

# LIST OF FIGURES

---

## CHAPTER 2

- Fig. 2.1. Mechanism of action of probiotics; adapted from Ng et al. 11

## CHAPTER 3

- Fig. 3.1. Sample collection sites 35
- Fig. 3.2. Clustering structure of lactic acid bacteria isolates and indicator strains. Dendrogram constructed using Ward Linkage, SPSS; version 18. 39
- Fig. 3.3. Cluster analysis of *Bacilli* based on biochemical characteristics. Dendrogram constructed using Ward Linkage, SPSS; version 18. 42
- Fig. 3.4. Pie diagram showing microbial diversity of fermented foods based on biochemical characteristics 43
- Fig. 3.5. Box plot showing probiotic properties of isolates from different origin: (A) Acid tolerance, (B) Bile tolerance and (C) Hydrophobicity. Abbreviations: MS: fermented mustard seeds, FV: fermented fruits and vegetables, SS: fermented soybean, BS: Fermented bamboo shoot and W: alcoholic products 44
- Fig. 3.6. Matrix Hierarchical Cluster Analysis of the isolates and the reference strains. Lane 1: Acid tolerance, lane 2: hydrophobicity and lane 3: bile tolerance. 45

## CHAPTER 4

- Fig. 4.1. Phylogenetic tree showing DS1 with closely related species based upon 16S rRNA sequences. Bootstrap values (1,000 replicates) are indicated at branch nodes. 58
- Fig. 4.2. Phylogenetic tree showing AMDK2 with closely related species based upon 16S rRNA sequences. Bootstrap values (1,000 replicates) are indicated at branch nodes. 58

- Fig. 4.3. Probiotic adhesion to Caco-2 cell line; (A) control Caco-2 cell, Gram staining depicting adhesion of (B) *P. pentosaceus* DS1 and (C) *L. monocytogenes* MTCC 1407 observed under 100X, (D) Quantitative representation of adhesion showing percentage adhesion expressed as bar diagram. 60
- Fig. 4.4. (A) Amplification of pediocin gene; lane 1: 1Kb+ ladder, lane 2: Amplicon of size 406 bp. (b) Phylogenetic tree showing *P. pentosaceus* DS1pediocin with closely related sequences. 61
- Fig. 4.5. (A) Multiple sequence alignment of *Pediococcus pentosaceus* DS1 pediocin gene (B) Secondary structure deduced from PSIPRED server. 62
- Fig. 4.6. Antimicrobial activity of culture supernatant against *Listeria monocytogenes* MTCC 839; (A) control (untreated), (B) pH neutralized, (C) catalase treated, (D) proteinase K treated and (E) antibiotic (ampicillin) 63
- Fig. 4.7. (A) Autoaggregation abilities of probiotic and pathogens, (B) Bacterial adhesion to hydrocarbon assay; different letters signify statistical difference ( $p < 0.05$ ) as calculated by Tukey's Multiple Comparison test, OriginPro 8.5. Abbreviations: 839- *L. monocytogenes* MTCC 839, AMDK2- *L. monocytogenes* strain AMDK2, DS1- *Pediococcus pentosaceus* DS1 64
- Fig. 4.8. Probiotic- mediated competition, exclusion and displacement of *L. monocytogenes* strains, different letters signify statistical difference ( $p < 0.05$ ) as calculated by Tukey's Multiple Comparison test, OriginPro 8.5. Abbreviations: 839- *L. monocytogenes* MTCC 839, AMDK2- *L. monocytogenes* strain AMDK2, DS1- *Pediococcus pentosaceus* DS1 64
- Fig. 4.9. Scanning electron micrograph (SEM) of biofilm formed by *L. monocytogenes* MTCC 839 and *L. monocytogenes* AMDK2, (A) MTCC 839 control, (B) *L. monocytogenes* MTCC 839 treated, (C) *L. monocytogenes* AMDK2 control and (D) *L. monocytogenes*AMDK2 treated. 66
- Fig. 4.10. (A) Adhesion and (B) invasion inhibition of *L. monocytogenes*, student's t-test, unpaired, OriginPro 8.5. Abbreviations: 839- *L. monocytogenes* MTCC 839, AMDK2- *L. monocytogenes* strain AMDK2.  $P < 0.05$  signifies statistical difference. 67

- Fig. 4.11 Modelled growth curves of (A) *L. monocytogenes* MTCC 839 and (B) *L. monocytogenes* AMDK2, fitted using the DMfit curve-fitting program; version 3.5. 68

