

***In vitro* Antimicrobial Activity of *Lactococcus Lactis sub spp lactis* KG8 isolated from Sri Lankan Finger Millet**

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1. Abstract

Probiotic potential Lactic Acid Bacteria (LAB), *Lactococcus Lactis.sub spp lactis* KG

8 isolated from Sri Lankan finger millet variety *Raavana* was evaluated (*In vitro*) for its antimicrobial activity against fourteen human pathogens including Sensitive and Multi Drug Resistant(MDR) organisms; *Escherichia coli* ATCC 2592 and ATCC 35218,

Staphylococcus aureus ATCC 6571, EMRSA 17 COCR and EMRSA 16 NCTC 13143, *Enterococcus faecalis* ATCC 49532 and ATCC 700802, *Streptococcus mutans* ATCC 25175, *Streptococcus pyogenes* ATCC 700294, *S. sanguinis* ATCC 10556, *Streptococcus salivarius* ATCC 13419, *Salmonella enterica* ATCC 700408, *Acinetobacter baumannii* ATCC 17978 and *Shigella flexenari* ATCC 12022. Agar well diffusion technique was applied in triplicate. Imipranum (10µg) was used as positive control and inhibition zone diameters were measured, and experiment was repeated thrice. Among the sensitive pathogens *Shigella flexenari* ATCC 12022 was significantly inhibited by *L. Lactis.sub spp lactis* KG 8 whereas, *E. coli* ATCC 2592 and *Streptococcus pyogenes* ATCC 700294 were moderately inhibited at the tested concentrations. However, *Streptococcus sanguinis* ATCC 10556 and *Streptococcus mutans* ATCC 25175 demonstrated resistance to *L. Lactis.sub spp lactis* KG 8. It was observed that, among the MDR pathogens, *L. Lactis.sub sp lactis* KG 8 could significantly inhibit *S. aureus* 16 EMRSA NCTC 13143 and *Salmonella enterica* ATCC 700408 and showed moderate antimicrobial activity against *Escherichia coli* ATCC 35218. Furthermore, *Enterococcus faecalis* ATCC 700802 demonstrated resistance to *L. Lactis.sub sp lactis* KG 8 at the tested concentrations. The *In vitro* antimicrobial activity of *Lactococcus Lactis.sub spp lactis* KG 8 indicates its ability to produce antimicrobial compounds and, therefore, it's reported as a potential candidate for use in probiotic starter cultures.

Keywords: Antimicrobial activity, Probiotics, Sri Lankan finger millet

Introduction and research problem/issue

Probiotics are defined as "live microorganisms, which when administered in adequate amounts confer a health benefit on the host" by the Food and Agriculture Organization

& World Health Organization. Lactic Acid Bacteria (LAB) are widely recognized among probiotic bacteria that are gram positive, non spore forming and catalase negative organisms. Due to the increasing interest in the application of probiotics and their metabolites as an alternative strategy for treatment and prevention of infections, testing antimicrobial activity of probiotic candidates against human pathogens is important. In addition, antimicrobial metabolites of probiotic LAB in prevent food spoilage, extend shelf life stability of food and enrich food with bioactive properties. Currently consumer demand exists for non dairy probiotic products in beverages, supplements, capsules and freeze-dried preparations. Probiotics require a substrate (prebiotic) to grow and multiply; therefore, cereals rich in prebiotics play a major role as substrates for many non dairy probiotic products. Finger millet (*Eleusine coracana*) is rich in carbohydrates, dietary fiber, minerals, and sulfur containing amino acids when compared with rice, the current major staple food in South Asia. Due to the presence of water-soluble fibers, oligosaccharides and resistant starch, finger millet fulfils the prebiotic effects and therefore can stimulate the growth of probiotic bacteria. Although, a fair of research is available on fermentation of finger millet using different starter cultures to develop functional foods in Africa and Asia, there is a paucity of data on Sri Lankan varieties. Therefore the objective of this research is to investigate the *In vitro* antimicrobial activity of *Lactococcus Lactis.sub spp lactis* KG 8 strain isolated from Sri Lankan Finger Millet variety *Raavana* against selected human pathogens.

