

Functional and Genomic Characterization of Buffalo-Derived Probiotics Against Carbapenem-Resistant Enterobacterales

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Introduction: The rise of antimicrobial resistance (AMR), particularly among carbapenem-resistant Enterobacterales, represents a major global health challenge. Probiotic bacteria such as *Lactobacillus* spp. may offer alternative strategies by inhibiting enteric pathogens and enhancing colonization resistance.

Objective: Evaluate the probiotic potential and antimicrobial activity of *Lactobacillus* strains isolated from buffalo milk against clinically relevant enteric pathogens. **Materials and Methods:** Fifty isolates were obtained from buffalo milk, of which 31 were identified by MALDI-TOF MS (*L. plantarum*, n=17; *L. fermentum*, n=8; *Lactobacillus* spp., n=6). Isolates were assessed for tolerance to acidic pH (2.5) and bile salts (2%), antimicrobial susceptibility, and biofilm formation. Antagonistic activity was evaluated against Enterobacterales isolates (n=10), including carbapenemase producers (NDM and KPC), using spot-on-lawn assays, liquid co-culture, and cell-free supernatant assays. **Results:** Most isolates exhibited high tolerance to acid and bile stress, maintaining growth comparable to controls after simulated gastrointestinal exposure. Several strains showed inhibitory activity against *Escherichia coli*, and *Klebsiella pneumoniae*, with clear inhibition zones observed. Co-culture assays indicated reduced enterobacterial growth in the presence of selected *Lactobacillus* strains. **Discussion:** These findings suggest that buffalo-derived *Lactobacillus* strains possess relevant probiotic traits and antimicrobial activity against resistant enteric pathogens. **Conclusion:** Buffalo milk represents a promising source of probiotic candidates with potential applications in controlling AMR pathogens. Additional analyses, including cell surface hydrophobicity, biofilm formation, hemolytic activity, and evaluation of anti-inflammatory effects in gut organoid models, are currently underway, alongside whole-genome sequencing to characterize functional attributes.

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