Type VI secretion systems of *Bradyhizobium* nodulating lupines

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The *Rhizobium*-legume symbiosis is highly specific and depends on several molecular signals produced by both partners. Some of these signals are bacterial proteins named effectors that are translocated into the plant cells by secretion systems similar to contractile nanomachines also called injectisomes (Deakin and Broughton, 2009). The injectisomes puncture and deliver the effectors into the target cell. One of these nanomachines, known as type VI secretion system (T6SS), was discovered recently and is reminiscent of phage injection machinery (Records, 2011). The role of these systems in legume endosymbiotic bacteria is mostly unknown, and this work presents the initial study of T6SSs from different bradyrhizobia. T6SSs have been identified in draft genomic sequences from *Bradyrhizobium* strains isolated from *Lupinus* spp. thriving in the Iberian Peninsula. In all cases, the genes encoding T6SSs were grouped and showed, in most cases, a high degree of conservation among genes encoding the structural components of the system. *Bradyrhizobium* sp. strain ISLU101 isolated from *L. angustifolius*, contains two clusters of genes involved in the formation of T6SS. One of such systems, designated as T6SS-1, contains 17 genes and shows a high degree of conservation regarding genes of *B. diazoefficiens* USDA110. The other one, T6SS-2, contains 16 genes flanked by insertion element sequences. Amino acid similarity between equivalent proteins encoded in both clusters is only about 40-50 %. A phylogenetic analysis based on the concatenation of sequences of several T6SS proteins was performed, and results indicate a clear separation of T6SS-2 from most rhizobial T6SSs. ISLU101 T6SS mutant derivatives in genes *impO*, *impC1* and *impC2* were generated by single homologous recombination of amplified internal fragments from the respective genes cloned into the suicide vector pK18*mobsac*. The symbiotic behaviour of mutants was examined with *L. angustifolius*. Results showed no effect of *impC1* and *impC2* mutations, while the *impO* mutant generated smaller plants with a mixture of white/red nodules. These results suggest that T6SSs may play a role in the *Bradyrhizobium*-lupines symbioses.

References

Deakin & Broughton, 2009. Symbiotic use of pathogenic strategies: rhizobial protein secretion systems. Nat Rev Microbiol, 7, 310-320

Durán, D.. Rey, L., Sánchez-Cañizares, C., Navarro, A., Imperial, J., Ruiz-Argueso, T. 2013. Genetic diversity of indigenous rhizobial symbionts of the *Lupinus mariae-josephae* endemism from alkaline-limed soils within its area of distribution in Eastern Spain. Syst. Appl. Microbiol. 36, 128– 136

Records A., 2011. The Type VI Secretion System: A Multipurpose Delivery System with a Phage-Like Machinery. MPMI 24, pp. 751–757

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