ABSTRACT CUM SUMMARY OF THE THESIS

Thesis title: Studies on native *Lactobacillus* isolates for their potential use against gut dysfunction

Probiotics are living microorganisms which confer health benefits on the host when administered in sufficient amounts. Several bacterial species are used as potential probiotics. Most of them belong to the genera *Lactobacillus, Bifidobacterium* and *Streptococcus*. The health benefits established by these bacteria include stabilization of indigenous microflora, protection against intestinal infection, non-specific enhancement of the immune system, alleviation of lactose intolerance, reduction of serum cholesterol, prevention of cancer and cardiovascular diseases. The enhancement of epithelial barrier function is also one of the proposed mechanisms by which certain probiotic organisms may confer health benefits. Increased intestinal permeability or "leaky gut" is recognized as having a role in the pathophysiology of a variety of gastrointestinal disorders and is observed in inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), celiac disease, and early stages of colon cancer. Altered permeability also causes cytokine induced changes in the tight junction and a vicious cycle of mucosal barrier dysfunction and inflammation, increase in the load of bacterial and dietary antigens in the lamina propria. In ulcerative colitis, activation of mucosal immune system leads to impaired epithelial barrier function and tissue destruction with relapsing diarrhoea. The barrier function is maintained by the mucosal layer as well as junctional complexes such as adherence junctions and tight junctions (TJ). Enhancement in barrier integrity is associated with changes in the TJ structure via alteration in TJ protein expression and distribution. Some probiotics are known to

improve the intestinal epithelial barrier by altering the expression or distribution of TJ proteins. The modulation of epithelial barrier function by probiotic is a highly relevant target for novel therapeutic or prophylactic treatments against a range of diseases. Since the probiotic properties and claimed health benefits possessed by different bacteria varies widely, it becomes necessary to screen and characterize numerous strains in order to obtain probiotics with a particular health benefit. So the aim of the study was to isolate lactobacilli from human stool samples and fermented foods of Indian origin and assess them for their potential use against gut dysfunction.

The health benefits upon consumption of fermented foods containing lactobacilli have a long history. Human gastrointestinal tract harbours several bacterial species amongst which lactobacilli are from one of the major groups. In the present study, lactobacilli were isolated from fermented foods and human stool samples of Indian origin. The isolates were confirmed by 16S-23S rRNA gene intergenic region amplification and subsequent sequencing. The organisms were identified by BLAST analysis and the sequences were submitted to Genbank. When used as a probiotic, lactobacilli are commonly delivered in a food system, therefore, they must possess the ability to resist the digestion process in the stomach and intestine. So the isolates were analysed for their ability to resist acid and bile. To evaluate the isolates, the established probiotic strain *L. rhamnosus* GG (LGG) was incorporated as a control in the study. After travelling through this harsh environment, the organism colonizes the epithelium of the lower intestinal tract. The survival of probiotic organisms in the gut depends on the colonization factors that they possess as well as on the organelles which facilitate them to resist the antibacterial mechanisms that operate in the gut. In addition to the antibacterial mechanisms, they need to avoid the effects of peristalsis, which tend to flush out bacteria with food. This can be achieved either by immobilizing themselves

or by growing at a much faster rate than the rate of removal by peristalsis. Thus, strains selected for use as probiotic bacteria should be able to tolerate acid and bile acids, attach to the epithelium, and grow in the lower intestinal tract before they can start providing any health benefits.

To evaluate their ability to survive in the acidic environment, the isolates were incubated in acidic pH of 2.5 for 2 h and their survival rate was found to be in the range of 69% to 81%. Amongst them, *L. fermentum* FA-5 and *L. helveticus* FA-7 had significantly higher survival rate than the standard probiotic strain LGG. When the survivability of the isolates was assessed at 0.3% and 1% bile concentration, *L. fermentum* strains GKI-1, FA-1 and FA-5 had significantly higher survivability than that of the standard strain LGG at both the concentrations. Further, these isolates were analysed for their antimicrobial activity against both Gram-positive and -negative bacteria and susceptibility towards different antibiotics. *L. fermentum* GKI-1 inhibited the growth of most of the test bacteria except *E.coli*. All the isolates exhibited less susceptibility to ofloxacin and cloxicillin antibiotics. Moreover, *L. fermentum* isolates FA-1, FA-5 and GPI-3 were found to be less susceptible to all the antibiotics.

The adherence of bacteria to the surface of intestinal mucosa is a key process for colonization and persistence in gastrointestinal tract (GIT). The isolates were assessed for their adhesion to the intestinal epithelial cell-lines, Caco-2 and HT-29. It was observed that adhesion of *L. fermentum* FA-5 to HT-29 cells was significantly higher (17.5 lactobacilli/HT-29 cell) than that of the standard probiotic strain LGG, while *L. fermentum* GKI-1 exhibited adhesion similar to that of LGG on HT-29 cells. Moreover, the adhesion of *L. fermentum* strains FA-5, IIS11.2 and GPI-3, and *L. helveticus* FA-7 to Caco-2 cells were similar to that of the LGG. Lactobacilli are known to protect the host from pathogenic infection by inhibiting the adhesion of pathogens to the intestinal

wall. So, further studies were conducted to assess the effect of these lactobacilli strains on the adhesion of enteropathogenic *E. coli* O26:H11 (EPEC) to these cell-lines. Different adhesion assays, such as adhesion inhibition assay, competitive inhibition assay and displacement inhibition assay, were designed to mimic different *in vivo* adhesion conditions. *L. fermentum* IIS11.2 significantly decreased the EPEC adhesion in HT-29 cells in all the three assays compared to LGG ($P<0.05$).

