

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.



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LYSYLPHOSPHATIDYLGLYCEROL SYNTHESIS UNDER ACID STRESS CONDITIONS IN THE MODEL BACTERIUM Sinorhizobium Meliloti 2011

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The nitrogen-fixing capacity of rhizobia allows their use to introduce nitrogen into arable soils through symbiotic association with legumes. This symbiosis is exposed to various abiotic stresses, such as soil acidity, which limit crop production worldwide. In particular, S. meliloti is very sensitive to low pH, although it is capable of developing a resistance phenotype called acid tolerance response (ATR), which generates increased resistance to acid shock. This phenotype is complex and is given by a multigenic response associated with changes, among others, in protein and lipid synthesis linked to membrane components. Membrane composition changes under stress conditions, and the concentration of the lipid lysylphosphatidylglycerol (LPG) increases in several species of rhizobia growing at low pH. Its synthesis requires the enzymes encoded by the IpiA and acvB genes, which act in a coordinated manner to secrete lysine into the extracellular medium to buffer the effect of acidity. These genes are generally found as operons; however, in S. meliloti 2011, this operon presents structural particularities that differentiate it from the rest of the rhizobia. The acvB gene is divided into two ORFs: SMc00612 and SMc00613 (acvB). The C-terminal domain retains the catalytic activity of the enzyme, but loses the signal peptide, which directs the protein to the membrane. Thus, the enzyme would be expected to lose its membrane localization, preventing the release of lysine to the extracellular medium and, therefore, the neutralization of the acidic conditions of the medium. On the other hand, in the synorhizobial species in which this mechanism has been studied, the presence of the lipid in the cell membranes has not been observed, so it is hypothesized that this system would either not be functional or that its synthesis-degradation activity would be high and would not allow the accumulation of LPG in the membranes. To study the functionality of this system in the defense against acid stress in S. meliloti 2011, insertional mutagenesis of each gene was carried out and their growth at different pH and the alteration of membrane permeability of these mutant strains were analyzed. The results revealed that disruption of the *lpiA* and *acvB* genes produced phenotypes that were more susceptible to acidity. Due to their location in the operon, and to avoid polar effects, the N-terminus of SMc00612, which did not show increased sensitivity to pH, was deleted, suggesting that this gene would be inactive. Regarding the alteration of membrane permeability, a higher permeability was determined in the IpiA and acvB mutant strains. The results

obtained indicate that the *lpiA-acvB* system in *S. meliloti* is functional under low pH conditions, and suggest the inactivation of SMc00612 in this system.

Palabras clave: Sinorhizobium Meliloti - Acid Tolerance Response - Lipid Membrane