

ABSTRACT

The study of microorganisms that produce naturally occurring bioactive compounds with antimicrobial and antibiofilm effects has sparked much interest in the microbial bioprospecting of fermented foods. Based on morphological and biochemical and molecular studies, two *Bacillus* strains, which were isolated from traditionally fermented meat, were identified as *Bacillus licheniformis*. All the isolates were also identified with robust biofilm formation characteristically of the *Bacillus* genus; being Gram-positive, catalase-positive, and spore-forming rods after seven days of incubation. Their optimal optical densities were 1.55 (DB) and 0.94 (AZ).

Although agar well diffusion assays indicated nonsignificant zones of inhibition and antimicrobial activity of $\geq 50\%$ v/v was confirmed with broth microdilution. Both ECM and WCM metabolites inhibited *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* in a dose-dependent manner, and biofilm inhibition was 70% at $2\times$ MIC. WCM extracts inhibited *E. coli* more than 70% which was the strongest inhibition and the ECM extracts inhibited overall biofilm formation by 67.5 and 72%. In addition, all extracts at $2\times$ MIC greatly affected the dispersal of preformed biofilms, which was more than 70% reduction across all pathogens.

A metabolite profile distinctively built included diketopiperazines [cyclo(L-prolyl-L-valine)], 11.59% quinoline, interesting phenolic acids like 2,5-dihydroxybenzoic acid, levomenthol, and heptyl succinate among others. Described in the literature, these compounds were linked to quorum signaling inhibiting, antioxidative, and antimicrobial properties.

It would seem that *B. licheniformis* and *B. paralicheniformis* shutdown the stereotype of being used solely as a source of bacitracin. It was proposed that the strains are being waste and that the strains could be used for be used for natural food preservation and for the prevention of biofilm formation. Of note, these compounds are a good candidate for multifunctional bioactive metabolites. The studies noted that the bioactive metabolites require further optimization and molecular characterization for target biomedical and industrial applications.