

TITLE: ANTI-BIOFILM ACTIVITY OF LIPOPEPTIDES PRODUCED BY A *Bacillus vallismortis* STRAIN

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Production of biofilm is a bacterial strategy to survive from harsh conditions and external damage. Since biofilms may cause several industrial damages, such as oil pipelines obstruction or can lead to corrosion in equipment used in oil and gas, there is an urgency to find methods to defeat them. Nevertheless, biofilms are difficult to eradicated and resist to many conventional biocides. As an alternative to eliminate biofilms, lipopeptide biosurfactants stand out due to their broad antimicrobial activity. Hence, this study aimed to evaluate antibiofilm activity of the lipopeptide mixture produced by a *Bacillus vallismortis* strain. Lipopeptide production was performed by growing the bacterial strain in mineral medium, for 48 h at 37 °C and 150 rpm. After that, lipopeptide was extracted by acid precipitation from the cell-free supernatant, freeze-dried and subsequently used in the antibiofilm microtiter assays. For the antibiofilm activity, lipopeptide mixture was tested in concentrations between 50 and 400 µg/L for inhibition of the biofilm formation of a *Bacillus sp.* strain isolated from a gasoline tank. For the biofilm production the strain was grown in TGE broth for 18h and 100 µl were transferred to 96 well microplates. After that, 50 µl of the lipopeptides were added and the plates were incubated at 37 °C for 24 h. After the incubation period, the wells were washed with PBS-tween, followed by ethanol, distilled water and 0.3% crystal violet. After the stain removal, 33% acetic acid was added to each well and the solution was transferred to a new 96 well microplate and the OD_{595nm} was taken. Ampicillin 100 µg/mL was used as positive control and sterile distilled water as negative control. The lipopeptide mixture was also characterized by mass spectrometry by dissolving it in methanol and analyzing in ESI-Q-TOF MS in the positive ion mode with internal calibration. Cell-free supernatant of *B. vallismortis* inhibited 64% of biofilm whereas the purified lipopeptides at concentration of 100 µg/L inhibited 97%. Mass spectrometry showed the presence of isoforms belonging mainly to the iturin and fengycin families. Our results highlight the potential of the lipopeptide mixture of a *B. vallismortis* strain for biofilm inhibition, even in very low concentrations or in unpurified form.

Keywords: Biofilm inhibition, Biosurfactant, microtiter assay

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