enzyme selection and validation of ARDRA-based phylotyping of Bacillus subtilis phylogeny group 99 – 103

4.2.3 Development of ITS-RFLP method for distinct differentiation of frequently misidentified Meyerozyma guilliermondii (predominant cultivable yeast associated with bamboo shoot fermentation) from Meyerozyma caribbica 103 – 120

4.2.4 Development and optimization of metagenomic DNA 121 – 125
4.2.5 Optimization of microbial community profiling by PCR-DGGE

4.3 MICROBIAL ECOLOGY OF SHORT-DURATION NATURAL BAMBOO SHOOT FERMENTATION–

Soidon

4.3.1 Changes in viable microbial population and environmental factors over time during soidon fermentation

4.3.2 Diversity and dynamics of culturable microorganisms during soidon fermentation

4.3.3 Culture-independent metagenomics-based microbial community structure and dynamics during soidon fermentation

4.3.4 Bacterial population dynamics during soidon fermentation analyzed by qPCR

4.4 MICROBIAL ECOLOGY OF LONG-DURATION NATURAL BAMBOO SHOOT FERMENTATIONS– SOBUM, HIKHU AND SELE

4.4.1 MICROBIAL COMMUNITY STRUCTURE AND POPULATION DYNAMICS DURING TRADITIONAL SPONTANEOUS SOBUM FERMENTATION
4.4.1.1 Changes in viable microbial population and environmental factors over time during *soibum* fermentation

4.4.1.2 Culturable microbial communities and dynamics during *soibum* fermentation

4.4.1.3 Culture-independent metagenomics-based microbial community structure and dynamics during *soibum* fermentation

4.4.1.4 Quantification of microbial community by qPCR

4.4.2 MICROBIAL COMMUNITY STRUCTURE AND POPULATION DYNAMICS DURING SPONTANEOUS *HIKHU* FERMENTATION

4.4.2.1 Changes in viable microbial population and environmental factors over time during *hikhu* fermentation

4.4.2.2 Culturable microbial communities and dynamics during *hikhu* fermentation

4.4.2.3 Culture-independent metagenomics-based microbial community structure and dynamics during *hikhu* fermentation

4.4.2.4 Quantification of microbial community by qPCR

4.4.3 MICROBIAL COMMUNITY STRUCTURE AND POPULATION DYNAMICS DURING SPONTANEOUS *SELE* FERMENTATION
4.4.3.1 Changes in viable microbial population and environmental factors over time during sele fermentation 221 – 222

4.4.3.2 Culturable microbial communities and dynamics during sele fermentation 222 – 226

4.4.3.3 Culture-independent metagenomics-based microbial community structure and dynamics during sele fermentation 226 – 238

4.4.3.4 Quantification of microbial community by qPCR 238 – 239

4.5 COMPARATIVE MICROBIAL ECOLOGY—DEFINING THE CORE MICROBIOTA OF NATURAL BAMBOO SHOOT FERMENTATION 240 – 252

4.5.1 Variation in the microbial community structure between different fermented bamboo shoots 240 – 247

4.5.2 Core microbiota of bamboo shoot fermentation 247 – 252

4.6 GENETIC DIVERSITY, POPULATION STRUCTURE AND FUNCTIONAL PROPERTIES OF Lactobacillus plantarum—A DOMINANT CORE MICROBIOTA OF NATURAL BAMBOO SHOOT FERMENTATION 253 – 285

4.6.1 Chromosomal DNA restriction analysis revealed high genetic diversity and food-type specific clonal selection of Lactobacillus plantarum population 253 – 258

4.6.2 MLST based evolutionary relationship analysis 259 – 273
revealed clonal population structure of *Lactobacillus plantarum* with two foundation clonal complexes and evidence of subspeciation

4.6.3 Excess of low frequency variation without recombination indicated recent selection sweep during *Lactobacillus plantarum* evolution

4.6.4 Phenotypic grouping largely linked with the genotypic clusters

CHAPTER 5 DISCUSSION 286 – 301

CHAPTER 6 SUMMARY 302 – 306

CHAPTER 7 CONCLUSION 307 – 309

REFERENCES 310 – 347

PUBLICATIONS AND PAPERS PRESENTED IN CONFERENCES 348 – 349