**Probiotic properties of Bacillus strains isolated from stingless bee (Heterotrigona itama) honey collected across Malaysia**


ABSTRACT

This study aimed to isolate, identify, and evaluate the probiotic properties of Bacillus species from honey of the stingless bee Heterotrigona itama. Bacillus spp. were isolated from five different H. itama meliponicultures, and the isolates were characterized through Gram-staining and a catalase test. Tolerance to acidic conditions and bile salt (0.3%), hydrophobicity, and autoaggregation tests were performed to assess the probiotic properties of the selected isolates, B. amyloliquefaciens HTI-19 and B. subtilis HTI-23. Both Bacillus isolates exhibited excellent antimicrobial activity against both Gram-positive and Gram-negative bacteria and possessed significantly high survival rates in 0.3% bile solution for 3 h. Their survival rates in acidic conditions were also comparable to a commercial probiotic strain, Lactobacillus rhamnosus GG. Interestingly, the hydrophobicity and autoaggregation percentage showed no significant difference from L. rhamnosus GG, a commercial probiotic strain. The results from this study suggest that B. amyloliquefaciens HTI-19 and B. subtilis HTI-23 isolated from stingless bee honey have considerably good probiotic properties. Therefore, more studies should be done to investigate the effects of these bacteria cultures on gastrointestinal health.



**Keyword:** Stingless bee honey; Probiotic Bacillus strains; Molecular identification; Antimicrobial activity; Pathogenic bacteria