Abstract

The objectives of this study were to isolate and identify thermotolerant protease producing *Bacillus* and *Lactobacillus* from raw milk and their antimicrobial activity. By spread plate method, there were 41 and 40 isolates of *Bacillus* and *Lactobacillus* respectively. LB supplemented with 2% skim milk was used for selection of thermotolerant protease producing bacteria and the culture condition was performed at 50 °C after incubation for 24 hours. Ten out of forty-one isolates showing clear zone diameter of more than 10 mm were selected and evaluated for the presence of protease activity. BA26 and BA27 exhibited the maximum specific protease activity with 12.278 and 12.058 U/mg protein towards 1.5% casein, respectively. Moreover, the enzyme was more specific to casein than gelatin. *Bacillus* sp. may be a source of extracellular protease and that producing thermostable enzyme at high temperature. Eighty-one bacterial colonies isolated from raw milk were evaluated for antimicrobial activity by agar well diffusion method. Nine isolates (BA08, BA16, LA09, LA10, LA13, LA16, LA18, LA19 and LA20) revealed antimicrobial activity against *Staphylococcus aureus* TISTR 517 and *Escherichia coli* TISTR 887 which exhibited the inhibition zones of more than 10 mm. Based on carbohydrate fermentation patterns, biochemical characteristics and Gram staining features, BA26 and BA27 which producing the maximum thermotolerant protease activity were identified as *Brevibacillus* non reactive. According to the maximum inhibition zone of antibacterial activity, BA08, BA16, LA10 and LA16 were
classified as *Brevibacillus laterosporus* and *Geobacillus thermoglucosidasius*, respectively. LA10 and LA16 were classified as *Lactobacillus plantarum* 1. The thermotolerant protease producing *Bacillus* and antibacterial activity producing *Bacillus* and *Lactobacillus* isolated from raw milk may have potential applications in food, tannery and pharmaceutical industries.