

Isolation and functional characterisation of honey bee gut bacteria with probiotic, antimicrobial, and antioxidant properties

Suneeta Panicker*, Rashi Sahu, Joel Varghese and Sanjeevani Wandre

Department of Microbiology, Dr. D.Y. Patil Arts,
Commerce and Science College, Sant Tukaram Nagar, Pimpri, Pune 411018, India

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Probiotics are live microorganisms that confer health benefits to the host, particularly by modulating the gut microbiota. Honey bees possess a unique gut microbiome that may harbour novel probiotic strains with potential therapeutic applications. This study isolated and characterised probiotic bacteria from the gut of two honey bee species (*Apis mellifera* and *Tetragonula iridipennis*). *Lactobacillus casei* Shirota (Yakult) was used as a probiotic positive control. Out of the total isolates, 12 were analysed for probiotic traits, including bile and acid tolerance, starch and arginine hydrolysis, antimicrobial activity, beta-galactosidase activity, antioxidant activity, and adhesion ability. All isolates were negative for amylase and arginine dihydrolase but positive for Bile Salt hydrolase. Every isolate showed antibacterial activity, with three of them being more promising. Only 7 isolates demonstrated antifungal activity. Ten isolates were positive for β -galactosidase. Four isolates exhibited antioxidant activity comparable to that of the *Lactobacillus casei* Shirota. The control strain exhibited a hydrophobicity of 73.54%, while the highest hydrophobicity of 43.80% was observed in one of the gut isolates. Nine isolates exhibited apparent inhibitory effects against *E. coli* in non-neutralised supernatant, confirming that lactic acid is a potential antimicrobial substance. The isolates demonstrate potential probiotic properties based on *in vitro* evaluations, comparable to those of *Lactobacillus casei* Shirota.

Keywords: *Apis mellifera*, Honey bee gut, Lactic acid bacteria, Probiotic properties, Probiotics, *Tetragonula iridipennis*
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