

CCCP INHIBITES EFFLUX PUMP PRODUCTION BY REDUCING BIOMASS, PROTEASE, AND SIDEROPHORE PRODUCTION IN MATURE BIOFILMS OF *B.pseudomallei*.

B. pseudomallei is a Gram-negative, saprophytic bacterium found mainly in soil and water causing melioidosis in tropical and subtropical regions, including northern Australia, Southeast Asia, and Northeast Brazil. Several virulence factors are produced by *B. pseudomallei*, including biofilms, extracellular proteases, and siderophores. Biofilms are associated with chronic infection, antimicrobial resistance, and relapse of the infection. While proteases contribute to hosting cell invasion, siderophores sequester iron for bacterial metabolism. Carbonyl cyanide m-chlorophenylhydrazone (CCCP) is a classic inhibitor of efflux pumps, interfering with biofilm formation. The objective of the study is to evaluate the effect of the efflux pump inhibitor CCCP on the biofilm structure and production of virulence factors of *B. pseudomallei*. Eight clinical and eight environmental strains of *B. pseudomallei* were used. Biofilms were grown in microplates containing 175 μ L of 1% BHI-Glucose broth and 25 μ L of inoculum at 1.5×10^9 cfu/mL. Then, the microplates were incubated at 37 °C for 48 hours. After this period, the biofilm supernatant was discarded and 200 μ L of BHI-Glucose broth, with or without CCCP at 67, 133, and 530 μ M, were added to the wells containing adhered cells, and the plates were incubated for another 24 hours. The strains were grown in triplicate for each growth condition and the tests were performed at two different times. After this period, the viability of the biofilm was evaluated with resazurin and the biomass with crystal violet. Additionally, the biofilm supernatant was removed and centrifuged, 500 μ L was added to 500 μ L of azoalbumin to analyze protease activity, and 100 μ L was mixed with CAS solution to analyze siderophore production. The plates were read in a spectrophotometer. It was observed that CCCP at the highest concentration significantly reduced the biofilm biomass, as well as the activity of proteases and siderophores was also reduced at the highest concentration ($P < 0.05$), both when compared with the control. Finally, CCCP-mediated inhibition of efflux pumps in the *B. pseudomallei* biofilm interferes with the structure of the biofilm, making it more vulnerable to antimicrobial agents.