

Abstract

Bacterial resistance gives persistent challenges in the infectious diseases field, driving the search for novel effective strategies. This study investigates the antibacterial potential of endophytic bacteria isolated from *Piper crocatum*, a medicinal plant used as a therapeutic agent. Surface sterilization was performed prior to the isolation of endophytic bacteria, followed by characterization based on morphological and biochemical properties. The isolates were cultivated to obtain crude extracts and secretome containing secondary metabolites. Before testing the extracts and secretome, preliminary antibacterial screening was conducted using the cross-streaking against three Gram-positive and three Gram-negative bacteria. Subsequently, the antibacterial activity of the secondary metabolite extracts and secretome were evaluated against two Gram-positive and two Gram-negative bacteria using the disc diffusion assay at different concentrations. The endophytic bacteria were successfully isolated and characterized from *Piper crocatum*, with the isolates identified as *Enterobacter sp.*, *Klebsiella sp.*, and *Rahnella sp.*. The cross-streaking demonstrated reduced bacterial growth and clear zone of antagonistic activity against selected Gram-positive and Gram-negative bacteria, indicating the antibacterial potential. However, the secondary metabolite extraction and secretome did not demonstrate detectable antibacterial activity under tested conditions. Therefore, the findings partially support the hypothesis that endophytic bacteria from *Piper crocatum* possess antibacterial. Additionally, further optimization of sterilization methods, the use of freshly prepared bacterial stocks, consistent in growth curve analysis, and the other tests such as antibiofilm assays, phytochemical screening, LC-MS, and membrane permeability assays or SEM/TEM analysis are recommended to better evaluate the antibacterial compounds.

Keywords: Endophytic bacteria, *Piper crocatum*, antibacterial, cross-streaking, disc diffusion