ABSTRACT OF THE Ph.D. THESIS

The growing interest in health and wellness of human beings has given emphasis to research studies in the areas of probiotics and prebiotics as well as the recent concept of synbiotics. Biotechnological approaches have enabled in enhancing the nutritional/health status of many of these foods through the interaction between prebiotics and probiotics. The use of potent microbial cultures with desirable traits could have better applications in obtaining quality consistent products. This could be achieved through the use of desirable cultures of lactic acid bacteria, bifidobacteria and yeasts with enhanced desirable attributes with focus on characterization and application of potent beneficial attributes.

Three well-characterized natural isolates of Lactobacillus plantarum MTCC 5422, Bifidobacterium adolescentis MTCC 5423 and Saccharomyces cerevisiae MTCC 5421 were obtained from a diverse range of foods and related samples. The cultures of Lb. plantarum and Bif. adolescentis showed positive PCR amplification with oligonucleotide primers targeting genus specific 16S rRNA for Lactobacillus and fructose-6-phosphate phosphoketolase for Bifidobacterium. Similarly, species specific positive amplification in PCR was observed with primers of phytase (acid phosphatase) in Sac. cerevisiae and α-D-galactosidase and bile salt hydrolase in Lb. plantarum and Bif. adolescentis. The cultures of Lb. plantarum and Bif. adolescentis exhibited a broad spectrum antibacterial activity against selected foodborne pathogenic bacterial species and tolerance to acid and bile. Genetic relatedness based on selected attributes revealed almost 90-95% homology with other closely related species and genera in the phylogenetic tree.

Growth medium formulated based on soy whey broth enabled the culture of Sac. cerevisiae MTCC 5421 to produce a maximum of 198 U/ml of phytase activity in 36 h of growth. Similarly, Lb. plantarum MTCC 5422 was able to elaborate 10.6 U/ml of α-D-galactosidase and 13.4 AU/ml of antibacterial activities in 36 h. Optimization of cultural
attributes by central composite design and therein generated response surface plots revealed the range of variables within the defined factors for obtaining appreciable levels of activities of desirable attributes in the respective microbial cultures.

The extracellular phytase elaborated by Sac. cerevisiae MTCC 5421 in a formulated soy whey broth medium was partially purified with a 5.6 fold purification and activity of 20.4 U/ml and specific activity of 63.7 U/mg, protein. The partially purified phytase was able to completely reduce phytic acid in flours of pearl millet, whole wheat and refined wheat. The partially purified intracellular α-D-galactosidase of Lb. plantarum MTCC 5422 exhibited an activity of 1.2 U/ml and specific activity of 12 U/mg, protein. The incubation of α-D-galactosidase treated flours of green gram, cow pea, double bean and soy bean for 2 h at 45°C resulted in the reduction of raffinose and stachyose in the range of 50-74% and 66-85%, respectively.

A probiotic soy curd was prepared with cow milk added with soy protein isolate and inoculated with the potent cultures of Lb. plantarum MTCC 5422 and Bif. adolescentis MTCC 5423 along with desirable quantity (10 U/ml) of partially purified α-D-galactosidase of Lb. plantarum. The probiotic soy curd revealed a decrease in levels of flatulence causing oligosaccharides (FCOs) to an extent of 90-99% that could be attributed to the action of α-D-galactosidase on stachyose and raffinose present in soy. HPLC profile of short chain fatty acids (SCFAs) in probiotic soy curd revealed that lactic acid was in higher quantity, followed by acetic acid, propionic acid and butyric acid. The formation of SCFAs is dependent upon a favourable growth of Lactobacillus sp. and Bifidobacterium sp. utilizing the available carbohydrates as the substrate.

Similarly, pearl millet-based rabadi, a traditional fermented beverage was prepared by incorporating partially purified extracellular phytase of Sac. cerevisiae MTCC 5421 prior to the process of fermentation. The product was inoculated with probiotic cultures of Lb. plantarum MTCC 5422 and Bif. adolescentis MTCC 5423. The
experimental sample showed complete hydrolysis of phytate during the fermentation period of 6 to 12 h. The profile of SFCAa showed the following pattern with butyric acid being highest, followed by acetic acid, propionic acid and lactic acid.

In another popular wheat based fermented and baked product namely nan, maida dough treated with phytase of Sac. cerevisiae MTCC 5421 for 60 min at 50°C resulted in the product with almost 95% reduction of phytic acid. The products prepared with beneficial attributes of selected microbial cultures were microbiologically stable with the absence of any viable populations of Bacillus cereus, Escherichia coli and Staphylococcus aureus.

Research studies did established the health benefits through prebiotic-probiotic interactions. However, it becomes important to characterize the desirable attributes in the potent cultures with appropriate biochemical and molecular markers. Overall, it should lead to appreciable viable populations of probiotic cultures in the gastrointestinal tract for a better human health.