Sociedad Argentina de Microbiología General

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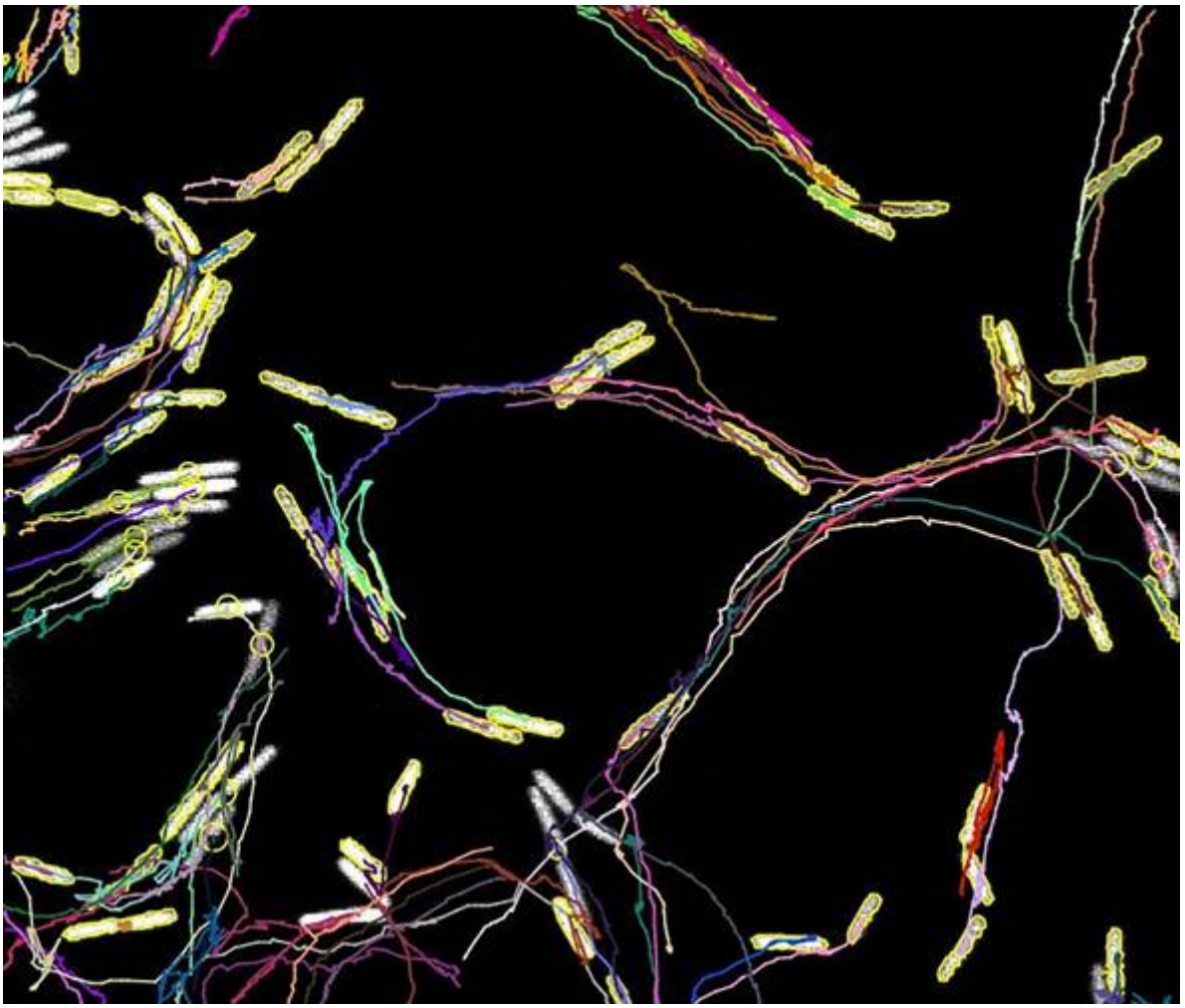

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

INVASIVENESS OF *Pseudomonas aeruginosa* IN LUNG EPITHELIAL CELLS ENHANCED BY HYPERMUTABILITY-DRIVEN ADAPTATIONS

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Pseudomonas aeruginosa (PA) is an opportunistic pathogen linked to chronic infections, especially in cystic fibrosis patients. Although traditionally classified as an extracellular pathogen, recent findings suggest PA can invade and persist in epithelial cells, contributing to immune evasion and antibiotic resistance. The precise molecular mechanisms behind this invasive phenotype remain unclear, highlighting the need to investigate the adaptive mutations PA develops within the host. Our research evaluated the invasiveness and persistence of PAO1 wt and PAO1 ?mutS strains in human A549 lung epithelial cells. Using an experimental evolution model, we conducted ten successive infection rounds, quantifying intracellular bacteria through antibiotic exclusion assays and confocal microscopy. The hypermutator PAO1 ?mutS strain showed a significantly enhanced invasive capacity compared to the wild-type, with this increase becoming more pronounced over time. Confocal microscopy revealed a substantial rise in both the percentage of invaded cells and the number of internalized bacteria per cell in the evolved hypermutator populations. Additionally, electron microscopy showed hypermutator strains escaping endolysosomal vesicles to reside in the cytosol, unlike wt strains confined within vesicles. This likely provides an adaptive advantage, enabling them to evade lysosomal degradation and replicate more efficiently within host cells, enhancing persistence. Subsequent analysis focused on phenotypic characterization of the evolved populations. Hypermutator strains demonstrated a marked reduction in virulence, evidenced by diminished cytotoxicity in *Caenorhabditis elegans* and *Arabidopsis thaliana* infection models. These populations also showed reduced production of virulence factors like quorum sensing signals, motility, pyoverdine, and pyocyanin, while biofilm formation remained unchanged. In the final phase of our study, a comparative genomic analysis identified molecular bases underlying the observed adaptations. Whole-genome sequencing of evolved hypermutator population revealed 851 sequence variations in coding regions, with 23% associated with virulence factors. Notably, mutations affecting the Type VI Secretion System (T6SS) were identified across all three of its modules (HSI-1,

HSI-2, HSI-3), indicating a potential shift in virulence regulation. These mutations, coupled with changes in key regulatory genes such as *gacS* and *ladS*, suggest reprogramming of virulence pathways that enhance intracellular persistence and invasiveness while reducing cytotoxicity. This differential regulation of the T6SS likely underpins the increased invasiveness observed in the hypermutator populations. Overall, these findings underscore the role of hypermutability in driving PA's adaptation to intracellular environments. Further studies, including functional validation of the identified mutations, are needed to fully elucidate the molecular mechanisms involved in this process

Palabras clave: Invasiveness - Hypermutability - Pseudomonas aeruginosa - Lung epithelial cells - Virulence factors