## CHAPTER 5

- Fig. 5.1. Phylogenetic tree showing *Bacillus* sp.NK7 with closely related species based upon 16S rRNA sequences. Bootstrap values (1,000 replicates) are indicated at branch nodes. 82
- Fig. 5.2. Probiotic adhesion to Caco-2 cell line; (A) control Caco-2 cell, Gram staining depicting adhesion of (B) *Bacillus* sp. NK7 and (C) *Lactobacillus plantarum* MTCC 1407 observed under 100X, (D) Quantitative representation of adhesion showing percentage adhesion expressed as bar diagram. 85
- Fig. 5.3. Single factor experiments showing effect of different factors on antimicrobial activity against *Bacillus cereus* 430; effect of (A) time, (B) Temperature, (C) pH, (D) inoculum size, (E) carbon source and (F) nitrogen source.\*indicates statistical difference at  $P < 0.05$  86
- Fig. 5.4. Optimization of different parameters antimicrobial activity; (A), (B) and (C) are the Design- Expert plots depicting effect of different variables in activity, (D) Multiresponse optimization showing desirability ramps for numerical optimization of parameters for antimicrobial activity 89
- Fig. 5.5. (A) Bacteriocin activity at different time intervals and zones of inhibition against *Bacillus cereus* MTCC 430 observed in (B) optimized and (C) unoptimized media. 90
- Fig 5.6. The inhibitory activity of BLIS on the growth of *B. cereus* MTCC 430. 91
- Fig. 5.7. (A) Native PAGE of bacteriocin and (B) in gel activity against *Bacillus cereus* MTCC 430 92
- Fig. 5.8. Effect of enzymes, pH and temperature on bacteriocin action. \*indicates significant difference ( $P < 0.05$ ). 93
- Fig. 5.9. Pattern of hemolysis on blood agar; (A) *Bacillus* sp. NK7 and (B) *Bacillus cereus* MTCC 430 94

## CHAPTER 6

- Fig. 6.1. Identification of the strain *C. tropicalis* BSS7. (A) Colony morphology in Hichrome *Candida* differential agar. (B) Molecular typing using ITS (internal transcribed spacer)-primer (Lane 2) and 26S rRNA gene D1/D2 primer (Lane 3); Lane 1 indicates GeneRuler 1 Kb Plus DNA ladder (Fermentas). Phylogenetic tree showing the position of BSS7 with closely related species based upon (C) 5.8S ITS rRNA and (D) D1/D2 26S rRNA sequences. Bootstrap values (1,000 replicates) are indicated at branch nodes. 107
- Fig. 6.2. Phylogenetic tree showing the position of *L. paracasei* D6 with closely related species based upon 16S rDNA sequence. Bootstrap values (1,000 replicates) are indicated at branch nodes. 108
- Fig. 6.3. Antifungal activities of *L. paracasei* D6 against (A) *C. tropicalis* BSS7 and (B) *C. albicans* MTCC 3017 visualized as halos in agar overlay method. MIC values of *Lactobacillus* metabolites against (C) *C. tropicalis* BSS7 and (D) *C. albicans* MTCC 3017 respectively. 109
- Fig. 6.4. Biofilm inhibition assay. (A) Biofilm inhibition by various concentrations of *Lactobacillus* metabolites; bars with red borders indicate BIC<sub>50</sub>. Biofilm formation by *C. tropicalis* BSS7 on microtitre plates (B) without treatment and (D) treatment with BIC<sub>50</sub>. Biofilm formation by *C. albicans* MTCC 3017 (C) without treatment and (E) treatment with BIC<sub>50</sub> as observed under light microscope (20X). 110
- Fig. 6.5. Germ tube inhibition assay. (A) *C. tropicalis* BSS7 control, (B) *Candida albicans* MTCC 3017 control, (C) *C. tropicalis* BSS7 treated with BIC<sub>50</sub> and (D) *C. albicans* MTCC 3017 treated with BIC<sub>50</sub>, as observed under light microscope (40X). 111
- Fig. 6.6. *Candida* adhesion inhibition assay. (A) Uninfected Caco-2 cell, (B) Caco-2 cells + BSS7 (control 1), (C) Caco-2 cells+ MTCC 3017 (control 2), (D) Caco-2 cells + BSS7 + BIC<sub>50</sub> of AFM (test1 ), (E) Caco-2 cells + MTCC 3017 + BIC<sub>50</sub> AFM (test 2) and (F) Spectrophotometric adhesion inhibition assay; different letters signify statistical difference ( $P<0.05$ ), estimated by Turkey's Multiple comparison test, GraphPad Prism, Ver 5.0 112

