Chapter 6: Summary
6. Summary

Fresh vegetables are the yet let explored source for isolation of LAB; this thesis work successfully exploited for isolation of LAB from one of the unconventional source that is fresh vegetables. The isolates were obtained from fresh vegetables which were collected from the fields of Pune region. Tomato, cauliflower, gherkins and fenugreek were found as favourable vegetables for LAB colonization. Primary characterization and identification revealed that, all isolates from this study are closely related to the genera *Lactobacillus, Enterococcus, Lactococcus, Leuconostoc, Pediococcus* and *Weissella*. Probiotic potential of the isolates was tested in artificial intestinal conditions; the tolerance to acidic pH, inhibitory bile salts and pancreatic enzymes. Lactic acid bacterial isolates from vegetable origin successfully survived in artificial intestinal conditions proving their suitability as probiotic. The plant probiotic potential is successfully tested and compared with isolates from different sources such as human origin and fermented food. Plant probiotics found at par in comparison to standard probiotics from human origin *Lactobacillus plantarum* MCC 2156 and selected isolates from fermented food source. Isolates with probiotic potential further tested for the additional bioactive potential such as, antioxidant activity by two methods TBARS and Comet assay. Several isolates showed antioxidant synthesis and displayed the ability to inhibit lipid peroxidation indicating it’s application in food preservation which will be helpful to enhance shelf life. LAB isolated found suitable in offering protection against genotoxic stress. Best isolates offering protection in DNA damage due to oxidative stress were selected for probiotic formulation preparation. The vegetable isolate *Lactobacillus plantarum* AG40V, *Enterococcus* sp. ID11V and *Enterococcus* sp. ID19V proved its safety with least percent. Two isolates from other sources *Lactobacillus* sp.HO17HF and *Lactobacillus* sp.SK2FB were selected which offered best protection. Overall, the isolates were found non genotoxic and protect host from oxidative damage. The Lactic acid bacterial isolates successfully inhibited the plant and human pathogens indicating its future application in food preservation. SCFAs have been pointed out as the link between diet, gut microbiota, and host energy metabolism. SCFA act as the immunemodulator and maintain gut homeostasis. Isolates from present study synthesizes SCFA indicating its health application. According to FSSAI and WHO guidelines the probiotic strain should be assessed for the safety. Safety assessment of the selected isolates was done by antibiotic sensitivity testing. Newly isolated strains before use in the probiotic fortified food should be known for
sensitivity pattern against different antibiotics. The five isolates *Lactobacillus plantarum AG40V*, *Enterococcus* sp. ID11V and *Enterococcus* sp. ID19V, *Lactobacillus* sp.HO17HF and *Lactobacillus* sp.SK2FB which was selected for the preparation of probiotic formulation was tested for antibiotic sensitivity. One of the emerging threat of antibiotic resistance can be prevented by the use of these isolates.

Further these selected strains were tested *ex-vivo* to investigate gene modulatory effects and therapeutic application on human colon epithelial cell line, Colorectal adenocarcinoma [Caco2 (ATCC®HTB37™)]. The results obtained explains that, the metabolites secreted by *Lactobacillus plantarum AG40V*, *Enterococcus* sp. ID11V and *Enterococcus* sp. ID19V are down regulating expression of genes which enhance inflammation and upregulation of genes which are involved in apoptosis process; confirms the beneficial effects the probiotic bacteria can have on colorectal cancer and inflammatory diseases and disorders. Probiotic bacteria from the genera *Lactobacillus* and *Enterococcus* demonstrated expression of beneficial genes in colorectal cancer cells which points out the health benefits of these bacteria.

To compare the probiotic potential of bacteria isolated from plants, humans, and fermented foods, four probiotic formulations were prepared with *Enterococcus* sp. ID11V (from vegetables), *Enterococcus* sp. ID19V (from vegetables), *Lactobacillus* sp. HO17HF (from human origin) and *Lactobacillus* sp. SK2FB (from fermented food). All four probiotic formulation found stable on storage at 4° C for 60 days. There was no change in appearance, color and bacterial count of the probiotic formulations indicating the stability of LAB cells and formulation ingredients.

Safety and efficacy testing of any drug or formulation is the primary requisite as it would be the ultimate product for human and/or animal implications. The safety assessment was done in two ways acute toxicity and sub-acute toxicity. In line to *in vitro* test the probiotic formulation found safe on Sprague-Dawley rat. All four probiotic formulations found safe in acute and sub-acute oral toxicity study. In sub-acute toxicity study the faecal analysis; probiotic formulation successfully reduced the pathogen *E.coli* and *Klebsiella sp.* increasing amount of beneficial flora. No significant difference was observed in haematology parameters in male and female rats fed with probiotic formulations, compared to control male and control female respectively. In addition to safety the probiotic formulation was also successfully reduced chemically induced inflammation compared to standard drug (Prednisolon).
Apart from health application, isolates from this study found applicable in food preservation. One of the potent strain *Lactobacillus plantarum* AG40V successfully reduced the pathogenic attack and increased the shelf life of fresh cut salad and sprouted mung beans. Metabolites synthesized by the LAB isolates proves it nutraceutical property and health application.

Overall prepared probiotic formulations using Lactic acid bacterial isolates from fresh vegetables found safe, non-toxic, protecting host against oxidative damage, pathogenic attack and reducing intestinal inflammatory conditions, synthesizing SCFA which will have beneficial role to mankind.