



ABSTRACT

The aim of present study was to investigate the antimicrobial activity of honey bee (HB) gut microbiota. Honey bees (*Apis Cerana*) were collected from local bee keeper from District Lahore, Punjab, Pakistan. Total 12 HB gut isolates were characterized morphologically and biochemically. These strains were further screened for their antimicrobial activity by primary and secondary screening against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus licheniformis* and *Salmonella* spp. using cross streak and agar well diffusion method respectively. In preliminary screening, among 10 Gram positive HB isolates, gut isolates B1 and M7 showed highest zones of inhibition (ZI; 11 ± 0.33 , 7 ± 0.33 mm; $p \leq 0.05$) against *E. coli*. HB isolates B5 and B6 showed highest ZI (10 ± 0.66 mm; $p \leq 0.05$) against *K. pneumoniae* and both M4, M5 showed highest ZI against *P. aeruginosa* (7 ± 0.33 , 8 ± 0.57 ; $p \leq 0.05$). Six HB isolates exhibiting good antimicrobial activity via primary screening were further subjected to secondary screening which revealed that among all the isolates, M4 and B1 showed highest inhibition against all pathogens and their significantly highest activity was against *E.coli* (ZI; 14 ± 0.330 , 13 ± 0.577 mm; $p \leq 0.05$). Additionally, only M4 and M7 were able to inhibit growth of *P. aeruginosa* (ZI; 11 ± 0.881 , 8 ± 0.331 mm; $p \leq 0.05$). 16S rRNA sequencing revealed these isolates as *Bacillus tequilensis* (Accession No: MN713819) and *B. cereus* (Accession No: MW255303). Antibiotic susceptibility testing revealed that *B. tequilenses* showed resistance to erythromycin and sensitivity to ampicillin and rifampicin. While *B. cereus* was sensitive to all antibiotics. Physiological characterization revealed that both strains showed best growth at pH 7 and temperature 37°C . High performance liquid chromatography (HPLC) was also performed for isolate B1 and M4 where isolate B1 showed 11 peaks on chromatogram and isolate M4 showed 8 peaks indicating possible antimicrobial compounds (lactic acid, acetic acid, formic acid, bacteriocin, diacetyl, hydrogen peroxide, benzoic acid, cinnamic acid, flavonoids, fatty acids and anesthetics) which had showed excellent inhibitory effect in primary and secondary screening. These results suggest that the gut of honey bee is a promising source of bacterial strains producing metabolites with significant antimicrobial activity.