TABLE OF CONTENTS

CHAPTERS	PAGE NO.
1.Introduction	1-4
2.Review of Literature	5-22
2.1 Fermented foods and fermented soyabean products	5-6
2.2 *Tungrymbai*-as a fermented soyabean food	6-7
2.3 Other fermented soyabean products	7-10
2.4 Biochemical properties of *tungrymbai* and related products	10-11
2.5 Health benefit effect	11
2.5.1 Health benefits of fermented soyabean	11-12
2.5.2 Health benefits of probiotics	12-13
2.6 Isolation and identification of probiotic bacteria	13-20
2.7 Mechanism of action of probiotics	20-21
2.8 Mode of action of probiotics	22
3. Materials and Methods	23-39
3.1 Materials	23
3.2 Enumeration and isolation of microbes	23-24
3.3 Study of probiotic properties of the isolates	24
3.3.1 Screening for growth on simulated gastric condition (Acid tolerance)	24
3.3.2 Bile tolerance	24
3.3.3 Detection of antibacterial property	25
3.3.4 Antibiotic susceptibility	25
3.3.5 Gelatin hydrolysis activity	25
3.3.6 Haemolysis activity	26
3.3.7 *In vitro* cholesterol lowering property	26
3.4 Identification of the isolates by phenotypic and genotypic methods	26
3.4.1 Phenotypic identification of the isolates	26
3.4.1.1 Gram's staining and microscopic examination	26-27
3.4.1.2 Catalase test	27
3.4.1.3 Triple sugar iron test	27-28

3.4.1.4 Starch hydrolysis	28
3.4.1.5 Carbohydrate fermentation	28-29
3.4.1.6 Hydrogen sulphide production and motility	29
3.4.1.7 The IMViC test	29-30
3.4.1.8 Casein hydrolysis	30-31
3.4.1.9 Gelatin hydrolysis	31
3.4.1.10 Urease activity	31-32
3.4.1.11 Nitrate reduction	32
3.4.2 Genotypic identification of the isolates	32
3.4.2.1 Identification of unknown microorganisms based on 16S rDNA sequence analysis	32-33
3.4.2.2 Procedure for amplification of bacterial DNA using PCR	33
3.4.2.2.1 Bacterial culture	33
3.4.2.2.2 Isolation of genomic DNA	33-34
3.4.2.2.3 Set up of PCR reactions	34-35
3.4.2.2.4 Purification of PCR products	35-36
3.4.2.3 Electrophoresis of PCR product	36-38
3.4.2.3.1 Observation of the gel in a Gel Doc system	38
3.4.2.4 Sequencing of the PCR-amplified 16S rDNA	38
3.4.2.5 Submission of sequences to NCBI	39
3.4.2.5.1 Tool used for submission of sequences	39
3.4.2.5.2 Obtaining of accession number	39
3.4.2.6 Construction of phylogenetic tree	39
4. Results and Discussion	40-76
4.1 Bacterial strains and isolation	40
4.2 Effect of simulated gastric condition (Acid tolerance)	40-42
4.3 Effect of bile concentration	43-45
4.4 Gelatinase activity	46
4.5 Antibiotic susceptibility test	47-48
4.6 Detection of Antibacterial activity	49

4.7 Haemolysis activity	50
4.8 Cholesterol assimilation	51
4.9 Phenotypic identification by biochemical tests	52
4.9.1 Gram's staining and Microscopic examination	52
4.9.2 Catalase test	53
4.9.3 Triple sugar iron test	53
4.9.4 Starch hydrolysis	53
4.9.5 Hydrogen sulphide production and motility	53
4.9.6 Casein hydrolysis	54
4.9.7 Urease test	54
4.9.8 Nitrate reduction	55
4.9.9 Gelatin hydrolysis	55
4.9.10 IMViC test	55-58
4.9.11 Carbohydrate fermentation	59-61
4.10 Amplification of 16S rDNA by PCR	62-63
4.11 Sequences obtained after DNA sequencing	63-75
4.11.1 Sequence of BAS-TU1	64
4.11.2 Sequence of BAS-TU2	65
4.11.3 Sequence of BAS-TU3	66
4.11.4 Sequence of BAS-TU4	67
4.11.5 Sequence of BAS-TU5	68
4.12 Genomic identification of the isolates	76
4.13 Phylogenetic tree	77
5. Summary and Conclusion	78-79
5.1 Summary	78
5.2 Conclusion	79
5.3 Future Prospects	79
6. Bibliography	80-87
Appendix	88-89
Publications	90

