Sociedad Argentina de Microbiología General

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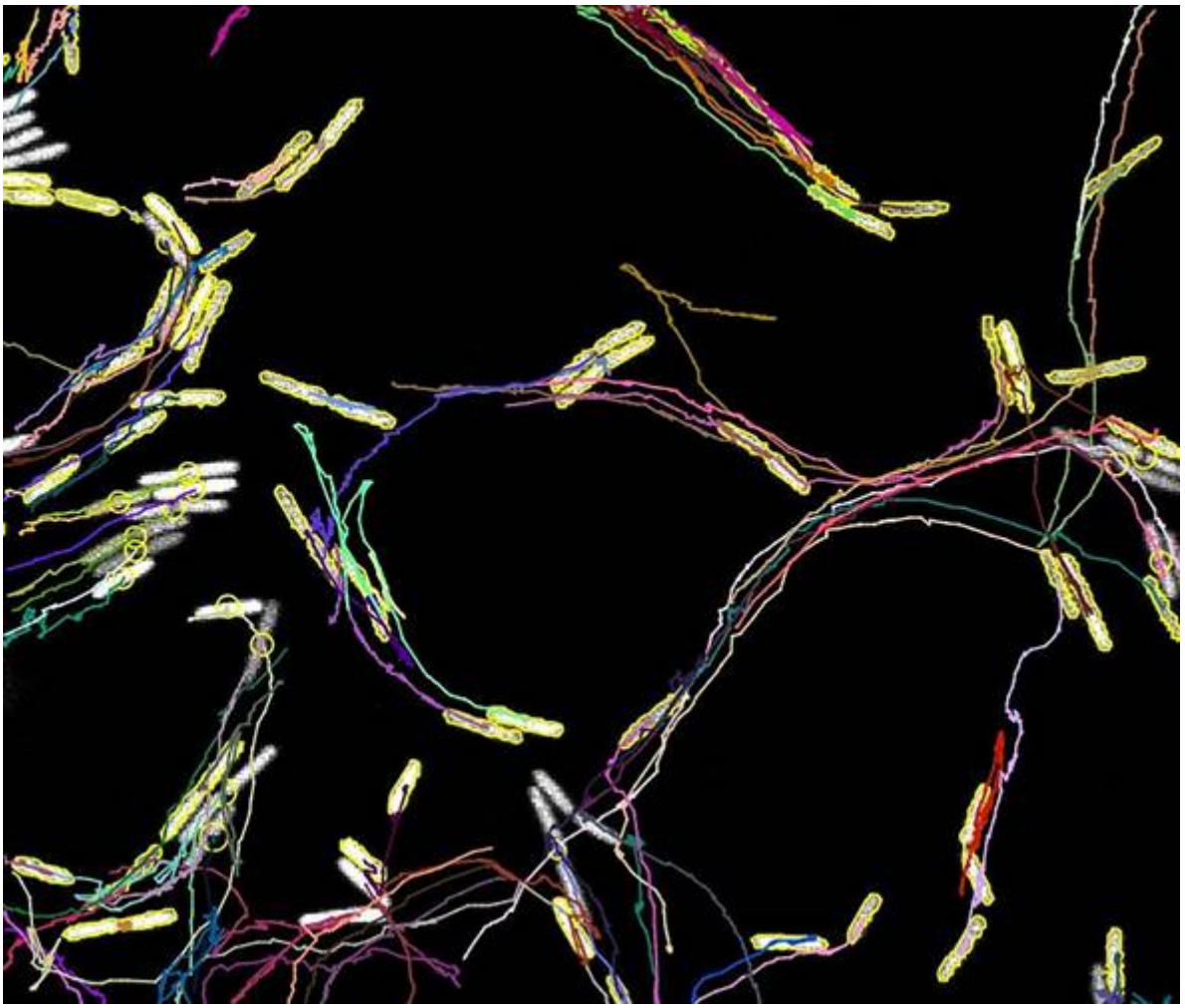

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

INVOLVEMENT OF LACTIC ACID SYNTHESIS IN THE GENERATION OF ATR(+) PHENOTYPES OF Sinorhizobium meliloti

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One of the problems of vital importance is the increasing degradation of agricultural soils due to the excessive application of agrochemicals that produce, among other consequences, changes in the surface pH of soils or eutrophication processes, substantially impairing crop development. It is a documented fact that the pH of soils in the Pampean area has been decreasing in the last decade, so it is necessary to implement management measures to ensure the sustainability of agrosystems. *Sinorhizobium meliloti* is able to establish a symbiotic relationship with alfalfa in which, as a final result, the bacterium is able to fix atmospheric nitrogen and supply it for the nitrogen nutrition of the crop. This symbiosis is highly sensitive to low pH; however, *S*. *meliloti* presents an adaptive phenotype, called ATR (Acid Tolerance Response) that allows it to improve its symbiotic behavior (competitiveness) when the symbiosis develops under conditions of moderate acidity. Previous characterization of ATR(+) phenotypes showed increased lactate production under moderate acidic growth conditions. To elucidate the role of this metabolite in ATR(+) phenotypes, insertional mutants were generated in the three putative genes involved in lactate production (*lldD1*, SMc01740; *lldD2*, SMc01712; *lldD3*, SMb20850). Using the SOEing PCR (Splicing by Overlap-Extension PCR) technique, three constructs with each of the in-frame deleted genes were generated in the pG18mob vector. These constructs were subcloned into the pK18mobsacB vector and transformed into *S. meliloti* 2011 to generate insertional mutants in each of the target genes. Their final growth was evaluated in Evans medium at different pH: pH7, pH 6.1 (moderate acidity) and pH 5.6 (growth limit). The results showed that all mutant strains decreased their growth at acidic pH (both pH 6.1 and pH 5.6). However, only the *lldD1* mutant strain showed similar growth to the wild-type strain Sme 2011 under neutral conditions, while the rest of the mutant strains also decreased their growth at pH 7. This suggests that only the expression of the lactate dehydrogenase enzyme encoded by the *lldD1* gene would be influenced by the pH of the medium and could be responsible for the increased lactate synthesis under acidic conditions. As a perspective, we plan to obtain the mutagenic deletions in all genes and their combinations (double mutants and triple mutants) in order to study the kinetics of growth and death of the bacterial strains altered in the synthesis of lactic acid, and also to evaluate the role of this metabolite in the development of symbiosis with alfalfa.

Palabras clave: Metabolism - Sinorhizobium meliloti - Stress