ABSTRACT

In this study, potential probiotic properties of selected isolates obtained from raw goat milk, fermented milk samples (Doi), and fermented soybean (Churpi) have been studied for their functional attributes and applications in food and feed. The samples were collected from Assam and Arunachal Pradesh, North-East India, considered to have unique environment and harbor a variety of novel microbial strains. North-East India falls under Indo-Myanmar biodiversity hot spot region and it favors a large number of organisms including microorganisms especially having food and medicinal significance. This region is inhabited by various indigenous communities having a rich food and cultural heritage. The diets of these ethnic groups include traditional fermented foods that are known to have health and therapeutic values. So far, many probiotic strains have been documented till now. However, search of novel strains that can contribute to human and animal nutrition as part of new functional foods remain to be explored. The processed foods that are high in fat or modern life styles that are full of strain can disturb the delicate balance of healthy bacteria in the digestive tract. Keeping this in mind, investigation of probiotics or live beneficial bacteria from traditional fermented food sources was conducted to meet the requirement of helpful microflora.

Thesis is divided into eight chapters which are briefly described below.

Chapter 1 describes the history of probiotic and its consensus definition. It also envisages a great scope in challenging today's health related issues. It focuses briefly on the application of probiotics for human and animal health. This chapter also introduces the ecological significance of sample collection area and available traditional fermented foods.

Chapter 2 illustrates the various types of fermented milk samples and fermented soybean products consumed in India and worldwide. This chapter describes the origin of fermented food and dominant microbes present in it. In addition, the health benefits imparted by these fermented food products have been also discussed. The two major probiotic groups, lactic acid bacteria (LAB) and the *Bacillus* probiotic are mentioned

in this section. The probiotic benefits and their mode of action is also explained briefly.

Chapter 3 deals with *Lactococcus lactis* AMD17 isolated from free range goat milk sample collected from Tezpur, Assam (India). The potential probiotic attributes based on functional traits such as resistance to simulated gastric acid and bile salts, antimicrobial activity and inhibition of pathogens adhesion to intestinal epithelium cell line Caco-2 cells were evaluated. The isolate significantly reduced the adherence of food borne pathogens *Listeria monocytogenes* AMDK2 (47.46 ± 0.17 %) to Caco-2 cells. Honey was used as an adjuvant of *L. lactis* AMD17 for preparation of dahi (curd) from buffalo milk and was found to support its survivability during storage (P <0.05). Sensory evaluation studies revealed dahi prepared with *Lactococcus lactis* AMD17 with addition of 3% honey exhibited highest score in taste and color. The texture characteristics were found superior to dahi prepared with only *Lactococcus lactis* AMD17. Moreover, the Nisin gene was amplified and showed a similarity of 100% to other NisR-producing *Lactococcus lactis* AMD17 strains. The present study suggests that dahi prepared using honey enriched milk with nisin-producing probiotic strain *Lactococcus lactis* AMD17 imparts health benefit and combats food borne pathogens possibly due to the antibacterial features of nisin peptide.

Chapter 4 reports the potential probiotic attributes of *L. plantarum* AMD6 isolated from fermented milk sample (Doi) and its cholesterol assimilation property. The bile salt hydrolase (BSH) gene responsible for the cholesterol-reducing activity was cloned and characterized. The recombinant AMD6 BSH-His showed a slight preference for glycine-conjugated bile salts. The cholesterol-lowering ability of *L. plantarum* AMD6 was investigated in high fat experimental diet induced rat model. The rats were divided into five groups fed on experimental cholesterol-enriched diets for 42 d. Interestingly, the probiotic fed treatment group, HFD+AMD6 (10^8 CFU/ml) exhibited a significant (P< 0.05) decrease of body weight as compared to normal and high fat diet animals after 42 d of experiment. Similarly, the HFD+AMD6 animal showed significant reduction of serum TC, TG, and LDL-C levels to 65.45, 123.30, and 16.89 mg/dl, respectively, after 6 weeks. In conclusion, AMD6 isolates have the potential to be explored as probiotics in the management of cardiovascular diseases.

