

TITLE: SPONGE-ASSOCIATED *Bacillus* ARE AN PROMISING SOURCE OF ANTIMICROBIAL AND BIOSURFACTANT SUBSTANCES

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ABSTRACT:

Bacillus are known to produce bioactive molecules with broad applications, and although found in different sites, it has been less explored when compared with other sponge-associated genera. In our previous work, two *Bacillus* strains isolated from marine sponges showed the production of antimicrobials against a wide range of antibiotic-resistant bacteria and a potential biosurfactant production. Therefore, this study aimed to assess the antimicrobial and biosurfactant potential from these strains, identified as *Bacillus pumilus* 64-1 and *Bacillus subtilis* 84-5, respectively. The complete genome of these strains was whole-sequenced (Illumina NextSeq500), assembled, and annotated. From antiSMASH and BAGEL 4 analysis, *B. pumilus* 64-1 genome (3.6 Mbp) appears to encode for at least 12 potential biosynthetic gene clusters (BGCs), with non-ribosomal peptide synthetases (NRPS), types I and III polyketide synthases (PKS) and one NRPS-T1PKS hybrid. In the latter, genes to bacillysin, a known antimicrobial agent, were identified. Two contigs encoding genes potentially related to the biosynthesis of ribosomally synthesized and post-translationally modified peptides (RiPPs), as amylocyclicin, sactipeptides, and the bacteriocin UviB precursor were found. *B. subtilis* 84-5 genome (4.12 Mbp) harbored 11 potential BGCs, including types I and III PKS, NRPS, and surfactin, the most powerful and effective lipopeptide-type biosurfactant known so far. In addition, two contigs encoding genes potentially related to the biosynthesis of RiPPs, as linear azol(in)e-containing peptides, subtilisin A, sactipeptide, and a quorum-sensing peptide pheromone ComX4 were detected. Meanwhile, bioactive extracts were obtained from the *B. pumilus* 64-1 strain using organic solvents and analyzed in a thin layer chromatography followed by antimicrobial bioassays. The methanolic extract showed a minimum inhibitory concentration of 29.6 µg/mL with bacteriostatic activity against *Staphylococcus aureus* cells. From the biosurfactant screening tests, *B. subtilis* 84-5 likely produces substances with biosurfactant and bioemulsifier properties: emulsification index of 5% in mineral oil and 25% in *n*-hexadecane and positive results in cell hydrophobicity and drop collapse tests. A surface tension of 38.06 ± 0.36 mN/m was verified for the cell-free supernatant. These results endorse the relevance of sponge-associated *Bacillus* as potential sources of biotechnologically-relevant antimicrobials and biosurfactants.

Keywords: *Bacillus*, antimicrobial, biosurfactant, genome.

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