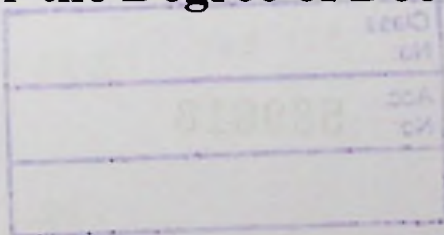




**Isolation and characterization of  
probiotic lactic acid bacteria  
from selected varieties of  
*Elucine coracana* and *Musa* species  
of Sri Lanka**

**A thesis submitted to the Faculty of Science,  
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## Abstract

Non-dairy probiotics are gaining recognition in commercial food applications as they are known to confer functional health benefits to the host. They differ from dairy probiotic food as they are lactose and fat free, non-allergen substrates that are rich in prebiotics. Lactic acid bacteria are the most predominantly known probiotics in foods. The present study aims to isolate, identify and characterize probiotic LAB from substrates of non-dairy origin; fermented flour of finger millet (*ravi*, *raavana* and *oshadha* varieties) and banana (*ambul nadee*, *ambum*, *seeni parakum*, *kolikuttu*, *kandula*, *seeni*, *anamalu*, *rathkesel* and *nethrappalam* varieties) grown in Sri Lanka.

Two hundred and forty bacterial colonies were isolated from fermented flour of above varieties of finger millet and banana. All isolates were phenotypically and biochemically characterized. Twenty five isolates which possess typical LAB phenotypic and biochemical characteristics were identified using 16S rRNA sequencing, their partial sequence was deposited in NCBI genbank and phylogenetic relationships were predicted. The new LAB strains, fifteen from finger millet; *Paenibacillus* species (MF480545.1), *Bacillus cereus* (MF480550.1), *Streptococcus lutetiensis* (MF574476.1), *Brevibacillus borstelensis* (MF480552.1), *Lactobacillus plantarum* (MF405176.1), *Lactobacillus fermentum* (MF033346.1), *Bacillus cereus* (MF480468.1), *Lactobacillus fermentum* (MF405134.1), *Lactococcus lactis* subspecies *lactis* (MF480428.1), *Enterococcus faecium* (MF420431.1), *Bacillus cereus* (MF754478.1), *Bacillus cereus* (MF574479.1), *Bacillus cereus* (MF574477.1), *Pediococcus acidilactici* (MF480434.1) and *Enterococcus lactis* (MF574475.1) and ten from banana; *Enterococcus durans* (MF405179.1), *Enterococcus gallinarum* (MF480436.1), *Lactobacillus plantarum* (MF405177.1), *Weissella cibaria* (MF480445.1), *Enterococcus hirae* (MF480429.1), *Enterococcus faecium* (MF574466.1), *Lactobacillus curieae* (MF405178.1), *Enterococcus durans* (MF480435.1), *Pediococcus acidilactici* (MF480433.1) and *Enterococcus faecium* (MF480430.1) were further investigated for survival in simulated gastric conditions, acid, bile, salt, temperature, phenol and gastric juice and *in-vitro* safety attributes. Their cell aggregation and adhesion activity were studied *in-vitro* and the best strains were evaluated for *in-vitro* bio-activity; anti-bacterial, anti-cancer, anti-oxidant, bile salt hydrolysis and cholesterol assimilation. Above LAB strains were further evaluated for repeated dose 14 days acute and 8 week sub-chronic oral toxicity in wistar rat models. Compatibility of LAB strains with their isolating substrate was investigated by formulating probiotic beverages and by studying the viability of LAB cells in the fermentation medium and physio chemical properties. Among the 25 LAB strains, five from finger millet, *L. plantarum*, *L. fermentum*, *L. lactis* sub species *lactis*, *E. faecium* and *P. acidilactici* and three from banana *E. durans*, *E. faecium* and *L. curieae* exhibited superior survival in simulated gastric conditions and were free from virulence causing enzymes and did not demonstrate major antibiotic resistance pattern *in-vitro*. *Enterococcus durans* demonstrated highest auto-aggregation of  $76.53 \pm 0.59\%$ , *L. fermentum* and *E. durans* exhibited the highest co-aggregating activity of  $67 \pm 0.21\%$  and *E. durans* exhibited highest adhesion of  $72.5 \pm 5.90\%$  and  $74.16 \pm 4.89\%$  to HCT-116 and HT-29 cell lines, respectively. All the above LAB strains could inhibit  $\geq 4$  drug sensitive and  $\geq 3$  multi



drug resistant human pathogens. While intracellular cell free extracts of *L. fermentum* exhibited the lowest IC<sub>50</sub> values of  $151.98 \pm 2.25$  and  $203.60 \pm 19.90$  against HCT-116 and HT-29 cell lines, respectively. Highest 2, 2, diphenyl-1-picrylhydrazyl free radical and hydroxyl radical scavenging activity was exhibited by intracellular cell free extract of *L. lactis* sub species *lactis* ( $54.33 \pm 0.88\%$  and  $23.10 \pm 1.20\%$ ) and it survived in hydrogen peroxide up to 4 h, at the concentration of 500  $\mu\text{g/ml}$ . All strains exhibited bile salt hydrolysis activity *in-vitro* and none could assimilate > 10% cholesterol. In 14 days repeated dose acute oral toxicity study, no treatment-related sign of toxicity, mortality or abnormal hematology and biochemistry of rat blood were observed in both male and female rats who received  $10^8$ ,  $10^{10}$  and  $10^{12}$  CFU/ml of LAB strains. No gross histopathological changes and bacterial translocation was observed in kidneys, livers and intestines. After eight week sub-chronic toxicity study, animals receiving LAB strains except  $10^{12}$  CFU/ml repeated oral dose of *E. durans*, others did not demonstrate treatment-related sign of toxicity, mortality, abnormal hematology and blood chemistry or gross histopathological changes. Therefore, the toxicity study concluded that *L. plantarum*, *L. fermentum*, *L. lactis*, *E. faecium* (two strains), *P. acidilactici* and *L. curieae* are safe up to 8 weeks in wistar rats at tested doses. Probiotic beverages formulated using LAB strains *L. plantarum* and *L. curieae* exhibited compatibility with their respective prebiotic substrates from which the strains were initially isolated; finger millet (*ravi*) and banana (*ambul nadee*), respectively, indicating their potentiality to carry out further development in to non-dairy probiotic food. This is the first report on isolation of probiotics from finger millet and banana varieties grown in Sri Lanka. This is also the first report on new probiotic strains with proven bio activity (*in-vitro*) and no toxicity (in rats) in Sri Lanka.