Chapter 5 deals with the microorganism showing probiotic attributes and hydrolyzing carboxymethyl cellulose isolated from traditional fermented soybean (Churpi) and identified as *Bacillus amyloliquefaciens* by analysis of 16S rRNA gene sequence and named as *B. amyloliquefaciens* AMS1. The potentiality of this isolate as probiotic was investigated *in vitro* and it showed gastrointestinal transit tolerance, cell surface hydrophobicity, cell aggregation and antimicrobial activity. The isolate was found to be non-hemolytic which further strengthens its candidature as a potential probiotic. The maize straw digestion was confirmed by scanning electron microscopy studies. The isolate was able to degrade filter paper within 96 hours of incubation. This study explores the possibility of combining the cellulose degrading ability of a microbe with its probiotic attributes to enhance gut health of animal and digestibility of the feed.

Chapter 6 reports the identification of cellulase encoding gene from potential probiotic *Bacillus amyloliquefacies* AMS1 and its *in-silico* studies. Further, cloning of endoglucanase gene and its heterologous expression in *E. coli* BL21 (DE3) has been investigated. The purified endoglucanase enzyme was found to be thermo–active and enhanced activity was observed in the presence of manganese salt. A full length endoglucanse gene of AMS1 was amplified and yielded approximately 1500 base pairs. The ORF encoded for a protein of 499 amino acid residues with the molecular weight (Mw) and isoelectric point (pI) predicted to be 55.23 kDa and 7.65, respectively. The amino acids sequence consists of a Glycosyl hydrolase family 5 domain (50–296 bp); Imm51 core (Immunity protein; 110–166 bp) fused to a CBM-3 (cellulose binding domain; 356–436 bp) at its C terminal. Docking result showed that CMC binds to endoglucanase AMS1 with a binding energy of -7.97648 kJ mol^{-1} and showed the presence of 12 putative residues of endoglucanse AMS1 that contact with CMC through hydrophobic interaction (Asn134, Lys33, His131, Gln297, Lys296 and Trp69) and hydrogen bonding with Ala98, Asp99, Thr97, Ala36, His65. The purified recombinant endoglucanase protein was characterized and shows maximum activity within a pH range (4.0-7.0) with an optimum at pH 5.0 (0.41 U/ml). It also showed maximum activity within a broad range of temperature (10 °C - 90 °C) with an optimum at 50 °C (0.43 U/ml). The purified enzyme was characterized and utilized for saccharification of acid pretreated maize straw as the enzymes have shown

potential for the agri-biotechnological processes and could be employed as animal feed.

Chapter 7 presents a study on the probiotic attributes of *Bacillus subtilis* AMS6 isolated from fermented soybean (Churpi). This isolate exhibited tolerance to low pH (pH 2.0) and bile salt (0.3 %), capability to autoaggregate and coaggregate. AMS6 also showed highest antibacterial activity against the pathogenic indicator strain *Salmonella enterica typhimurium* (MTCC 1252) and susceptibility towards different antibiotics tested. The isolate was effective in inhibiting the adherence of food borne pathogens to Caco-2 epithelial cell lines, and was also found to be non-hemolytic which further strengthen the candidature of the isolate as a potential probiotic. Further studies revealed *B. subtilis* AMS6 showed cellulolytic activity (0.54 ± 0.05 filter paper units mL^{-1}) at 37 °C. The isolate was found to hydrolyze carboxymethyl cellulose, filter paper and maize (*Zea mays*) straw. The maize straw digestion was confirmed by scanning electron microscopy studies. The isolate was able to degrade filter paper within 96 hours of incubation. A full length cellulase gene of AMS6 was amplified consisting of 1499 nucleotides. The ORF encoded a protein of 499 amino acids residues with a predicted molecular mass of 55.04 kDa. The amino acids sequence consisted of a glycosyl hydrolase family 5 domain at N-terminal; Glycosyl hydrolase catalytic core and a CBM-3 cellulose binding domain at its C terminal. The study suggests potential probiotic *Bacillus subtilis* AMS6 as a promising candidate envisaging its application as an animal feed additive for enhanced fiber digestion and gut health of animal.

Chapter 8 contains summary and future prospects of the work.