Sociedad Argentina de Microbiología General

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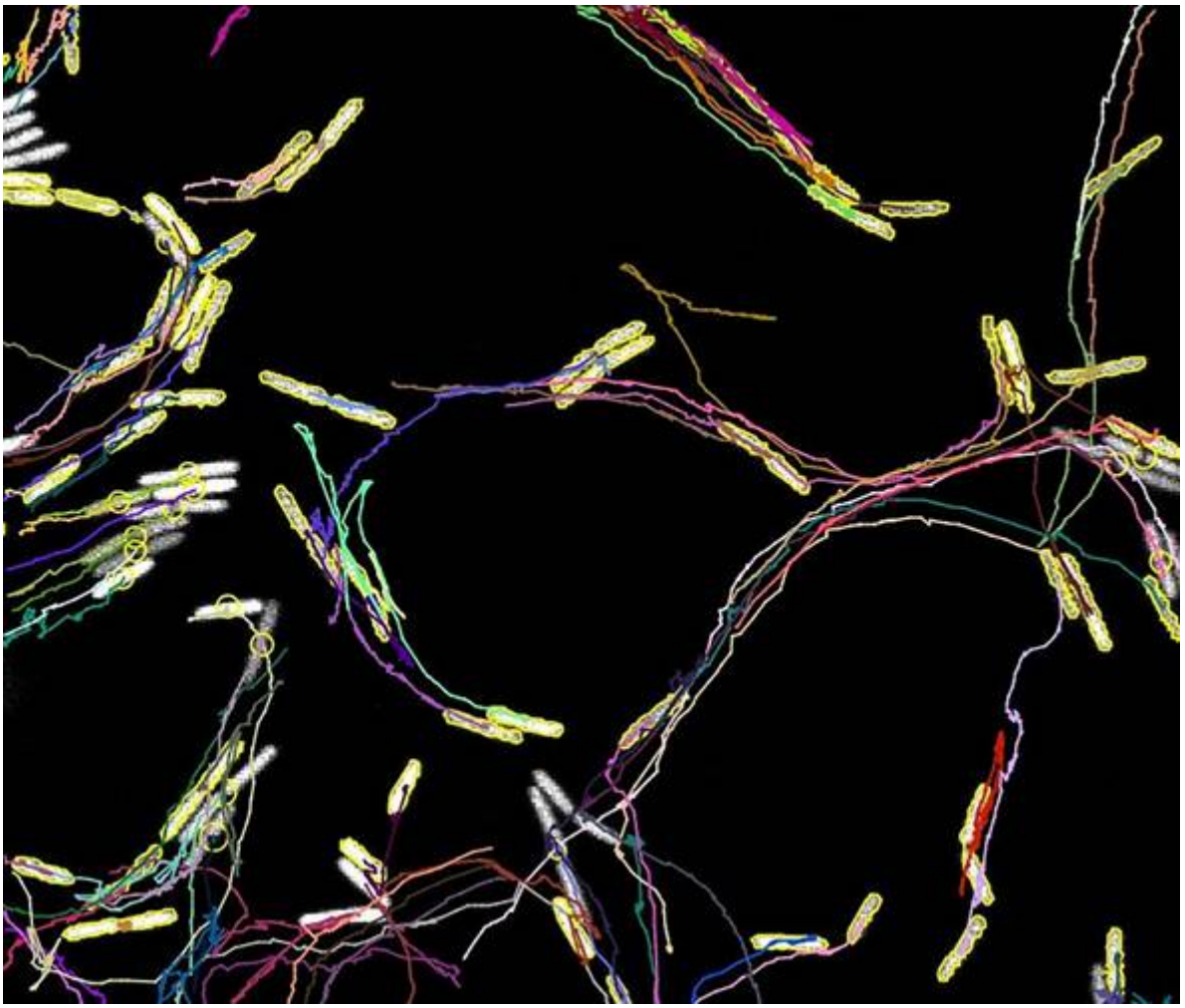

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

ß-LACTAM CYSTEINE ACYLATION OF A NOVEL BACTERIAL RESISTANCE SYSTEM FROM Vibrio parahaemolyticus

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The emergence of ?-lactam-resistant bacteria represents a significant public health challenge worldwide. Resistant strains of the *Vibrio* genus, the causative agents of acute intestinal infections, have been reported globally, posing a serious threat to human health. A ?-lactam antibiotic resistance system has recently been identified in *Vibrio parahaemolyticus*. This resistance is mediated by a two-component system comprising a membrane-associated histidine kinase (VbrK) and a cytoplasmic response regulator (VbrR). Upon exposure to ?-lactam antibiotics, this system activates the expression of a CARB-type ?-lactamase. Our objective is to elucidate the molecular mechanism underlying antibiotic detection and signal transduction that leads to resistance. Topology and domain predictions of VbrK indicate the presence of a putative signal peptide, a putative periplasmic sensor domain, a transmembrane ?-helix, and a cytoplasmic C-terminal catalytic and ATP-binding domain. While ?-lactam antibiotics are proposed to be the signal detected by VbrK, this remains controversial. The crystal structure of the kinase sensor domain (VbrKSD) has been determined, revealing a disulfide bond between C86 and C107. However, the structure of the domain in complex with ?-lactams remains unknown. It has been shown that Cysteine 86, located at the distal region of the sensor domain and the putative antibiotic binding site, undergoes nitrosylation, which activates the kinase in response to nitrite. To investigate the recognition of ?-lactams by VbrK, we optimized the expression and purification of VbrKSD and confirmed the predicted signal peptide cleavage site via mass spectrometry. We employed Ellman's assay, cysteine labeling with DCIA and saturation transfer difference (STD) NMR spectroscopy to study the correlation between the oxidation state of the four cysteine residues in VbrKSD and ?-lactam binding. Additionally, we used an SDS-PAGE assay with the fluorescent antibiotic Bocillin FL to determine whether the sensor domain forms a covalent adduct and the residues involved. In addition, we characterized ?-lactam ring hydrolysis by monitoring the absorbance change at 495 nm of the ?-lactam probe nitrocefin. Our findings indicate that the sensor domain must be in a reduced state to interact with ?-lactams. In its reduced state, the domain undergoes acylation by the fluorescent ?-lactam Bocillin FL, and based on experiments with different cysteine mutants, we

provide evidence that the acylation sites are C86 and C107. Furthermore, we determined that nitrocefin hydrolysis follows a product-inhibited kinetic model. Understanding the interaction between VbrKSD and ?-lactams is crucial for developing inhibitors of bacterial resistance. Here, we deepen our understanding of this interaction and provide an explanation for the existing controversies in the literature.

Palabras clave: Vibrio parahaemolyticus – VbrKR – ?-lactam resistance - NMR