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Research Paper

Evaluation of Antimicrobial and Anti-Biofilm activities of *Lactobacillus* Species isolated from Kefir Against Foodborne Pathogens *Staphylococcus Aureus* and *Salmonella Enterica* Serovar Typhimurium

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Extended Abstract

Introduction The increasing threat of antibiotic-resistant foodborne pathogens and the limitations of chemical preservatives in food safety have emphasized the need for natural, effective biocontrol strategies. Kefir, a traditional fermented beverage made from kefir grains, harbors a complex symbiotic community of yeasts and lactic acid bacteria (LAB), particularly *Lactobacillus* species. These bacteria are renowned for their probiotic properties and their ability to produce antimicrobial compounds such as lactic acid, hydrogen peroxide, and bacteriocins. In addition to inhibiting pathogen growth, certain *Lactobacillus* strains exhibit the unique ability to disrupt or prevent the formation of biofilms structured microbial communities that are notoriously resistant to antibiotics and disinfectants. The present study aims to isolate and identify *Lactobacillus* strains from kefir and evaluate their antimicrobial and antibiofilm activity against two major foodborne pathogens: *Staphylococcus aureus* and *Salmonella* Typhimurium.

Methods 10 grams of commercial kefir grains were fermented in sterilized milk, and serial dilutions were cultured on MRS agar under anaerobic conditions. Colonies were selected based on morphology and further screened for catalase activity, Gram staining, and rod-shaped appearance to confirm their identity as *Lactobacillus*. 10 isolates were preserved and subjected to functional characterization. The antimicrobial activity of the isolates was evaluated using the well diffusion method on Mueller-Hinton agar against *S. aureus* ATCC 25923 and *S.* Typhimurium ATCC 14028. Supernatants from 24-hour *Lactobacillus* cultures were sterilized and added to wells inoculated with each pathogen. The inhibition zones were measured after 24-hour incubation at 37°C. Antibiofilm activity was assessed using the crystal violet microplate assay. Pathogens were co-incubated with sterile *Lactobacillus*

supernatants in 96-well plates for 24 hours. After biofilm fixation and staining, optical density at 590 nm was measured to calculate the percentage inhibition relative to control wells. Molecular identification of the most effective strains was performed using 16S rRNA gene sequencing. Genomic DNA was extracted, amplified via PCR, and sequenced. Sequences were analyzed using BLAST and phylogenetic relationships were constructed with MEGA software. Carbohydrate fermentation patterns were determined using phenol red-based sugar fermentation assays for a range of sugars (e.g., glucose, fructose, lactose, maltose, mannitol, etc.).

Results and Discussion A total of 23 bacterial isolates were recovered from kefir, of which 10 were confirmed as catalase-negative, Gram-positive rods indicative of *Lactobacillus*. These isolates displayed typical creamy-white, moist colony morphologies on MRS agar. All isolates exhibited inhibitory activity against both pathogens, although with varying efficacy. The inhibition zone diameters ranged from 11.6 to 17.1 mm for *S. aureus* and from 9.2 to 15.9 mm for *S.* Typhimurium. Strain Lb7 demonstrated the highest antimicrobial activity against both pathogens, with significantly larger inhibition zones ($p < 0.01$). Strains Lb4 and Lb6 also showed strong antagonistic activity. All isolates showed the capacity to reduce biofilm formation by the tested pathogens. The highest inhibition was observed for Lb7 (63.3% for *S.* Typhimurium, 62.1% for *S. aureus*) and Lb4 (59.9% and 60.4%, respectively). In contrast, Lb5 and Lb8 showed significantly lower biofilm inhibition (below 45%). Most strains exhibited comparable antibiofilm activity against both pathogens, although Lb3 and Lb9 showed a statistically significant difference in efficacy between the two. Four highly active strains (Lb4, Lb6, Lb7, and Lb10) were selected for 16S rRNA gene sequencing. BLAST analysis confirmed that these strains belonged to *Lactobacillus rhamnosus* and *Lactobacillus paracasei*. Phylogenetic trees confirmed their taxonomic placement within the genus *Lactobacillus*. The selected isolates exhibited diverse carbohydrate fermentation patterns. Lb4 showed the broadest metabolic capacity, fermenting all tested sugars. This metabolic versatility could contribute to its effective colonization and antagonistic activity in diverse environments. This study demonstrated the potent antimicrobial and antibiofilm properties of *Lactobacillus* strains isolated from kefir. These results support previous findings highlighting the inhibitory effects of LAB-derived metabolites against foodborne pathogens. Notably, the efficacy was higher against the Gram-positive *S. aureus*, likely due to the absence of an outer membrane that limits antimicrobial penetration in Gram-negative bacteria. Differences in biofilm inhibition among isolates suggest variation in the production of bioactive compounds such as organic acids, biosurfactants, hydrogen peroxide, and bacteriocins. The high efficacy of Lb7 and Lb4 marks them as promising candidates for applications in food preservation and safety. Moreover, the use of molecular tools allowed accurate species-level identification, enhancing reproducibility and potential regulatory acceptance for future applications. The study also highlights the advantages of targeting biofilm formation often a major contributor to chronic contamination and resistance rather than focusing solely on planktonic bacterial inhibition. This dual-action capability (antimicrobial and antibiofilm) makes certain *Lactobacillus* strains particularly valuable as natural biopreservatives.

Conclusion The findings of this study underscore the significant potential of kefir-derived *Lactobacillus* strains, particularly *L. rhamnosus* and *L. paracasei*, in inhibiting the growth and biofilm formation of major foodborne pathogens. Their natural origin, probiotic benefits, and efficacy against resistant microbial structures like biofilms highlight their applicability as safe and effective alternatives to chemical preservatives in food systems. Future research should explore formulation, safety evaluation, and in vivo efficacy to fully harness these strains in industrial and clinical settings.

Keywords: *Lactobacillus,* kefir, *Staphylococcus aureus, Salmonella enterica* serovar Typhimurium, antibiofilm, antibacterial

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