Sociedad Argentina de Microbiología General

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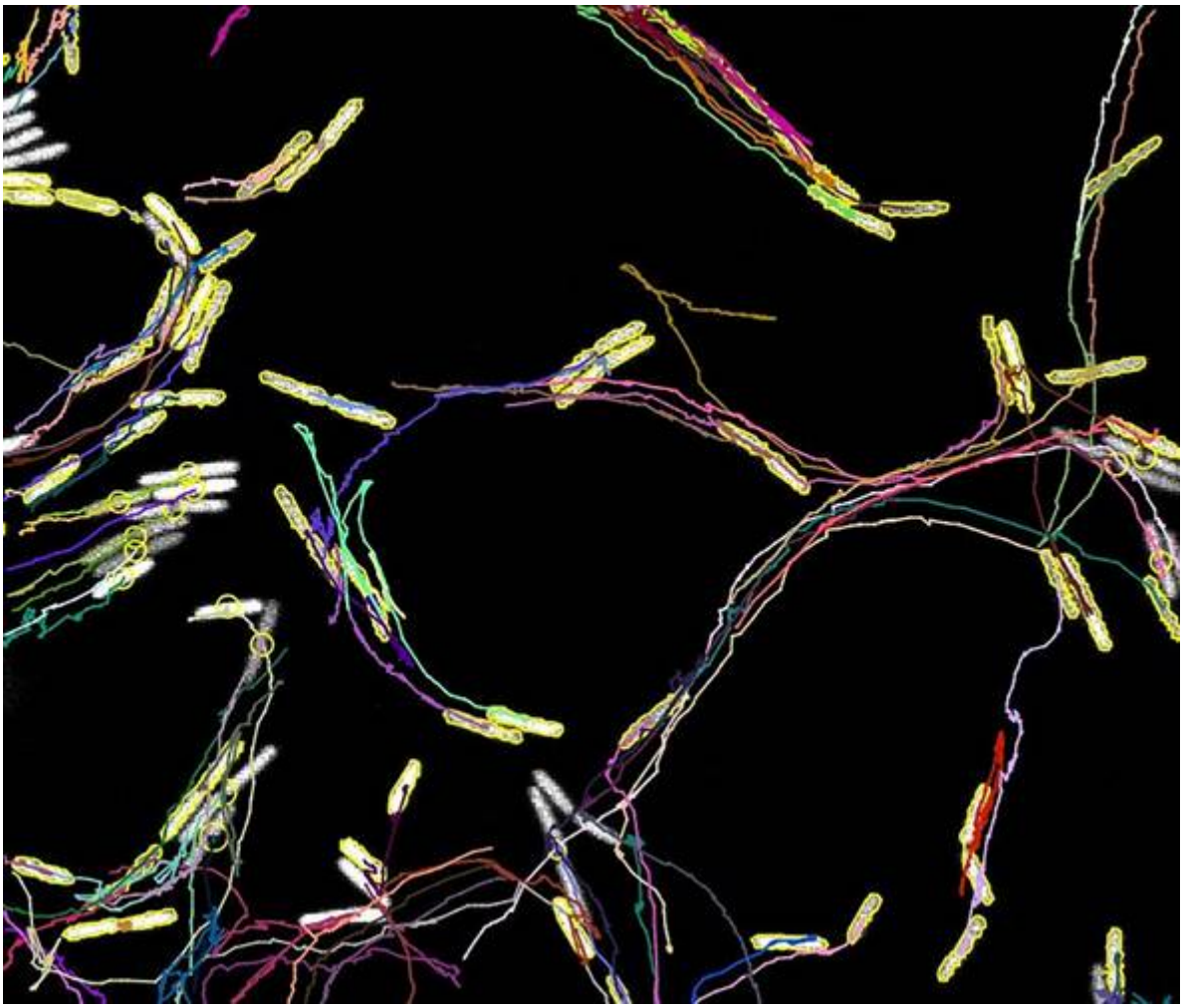

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

IMPACT OF *Staphylococcus aureus hssR* MUTATION ON THE INTERACTION WITH *Pseudomonas aeruginosa*

Galeano, M.B.[1,2]-Palomino M.M.[1.2]-Tribelli P.M.[1.2]

1) IQUIBICEN CONICET-Ciudad Autónoma de Buenos Aires-Buenos Aires-Argentina
2) Dpto. Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA-Ciudad Autónoma de Buenos Aires-Buenos Aires-Argentina.
Contacto: marianabeleng@gmail.com

S. aureus (SAwt) and *P. aeruginosa* (PA) can coinfect patients, interacting competitively or cooperatively, driven by metabolism. Iron, particularly as heme, is vital for bacterial survival during infection. However, iron accumulation can lead to oxidative stress. In SAwt, heme homeostasis is regulated by the HssSR system. Previous studies showed the *hssR* mutant grew like the wild type in low oxygen with hemin but had reduced survival with PA. This study analyzed oxidative stress and organic acid production under aerobic and microaerobic conditions to better understand the *hssR* phenotype. Mono and cocultures were performed in artificial sputum medium (ASM) for 24h with or without hemin. Flow cytometry with 2',7'-dichlorofluorescein was used to measure reactive oxygen species (ROS). The *hssR* mutant exhibited altered ROS production in co-culture, being 10-fold higher with hemin under aerobic conditions. No significant ROS differences were observed under microaerobic conditions. ASM mimics the lung environment, containing amino acids as carbon sources. High-performance liquid chromatography was used to quantify six organic acids: citrate, pyruvate, succinate, lactate, formate, and acetate. In USA300 monocultures, organic acid production was similar with or without hemin under aerobic conditions. However, under microaerobic conditions, lactate increased 3.5-fold without hemin, with no significant changes in other acids. For the *hssR* mutant, acetate was 2.6-fold higher with hemin under aerobic conditions, and lactate was 1.6 times higher with hemin under microaerobiosis. In SAwt-PA cocultures, lactate levels were 2-fold higher, and acetate was 1.5-fold higher without hemin under aerobic and microaerobic conditions, respectively. In PA-*hssR* aerobic cocultures, lactate was 11-fold higher without hemin, while acetate was 1.5-fold higher with hemin. Under microaerobiosis, acetate production in *hssR* was the highest, with no significant differences between hemin conditions. In summary, in aerobic monocultures, the wild type showed a consistent organic acid profile with or without hemin, while the *hssR* mutant had increased acetate production with hemin, indicating a more fermentative state. Under microaerobic conditions, the wild type showed a fermentative profile without hemin, with a significant decrease in lactate with hemin. The *hssR* strain had lower organic acid levels than the wild type, except for lactate, which increased with hemin, indicating a unique metabolic pattern. In cocultures, organic acids were produced or

consumed by both strains. With hemin, acetate increased in SAwt-PA cocultures regardless of oxygen tension, while in *hssR*-PA cocultures, acetate showed the opposite pattern under aerobic conditions. These results suggest that the *hssR* mutation alters not only hemin homeostasis but also SA's metabolic profile and survival in the presence of PA. Further studies will explore the role of *hssR* in survival, stress resistance, and virulence.

Palabras clave: Staphylococcus aureus-Pseudomonas aeruginosa-hssR-iron metabolism-interaction.