Modulation of *Bulkholderia cenocepacia* virulence in response to environmental factors of lung tissue in cystic fibrosis patients

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This work aims to study the modulation of virulence factors produced by *Burkholderia cenocepacia* under conditions associated with mucus characteristic of Cystic Fibrosis (CF), such as high osmotic pressure, high iron concentrations and the presence of mucolytic agents used in the treatment of CF, for instance, N-acetyl – cysteine (NAC). For analysis of virulence factors, an ET-12 clone of *B. cenocepacia* was employed. Different concentrations of these substances were used: NaCl (0.1M, 0.3M, 0.4M and 0.5M), Ferric Citrate (or Ammoniacal Citrate) at 1, 10µM and NAC (0.5, 1.0 and 2.0 mg/mL). Phenotypic tests were performed to confirm the characteristics of the strain. The agar diffusion technique and a growth curve were applied to study the effect of culture conditions. Virulence expression was assessed through biofilm formation assay and proteomic analysis using intracellular protein extracts and culture supernatant. In the screening performed by the agar diffusion technique, there was no inhibition. A growth curve to evaluate the behavior of ET-12 determined that the bacteria takes, on average, 6 hours to reach the log phase. It does not grow in the presence of NAC (2.0mg/mL), EDTA (10mM) and 0.4M/ 0.5M of NaCl, besides its growth is stimulated by iron at 1µM and 10 µM. On the other hand, biofilm formation was slightly reduced in presence of iron (1 and 10 µM), decreased in a dose-dependent manner at high osmotic pressures (0.1M, 0.3M, 0.4M and 0.5M NaCl), and was completely inhibited in the presence of EDTA (10 mM). The NAC inhibited the biofilm expression at concentrations of 0.5mg/mL and 2.0mg/mL, and surprisingly, it seemed to stimulate this process at the concentration of 1.0mg/mL ($p \leq 0.05$). Furthermore, ET-12 protein extracts from ET-12 (secreted) and cellular (intracellular) culture supernatants are being obtained in LB containing 0.4M NaCl, 10µM ferric citrate and 1mg/mL of NAC for the analysis of expressed proteins in these conditions. In general, iron, EDTA, NAC and NaCl negatively modulated biofilm expression, the main virulence factor of ET-12. This preliminary data should be confirmed by further testing. The results obtained will contribute to a better

understanding of the mechanism of pathogenesis and to improve the infection control and treatment of CF patients.

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