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Abstract

The present study aimed to investigate the antioxidant activity, antibacterial and anti-biofilm effects of *Lactobacillus* isolates against *Salmonella* Typhimurium and *Staphylococcus aureus*. The cell-free supernatant (CFS) of five probiotics isolates (including *Lactobacillus acidophilus, L. animalis, L. leichmannii, L. fermentum,* and *L. reuteri*) was prepared. The antioxidant activity of CFSs was determined using two methods (ABTS and DPPH), and their antibacterial activity was determined by measuring the optical density of the bacterial suspension. CFS of lactobacilli was used to inhibit and remove the biofilms of the pathogens. The anti-biofilm effects of living cells of *Lactobacillus* were also evaluated in three conditions of co-culture of *Lactobacillus* with the pathogen (competition), pre-culture of *Lactobacillus* (exclusion), and post-culture of *Lactobacillus* (displacement) in order to inhibit and removal of biofilm of the pathogens. The results of antioxidant tests showed that the CFS of *L. reuteri* with 94.97% ABTS radical scavenging activity, and CFS of *L. fermentum* with 47.19% DPPH radical scavenging effect had the highest antioxidant potential. The results of antibacterial activity showed that the CFSs of *L. leichmannii* and *L. fermentum* significantly (P<0.05) inhibit the growth of *Salmonella* Typhimurium and *S. aureus*. The results of biofilm inhibition test showed that CFS of *L. acidophilus* had the greatest inhibitory effect on *S. aureus* biofilm with reduction of 2.97 log cycles, While CFSs of lactobacilli had a partial inhibitory effect on *Salmonella* Typhimurium biofilm, and CFS of *L. reuteri* had the greatest effect with a decrease of 0.48 log cycles compared to the others. Based on the results of biofilm removal test, CFS of *L. acidophilus* flocculation with the ability to remove biofilm by 4.14 log cycles had the greatest effect on *S. aureus* biofilm. On the other hand, CFS of *L. leichmannii* with a reduction of 4 log cycles, showed the strongest effect in removing *Salmonella* Typhimurium biofilm. The results of the competition test indicate that all lactobacilli were able to reduce *S. aureus* biofilm in about 1 log cycle compared to the control. However, the results of the competition test for *Salmonella* indicated that lactobacilli had a significant but partial effect in inhibiting the *Salmonella* biofilm. The results of exclusion test revealed that the formation of biofilm by all *Lactobacillus* strains could significantly prevent the formation of *S. aureus* biofilm and the greatest reduction (2.32 log cycles) was caused by *L. leichmannii*. However, the results showed the insignificant effect of all *Lactobacillus* strains in preventing of *Salmonella* biofilm formation cycles. The results of the displacement test showed that the studied lactobacilli had a significant but minor effect on the biofilm of *S. aureus,* and the greatest effect was related to *L. animalis* with a decrease of 0.45 log cycles. However, *L. animalis* showed the greatest effect in removing *Salmonella*

Typhimurium biofilm with a decrease of 1.24 log cycles. In conclusion, the results of this research showed that the studied lactobacilli and especially their cell-free supernatants have significant antioxidant, antibacterial and anti-biofilm effects, and have the potential to be used in the food industry as natural and safe compounds.

Key words: *lactobacillus, salmonella, staphylococcus*, biofilm, antioxidant, antibacterial, anti- biofilm.