Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

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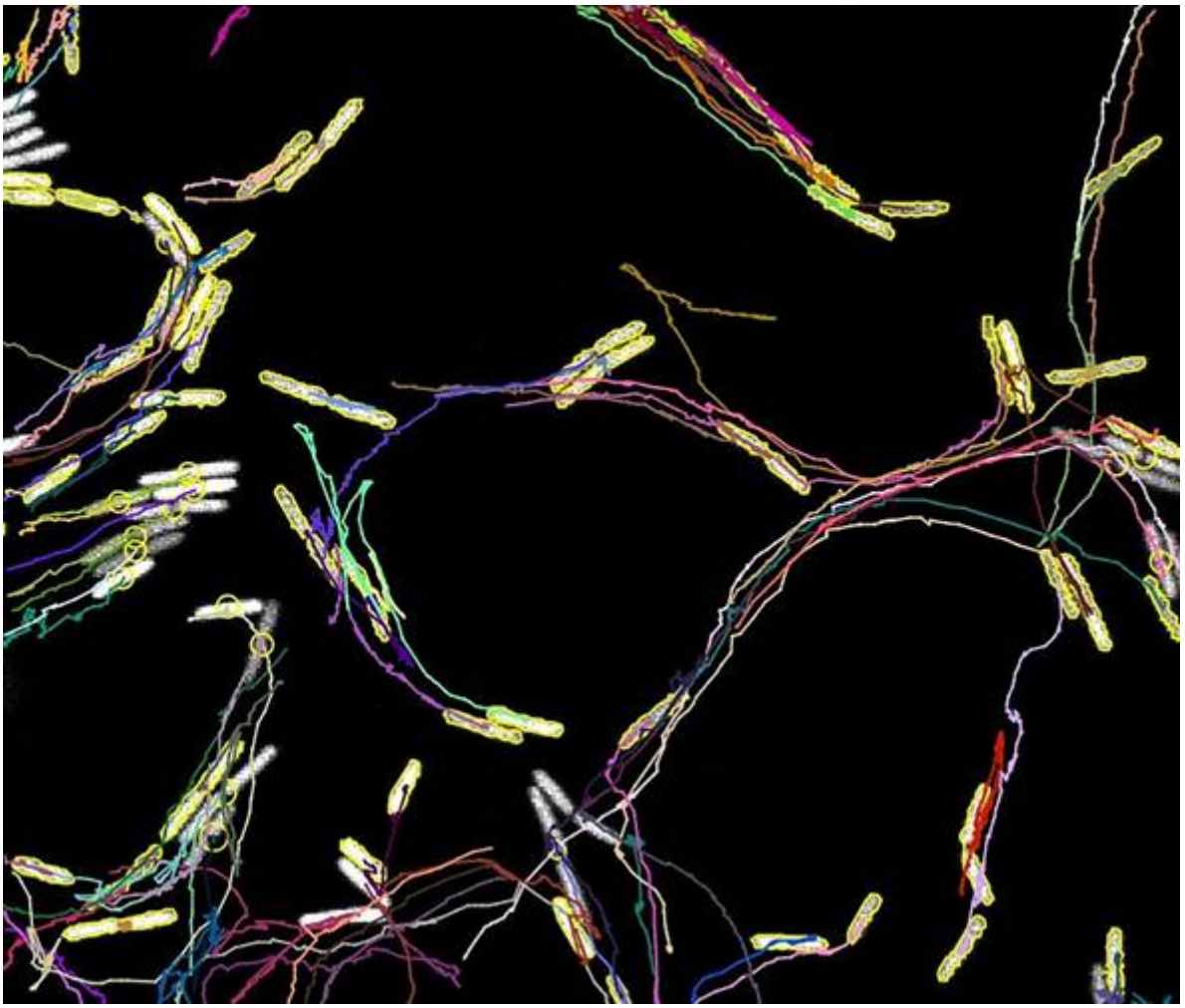

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

207 - Microbiología de las Interacciones

ROLE OF NCR-RELATED PEPTIDASES IN THE *Rhizobium favelukesii* – *Medicago* SYMBIOSIS

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Legumes can generate two types of nitrogen-fixing nodules depending on both the host plant and the symbiont. In indeterminate nodules, bacteria undergo terminal differentiation into bacteroids, an irreversible state in which they are able to fix atmospheric nitrogen into ammonia. In *Medicago truncatula* nodules, the differentiation process is controlled by nodule-specific cysteine-rich peptides (NCRs). The NCRs are secreted by the plant and translocated to the bacterial cytosol, where they can be cleaved by bacterial peptidases, as a defense response to the antimicrobial activity of those peptides. Two peptidases involved in the development of nitrogen-fixing nodules have recently been described: HrrP and SapA. Both proteins showed peptidase activity against NCRs. Expression of *hrrP* (host range restriction peptidase) in *S. meliloti* B800 inhibits nitrogen fixation in *M. truncatula* A20. On the other hand, overexpression of *sapA* generates plants with lower levels of nitrogen fixation. *Rhizobium favelukesii* LPU83 was isolated from acid soils of Argentina and can nodulate *M. truncatula*. Even though this bacterium presents a symbiotic plasmid with the necessary genes to establish an effective symbiosis, it is inefficient in nitrogen fixation. In this work, we sought to identify and analyze the role of homologous genes to *hrrP* and *sapA* peptidases in the symbiosis between LPU83 and *M. truncatula* in order to evaluate their implication in the symbiotic phenotype of the strain. Genes homologous to *hrrP* and *sapA* were found in the symbiotic plasmid and chromosome of LPU83. Mutants in these genes were generated and their symbiotic phenotype was evaluated in *M. truncatula* A20. Our results showed that both single and a double mutant on these genes did not exhibit changes in their symbiotic phenotype compared to controls, with no significant differences in shoot dry weight. Previous research indicated that overexpression of *hrrP* in *S. meliloti* negatively affected symbiosis, resulting in ineffective nitrogen fixation in plants with white, small nodules. To study the impact of LPU83 peptidases, the homologous genes were cloned into replicative plasmids in rhizobia under the promoter of the *hrrP* gene and transferred to *S. meliloti*. However, overexpression of LPU83 peptidases showed no differences with the strain carrying the empty plasmid, with plants exhibiting green leaves and pink, elongated nodules. Confocal microscopy of these nodules revealed that even though the plants fix nitrogen, there were more dead bacteria inside the nodules infected with overexpressed LPU83 peptidases. Overall, these findings suggest that while the *hrrP* gene plays a crucial role in nodulation and nitrogen fixation in

S. meliloti, the peptidases from LPU83 do not significantly impact on the symbiotic effectiveness. Further research is needed to explore and clarify the special phenotype observed in LPU83.

Palabras clave: Symbiosis – Rhizobia – Nodules