Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024
Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.

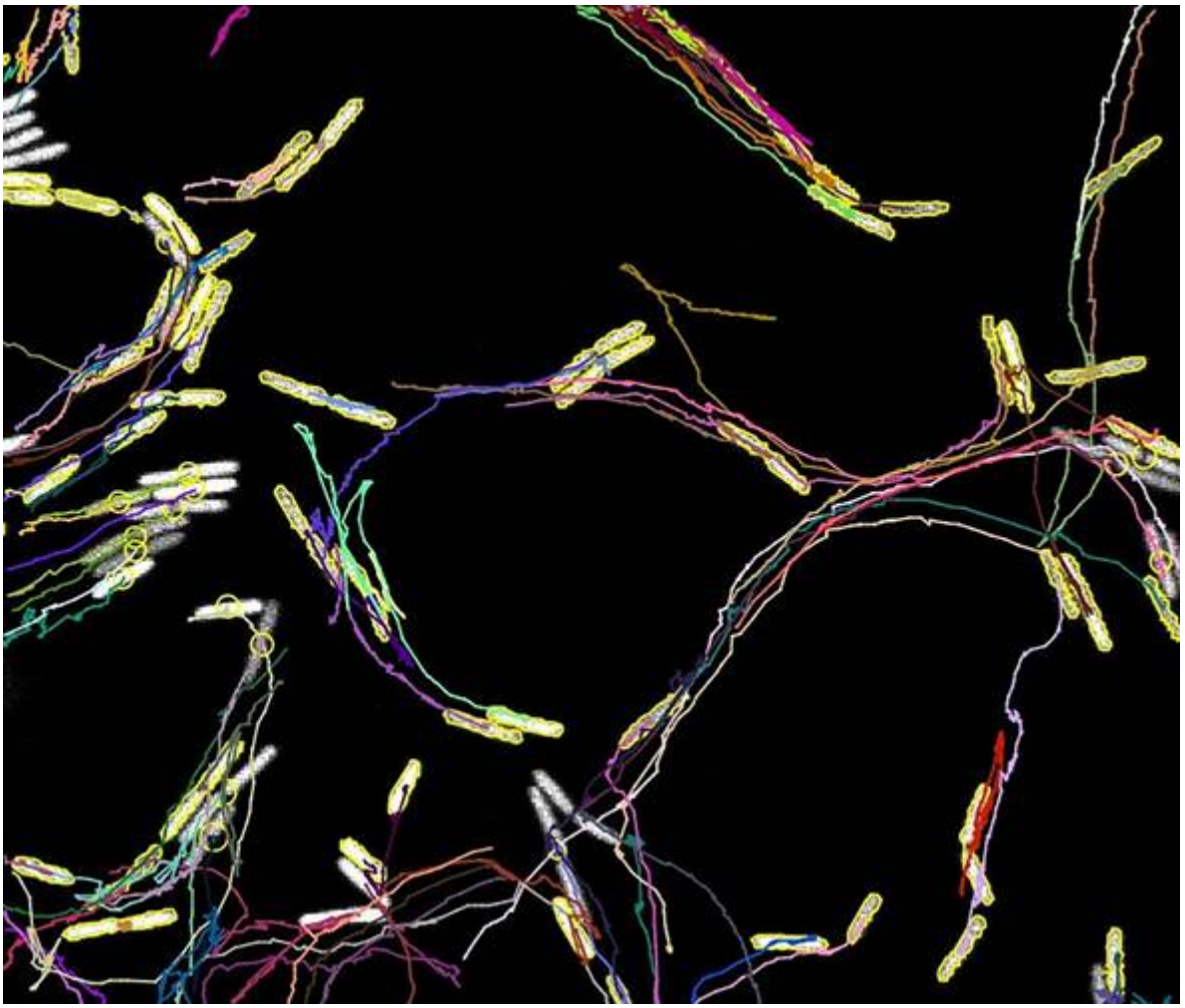

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

CHARACTERIZATION OF INTRACELLULAR TRAFFIC OF *Serratia marcescens* AND THE ROLE OF THE ShlA HEMOLYSIN

Arango Gil, Brayan Stiven - García Véscovi Eleonora

Laboratorio de Patogénesis Bacteriana - Instituto de Biología Molecular y Celular de Rosario, CCT-CONICET-Rosario - Santa Fe - Argentina
Contacto: arango@ibr-conicet.gov.ar

Serratia marcescens (Sma) is an opportunistic bacterial pathogen associated with a wide range of human infections. Recently, it has emerged as a significant public health concern, particularly for immunocompromised individuals and neonates. This bacterium possesses virulence factors that enable it to invade and proliferate within epithelial cells inside non-acidic, non-degradative vacuoles known as *Serratia* Containing Vacuoles (SeCVs). These SeCVs are autophagic vacuoles decorated by LC3 (an autophagic marker). A key virulence factor of *Serratia* is the hemolysin ShlA, which triggers an early autophagic response prior to bacterial internalization and is crucial for bacterial egress into the extracellular environment. The objective of this work was to evaluate the role of ShlA in the formation and maturation of SeCVs. To track and visualize SeCVs, CHO cells stably transfected with GFP-LC3 were used. To detect bacteria by fluorescence microscopy, both Sma wild-type and a ?shlBA mutant strains harboring a plasmid encoding mCherry were used. We employed both fixed-cell and *in vivo* time-lapse confocal imaging to track the formation and maturation of SeCVs. We evaluated two time intervals: early infection (0-120 minutes post-infection) and late infection (120-360 minutes post-infection). At 20 minutes p.i. most intracellular bacteria did not recruit LC3. However, from 40 minutes p.i onwards, a significant LC3 accumulation around the WT strain and the formation of large LC3-decorated vacuoles enclosing bacteria was observed. Time-lapse confocal imaging revealed the formation of long, LC3-labeled tubular filaments extending from and retracting into SeCVs, a phenomenon previously unreported in *Serratia* intracellular traffic. In contrast, LC3 recruitment to SeCVs in the ?shlBA strain was slower, and no large vacuole formation was detected. However, the formation of filaments was observed around SeCVs in this strain, albeit with lesser magnitude than those observed in the WT strain. In conclusion, ShlA hemolysin facilitates the rapid recruitment of LC3 to SeCVs in the WT strain following bacterial internalization into the host cell and contributes to the formation of LC3-labeled filaments that extend from the SeCVs throughout the cell. These findings elucidate a novel role for ShlA in the intracellular trafficking dynamics of *Serratia marcescens*, enhancing our understanding of its pathogenic mechanisms.

Palabras clave: Intracelluar-Traffic, ShlA, Autophagy, LC3-filaments