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Understanding Host-Pathogen Interactions of *Pseudomonas aeruginosa* with Lung Epithelial Cells

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Pseudomonas aeruginosa is a Gram-negative opportunistic bacterial human pathogen that is responsible for severe acute and chronic infections worldwide. The ubiquitous nature of *P. aeruginosa* and ability to survive on abiotic surfaces, makes it one of the leading causes of nosocomial infections [1, 2]. Due to its capability to adapt, survive and possess resistance to multiple broad- spectrum antibiotics, *P. aeruginosa* infections are posing a serious public health threat with approximately 51,000 healthcare associated infections in US every year (Center of Disease Control, 2019 report). It is one of the primary organisms that infects airways of cystic fibrosis (CF) patient's lungs and a leading cause of morbidity and mortality [3]. CF is a genetic disorder that occurs because of the defect in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. CFTR is responsible for regulating the flow of chloride ions in the lung secretions, aiding in mucocilliary clearance. In CF, the mutations in CFTR disrupt its functioning leading to decreased chloride excretion and deposition of thick mucus in the lung airways [4]. Pulmonary fluids of CF patients contain an elevated concentration of ions such as Na^{+}, Mg^{2+}, and Ca^{2+} [5]. Studies suggest that Ca^{2+}, an important cellular signaling molecule, is known to enhance the virulence capability of *P. aeruginosa* [6], however little is known about how it regulates the host-pathogen interactions of *P. aeruginosa* with lung epithelial cells.

We aim to understand how Ca^{2+} affects the adherence of *P. aeruginosa* PAO1 (lab strain) and FRD1 (CF pulmonary isolate) with A549 alveolar lung epithelial cells in the low (0.5mM) and high (5mM) Ca^{2+} conditions. Immunofluorescence microscopy and scanning electron microscopy (SEM) were used to test for adherence of *P. aeruginosa* on the surface of epithelial cells. *P. aeruginosa* strains and epithelial cells were cultured separately in their respective media, followed by seeding of epithelial cells on glass coverslips in tissue culture treated plates. At a multiplicity of infection of 50 bacteria/cell, epithelial cells were infected with *P. aeruginosa* for 2 hours, stained for indirect immunofluorescence (IF) and visualized using Leica DM16000B fluorescent microscope. Simultaneously, the coverslips were prepared for SEM and visualized using FEI Quanta 600 field-emission gun Environmental SEM. A significant increase in the adherence of *P. aeruginosa* strains was observed in the presence of high Ca^{2+} by IF as well as by high- power SEM (Fig 1). Additionally, increased Ca^{2+} concentration led to enhanced biofilm formation by FRD1 on the surface of A549 (Fig 2). Taken together, this study provides significant insights regarding the effects of Ca^{2+} on the colonization and biofilm production of *P. aeruginosa* during infection using different microscopy techniques, the knowledge of which is important for understanding the enhanced virulence of *P. aeruginosa* in CF.

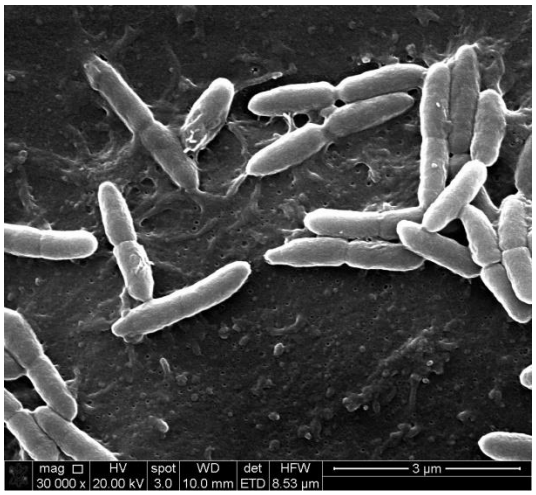

Figure 1: PAO1 adheres to the surface of A549 cells as observed using high throughput Scanning Electron Microscopy (SEM).

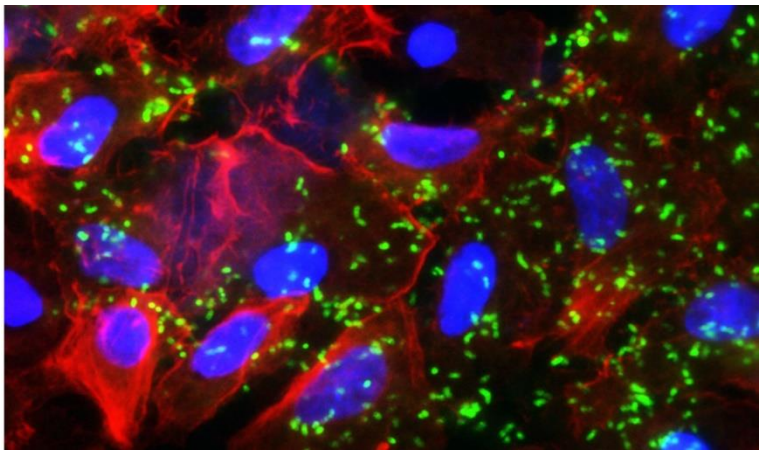

Figure 2. *P. aeruginosa* FRD1 adhering to the surface of A549 alveolar lung epithelial cells. Shown are *P. aeruginosa* (green), host nuclei (blue) and actin (red).

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