LIST OF TABLES

TABLES	PAGE NO.
4.1 Place of collection of *tungrymbai* in Meghalaya, India with their LAB count	40
4.2 Effect of simulated gastric condition on the growth of the 40 isolates, isolated from *tungrymbai,* Meghalaya, India	42
4.3 Bile tolerance test of the selected isolates from *tungrymbai*, Meghalaya, India	45
4.4 Gelatinase activity of the selected isolates from *tungrymbai*, Meghalaya, India	46
4.5 Antibiotic susceptibility test of the selected isolates from *tungrymbai*, Meghalaya, India	48
4.6 Antibacterial activity of the selected isolates from *tungrymbai*, Meghalaya, India	49
4.7 Haemolysis activity of the selected isolates from *tungrymbai*, Meghalaya, India	50
4.8 Cholesterol assimilation of the selected isolates from *tungrymbai*, Meghalaya, India	51
4.9 Biochemical tests of the selected isolates from *tungrymbai*, Meghalaya, India	58
4.10 Carbohydrate fermentation of the selected isolates from *tungrymbai*, Meghalaya, India	61
4.11 Accession numbers obtained for the submitted sequences	76

LIST OF FIGURES

FIGURES	PAGE NO.
2.1 Representation of various functions and health benefits of probiotics	13
2.2 Mechanism of action of probiotics	21
2.3 Mode of action of probiotics	22
3.1 Sample of *tungrymbai* collected from Shillong, Meghalaya, India	23
4.1(a) Bile tolerance test of the isolates 4.1(b) Plating done for bile tolerance test	44
4.2 Representing antibiotic susceptibility test indicating zones of inhibition	47
4.3(a & b) Microscopic view of the isolates after Gram's test	52
4.4 Triple sugar iron test indicating yellow butt and slant in all the isolates	53
4.5 Representing positive reaction for casein hydrolysis for BAS-TU3	54
4.6 Urease test for identification of isolates	54
4.7 Nitrate reduction test for identification of isolates	55
4.8 Methyl red test for identification of isolates	56
4.9 Voges proskauer test for identification of isolates	56
4.10 Citrate utilization test for identification of isolates	57
4.11Carbohydrate fermentation test for identification of isolates	60
4.12 Agarose gel being observed under UV illumination	62
4.13 PCR amplified bands under the Gel-Doc system	63
4.14 Base count of BAS-TU1	69
4.15 Base count of BAS-TU2	69
4.16 Base count of BAS-TU3	69
4.17 Base count of BAS-TU4	70
4.18 Base count of BAS-TU5	70
4.19 Electropherogram data of BAS-TU1	71
4.20 Electropherogram data of BAS-TU2	72

4.21 Electropherogram data of BAS-TU3	73
4.22 Electropherogram data of BAS-TU4	74
4.23 Electropherogram data of BAS-TU5	75
4.24 Phylogenetic tree	77

LIST OF ABBREVIATIONS

BAS-TU	Barasa Malakar, Arup Jyoti Das, Sankar Chandra Deka – Tezpur University
LAB	Lactic acid bacteria
CFU	Colony forming units
ATCC	American type cell culture
MTCC	Microbial type cell culture
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
PCR	Polymerase chain reaction
UV	Ultraviolet
Gel-Doc	Gel-Documentation
NCBI	National Centre for Biotechnology Information
BLAST	Basic Local Alignment Search Tool
MEGA	Molecular Evolutionary Genetic Analysis
A	Adenine
G	Guanine
C	Cytosine
T	Thymine