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THE *tamB* MUTANT OF *Rhizobium leguminosarum* IS AFFECTED IN CELL ENVELOPE HOMEOSTASIS.

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Rhizobium leguminosarum is an alphaproteobacteria soil bacterium that develops a symbiotic relationship with legume plants, resulting in nitrogen-fixing root nodules. Changing from free-living to endosymbiont lifestyles involves survival and adaptation to environmental fluctuations in which bacterial envelope is critical. The TAM system (Translocation and Assembly Module) of *E. coli* and other gammaproteobacteria has been proposed to play a role in the assembly in the outer membrane (OM) of a subset of outer membrane proteins (OMPs). The TAM complex consists of the OMP TamA and TamB, which spans in the periplasm and is anchored to the inner membrane through its N-terminal end. *Brucella* is a facultative intracellular animal pathogen that is closely related to *Rhizobium*. Our group showed that the TamB homologue of *Brucella suis* (MapB) is required for cell envelope integrity, cell division and full virulence, suggesting a general role in cell envelope homeostasis. To study the role of TAM in rhizobia, we generated a deletion mutant in the gene encoding the MapB homologue (RL4382) of *R. leguminosarum* bv. *viciae* 3841 (Rlv 3841). Bacterial growth showed no differences between $\Delta tamB$ mutant and wild type strain. Besides, TEM observation of cells using negative staining suggested that cell division is not altered in the mutant and no differences in the sensitivity to the polycationic peptide Polymyxin B were observed between both strains. However, upon exposure to lysozyme (0.01 mg/ml), the mutant exhibited a marked increase in cell lysis compared to the parental strain, a phenotype that was complemented with the *tamB* gene cloned into pBBR1MCS2. Besides, the tolerance to several membrane disrupting agents such as the detergent Triton X-100 (0.5 %), the calcium chelating EDTA (10 mM) and the hydrophobic antibiotic Vancomycin (0.4 mg/ml) was decreased in the $\Delta tamB$ mutant, further confirming that the envelope integrity is altered in the TamB-deficient strain. Our observations suggest that TamB plays a conserved and critical role in cell envelope homeostasis in alphaproteobacteria. Further biochemical and genetic studies are required to give insight into the role of TAM in *R. leguminosarum* and other alphaproteobacteria in cell envelope biogenesis.

Palabras clave: TAM SYSTEM-ENVELOPE-RHIZOBIUM-MEMBRANE-HOMEOSTASIS