Among all the isolates, *L. fermentum* FA-5 followed by *L. fermentum* FA-1 were found to best in most of the probiotic attributes.

In the next chapter, these isolates and a few other isolates from our laboratory were analysed for their ability to neutralize the effect of enteropathogenic *E. coli*-induced epithelial barrier dysfunction *In vitro* using HT-29 and Caco-2 cells. The other isolates obtained from our laboratory were already characterised for various probiotic properties. Barrier dysfunction was measured by transepithelial electrical resistance (TEER) and permeability for macromolecules across the monolayers. Intestinal epithelial cells cultured on the transwell inserts were pre-infected with EPEC for 4 h to induce barrier disruption, which was followed by treatment with different lactobacilli strains up to 18 h. Amelioration in the TEER reduction and the increased permeability was observed by all the strains at different time intervals. *L. plantarum* GRI-2 exhibited maximum reversal in TEER of both the monolayers after 18 h of treatment. In the permeability assay, using Caco-2 monolayer, highest reduction in the permeability was observed when monolayers were treated with *L. fermentum* GPI-3 for 4 h and with *L. fermentum* FA-1 for 18 h. These strains were further analysed for their effect on the expression of mRNA encoding TJ proteins and the distribution of these proteins in the EPEC-infected intestinal epithelial cells. The results from qRT-PCR and immunofluorescence staining indicated that most of the strains had no effect on the

transcription of TJ proteins except *L. helveticus* FA-7. The immunofluorescence staining revealed that *L. fermentum* FA-1 and *L. plantarum* GRI-2 completely redistributed all the TJ proteins from the cytoplasm to the cell periphery whereas the other strains partially redistributed some or the other TJ proteins to the cell-periphery. Only *L. fermentum* FA-7 significantly increased the mRNA expression of claudin-1 (3.0-fold) and ZO-1 (2.5-fold) and distributed it to the cell periphery while the rest of the strains redistributed the already existing proteins from the cytoplasm to the cell boundaries as observed by immunofluorescence assay.

Furthermore, the study was extended to assess the effects of these strains in (Dextran sodium sulphate) DSS-induced colitis model of mice. DSS-induced colitis in mice exhibit symptoms which are similar to the human ulcerative colitis. In the present study, colitis was induced in the mice by administration of 3% DSS for 7 days. The colitis-induced mice were then treated with different lactobacilli (10^9 CFU) by intragastric administration for the next 14 days (5 mice in each group). The mice without DSS administration were used as control group. After 21 days of experiment, mice from each group were sacrificed followed by assessment for the improvement in colitis associated symptoms, such as weight loss, colon shortening, histological damage in the colonic tissue, and altered gene expression of TJ proteins. Mean weight change in the lactobacilli treated mice was significantly different (ranging from -11.5% to 12%) from that of the DSS group of mice (-26%). Isolates *L. fermentum* FA-1 and FA-5, *L. helveticus* FA-7, and *L. plantarum* GRI-2 significantly reduced inflammatory induced colon shortening in the mice. qRT-PCR analysis of TJ proteins (claudin-1, occludin and ZO-1) revealed that *L. fermentum* GPI-3 and *L. helveticus* FA-7 significantly increased the levels of mRNA expression specific for claudin-1 compared to DSS mice. Unlike *In vitro* studies, *L. helveticus* FA-7 significantly increased the levels of mRNA

expression specific for occludin in the mice, while no significant effect was observed on the levels of mRNA expression specific for ZO-1 compared to DSS-induced colitis mice. Significant increase in the levels of mRNA expression specific for ZO-1 was observed in the colitis-induced mice treated with *L. fermentum* FA-1 compared to DSS-group of mice. The strains used in the present study were also able to repair the DSS-induced histological damage in the colonic tissue, as indicated from the microscopic observations of the colonic sections and the associated reduction in the histological scores. The highest significant reduction in the histological score was observed with mice treated with LGG (2.6) followed by *L. fermentum* FA-1 (4.1) and *L. plantarum* GRI-2 (4.0) compared to DSS-group of mice (10).

CONCLUSIONS:

Amongst the *Lactobacillus* strains isolated from human stool samples and fermented food samples of Indian origin, *L. fermentum* FA-5 followed by *L. fermentum* FA-1 exhibited significantly higher probiotic attributes compared to the established probiotic strain LGG. Further, *L. fermentum* FA-1, *L. helveticus* FA-7 and *L. plantarum* GRI-2 showed significantly comparable and/or higher capabilities to improve the gut dysfunction in most of the attributes examined under *in vitro* and *in vivo* study.