SUMMARY

In the present study, lactic acid bacteria were isolated from South Indian fermented foods Kallapam, Koozhu and Mor kuzhambhu. A total of six LAB strains were screened based on antibacterial activity. These strains were characterized by morphology, biochemical assays, carbohydrate fermentation patterns and phylogenetic classification based on 16S rDNA approach. Identification by 16S rDNA sequence homology of the isolates revealed the presence of Weissella paramesenteroides, Lactobacillus plantarum and Lactobacillus fermentum. Lactobacillus plantarum AS1 isolated from Kallapam showed maximum antibacterial activity among selected strains. Furthermore, it showed tolerance at different temperatures (10-45 °C) and pH (4.4-10.0) conditions. Therefore, Lactobacillus plantarum AS1 was preferred over other LAB strains for further study. Similarly, Streptococcus phocae PI80 and Enterococcus faecium MC13 isolated by from the gut of Penaeus indicus and Mugil cephalus respectively were employed in this study. These two LAB strains were isolated by previous researcher in the laboratory. Antibacterial protein bacteriocins of the above three selected strains were characterized and purified to homogeneity. Purification of bacteriocin protein was achieved by application of ultra-filtration, ammonium sulphate precipitation, dialysis, sephadex G-25 gel exclusion chromatography and reverse phase-high performance liquid chromatography in a step-wise manner. Furthermore, mass of bacteriocins were determined by mass assisted laser desorption ionization-mass spectrometry. Molecular mass of S. phocae PI80, E. faecium MC13, and L. plantarum AS1 were identified to be 9.26 kDa, 2.15 kDa, and 3.35 kDa respectively. All the three bacteriocins showed broad antibacterial spectrum towards Gram-positive and Gram-negative bacteria. Above selected strains L. plantarum AS1, S. phocae PI80 and E. faecium MC13 were screened for probiotic properties in in vitro conditions. They were subjected to bile
salt tolerance, artificial gastric juice tolerance, cholesterol reduction and HT-29 cell line colonization assay. All three strains were able to tolerate bile salt at the concentration equivalent to that found in human gut. However, *L. plantarum* AS1 and *E. faecium* MC13 showed delayed growth response. During artificial gastric juice tolerance assay, *L. plantarum* AS1 survived up to 24 h at pH 2.0. However, *S. phocae* PI80 and *E. faecium* MC13 could survive only up to 3 h and 2 h respectively. Similarly, *L. plantrum* AS1 assimilated more cholesterol compared to other two strains. In case of HT-29 binding assay all three LAB strains colonized HT-29 cell line but again *L. plantarum* AS1 was efficient compared to other two. The strain *L. plantarum* AS1 was selected for medical application study as this was isolated from food and was efficient compared to LAB used in this study. *L. plantarum* AS1 was incubated with HT-29 adenocarcinoma cell line to assess its adhesion potency and examined for its inhibitory effect on the cell attachment by an enterovirulent bacterium *Vibrio parahaemolyticus*. *L. plantarum* AS1 attached efficiently to HT-29 cells as revealed by scanning electron microscopy and bacterial adhesion assay. *L. plantarum* AS1 significantly reduced *V. parahaemolyticus* attached to HT-29 cells by competition, exclusion and displacement mode. *L. plantarum* AS1 seems to adhere to human intestinal cells via mechanisms that involve different combinations of carbohydrate and protein factors on the bacteria and eukaryotic cell surface. Furthermore, *L. plantarum* AS1 was studied for its anti-colorectal cancer effect over 1,2-dimethylhydrazine subjected Wistar rats. Rats were divided into six groups consisting six rats each based on the type of treatments received. At the end of study period of 26 weeks all the rats were sacrificed and colon tissues and plasma were used for biochemical assays. Further, tissues were preserved in buffered formalin and subjected to histology. In this study, an increased level of lipid peroxide (LPO) products and increased activities of antioxidant enzymes (superoxide dismutase, catalase and glutathione-S transferase).
and marker enzymes (alkaline phosphatase and acid phosphatase) in colon and plasma of cancer-bearing animals have been observed. *L. plantarum* AS1 was supplemented either before initiation or during initiation and selection/promotion phases of colon carcinogenesis and was found to be effective in altering lipid peroxidation and antioxidant enzyme activities and marker enzymes to a statistically significant level measured either in the colon and in the plasma. These alterations inclined towards normal in a time-dependent manner on *L. plantarum* AS1 supplementation. The mean tumor volume diameter and total number of tumors were found to be statistically decreased in *L. plantarum* AS1 pre & post treated rats. Furthermore, histological examination shows remarkable difference between control and treated groups. The *in vitro* antioxidant assay shows that *L. plantarum* AS1 has promising antioxidant property. These results demonstrate that *L. plantarum* AS1 strain can modulate the development of DMH-induced rat colon carcinogenesis through an antioxidant-dependent mechanism. Overall, study demonstrated the probiotic property of *L. plantarum* AS1 and its disease prevention capability. Also, *S. phocae* PI80 and *E. faecium* MC13 could be used as aquaculture probiotics as they showed antibacterial activity against aquaculture pathogens *Vibrios*, and *Aeromonas*. The bacteriocin protein of above LAB strains could be employed for biopreservation of seafoods and cheeses.