Summary and Concluding Remarks

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The purpose of the study was to screen human origin probiotic *Lactobacillus* strains, as the gut microbiota varies among individuals, depending on factors such as diet, lifestyle and genetic factors. Lactobacilli is a diverse group of bacteria, to which we are exposed in our day-to-day life as they are commonly present in foods such as fruits, vegetables, and fermented foods. Some *Lactobacillus* species that reside in the gastrointestinal tract of mammals and vagina of humans and animals are associated with the well-being of the host. There are numerous probiotic strains available for commercial use, but isolation and characterization of novel strains is still a fascinating research area particularly in India. Therefore, one of the aims of the work was to characterize autochthonous human origin *Lactobacillus* strains, for their probiotic properties and evaluation with reference to well-documented standard strains *Lactobacillus rhamnosus* GG, *L. rhamnosus* 231 and *L. casei* DN 114 001 (Actimel).

- Forty-three isolates that were Gram-positive rods, arranged singly or in pairs, non-motile and catalase negative were considered *Lactobacillus* strains isolated from infant feces and vaginal mucosa of healthy female. According to the classification scheme, they belong to the *Streptobacterium* group and facultative heterofermenter. The isolates were tentatively identified on the basis of sugar fermentation pattern as *Lactobacillus rhamnosus* strains. Out of 43 isolates, 3 isolates obtained from vaginal samples Vc, Vd2, Vd6 and Fb isolated from human infant fecal sample exhibited tolerance to all the prescribed conditions pH 2, bile salt (4%), and phenol (0.6%). 14 isolates were able to tolerate pH 2, 8 isolates were able to tolerate 4% bile salt, 26 isolates were able to tolerate 0.6% phenol and all the isolates were able to grow in the presence of 6% NaCl. Moreover, these isolates exhibited broad antimicrobial spectrum against gastrointestinal and food-borne pathogens. *L. rhamnosus Fb* and *L. rhamnosus* Vc identified by 16S rDNA sequence analysis, isolated from two different habitats were selected for further studies, as they exhibited probiotic properties.

- *L. rhamnosus* Fb and *L. rhamnosus* Vc were evaluated for their functional, safety and technological aspects, selection criteria for novel probiotic strains. These strains are able to tolerate pH 2 and 4% bile salt and grow in 10% NaCl and 0.6% phenol. In

addition, exert transit tolerance to simulated oral-gastrointestinal fluids. The cell surface of *L. rhamnosus* strains is hydrophilic in nature as revealed by bacterial adhesion to hydrocarbons (BATH) assay. Despite this, *L. rhamnosus* strains showed strain-specific mucin adherence, autoaggregation and coaggregation abilities. *L. rhamnosus* strains produce bile salt hydrolase (BSH) and β-galactosidase activities and exhibit antimicrobial activity against food-borne and gastrointestinal pathogens, as well as *Candida* and *Aspergillus* spp. *L. rhamnosus* strains have similar antibiotic susceptibility pattern, and non-susceptibility to certain antibiotics is intrinsic or innate. These strains are neither haemolytic nor producer of biogenic amines such as histamine, putrescine, cadaverine and tyramine. Lyophilized cells of *L. rhamnosus* Fb exhibited probiotic properties demonstrating potential of the strain for technological suitability and in the preparation of diverse probiotic food formulations.

- Antimicrobial activity of *L. rhamnosus* Fb against food-borne and gastrointestinal pathogens is partially attributed to thermostable low molecular weight peptides with molecular mass of 6kDa *ca*. Thermostability (100°C, 60 min), pH sensitivity (2-9), and sensitivity to proteolytic enzymes (Proteinase K, trypsin and pepsin) of these LMW antimicrobial peptides was observed to be test organism specific. EPC is a mixture of antimicrobial peptides, since the antimicrobial spectrum of the EPC is intimately associated with the growth phase and their antimicrobial activity spectrum changes in fractions obtained from gel permeation chromatography. Storage of the EPC at 4°C for ≥180 days remains unchanged except against certain test organisms.

- *Lactobacillus rhamnosus* strains were evaluated for their antigenotoxic and antimutagenic activities against two direct acting mutagens (i) 4-nitroquionoline-1-oxide – widely used for cancer related studies and (ii) *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine – endogenously formed mutagen using distinct microbial assays SOS-Chromotest and Ames test. Binding of mutagens by Lactic acid bacteria is one of the mechanisms responsible for their antimutagenic activity. Therefore, these strains were determined for their potential to bind with chemically different classes of promutagens (i) Acridine orange – used as food colorant and toxicant found in environment and (ii) 2-amino-3, 8-dimethylimidazo-[4, 5-*f*]-quinoxaline, a food-borne carcinogen.

➢ Non-growing viable cells of lactobacilli biotransformed and detoxified 4-NQO simultaneously. Biotransformation of 4-NQO was evidenced from the UV spectral modifications and HPLC analysis. 4-NQO biotransformation was associated with concomitant reduction of genotoxicity (80-96%) and mutagenicity (69-89%). Biotransformation rates of 4-NQO is dependent on pH, cell density, and incubation time. Moreover, heat-killed cells did not biotransform 4-NQO. Biotransformation rates increased with 4-NQO concentration although it varied with strains. Simulated gastro-intestinal fluid treated cells retained the 4-NQO biotransformation ability. Cell wall peptidoglycan, carbohydrates, and proteins play an important role in the binding of 4-NQO by *L. rhamnosus* Fb.

➢ *In vitro* studies revealed that co-incubation of MNNG with viable cells of *L. rhamnosus* Vc resulted in the modification of UV spectrum of the parent compound and accompanied with reduction in genotoxicity (69%) and mutagenicity (61%) evaluated by SOS-Chromotest and Ames test respectively. Antigenotoxic activity is strictly associated with viable bacterial cells, and correlated to cell density, and incubation time and dependent on pH and MNNG concentration.

➢ Viable and heat-killed cells of *Lactobacillus* strains bind AO instantaneously, predominantly through extracellular cell wall components. Binding of AO by the cells depends on the pH, cell-density, and AO concentration. Cells treated with HCl, NaOH, $MgCl_2$ and $CaCl_2$ have reduced capacity to bind AO implicating the role of carbohydrates, teichoic acids, and proteins. AO adsorption involves hydrophilic interactions, as urea treatment did not alter AO binding. Simulated gastrointestinal fluid treatment does not affect the binding of AO.

➢ Resting viable cells of *Lactobacillus rhamnosus* strains 58-63% adsorbed MeIQx and biotransformed MeIQx as evidenced by UV spectral modifications and HPLC analysis. MeIQx biotransformation is higher in pH range 6-10. Binding of MeIQx involves hydrophobic interactions, cell wall components carbohydrates, proteins and teichoic acids. Moreover, surface-associated proteins play an important role in biotransformation of MeIQx.

➢ Freezing did not alter the viability of the cells, although 16% reduction occured following the lyophilization process. Cells were also lyophilized with carrier media

such as skim milk and sugars (glucose, lactose and sucrose), but it did not have significant influence on the viability during lyophilization process but the carrier media provide protection during storage at 4°C particularly lactose and sucrose in retaining their probiotic properties. With the aim of developing functional food, chocolate was prepared using probiotic *L. rhamnosus* Fb and 48% of the cells remain viable after 1.5 years of storage at 4°C. Chocolates prepared with *L. rhamnosus* Fb, exhibited tolerance to gastrointestinal conditions as well as antigenotoxic activities against carcinogens 4-NQO and MNNG.