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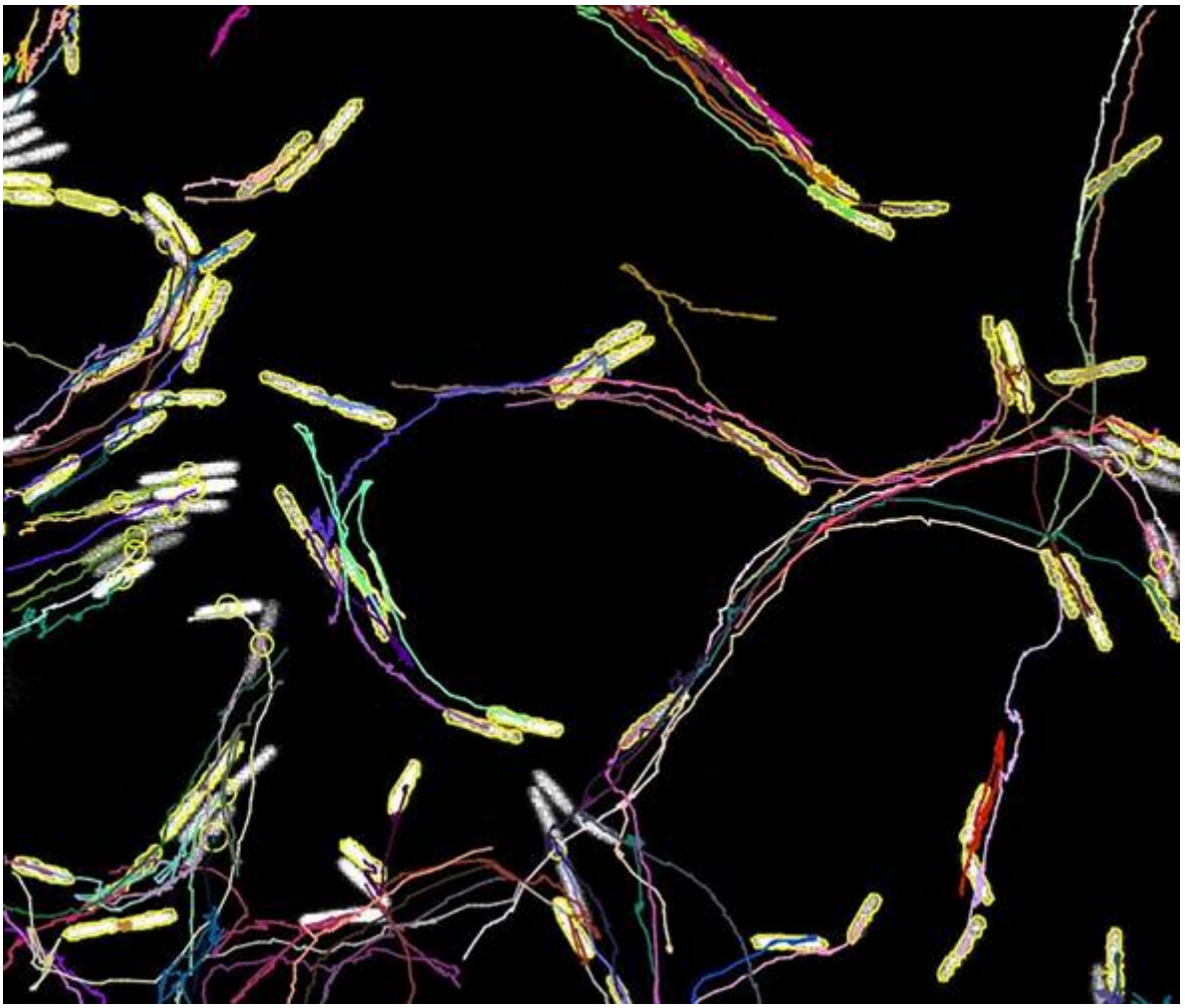

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

CELL ENVELOPE BIOGENESIS: ROLE OF AsmA-*LIKE* PROTEINS IN *Brucella suis*

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The cell envelope of Gram-negative bacteria is the main point of interaction between a pathogen and the host. It consists of three distinct layers: the cytoplasmic membrane (IM), a peptidoglycan, and an asymmetric outer membrane (OM) composed of phospholipid in the inner leaflet and lipopolysaccharide (LPS) glycolipid in the outer leaflet. The correct assembly of this complex structure is crucial for bacterial viability and pathogenicity. However, the precise mechanisms governing cell envelope biogenesis and homeostasis in diderm bacteria remain a challenge. *Brucella* is an intracellular pathogen that belongs to the Alphaproteobacteria group, characterized by unique surface properties that make it a furtive pathogen and particularly resistant to several host defence compounds and antimicrobial agents. In previous studies, we have identified and characterized MapB of *Brucella suis*, the homolog of TamB from gammaproteobacteria. TamB, the inner membrane component of TAM system, is a large AsmA-*like* protein mostly with ?-helical structure immersed in the periplasm but inserted in the IM by an N-terminal non-cleavable signal peptide. We demonstrated that the ?*mapB* mutant of *B. suis* exhibits increased sensitivity to lysozyme, Triton X-100, polymyxin B and displayed altered cell division, indicating that MapB plays a key role in cell envelope integrity. Although we observed an inefficient insertion of a subset of outer membrane proteins, our observations point out to a more general role of MapB in OM biogenesis. Bioinformatic analysis of *Brucella* genome revealed the presence of other proteins belonging to the AsmA-*like* family. We identified three proteins in addition to MapB: a highly conserved classical AsmA, a homolog to YhdP from *E. coli*, and a hypothetical conserved protein with no homologs in gammaproteobacteria that contains an AsmA domain and an AsmA2 domain. A deletion mutant (?*BR08*) for this hypothetical protein and a double mutant ?*BR08* ?*mapB* were generated by restriction-free cloning. This cloning technique is a PCR-based method for the creation of custom DNA plasmids, allowing the insertion of a sequence of interest, independent of restriction sites and/or ligation. To assess cell envelope integrity, we performed sensitivity assays to antibiotics and lysozyme. Our results indicate that the ?*BR08* strain shows no differences in resistance to disruptive agents compared with the wild type strain, under the studied conditions. However, ?*BR08* ?*mapB* exhibited additional phenotypes compared to the *mapB* single mutant, suggesting that there is a functional relationship between the *mapB* and *BR08* genes. We proposed that

MapB and the new AsmA-*like* protein play complementary or redundant functions in cell envelope biogenesis.

Palabras clave: Brucella suis- TAM system- AsmA-like proteins- restriction free cloning- cell envelope homeostasis