Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

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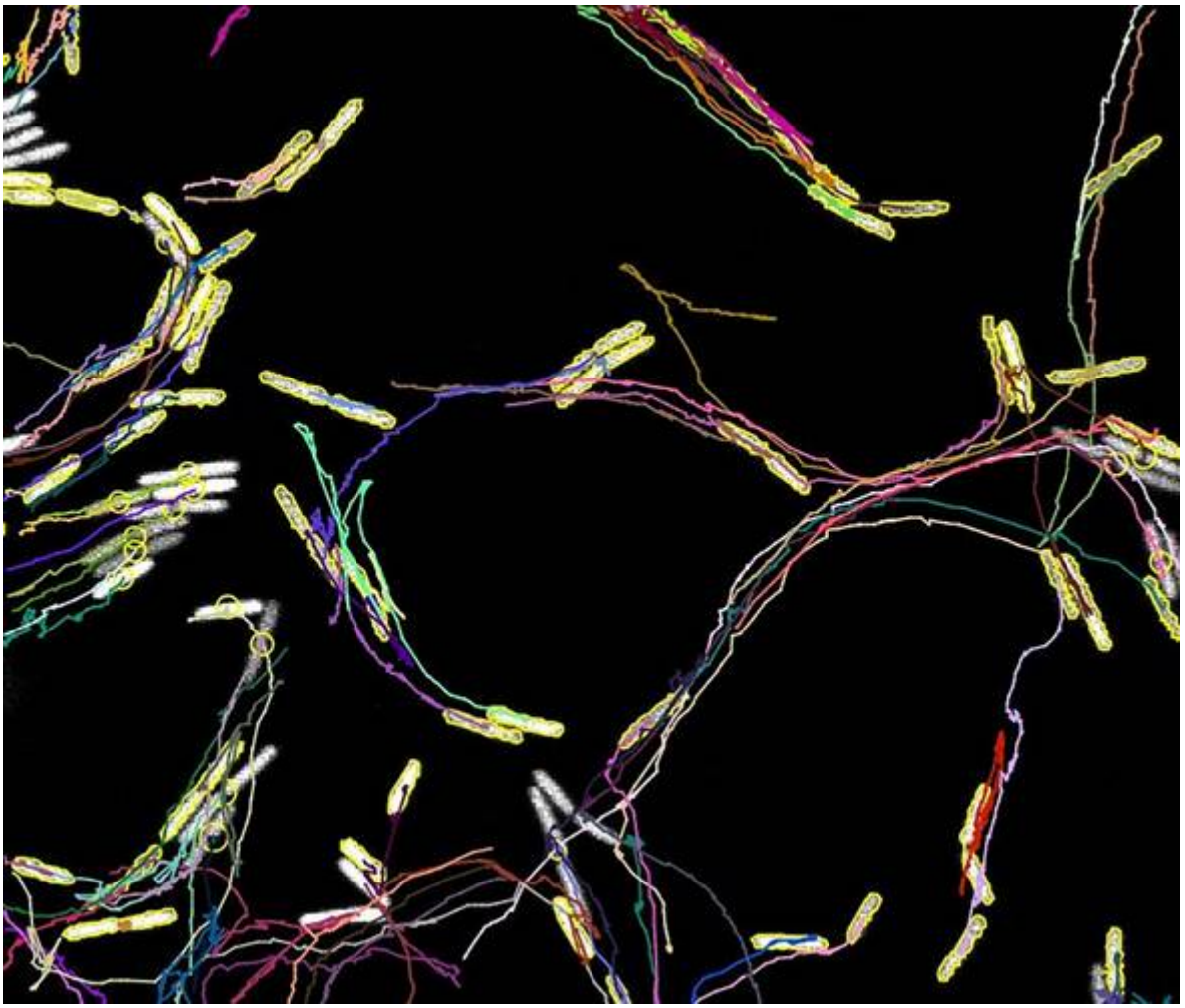

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

51 - Microbiología Molecular y Fisiología

GENERATION OF A BIOSAFETY LEVEL I REPORTER STRAIN OF THE bla SYSTEM IN Staphylococcus aureus

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Methicillin-resistant *Staphylococcus aureus (*MRSA) is a human pathogen considered by the World Health Organization as one of the most significant global clinical threats. This is due to the multidrug resistance exhibited by this "superbug" and the limited number of new drugs in advanced stages of clinical development to treat MRSA infections. MRSA exhibits two mechanisms of resistance to ?-lactam antibiotics. The first is the production of a serine ?-lactamase (PC1) encoded in the *bla* operon. PC1 catalyzes the hydrolysis of the ?-lactam ring, thereby inactivating it. The second mechanism involves the production of an auxiliary penicillin-binding protein, PBP2a, which has low affinity for ?-lactams and is encoded in the *mec* operon. The expression of PC1 and PBP2a is induced by ?-lactams through a mechanism that is not yet fully understood. An accessory regulatory protein, MecR2, has recently been characterized in the mec system. In the case of the *bla* system, it has been proposed that phosphorylation is a requisite for activation. In this work we engineered a Biosafety Level I reporter strain of the *bla* system which could be used as a sensor for ?-lactam antibiotics and for the selection of inhibitors targeting this system. The reporter gene gfp (green fluorescent protein) was cloned under the control of the P*blaZ p*romoter, the operator region of the bla operon, and the regulatory genes *blaR1* and *blaI* into vector pET24a(+). Induction of the system was evaluated in *E. coli* DH5? transformants. Cell growth was monitored simultaneously with the intensity of fluorescence emission of GFP, in the presence of different antibiotic concentrations. GFP expression level was also evaluated by Western blot. Growth of the reporter strain showed a basal level of GFP expression. Incubation with ampicillin and oxacillin showed a significant increase over time in the intensity of fluorescence emission, attributed to ?-lactam induced-GFP production. The augmented fluorescence correlated with an increased amount of GFP protein was detected by Western blot. Ampicillin-induced GFP expression saturated at an antibiotic concentration of 0.25-0.5 µg/mL (0.06-0.125 x MIC), while oxacillin-induced expression was linear up to 32 µg/mL (0.125 x MIC). Activation of the *bla* system in *E. coli* was comparable to activation in *S. aureus* RN4220. In conclusion, we demonstrated that the *bla* system can be reconstituted in *E. coli*, with a functional

sensor/transducer BlaR1 membrane protein, despite the difference in the membrane composition of these Gram-positive and Gram-negative strains. Besides, activation did not require additional *S. aureus* proteins. A successful *E. coli* reporter strain for the *bla* system has been generated, simplifying future functional studies and inhibitor screening.

Palabras clave: Staphylococcus aureus – bla system – reporter strain