Summary & Conclusion
5. SUMMARY AND CONCLUSION

A healthy human GIT is a quite complex microbial ecosystem that facilitates normal physiological functions of the host organism unless harmful and potentially pathogenic bacteria dominate it. In order for the intestine to function optimally, the 'balance' of the bacteria must be maintained and it seems to be increasingly difficult due to change in way of life. Several factors may alter the balance of the gut microflora towards high proportion of potentially harmful or pathogenic microorganisms, like clostridia. Predominance of these harmful organisms may be a cause of a number of clinical disorders. Microflora modulation towards beneficial organism, can occur through food stuffs that contain sufficient level of prebiotics that target the (potentially) health promoting flora i.e. lactobacilli and bifidobacteria. Therefore, maintenance of a good community structure in the intestine through increased high proportion of health promoting bacteria such as lactobacilli and bifidobacteria by systematic supplementation of the diet with probiotics, prebiotics or synbiotics. Hence, the present investigation was undertaken to select a Lactobacillus culture based on probiotic attributes and to prepare a freeze-dried synbiotic product incorporating the selected Lactobacillus culture and prebiotics (inulin and gum acacia). The major results obtained during course of this study are summarized as follows:

5.1 A total of 50 samples of food and fecal were used for the isolation of lactobacilli using Lactobacillus selection MRS agar as the growth medium.

5.2 Based on Gram staining technique and catalase negative reaction, 20 isolates were selected for further studies.
5.3 All the isolated cultures, when incubated in MRS broth at 15°C for 7d, showed the turbidity as the indication of microbial growth except two isolate. Similarly, when isolates were incubated at 45°C for 24-48h, the turbidity was observed only in one and thus representing their wide range of temperature tolerance indicating them to be from lactobacilli genera.

5.4 All the isolates were subjected to phenotypic characterization i.e. nitrate reduction, gas production from glucose, aesculin hydrolysis, arginine hydrolysis test and utilization of different carbohydrates and were confirmed as lactobacilli genera.

5.5 The isolates showed different types of sugar utilization pattern which were confirmed by comparing with the Bergey's Manual of Determinative Bacteriology. Twenty isolates were identified as *L. casei subsp.casei*, *L. brevis* of exogenous origin; and *L. fermentum* and *L. plantarum* from indigenous sources.

5.6 After final identification, total 4 cultures one from each species i.e. *L. casei subsp.casei*, *L. brevis* *L. fermentum* and *L. plantarum* were randomly selected for their *in vitro* evaluation of probiotic attributes.

- All selected cultures were screened, for their tolerance to simulated pH of the human stomach. Lactobacilli cultures at the rate of $10^9$ to $10^{10}$ cfu/mL were exposed to acidified distilled water (1.5, 2.0, 2.5 and 3.0). The number of surviving cells was determined immediately on exposure (0h) and after 1, 2 and 3h of incubating at 37°C. The pH 1.5 was observed to be inhibitory than other pH levels. The strain, *L. plantarum* gave best results as it could survive at pH 1.5 for 3h in simulated acidic conditions with a reduction of 5.1 log.
• In order to study bile tolerance, the cultures were exposed to Solutions of different ox bile concentrations (0, 1.0, 1.5, 2.0 and 3.0%) prepared in autoclaved distilled water. Viability of cultures was observed at 0, 3 and 12h. *L. plantarum* exhibited better bile tolerance than other three strains. This organism revealed most stable characteristics as the strain resisted the 1.0, 1.5 and 2.0% bile up to 12h of incubation with maintaining a consistent viable count more than 4 log cycles.

• The most important criteria for a potentially probiotic strain is supposed to be its ability to adhere to intestinal mucosa of the human GIT. As the cell surface hydrophobicity is a sign of the ability of cells to adhere to epithelial cells, the cultures were screened for their cell surface hydrophobicity on the basis of their adherence to the hydrocarbons; n-hexadecane, xylene and toluene. The wide variations were observed in their cell surface hydrophobicity ranging from 21.20 to 70%. The highest values of hydrophobicity were exhibited by the *L. plantarum* ranged from 46.40 to 70%.

