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## ABSTRACT

Probiotics are living bacteria that positively influence human health by maintaining intestinal microbial equilibrium. This study isolated, screened, and optimized probiotic lactic acid bacteria strains from yogurt, raw milk (i.e., cow, buffalo, and goat), cheese whey, and stool sample. Bacterial strains were grown at 37°C for forty-eight hours under anaerobic conditions on MRS agar. Sixteen strains were identified, purified, and characterized based on morphological, biochemical, and genotypic studies, such as gram staining, catalase test, and hemolytic test. To establish the probiotic qualities of each isolate, various tests, including NaCl, phenol, acid, and bile tolerance, were conducted. Eleven of the sixteen bacterial strains were rod- or cocci- or oval-shaped, gram-positive, catalase-negative, and non-hemolytic. Eleven isolates thrived in NaCl concentrations ranging from 2-10% and phenol concentrations ranging from 0.1%- 0.4%. Five of eleven isolates were resistant to acidic pH values (survival >50% at pH 3), and strain SS-2 demonstrated considerably greater tolerance to stomach pH than other isolates. Five isolates were resistant to bile (survival rate >50% at 0.3% bile salts). Five isolates were tested for antibacterial activity against *E. coli*, *S. aureus*, and *P. aeruginosa*. Only two isolates (SS-2 and Y-5) exhibited an antibacterial effect against *P. aeruginosa*, but all isolates exhibited against *S. aureus*. The antibiotic susceptibility of five isolates was examined using antibiotics, including Ampicillin, Amoxicillin, Gentamicin, Chloramphenicol, Ciprofloxacin, Amikacin, and Sulphomethazole. Two isolates were sensitive to Chloramphenicol, Amikacin, and Sulphomethazole. Each of the five isolates showed effective aggregation. Through 16S rRNA sequence analysis, *Limosilactobacillus Fermentum* was determined. Under various physiochemical circumstances, the production optimization of probiotic isolate Y-5 has been examined fruitfully. Using 14.15 g/l molasses as a carbon source, pH 7 (15.81 g/l), temperature 37°C (15.91 g/l), incubation duration 72 hours (21.15 g/l), inoculum size 10% (30.15 g/l), and Tryptone (39.61 g/l) as an organic nitrogen source the maximum biomass of isolate was obtained. The *Limosilactobacillus fermentum* was deemed a viable probiotic bacteria based on the results.