Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024
Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.

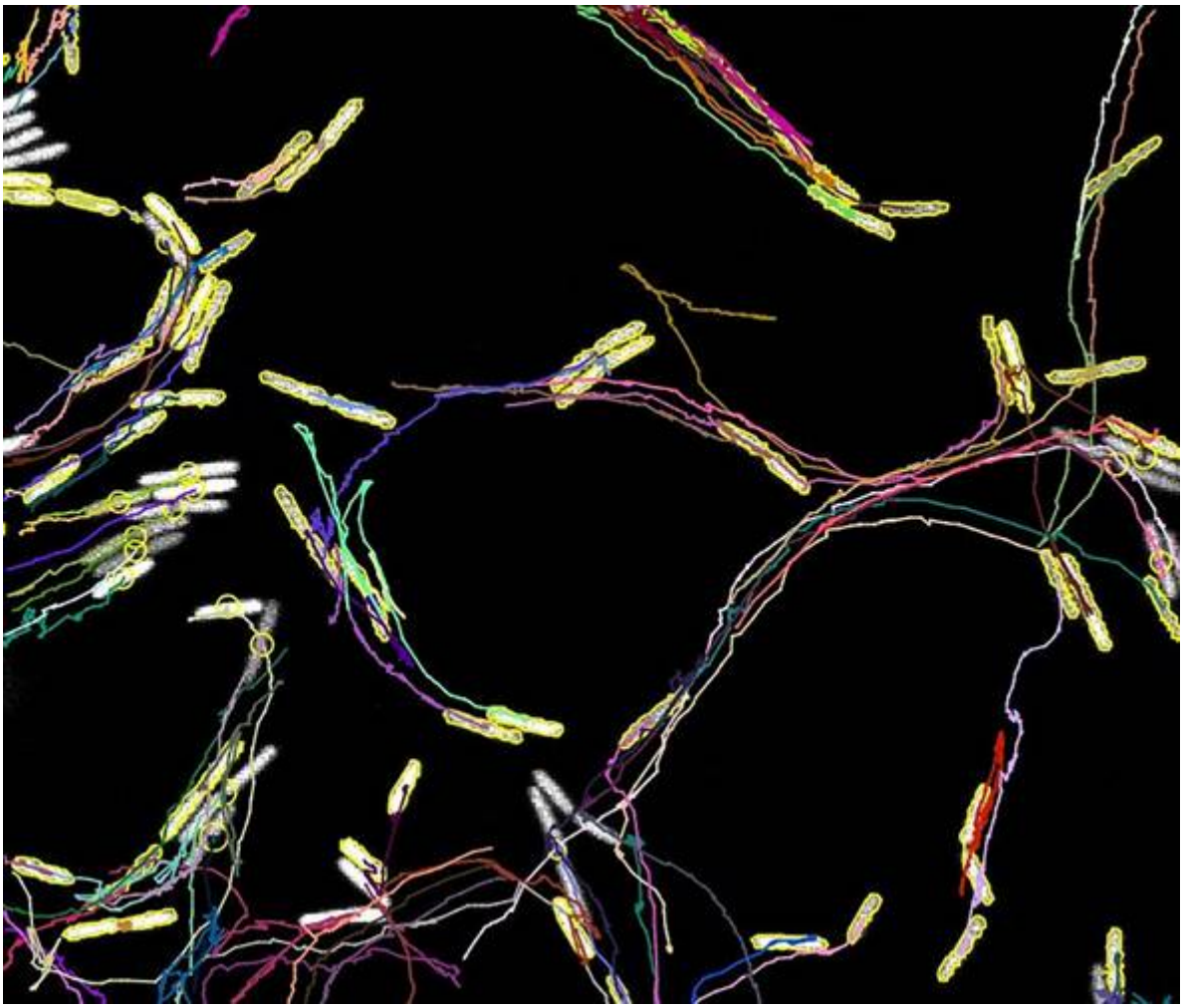

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

GROUPING PYOMELANINS BY PHISIOLOGICAL EFFECTS: INSIGHTS FROM Pseudomonas aeruginosa CLINICAL ISOLATES AND REFEERENCE STRAINS

Diaz Appella, Mateo Nicolas[1] - Li, Ningna[2] - Lopez, Nancy Irene[1,3] - Götz, Friedrich [2] - Tribelli, Paula Maria[1,3]

1) Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
2) Eberhard Karls Universität Tübingen Mathematisch-Naturwissenschaftliche Fakultät: Tubingen, Baden-Württemberg, DE
3) IQUIBICEN-CONICET, Buenos Aires, Argentina
Contacto: mateodiaz.appella@gmail.com

Pyomelanin is a heterogeneous polymeric pigment whose production is widely distributed among various microbial genera. In *Pseudomonas* species, its synthesis is associated with alterations in tyrosine catabolism, and recent studies have demonstrated that strains within the same species can exhibit structural diversity. This work aims to investigate whether such variability impacts the physiology of clinical isolates of *Pseudomonas aeruginosa* and their interaction with the host. We hypothesize that the structural diversity of pyomelanin will influence interactions with host cell lines. Initially, pyomelanin was isolated, purified, and quantified from four P. aeruginosa strains: two melanin-producing mutants constructed via CRISPR/nCas9 from reference strains *P. aeruginosa* PAO1 (PAO1 hmgA*) and PA14 (PA14 hmgA*), and two clinical isolates, one from a patient with acute infection (PAM) and another from a cystic fibrosis patient with chronic infection (PAmel4-7). It was found that PAO1 hmgA* was the highest pigment producer after 24 h of incubation in LB medium. Similarly, colony counts revealed that the reference strains PAO1 hmgA* and PA14 hmgA* showed the highest growth after 24 h of aerobic culture. Next, it was assessed whether the different pyomelanins induced differential cytokine production through ELISA detection assays, in two cell lines by adding melanin to a final concentration of 0.2 mg/ml: the human lung epithelial cell line A549 and the murine macrophage cell line RAW 264.7. In A549 cells, pyomelanins from clinical isolates induced higher IL-8 production. On the other hand, none of the pyomelanins induced IL-6 or TNF-alpha production in this cell line. In contrast, when RAW 264.7 cells were stimulated with these pigments, all pyomelanins induced similar levels of IL-6 production, but the clinical isolates induced lower TNF-alpha production. Subsequently, it was evaluated the differential cytotoxicity of the pyomelanins in the A549 cell line using an MTT assay. Various concentrations of each pyomelanin were tested, and no differences in toxic effects were observed among these pigments after 24 h of exposure. Finally, the antioxidant activity of purified pyomelanin was determined based on its ability to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals. The pyomelanins

from the reference strains exhibited the highest oxidative stress protection, while the clinical isolates showed lower scavenging capacity. These results allow us to categorize pyomelanins into two groups based on the observed physiological effects: those produced by the reference strains PAO1 hmgA* and PA14 hmgA*, and those from clinical isolates. Overall, it was demonstrated differential effects of the various pyomelanins. Thus, structural diversity in pyomelanin produced by strains of the same species can influence bacterial physiology and its interaction.

Palabras clave: : Pseudomonas aeruginosa – Pyomelanin – Antioxidant activity