

CHAPTER 8

SUMMARY AND FUTURE PROSPECTS

Summary

- Out of total 210 strains isolated from various fermented foods of Assam and Arunachal Pradesh, 99 were presumptively characterized as lactic acid bacteria, 52 as *Bacillus* and 31 were characterized as yeasts.
- Maximum 23.94% of the isolates were found to be similar to the *Lactobacillus* cluster, followed by *Bacillus* cluster (13.38%).
- Isolates from rice beer starter culture showed maximum viability in acidic conditions, whereas isolates from fermented mustard seeds showed maximum viability in bile (0.3%) and maximum hydrophobicity was shown by the isolates from fermented bamboo shoot products.
- Bacterial isolates D6, DS1, NK7, NL6 and DK6 and yeast isolate ARDMC1 showed maximum probiotic characteristics compared to other isolated strains.
- The potential probiotic bacterial isolate DS1 was found to produce bacteriocin like anti-microbial substances. On the basis of biochemical and molecular characteristics the strain DS1 was identified as *Pediococcus pentosaceus*. On analysis of its genome class IIA bacteriocin pediocin (GenBank accession number KT345707) with an YGNGV motif was identified in *P. pentosaceus* DS1.
- The biofilm formed by *L. monocytogenes* AMDK2 was found to be maximum excluded ($55.54 \pm 3.44\%$) when co cultured with *P. pentosaceus* DS1.
- Maximum adhesion inhibition to Caco-2 cell line was observed in case of *L. monocytogenes* AMDK2 (91.8%), whereas maximum decrease in invasion was in case of *L. monocytogenes* MTCC 839 (52.9%).
- Co-culture of *P. pentosaceus* DS1 with pathogenic bacteria *Listeria monocytogenes* in milk significantly decreased its growth.
- *Bacillus* sp. NK7 (GenBank accession number KY923226) isolated from fermented mustard showed maximum antimicrobial activity against food pathogen *Bacillus cereus*. Optimum culture conditions for maximum antimicrobial activity were initial pH 7.92, carbon source (fructose) 40 mg/ml and nitrogen source (ammonium citrate) 35.61 mg/ml.

- The substance responsible for antimicrobial activity in *Bacillus* sp. NK7 was found to be a bacteriocin like substance (BLIS) having an apparent size of 20KDa.
- The strain *Bacillus* sp. NK7 did not show antibiotic resistance and haemolysis.
- Antifungal metabolites of the strain D6 could inhibit biofilm and germ tube formation of *Candida*. Minimum biofilm inhibitory concentrations of the metabolites were found to be 0.875 mg/mL and 0.438 mg/mL against *Candida albicans* and *Candida tropicalis* respectively.
- The strain D6 was identified as *Lactobacillus paracasei* and obtained a GenBank accession number KJ867173.
- The antifungal metabolites of *L. paracasei* D6 could significantly ($P < 0.05$) lower the adhesion of *Candida* to human colorectal (Caco-2) cell line.
- The GC- MS analysis antifungal metabolites of *L. paracasei* D6 showed the presence of 6 the major constituents: lactic acid, α -Hydroxyisocaproic acid, benzoic acid, 6-Octadecenoic acid, Benzeneacetic acid and 2-hydroxy, 3-methyl butanoic acid with previously reported antifungal properties and food- grade applications.
- An optimal combination of *C. tropicalis* inoculum of 3.67 log units, antifungal metabolite concentration of 1.75 mg/mL and heat treatment of 1.46 min of led to the maximum inhibition of *C. tropicalis* in fruit juice.
- The strain ARDMC1 was identified as *Saccharomyces cerevisiae* (GenBank accession numbers KF414969 and KP233782) and showed good probiotic characteristics as good as the commercially available probiotic strain *Saccharomyces boulardii* used for medication.
- *S. cerevisiae* ARDMC1 showed in vitro cholesterol lowering properties which could reduce 41.52% of cholesterol concentration in cholesterol- supplemented media within 72 h of incubation.
- Wistar rats supplemented with probiotic *S. cerevisiae* ARDMC1 showed significant decrease in the LDL- cholesterol, VLDL-cholesterol, triglyceride and total cholesterol levels in serum ($P < 0.05$) when compared with only high fat diet (HFD)

fed rats. This was comparable to the effects of the commercially available drug 'Statin' used for controlling blood cholesterol.

- The atherogenic index (AI) and the LDL/VLDL ratio, which are known as the markers of cardiovascular diseases, were also found to be decreasing in case of probiotic- treated groups.

Future prospects

- Purification of bactericins from the strains *Pediococcus pentosaceus* DS1 and *Bacillus* sp. NK7.
- *In vivo* expression studies of *Candida* virulent factors in the presence or absence of antifungal metabolites produced by *Lactobacillus paracasei* D6.