

Rhamnolipid reduces biomass and increases protease activity of mature *Burkholderia pseudomallei* biofilms

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Burkholderia pseudomallei is a Gram-negative bacterium that inhabits soils and water and causes melioidosis. This disease is endemic to 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 produced by *B. pseudomallei*, including biofilms and extracellular proteases. Biofilms are associated with chronic infections, antimicrobial resistance and clinical relapse, 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 mature biofilms of *B. pseudomallei*. For such, 8 clinical and 8 environmental strains of *B. pseudomallei* from Ceará were used. Biofilms were grown in microplates, containing 175 µL of BHI-Glucose 1% broth, and 25 µL of an inoculum at 1.5×10^9 cfu/mL. Then, microplates were incubated at 37 °C, for 48h. After this period, biofilm supernatant was discarded, and 200 µL of BHI-Glucose broth, with or without rhamnolipid at 7.8, 78 and 780 mg/mL, were added to wells containing adhered cells, and plates were incubated for further 24 hours. Strains were grown in triplicate for each growth condition, and assays were performed at two different moments. After this period, biofilm viability was evaluated with resazurin and biomass was assessed through crystal violet staining. Additionally, biofilm supernatant was removed, centrifuged and added to 500 µL of azoalbumin, and incubated at 37 °C, for 3 hours, to analyze protease activity. Afterwards, the enzymatic reaction was stopped with trichloroacetic acid, NaOH was added and the solution was read at 440 nm. Rhamnolipid did not interfere with biofilm viability at any concentration, but it significantly reduced biofilm biomass at 78 and 780 mg/mL, when compared to biofilm growth control. Additionally, rhamnolipid increased biofilm protease activity at all concentrations, but it was only significant at 78 and 780 mg/mL. Rhamnolipid induced mature biofilm dispersion and increased biofilm protease activity, which may be an attempt to maintain the biofilm structure, overcoming the stress caused by this compound. Finally, further studies are necessary to evaluate the role of biosurfactants in the dispersion of *B. pseudomallei* biofilms.

Keywords: *Burkholderia pseudomallei*; rhamnolipid; mature biofilms; proteases