ABSTRACT

The present study deals with the isolation of microorganisms from selected fermented foods of Assam and Arunachal Pradesh and assessment of their probiotic attributes. Indigenous fermented foods were collected from the two largest states of North-East India, Arunachal Pradesh and Assam which share maximum area and a substantial population diversity. This part of the country also belongs to the Indo- Burma biodiversity hotspot region and home to different tribal communities which maintain a close relationship with nature. As a result, the preparation of fermented foods and beverages utilizing natural resources is ubiquitous in their culture. Apart from flavour enhancement and shelf- life improvement, fermented foods from this region are also known for their health- beneficial effects. So far, limited reports are available regarding the connection between health- benefits of traditional fermented foods and probiotics present in them. Considering the traditional knowledge about the health benefits of fermented foods, the study was carried out to assess the microbial diversity of fermented food collected from Assam and Arunachal Pradesh and to evaluate the probiotic properties of the culturable strains which show the potential to become a part of functional foods.

Thesis is divided into seven chapters which are briefly described below:

Chapter 1 is a brief introduction to the process of fermentation and the role of different types of microorganisms in the preparation of fermented foods. It also focuses on the importance of probiotics for health beneficial activities. This chapter also documented the diversity of fermented food prepared in different geographical regions of North-East India.

Chapter 2 contains review of literature dealing with definition of probiotics and their health benefits. Importance of the involvement of food matrices other than milk for carrying probiotics is also explained.

Chapter 3 describes the isolation of microbial strains from selected fermented foods of Assam and Arunachal Pradesh, India. The isolated microbial strains were characterized using biochemical techniques and clustered with previously identified probiotic strains. Out of total 210 strains isolated, 99 were presumptively characterized as lactic acid bacteria, 52 as *Bacillus* and 31 were characterized as

yeasts. All strains were characterized for probiotic characteristics and it was found that 5 isolates, i.e. D6, DS1, NK7, ARDMC1, NL6 and DK6 fulfilled the prerequisite criteria for probiotics and were clustered together with known probiotics.

Chapter 4 presents a study on the antilisterial activities of potentially probiotic strain *Pediococcus pentosaceus* DS1 isolated from fermented food. 16s rRNA gene sequence analysis confirmed the identity of the strain as *P. pentosaceus* DS. On genetic analysis class IIA bacteriocin pediocin expressing YGNGV motif was identified and its antilisterial activity was validated by well diffusion assay. When cocultured with *P. pentosaceus* DS1, in case of *L. monocytogenes* AMDK2 maximum biofilm exclusion (55.54 ± 3.44%) was observed, which could be visually observed by scanning electron micrographs. 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 MTCC 839 (52.9%). Coculture in UHT milk also significantly decreased microbial growth parameters of *Listeria* suggesting the efficiency of *P. pentosaceus* DS1 in the removal of *Listeria* contamination from food.

Chapter 5 aims at the evaluation of antimicrobial properties of strains isolated from an alkaline fermented mustard seed *kharoli,* and the optimization of culture conditions for maximum antimicrobial substance production. It was found that the strain *Bacillus* sp. NK7, as identified using 16S rDNA sequencing, showed antimicrobial activities against different pathogenic indicator strains. The optimized culture conditions for the antimicrobial substance production were found to be initial pH 7.92, carbon source (fructose) 40 g/L and nitrogen source (ammonium citrate) 35.61 g/L. Under these conditions, the strain showed maximum activity of 24.10 mm (zone of inhibition) against *Bacillus cereus,* which is a major food- borne pathogen. The antimicrobial substance was characterized and found to be a bacteriocin like substance (BLIS) with an apparent size of 20 KDa. The BLIS was stable at a wide range of temperature and pH and therefore it can be concluded that it has a potential to be used as a biopreservative in the food industry.

Chapter 6 deals with a *Lactobacillus* mediated approach for the inhibition of food spoilage isolate *Candida tropicalis* BSS7 showing potential virulent properties like biofilm and germ tube formation. Cell-free extract of *Lactobacillus paracasei* D6 exhibited minimum biofilm inhibitory concentration of 0.438 mg/mL against *C.*

tropicalis BSS7, which was found to be sufficient to inhibit its germ tube formation. The adhesion of *C. tropicalis* BSS7 to the epithelial Caco-2 cell line was also significantly reduced by the antifungal metabolites. GC-MS analysis of the exometabolites revealed the abundance of 6 different compounds with potential antifungal properties. An optimal combination of inoculum 3.67 log CFU/mL, 1.75 mg/mL of antifungal metabolite and 1.46 min of heat treatment at 70 °C led to the maximum inhibition of *C. tropicalis* in a fruit juice model. This is the first report of inhibition of virulence factors of *Candida* using antifungal metabolites of *Lactobacillus* in an *in vitro* model of intestinal epithelia. Moreover, the model for synergistic effect of *Lactobacillus* derived antifungal metabolites and temperature treatment on *Candida* inhibition also gives an idea about an alternative strategy for the prevention of food spoilage.

Chapter 7 aims at the investigation of probiotic attributes of *Saccharomyces cerevisiae* ARDMC1 isolated from traditional rice beer starter cake and its hypocholesterolemic effects on Wistar rats fed with high cholesterol diet. The indigenous isolate ARDMC1 showed potential probiotic characteristics such as tolerance to simulated gastrointestinal stress conditions, autoaggregation properties, and adhesion to intestinal epithelial cell line (Caco-2 cell line). In addition, ARDMC1 isolate exhibited *in vitro* cholesterol assimilation properties in media supplemented with cholesterol. Furthermore, administration of probiotic isolate to rats fed with hypercholesterolemic diet resulted in significant reduction of serum total cholesterol, low-density lipoprotein cholesterol and triglyceride at the end of 42 days. The present study envisages ARDMC1 as a promising starter culture for the preparation of functional foods with properties to combat cardiovascular diseases.

Chapter 8 contains the summary and future prospects of the present work.