Effects of rhamnolipid on growing biofilms of *Burkholderia pseudomallei*: an analysis of the biofilm biomass and protease production

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Burkholderia pseudomallei is a Gram-negative bacillus, saprophyte of soils and water, which causes melioidosis. This disease is endemic to Northeastern Brazil, specifically, the state of Ceará, where over 30 cases have been diagnosed, since 2003, when the first cases were reported. Several virulence factors are involved in the pathogenicity of *B. pseudomallei*, including biofilm formation and protease production. Biofilms are associated with chronic refractory infections and antimicrobial resistance, while proteases may contribute for host cell invasion. Rhamnolipid is a biosurfactant produced by *Pseudomonas aeruginosa*. It is known that biosurfactants are secreted by bacterial sessile cells to induce biofilm dispersion. Thus, this study aimed at evaluating the effects of rhamnolipid on biomass and protease production of growing biofilms of *B. pseudomallei*. For such, 8 clinical and 8 environmental strains of *B. pseudomallei* from Ceará were used. Biofilms were grown in 96-well microplates, containing 175 µL of BHI-Glucose 1% broth, with or without rhamnolipid at 0.78, 7.8 or 78 mg/mL, and 25 µL of a bacterial inoculum at $1.5x10^9$ cfu/mL. Then, microplates were incubated at 37 °C, for 48h. Strains were grown in triplicate for each growth condition, and the assays were carried out at two different moments. After this period, biofilm biomass was spectrophometrically evaluated through crystal violet staining. Additionally, the supernatant of each strain in sessile growth was removed, centrifuged and added to 500 µL of azoalbumin, and incubated at 37 °C, for 3 hours, for the analysis of protease activity. Afterwards, the enzymatic reaction was stopped by the addition of trichloroacetic acid and the solution was spectrophotometrically read at 440 nm, after adding NaOH. It was observed that rhamnolipid at any tested concentration did not significantly alter biofilm biomass, when compared to biofilm growth control. However, protease activity was significantly higher than biofilm growth control when biofilms were grown at 7.8 mg/mL and 78 mg/mL of rhamnolipid, but not at 0.78 mg/mL. Although rhamnolipid did not interfere with biofilm biomass, it induced protease activity in *B. pseudomallei* growing biofilms, which may be a mechanism to overcome rhamnolipid-induced stress and warrant biofilm formation. Finally, more studies are necessary to understand the role of biosurfactants in the maintenance of *B. pseudomallei* biofilms.

Keywords: *Burkholderia pseudomallei*; rhamnolipid; growing biofilms; protease production