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**BEHAVIORAL CHANGES OF *Pseudomonas aeruginosa* IN
RESPONSE TO APOPTOTIC CELLS: INSIGHTS FROM REAL-TIME
OBSERVATION OF TWITCHING MOTILITY AND TRACKING
ANALYSIS**

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Pseudomonas aeruginosa is a ubiquitous bacterium known for causing opportunistic infections in severely burned and wounded patients. It is also a major causative agent of chronic lung infections in individuals with cystic fibrosis. A common factor in these susceptible contexts is compromised or damaged epithelial tissue. Our research showed that when epithelial monolayers are infected, free-swimming *P. aeruginosa* attaches to apoptotic cells extruded from the epithelium, leading to the formation of stable bacterial clusters. Through a combination of experimental studies and mathematical modeling, we have identified that the initial adhesion to apoptotic cells and clustering are mediated by type IV pili (T4P) activity. T4P are surface-exposed filaments that rapidly extend and retract, generating active forces. In addition to their role in adhesion, T4P facilitate bacterial movement on surfaces, a phenomenon known as twitching motility. The ability to alter its motility strategy grants this bacterium versatility for invasion and virulence towards host tissues. Of note, twitching trajectories can be tracked. Consequently, we expanded our research to include the interaction between *P. aeruginosa* and apoptotic cells in this context. We monitored *P. aeruginosa* populations under microscopy as they engaged in twitching motion and, using segmentation and tracking analysis, examined their behavior. Bacteria in proximity to apoptotic cells exhibit an increased speed of movement. Under controlled conditions (without apoptotic cells), bacteria at the leading edge of the twitching zone exhibit an average speed of 0.07 ± 0.04 $\mu\text{m}/\text{sec}$. In contrast, when apoptotic cells are present, the average speed in that zone nearly doubles to 0.12 ± 0.05 $\mu\text{m}/\text{sec}$. This behavioral shift may suggest that the bacteria detect a molecule secreted by apoptotic cells and respond by increasing their motility. In addition, we observed a second behavioral shift: after bacteria contact apoptotic cells, they display a linear back-and-forth movement over a short distance, similar to a behavior reported for *P. aeruginosa* during its attack on other bacterial species' colonies. Additional experiments are underway to better understand these complex and relatively unexplored behaviors. In addition, to study the activity of T4P before and after contacting apoptotic cells, we are using a recently reported technique that involves cysteine substitution

within the major structural subunit of T4P. This technique allows to observe T4P dynamic activity in real time. We are currently investigating whether changes in T4P dynamics drive the observed behavioral shifts.

Palabras clave: *Pseudomonas aeruginosa* – Twitching motility – Apoptotic cells – Tracking – Type IV pili