Sociedad Argentina de Microbiología General

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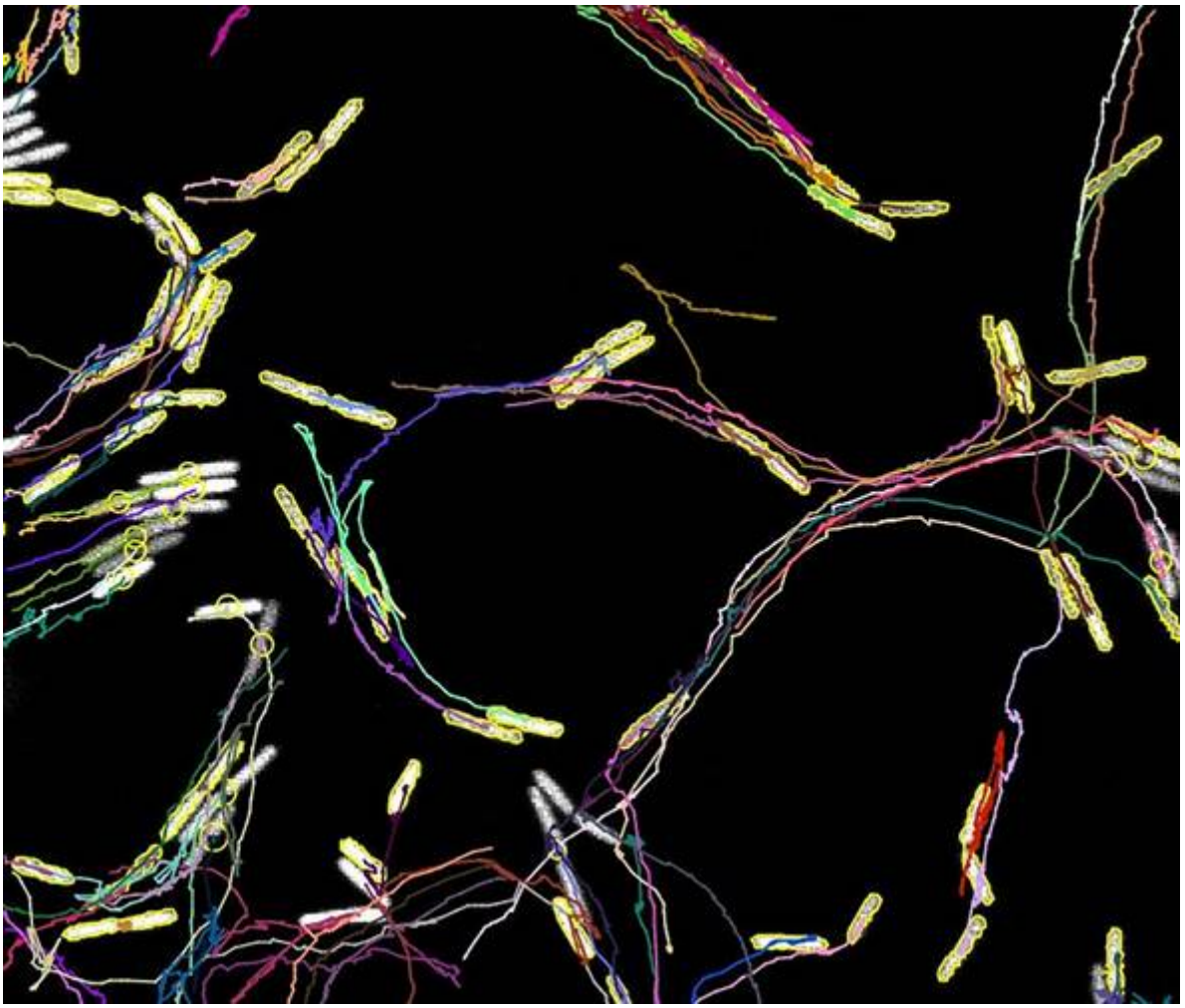

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UV OR NOT UV: STRAIN-DEPENDENT PYOMELANIN PROTECTION AGAINST UV-C RADIATION IN Pseudomonas SPECIES

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Pyomelanin, a type of melanin, is a polymeric pigment produced by several bacterial species and its production in *Pseudomonas* species is mainly due to alterations in the tyrosine catabolism and the accumulation of homogentisate. This pigment has been linked to increased resistance to various stressors like UV light, antibiotics, and reactive oxygen or nitrogen species. Additionally, it is proposed that melanin influence bacterial survival by increasing protection against contaminants and exhibits antimicrobial activity against fungi and bacterial species. Given the growing interest in utilizing bacterial traits for biotechnological applications, understanding how melanin production impacts bacterial physiology is relevant. We hypothesize that melanin production influences physiological traits in a strain-dependent manner. In this study we analyzed two *Pseudomonas* species: *P. extremaustralis* (Pex-wt), *P. protegens* Pf-5 (Pf5-wt) and their melanogenic variants obtained in our laboratory (Pex-Mel and Pf5-Mel, respectively). *P. extremaustralis* is an extremophile from Antarctica, with its melanogenic mutant created by knocking out a diguanylate cyclase gene using a mini-Tn5-derived transposon. *P. protegens* Pf-5 is a known plant growth-promoting bacterium (PGPB), and its melanogenic variant was generated using CRISPR/nCas9 by introducing a premature termination codon into the *hmgA* gene. Pf5-wt and Pf5-Mel didn't show significant differences in growth dynamics during a 24 h aerobic culturing in LB medium while Pex-Mel entered the decline phase earlier than Pex-wt. We purified and analyzed the UV-vis absorption spectrum of the melanin for both strains revealing differences especially in the UV region. The melanin produced by Pf5-Mel has an absorption local maximum at 251 nm which is absent in Pex-Mel's melanin spectrum. Subsequently, we investigated the role of melanin in UV protection and examined whether there were differences depending on the strain. Therefore, we washed cultures of the different strains, extracted pyomelanin was added in a concentration of 0.15 mg/ml and the suspensions were exposed to UV-C radiation (? = 253.5 nm). Survival curves were obtained and additionally different controls were performed including melanin-free cell suspensions, cultures supplemented with PBS (control) and a cross-supplementation assay in which Pf5-Mel suspension was supplemented with purified melanin from Pex-Mel. Our results indicated that both melanins, irrespective of the strain, confer UV protection. However, melanin from

Pex-Mel demonstrated a more prolonged protective effect. Finally, we perform a biofilm development assay in polystyrene multiwell plates. While Pex-wt and Pex-Mel showed comparable biofilm formation on plates, Pf5-Mel exhibited a significant decrease in biofilm formation. These results indicate that each strain produced pyomelanins with specific structural characteristics leading to different effects on bacterial physiology.

Palabras clave: Pseudomonas – Melanin – Bacterial Physiology