SUMMARY

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THESIS TITLE: Study of *Lactobacillus* isolates from human sources with regard to their beneficial physiological attributes

The gastrointestinal tract of mammals hosts diverse groups of commensal microbiota, which includes both aerobes as well as anaerobes. These bacteria live symbiotically within the human gastrointestinal tract and together the bacteria play an important role in the protection of the organism against harmful microrganisms and also strengthen the host's immune system. Probiotics are defined as “Live microorganisms that when administered in adequate amounts confer a health benefit on the host” (FAO/WHO 2001). Lactobacilli, bifidobacteria and *Streptococcus thermophilus* are Gram-positive rods, non-pathogenic and desirable members of intestinal tract. They are considered protective organisms and are thought to inhibit the growth of pathogenic organisms via the production of lactic acid and other metabolites. In order to persist in the host, probiotics must reach a suitable microhabitat and establish a proliferating population. Survivability and colonization in the digestive tract are desirable properties for any probiotic, which depends on several factors including the ability of the bacteria to tolerate acidic pH, bile toxicity of the digestive tract and on the adhesion of bacteria to intestinal cells and mucus.

Under the present study, isolation of *Lactobacillu*s has been carried out from human gut. The primary screening was carried out on the basis of Gram staining and biochemical character like presence of catalase. The isolates which showed Gram positive nature and catalase negative phenotype were further 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. After that they were checked for their resistance to acid (pH 2.5), bile tolerance (bile concentration at 0.3% and 1%), antibacterial activity and antibiotic susceptibility. Besides this, adhesion to Caco-2 and HT-29 monolayers and antagonism against an enteropathogen *E. coli* O26:H11 was also checked. These various probiotic properties were evaluated against standard probiotic *Lactobacillus rhamnosus* GG (LGG). Among the isolates, *L. salivarius* strains GPI-1(S), GPI-4, and *L. fermentum* GPI-7 showed significantly comparable and/or higher ability compared to LGG under all these different *in vitro* assays to characterize a strain as a probiotic.

Lactobacilli possess various health-promoting properties useful for both humans and animals. For this reason, there has been much interest in developing food products containing these bacteria as dietary adjuncts. Lactobacilli are frequently used in products for human consumption and can be found as probiotics in infant foods, cultured milks, and various pharmaceutical preparations. The health claims of ingesting live cells of lactobacilli could be due to several possible mechanisms, which may include restoration of normal intestinal flora and removal of pathogens, accumulation of their metabolites including organic acids in the intestine and enhancement in the normal functioning of digestive tract. One of the beneficial effects that have been suggested to result from human consumption of *Lactobacillus* is amelioration of symptoms of lactose intolerance. One of the glycosidases is β-galactosidase an enzyme that is widely used in dairy industry and it is produced by most lactobacilli. Low activity of β-galactosidase causes digestive

insufficiency, called lactose intolerance in most cases. The addition of lactobacilli producing high β-galactosidase enzyme as probiotic can be used for improvement of digestion of lactose in dairy products. Another beneficial effect is, their α-galactosidases, have been considered as an effective food additive to remove anti-nutrient oligosaccharides, which occur in pulse and soybean meal containing diets. α-galactosidases are exoglycosidases that helps to cleave the α-1, 6 linked galactose residues from α-D-galactosides such as melibiose, raffinose and stachyose, and branched polysaccharides such as galactomannans and galactoglucomannans. Since humans lack α-galactosidases in the intestinal mucosa, there is a chance for the formation of flatus due to the fermentation of such oligosaccharides by intestinal microflora. Therefore it is necessary to remove the oligosaccharides from food with the help of α-galactosidases. Some studies have shown that adding probiotics to pulse products can indeed reduce gastrointestinal discomfort due to gas. Other beneficial effect that has been suggested to result from human consumption of LAB is a reduction in serum cholesterol levels, as suggested by the results of several human and animal studies. The *in vitro* experiments have demonstrated that many LAB have an ability to reduce cholesterol level in the growth medium containing bile salts. These cholesterol-lowering effects can be partially ascribed to bile salt hydrolase (BSH) activity. BSH activity increases the rate of excretion of free bile acids. Such mechanism could be used in controlling serum cholesterol levels by colonic microbes. Thus, in the present study isolated lactobacilli were also evaluated for their above mentioned health promoting properties. Isolate *L. salivarius* GPI-1(S) and *L. plantarum* CS23 showed significant level of β-galactosidase production. They also performed equally well in cholesterol removal when *Lactobacillus* culture was used directly in broth as well

as when *Lactobacillus* fermented curd was used as inoculum, and they also showed higher level of α-galactosidase activity.

Several probiotic effects are mediated through immune regulation, particularly through establishing and maintaining a balance between pro- and anti- inflammatory cytokines. Hence the study of immunomodulatory properties of the probiotics is also very important. Lactobacilli have been shown to activate immune cells such as monocytes, macrophages, dendritic cells and polymorphonuclear cells (PMNs), which are important in antigen processing, presentation and activation of antigen-specific immune response, i.e. cell-mediated immunity. They mediate their immune-modulatory effects through the induction of regulatory cytokines, induction of T regulatory cells, modulation of APC promotion of epithelial function and development and by inhibition of pro-inflammatory cytokines. Defective maturation of immune competence in association with poor microbial stimuli may thus lead to dysregulation of both innate and adaptive immune systems; this may also be associated with various diseases or inflammatory conditions. In the present study underlying effect of different lactobacilli on immune cells like PBMCs, macrophages and PMNs (from blood of healthy individual) on non-pathological conditions was investigated by analyzing the array of cytokines and chemokines produced following incubation with lactobacilli. *L. salivarius* strains GPI-1(S) and GPI-4 treated PBMCs, macrophages and PMNs expressed significantly higher level of M2 marker or anti-inflammatory and low level of M1 marker or pro-inflammatory cytokine. Generation of anti-inflammatory cytokines is considered to be beneficial at the site of an infection. This study has therefore been able to select some *Lactobacillus* strains that have been demonstrated to behave better

than standard probiotic strain LGG in terms of their immunomodulatory properties, which can be further evaluated for safety and application for providing health benefit to human hosts.

Conclusions

Among the six *Lactobacillus* strains isolated from human gut, *L. salivarius* strains GPI-1(S) and GPI-4 showed significantly comparable and/or higher probiotic attributes compared to the established probiotic strain LGG. Further, isolate *L. salivarius* GPI-1(S) and *L. plantarum* CS23 were showed higher β-galactosidase production. They also performed equally well in cholesterol removal when *Lactobacillus* culture was used directly in broth as well as when *Lactobacillus* fermented curd was used as inoculum, and they also showed highest level of α-galactosidase. *L. salivarius* strains GPI-1(S) and GPI-4 also exhibited positive immunomodulatory effects. The findings need further confirmation by an *in vivo* study.

L. salivarius GPI-1(S) was identified as a novel probiotic strain with high *in vitro* colonization ability, and applications in the prevention and treatment of different diseases, and/or anti-inflammatory properties.