

## 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