Sociedad Argentina de Microbiología General

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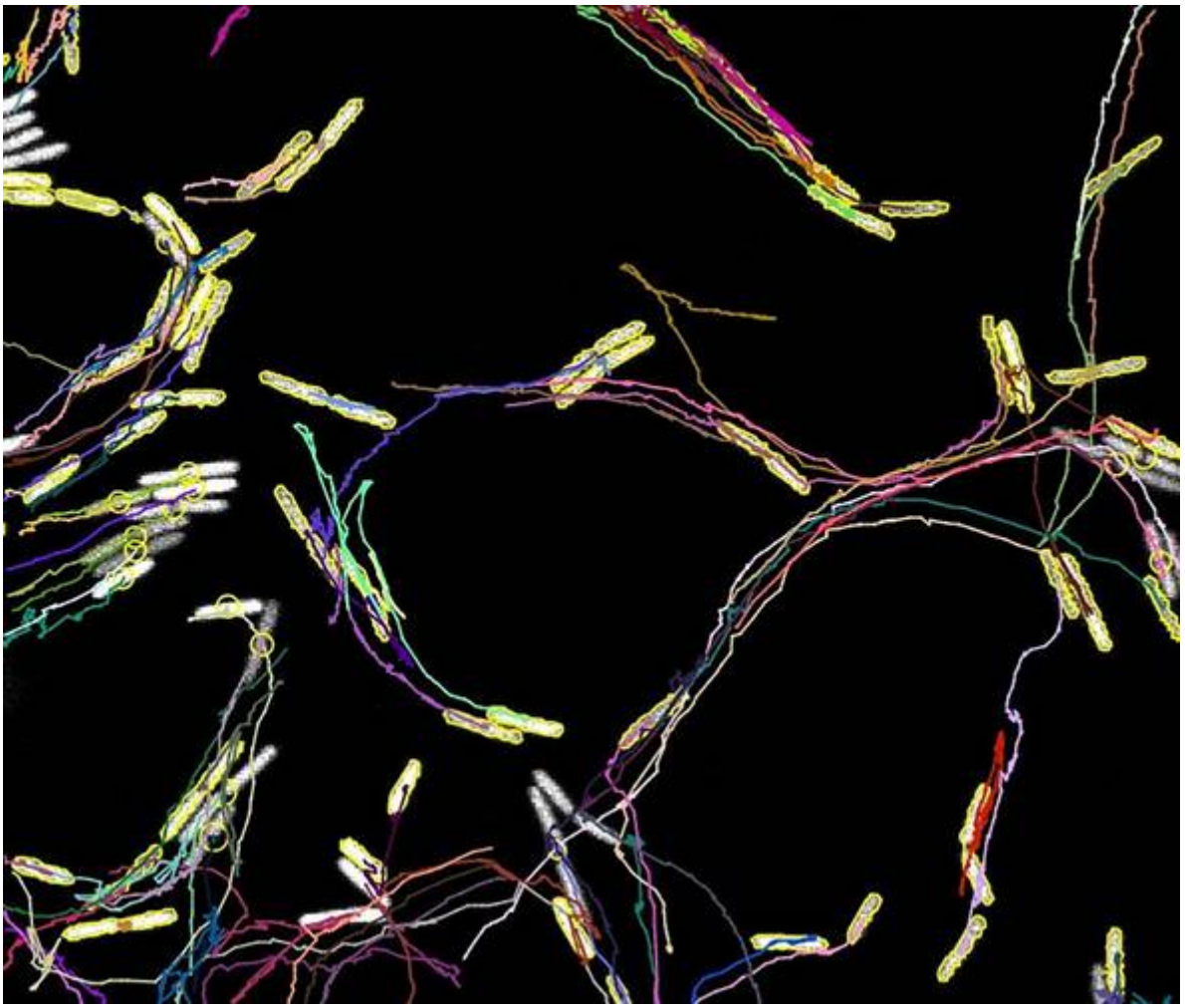

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ANALYSIS OF THE ROLE OF ETHANOL OXIDATION METABOLISM IN *Pseudomonas aeruginosa* IN INTERACTION WITH *Staphylococcus aureus*. TEMPERATURE IMPACT.

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Pseudomonas aeruginosa (PA) and *Staphylococcus aureus* (SA) are two opportunistic pathogenic bacteria in humans responsible for a wide range of infections. These species can cause coinfection in lungs of patients with cystic fibrosis, in hospital-acquired infections and chronic wounds. Our group performed a transcriptional profile of cocultures of these bacterial species at 37? and 39? to describe the effect of febrile temperatures on their interaction, revealing changes in the expression of genes involved in ethanol oxidation metabolism. In *Pseudomonas* species, ethanol oxidation is a secondary metabolic pathway where ExaA, ExaB and ExaC participate in energy generation when ethanol is used as a carbon source. This work focuses on the PA *exaA* gene, encoding ExaA a PQQ-dependent ethanol dehydrogenase, that was upregulated in PA-SA co-cultures at 39?. Our aim was to evaluate the influence of the ethanol oxidation pathway on the interaction between *P. aeruginosa* PAO1 (PAO) and *S. aureus* (USA300) at 39?. PAO *exaA* mutant was generated using a CRISPR-Cas9 editing vector. A growth curve in monocultures was performed for PAO WT and *exaA* at both 37°C and 39°C in TSB medium. The *exaA* strain showed similar growth to the WT despite the temperature. Additionally, we performed plate competence assays with SA at both temperatures in tryptic soy agar (TSA) or artificial sputum medium (ASM) using USA300 and four SA clinical isolates from cystic fibrosis patients. In TSA, competence between SA and PA strains was not influenced by temperature or the *exaA* mutation, except for one clinical isolate (called SU), which exhibited a decrease in competence with exaA only at 37°C, suggesting a role of *exaA* gene in competence depending on the SA strain. In ASM agar medium, no differences in PA-SA competence were observed for SA strains or temperature. However, in this medium, all SA strains were more resistant to PA compared to competence in TSA medium at both temperatures. Bacterial survival in mono or PA-USA300 cocultures under microaerobic conditions at 37? and 39? was assessed using TSB medium supplemented with 0.5% KNO3. Survival was determined by measuring CFU/ml in selective media: cetrimide for PA and TSA-NaCl for SA. Cell count for PAO and *exaA* showed similar results at both temperatures in mono and cocultures.

However, in cocultures, USA300 presented a decrease of 1-fold and 2-fold for PA-USA300 and *exaA*-USA300 at 39?, respectively. This difference was not observed at 37?.

Our results showed that *exaA* mutation did not alter growth or PA survival. However, at 39?, SA presented a decrease in CFU in the presence of the *exaA* strain, suggesting an alteration in the physiology of the *exaA* mutant strain only at 39?. This possible role is also in line with the differences between temperatures in competence of the clinical isolate observed only with the *exaA*. More experiments are necessary to understand the role of the ethanol oxidation pathway in PA-SA interaction.

Palabras clave: INTERACTION-ETHANOL OXIDATION-PSEUDOMONAS - FEVER-LIKE TEMPERATURES