ABSTRACT

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

A complex microbiota of more than a thousand different bacterial species with a population of about 10^{14} cells inhabits the oral cavity, gastrointestinal tract (GIT), upper respiratory tract, vagina and skin, with a major part of this microflora residing in the human gut. They are able to survive in the gastrointestinal (GI) tract and are safe for the consumers. The survivability and colonization in the digestive tract are considered critical to ensure optimal functionality and expression of health promoting physiological functions by probiotics. *Lactobacillus* is an important genus among LAB family and many *Lactobacillus* strains are widely used as probiotic bacteria. For survivability in the gut, the organism must tolerate acidic pH and bile toxicity of the digestive tract. In the context of their effective colonization, the ability to adhere to epithelial cells and mucosal surfaces has been suggested to be an important property of bacteria used as probiotics. They may also express antibacterial like substances against pathogens, besides competition for adhesion sites and inhibit the binding of pathogens to the mucosal surface. Probiotic lactobacilli possess various health-promoting properties useful for both humans and animals including, ability to combat lactose intolerance, to remove cholesterol and also have potential to improve legume oligosaccharide digestibility. Additionally, their immunomodulatory role has been one possible mechanism to protect the host from intestinal disease. In order to develop indigenous isolates that may be expected to fare better than non-indigenous isolates in view of local food habits, this study was aimed at isolating autochthonous lactobacilli from

human gut and check for their potential probiotic properties and their health promoting features. In order to achieve this, the objectives of this study were the following:

Objectives:

1. Isolation, identification and characterization of *Lactobacillus* strains from human sources.
2. Study production of β-galactosidase and α-galactosidase and cholesterol removal by *Lactobacillus* strains.
3. Study immunomodulating potential of *Lactobacillus* strains on PBMCs, PMNs and macrophages.

Six isolates were obtained from human gut and were studied for their probiotic attributes such as acid and bile tolerance, binding to intestinal cells, and antimicrobial activity. Isolates were also studied for their antibiotic susceptibility. These isolates were evaluated against standard probiotic *Lactobacillus rhamnosus* GG (LGG). Isolate *L. fermentum* GPI-6 showed the best survival profile (91% at 0.3% bile salt and 89% at 1% bile salt) as compared to LGG (81% at 0.3% and 1% bile salt). Isolates *L. plantarum* GRI-2 and *L. salivarius* GPI-4 showed best (no reduction/100% survival) survival rate at pH 2.5. The *L. fermentum* isolates GPI-7 and GPI-1(B) had the highest zone of inhibition (antimicrobial activity) against both Gram-positive and Gram-negative bacteria, followed by *L. salivarius* isolates GPI-1(S), GPI-4 and *L. plantarum* GRI-2. *L. salivarius* isolates GPI-1(S) and GPI-4, *L. fermentum* isolates GPI-1(B) and GPI-6 were found to be resistant (no zone of inhibition) to all the tested antibiotics. As expected, isolates showed strain specific

difference when comparing various attributes. Isolates GPI-4, and GPI-7 showed better adhesion to HT-29 while isolate GPI-4 adhered better to Caco-2 cells than LGG. However, when studying their ability to compete with *E. coli* O26:H11, isolates GPI-6 and GPI-7 significantly inhibited *E. coli* adhesion to both HT-29 and Caco-2 cells compared to LGG. Further, isolated lactobacilli were grown on MRS plates and β-galactosidase activity was determined using o-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate. Three isolates GPI-3 and GPI-6 and GPI-1(S) showed better activity than the standard strains LGG and *L. plantarum* ATCC 8014. The isolates showed variability in assimilating cholesterol during growth. Several showed excellent cholesterol lowering ability compared to standard strains. Isolate SCB being the highest acid producer (pH 4.38) also showed the highest cholesterol reduction compared to other strains including standard strains. The ability of these isolates to produce α-galactosidase was studied on the basis of p-nitrophenyl-α-D-galactopyranoside (PNPG) hydrolysis and the maximum α-galactosidase activity was found in isolate GPI-1(S) followed by FA-5 and FA-7. The most probiotic effects are achieved by immunomodulation ability of lactobacilli. Thus, the study of lactobacilli isolates to modulate the array of cytokines and chemokines was undertaken. *L. salivarius* strains GPI-1(S) and GPI-4 treated PBMCs/macrophages and PMNs expressed significantly higher level of anti-inflammatory or M2 marker and low level of pro-inflammatory or M1 marker. *L. fermentum* FA-1, *L. salivarius* strains GPI-1(S) and GPI-4 treated PMNs expressed significantly higher level of anti-inflammatory cytokines and low level of pro-inflammatory cytokines. Whereas *E. coli* O26:H11 infected PBMCs/macrophages and PMNs showed higher M1 profile and pro-inflammatory cytokines level respectively. This study showed that the M1/M2 markers and pro-

inflammatory/anti-inflammatory expression level in human PBMCs/macrophages and PMNs respectively, to lactobacilli can be different depending on the strains. In conclusion, isolate *L. salivarius* GPI-1(S) showed excellent results in all tested probiotic properties.