• Antimicrobial activity is thought to be an important means for probiotic bacteria to competitively exclude or inhibit activities of harmful or pathogenic intestinal microbes. For this, Lactobacilli cultures were tested for their antagonistic activity against indicator strains, *E. coli* MTCC443, *E. faecalis* MTCC439, *S. aureus* MTCC87, *B. cereus* NCDC240 and *S. typhimurium* NCDC113. All lactobacilli were effective against all other enteric organisms. However, among the lactobacilli cultures, *L. plantarum* produced maximum antibacterial activity against *S. typhimurium* NCDC113 with inhibition zones of 14.86mm in diameter.
5.7 A non-digestible food ingredient called prebiotics that beneficially affects the host by selectively stimulating the growth or activity of one or limited number of bacteria such as bifidobacteria, lactobacilli in the colon that can improve the host health. Inulin and gum acacia are the most commonly available prebiotics. The ability of lactobacilli cultures to utilize inulin and gum acacia were determined in carbohydrate free modified MRS broth with BCP, as the basal medium. All the cultures were able to utilize inulin and gum acacia. However, on the basis of highest \( \mu \) on inulin supplementation, the growth stimulatory effect of inulin on \( L. \) \textit{plantarum} was considerably higher than that on other three cultures.

5.8 On the basis of \textit{in vitro} probiotic attributes, it was accomplished that the overall performance of \( L. \) \textit{plantarum} was found best among the cultures of lactobacilli. Thus far, on the basis of its greatest performance in terms of pH tolerance, bile tolerance, cell surface hydrophobicity, antimicrobial activity and utilization of prebiotics, the culture \( L. \) \textit{plantarum} was selected for the preparation of synbiotic powder and further study.

5.9 In order to evaluate whether the incorporation of a prebiotics in the growth medium of the selected culture \( L. \) \textit{plantarum} has any enhancing effect on the antimicrobial activity of this culture, the antimicrobial activity of organism in glucose containing media was compared with that grown in prebiotics (inulin and gum acacia) containing media. However, no enhancing effect was observed on incorporating prebiotics in the growth medium, slightly the antibacterial activity exhibited by \( L. \) \textit{plantarum} grown in prebiotics containing media was lower than that of \( L. \) \textit{plantarum} grown in glucose added media.
5.10 Optimal performance of strains incorporated in synbiotic product must at first guarantee their potential to survive during freeze drying and retain the viability after a long storage. For that reason, the development of products in which a suitable level of viable probiotic cells are retained for a longer period is one of the key research and development area for probiotic foods. As prebiotics selectively enhance the multiplication of desirable bacteria, consumption of prebiotics along with probiotics might result in a competitive advantage for the probiotic. In the present study, a freeze-dried synbiotic formulation was prepared incorporating *L. plantarum* and prebiotics (inulin and gum acacia) using non fat dry milk as base material. The product contained very high numbers of viable counts in the range of 8 to 9 log cfu/g of viable lactobacilli cells, fulfilling the criteria of consisting viable populations of more than 7 log cfu/g to bring out the beneficial effect.

5.11 The achievement of probiotic food development relies on the maintenance of viability and activity of probiotic culture during storage. Therefore, viable counts of *L. plantarum* in the synbiotic preparation were examined at refrigeration and room temperatures for a period of 90d. The viable count in synbiotic powder remained in the range of 8 to 9 log cfu/g even after 90d of refrigerated storage; while in the case of room temperature distinct reduction by 5 to 6 log cfu/g was observed.
CONCLUSION

Developments in functional foods markets are being driven through the change in consumer's attitude and expectations by the use of fortified food with specific ingredients imparting certain health benefits. In this milieu, development of synergistic probiotic and prebiotic products (synbiotics) has a significant role. For the development of synbiotic foods, qualified active probiotic bacterial cultures are added in, which are capable in maintaining high cell numbers throughout its shelf-life. Moreover, the effectiveness of probiotic foods can be enhanced by exploiting synergistic interaction between functional ingredients. All these aspects were taken into consideration in the present study, in which a freeze-dried lyophilized synbiotic formula was developed incorporating L. plantarum, selected on the basis of its probiotic attributes under simulated GIT conditions of human being and prebiotics, inulin and gum acacia that were selected based on their effects on probiotic. The synbiotic product retained high and constant levels of the probiotic culture even after 90 d of refrigerated storage indicating a possible use of these beneficial microorganisms as food supplements. However, in vivo testing in mice and human volunteer trials are to be needed to reach a final conclusion as it is the ultimate way to reveal the health benefits in humans.