- Fig. 6.7. Identification of antifungal metabolites. (A): Relative abundance of compounds in antifungal metabolites of *L. paracasei* D6 identified by GC-MS. (B): Mass spectra of the most abundant compound. 113
- Fig. 6.8. Optimization of different parameters for the inhibition of *C. tropicalis* BSS7 in fruit juice. (A) DESIGN- EXPERT Plot showing effect of different concentrations of antifungal metabolites and treatment time for the inhibition of *C. tropicalis* BSS7 in fruit juice. (B) Multiresponse optimization showing desirability ramps for numerical optimization of parameters for the inhibition of *C. tropicalis* BSS7. 116

## CHAPTER 7

- Fig. 7.1. (A) Molecular typing of Sc and Sb isolates by using ITS (internal transcribed spacer)-primer (Lane 2 and 4) and 26S rRNA gene D1/D2 region (Lane 3 and 5); Lane 1 & 6 indicates GeneRuler™ 1 Kb Plus DNA ladder (Fermentas); Phylogenetic tree showing *S. cerevisiae* ARDMC1 with closely related species based upon (B) 5.8S ITS rRNA and (C) D1/D2 26S rRNA sequences. Bootstrap values (1,000 replicates) are indicated at branch nodes. 130
- Fig. 7.2. Adhesion to Caco-2 cell line as observed under inverted microscope (20X), a) Control, b) *S. cerevisiae* ARDMC1, c) *S. boulardii* and d) % Adhesion of probiotic isolate to Caco-2 Cell line 132
- Fig. 7.3. SEM image of *S. cerevisiae* ARDMC1 grown in YM broth without cholesterol, (B) *S. cerevisiae* ARDMC1 grown with cholesterol, (C) cholesterol assimilation by *S. cerevisiae* ARDMC1 spent broth and resting cells, (D) SEM image of *S. boulardii* grown in YM broth without cholesterol, (E) *S. boulardii* grown with cholesterol, (F) cholesterol assimilation by *S. boulardii* spent broth and resting cells. 133

## LIST OF TABLES

---

### CHAPTER 2

Table 2.1.	Definition of probiotics	8
Table 2.2.	Probiotic efficacy of different yeast strains	14
Table 2.3.	Isolation of different probiotic bacteria from non- dairy fermented products	15
Table 2.4.	Incorporation of probiotic bacteria into functional foods	16

### CHAPTER 3

Table 3.1.	Different types of fermented food collected from various places of Assam and Arunachal Pradesh	36
Table 3.2.	Physiological and biochemical characterization of lactic acid bacteria ( <i>Lactobacilli</i> )	38
Table 3.3.	Physiological and biochemical characterization of lactic acid bacteria ( <i>Cocci</i> )	40
Table 3.4.	Physiological and biochemical characterization of spore forming <i>Bacilli</i>	41

### CHAPTER 4

Table 4.1.	Tolerance to simulated gastrointestinal transit tolerance; (A) simulated gastric fluid tolerance, (B) simulated intestinal fluid tolerance	59
Table 4.2.	Estimation of microbial growth parameters	68

### CHAPTER 5

Table 5.1.	Antimicrobial spectrum of the strain NK7	83
Table 5.2.	Simulated gastrointestinal tolerance of probiotics; (A) simulated gastric fluid tolerance and (B) simulated intestinal fluid tolerance	84
Table 5.3.	Box- Behnken Design arrangement for the independent variables and the response	87

Table 5.4.	ANOVA for Response Surface Quadratic Model	88
Table 5.5.	Antibiotic susceptibility test	94

## CHAPTER 6

Table 6.1.	Predicted and experimental values of <i>C. tropicalis</i> inhibition	114
Table 6.2.	ANNOVA table for the quadratic model of inhibition	115

## CHAPTER 7

Table 7.1(A)	Simulated gastric fluid tolerance test	132
Table 7.1(B)	Simulated intestinal fluid tolerance test	132
Table 7.2.	Autoaggrgation, coaggregation and hydrophobicity of the isolate ARDMC1 and <i>Saccharomyces boulardii</i>	133
Table 7.3.	Effect of probiotic isolates on serum lipids level (mg/dL) and body weight (g)	138