3. Research Methodology

Lactococcus Lactis.sub spp lactis KG 8 was evaluated for its *in vitro* antimicrobial activity by performing the agar well diffusion assay. The test Lactic Acid Bacteria (LAB) strain was maintained in de Man Rogosa Sharpe (MRS) broth with 30% glycerol at -80

°C. Anti-microbial activity of *Lactococcus Lactis.sub spp lactis* KG 8 was evaluated against fourteen human pathogenic bacteria obtained from culture collection of International Centre for Chemical and Biological Sciences including Sensitive and Multi Drug Resistant (MDR) pathogens. The test

pathogens were activated and maintained in their selective broth mediums. Cell Free Supernatant (CFS) of *L. Lactis.sub spp lactis KG 8* was prepared by inoculating the culture in to sterile MRS broth , Incubating at 37 °C for 18 hrs followed by centrifugation at 10,000 g at 4 °C. The test pathogens including *Escherichia coli* ATCC 2592, *Escherichia coli* ATCC 35218 (MDR), *Staphylococcus aureus* ATCC 6571, *Staphylococcus aureus* EMRSA 17 COCR (MDR), *Staphylococcus aureus* EMRSA 16 NCTC 13143 (MDR), *Enterococcus faecalis* ATCC 49532, *Enterococcus faecalis* ATCC 700802 (MDR), *Streptococcus mutans* ATCC 25175, *Streptococcus pyogenes* ATCC 700294, *Streptococcus sanguinis* ATCC 10556, *Streptococcus salvarius* ATCC 13419, *Salmonella enterica* ATCC 700408 (MDR), *Acinetobacter baumannii* ATCC 17978, *Shigella flexenari* ATCC 12022 were selected for the study. Eighteen hr old test cultures (200 µl); which were adjusted to 0.5

MacFarland prior to the assay were inoculated into molten Nutrient Agar (NA) tubes. NA tubes with test pathogenic culture was poured to the sterile petri plates and allowed to solidify. Wells were made in solidified petri plates using sterile cork borer (10 mm diameter). Hundred micro liters of CFS of *L. Lactis.sub sp lactis KG 8* was added to each well and the plates were incubated at 37 °C for 24 hrs. After incubation, the plates were observed for the presence of inhibition zones and the diameters of inhibition zones were measured using a calibrated vernier caliper. The antimicrobial activity of *L. Lactis.sub sp lactis KG 8* was expressed as the mean inhibition zone diameter. Disc of Imipranum (10 mg l⁻¹) was used as a positive control. Experiments were repeated thrice.

4. Results and findings

Among the sensitive pathogens *Shigella flexenari* ATCC 12022 was significantly inhibited by *L. Lactis.sub spp lactis KG 8* whereas, *E. coli* ATCC 2592 and *Streptococcus pyogenes*

ATCC 700294 were moderately inhibited at the tested concentrations. However, *Streptococcus sanguinis* ATCC 10556 and *Streptococcus mutans* ATCC 25175 demonstrated resistance to *L. Lactis.sub spp lactis KG 8*. It was observed that, among the MDR pathogens, *L. Lactis.sub sp lactis KG 8* could significantly inhibit *S. aureus* 16 EMRSA NCTC 13143 and *Salmonella enterica* ATCC 700408 and showed moderate antimicrobial activity against *Escherichia coli* ATCC 35218.

Furthermore, *Enterococcus faecalis* ATCC 700802 demonstrated resistance to *L. Lactis.sub sp lactis* KG 8 at the test concentrations.

5. Conclusions, implications and significance

L. Lactis.sub spp lactis KG 8 demonstrated *In vitro* antimicrobial activity against both sensitive and MDR pathogens thus indicating its ability to produce antimicrobial compounds. However, further investigations on genotoxicity of the *Lactococcus Lactis.sub spp lactis* KG 8 need to be conducted prior to recommending as a starter culture in industrial fermentation.

6. References (Selected)

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3.4.4 Influence of Storage Temperature on the Quality Parameters of Wheat Flour during Short Term Storage