Sociedad Argentina de Microbiología General

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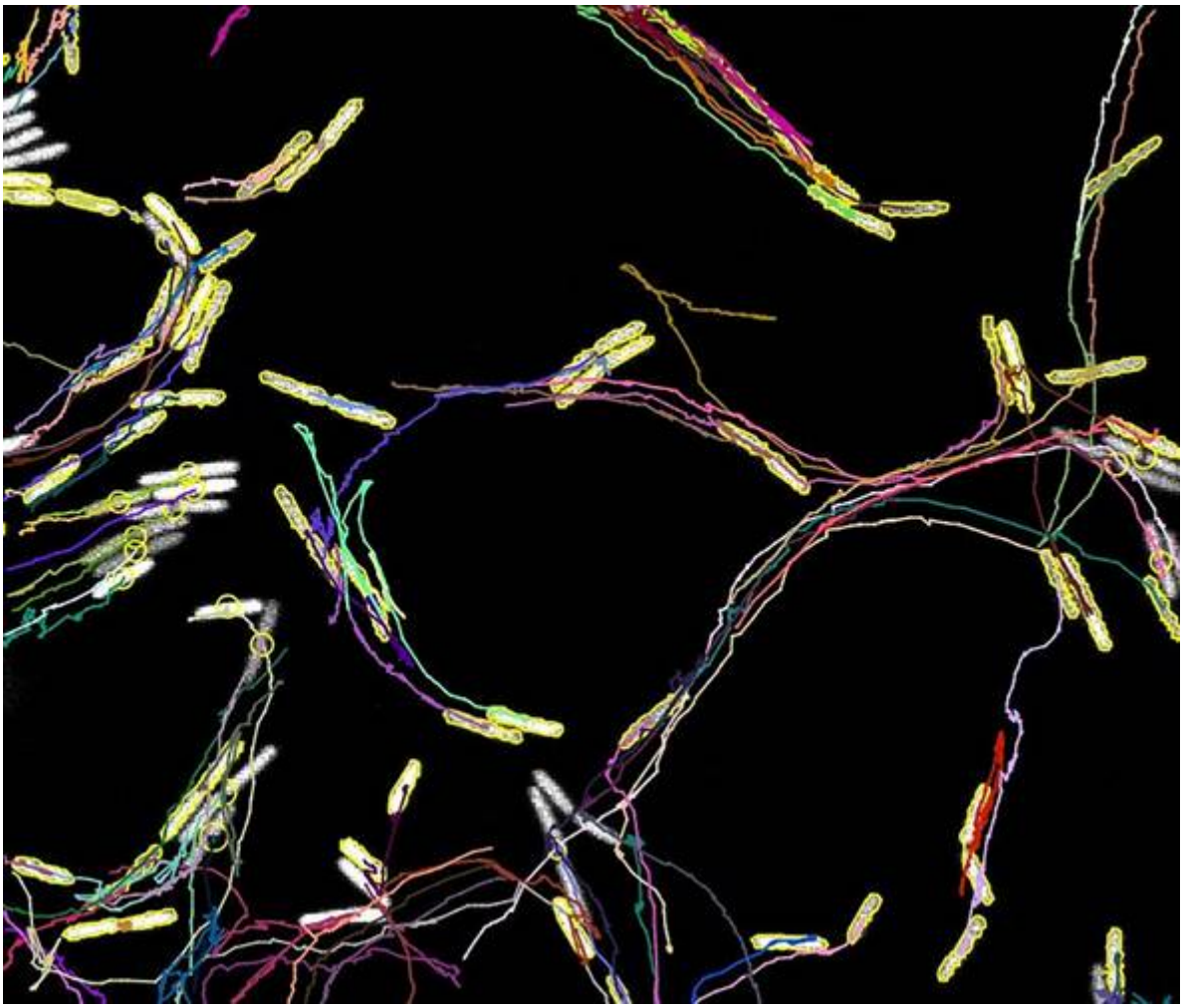

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

REGULATORY INFLUENCE OF SIGMA FACTOR ALGT AND ANTI-SIGMA MUCA ON ANAEROBIC ADAPTATION AND ACIDIFIED NITRITE RESPONSE IN Pseudomonas aeruginosa

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Pseudomonas aeruginosa is an opportunistic pathogen that causes both acute and chronic respiratory infections in cystic fibrosis (CF) patients. Its persistence in CF airways is due to its adaptability, leading to the emergence of phenotypes like the mucoid variant. This phenotype, driven by mutations in the *mucA* gene, correlates with a worse prognosis and indicates chronic infection progression. The mucA gene encodes an anti-sigma factor that regulates alginate production by sequestering AlgT, an alternative sigma factor that controls the alginate biosynthetic operon and other critical processes. The most frequent mutation responsible for mucoid conversion is a deletion of a G residue within a homopolymeric track of five Gs (G5426), also known as mucA22 allele, causing the truncation of MucA C-terminal periplasmic domain. Chronic infection progression is characterized by reduced oxygen tension, which supports microaerobic and anaerobic niches with increased nitrate (NO3-) and nitrite (NO2-). Consequently, *P. aeruginosa* shifts to anaerobic respiration, using NO3- and NO2- as terminal electron acceptors. Evidence shows that the mucoid *mucA22* is unstable under static aerobic conditions and reverts to a nonmucoid phenotype mainly through *algT* suppressor mutations, while it remains stable but highly sensitive to acidified nitrite (A-NO2-) under anaerobic conditions. We previously confirmed *mucA22* strain sensitivity to NO2- and showed that this phenotype is nearly restored to wild-type levels following algT deletion, suggesting a link to sigma factor deregulation. To elucidate the relationship between mucA mutations, the mucoid phenotype, and anaerobic metabolism, we employed quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) with Q-Exactive Orbitrap Mass Spectrometers to analyzed *algTmucA* (wild-type), ?*algTmucA22*, and *mucA22* mutant strains, both treated and untreated with A-NO2- under anaerobic conditions. Additionally, reversion tests under different culture conditions were performed to isolate revertants and assess their sensitivity to A-NO2-. Proteomic analysis revealed that the PAO1

mucA22 strain exhibits reduced NirS and NirF levels, which are key for NO2- reduction, and increased OprF levels, a porin involved in NO3- and NO2- diffusion, while the PAO1 ?*algTmucA22* mutant shows significantly higher Nir levels. This suggests that partial AlgT release in the PAO1 *mucA22* strain disrupts Nir expression, affecting denitrification and leading to NO2- accumulation and cellular toxicity. Revertants with suppressor mutations in *algT* or related pathways were the only ones resistant to NO2-, confirming that NO2- sensitivity is due to AlgT dysregulation and indicating that alginate overproduction and NO2- sensitivity are independent processes. This study advances the understanding of AlgT and MucA interactions in anaerobic metabolism and may help develop strategies to manage mucoid variants and improve CF patient outcomes.

Palabras clave: Pseudomonas aeruginosa - Cystic fibrosis - Anaerobic metabolism - Chronic infection