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## Abstract

The type VI secretion system (T6SS) is a nanosyringe that injects proteins into prokaryotic or eukaryotic cells, and it is encoded in the genomes of more than 25% of Gram-negative bacteria (1). We are studying the T6SS of *Rhizobium etli* Mim1 and *Bradyrhizobium* sp. A16, symbionts of *Mimosa affinis*/*Phaseolus vulgaris*/*Leucaena leucocephala* and *Lupinus micranthus*/*Lupinus angustifolius*/*Spartium junceum* respectively.

*R. etli* Mim1 contains a T6SS gene cluster organized in two divergent operons. When the T6SS is active, Hcp, a constituent of the secretory apparatus, can be detected in the extracellular medium (2). Hcp has been immunologically detected in the supernatant of Mim1 cultures. This protein was also detected in bean nodules extracted and in cultures grown in the presence of different legume exudates. The putative divergent promoters located between the two T6SS gene clusters were analysed by  $\beta$ -gal fusions. The results showed high levels of expression of the two promoters at high OD and low values at lower ODs. Mutants affected in structural genes induced white nodules with *P. vulgaris* and *L. leucocephala*.

On the other hand, mutagenesis of T6SS structural genes from LmcA16 strain produced different symbiotic phenotypes. An LmcA16 *tsC* mutant showed reduced levels of nitrogen fixation on *L. micranthus*, whereas the same mutant induced the formation of few white nodules on *L. angustifolius* and *S. junceum*.

Genes *tsp78* and *tsp79* are located between *hcp* and *vgrG* in Mim1 strain, where often effector/immunity pairs have been identified. In order to assess their functionality they had been expressed in *Escherichia coli* and growth was evaluated.

## T6SS of *Rhizobium etli* Mim1

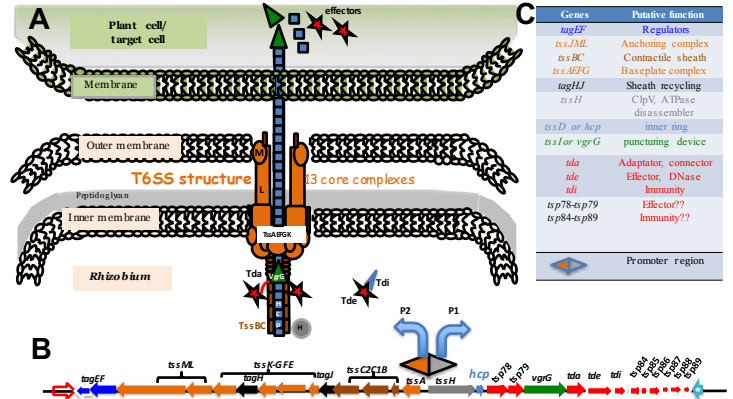
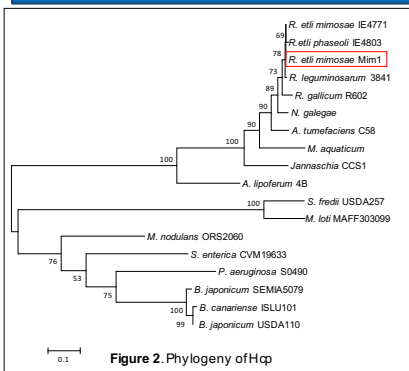
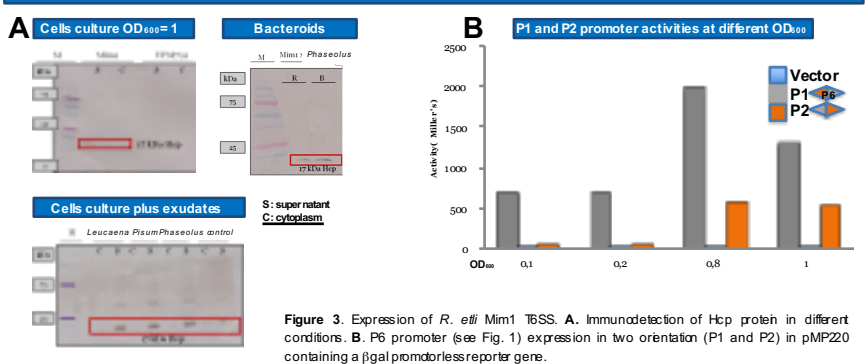


Figure 1. The T6SS of *Rhizobium etli* Mim1. A. Structure of the T6SS nanosyringe. B. Gene cluster. C. Function of T6SS genes

## Phylogeny of Hcp of *R. etli* Mim1

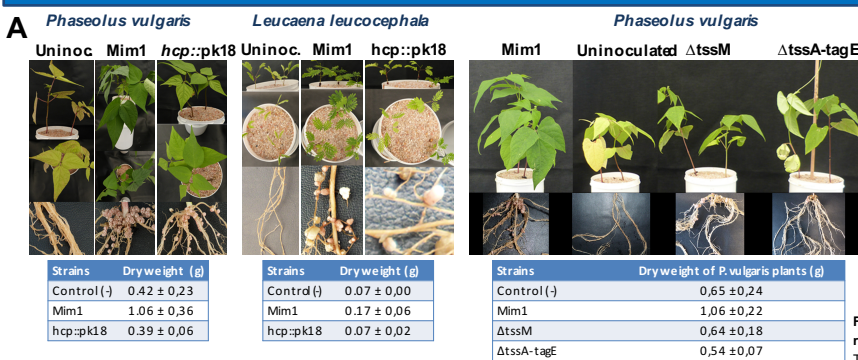


## Expression of *R. etli* Mim1 T6SS



## Symbiotic performance of T6SS mutants

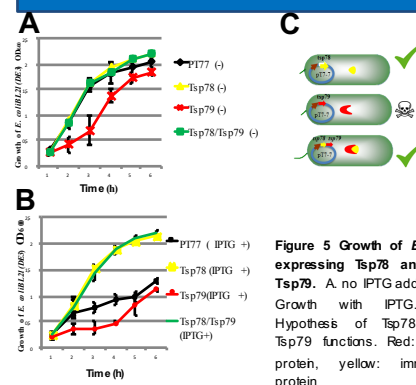
### *R. etli* Mim1 T6SS mutants



## Non structural genes in T6SS of *Rhizobium etli* Mim1

Gene	aa	Identities	Microorganism coding for similar genes	In T6SS
<i>tsp78</i>	360	309/358(86%)	<i>R. leguminosarum</i> <i>Sinorhizobium americanum</i> CCGM7 <i>Rhizobium galegae</i> bv. orientalis HAMB1 540	+
<i>tsp79</i>	308	239/298(79%)	<i>R. leguminosarum</i> <i>Sinorhizobium americanum</i> CCGM7 <i>Neorhizobium galegae</i>	+
<i>tsp84</i>	216	208/216(96%)	<i>Rhizobium phaseoli</i> N831 <i>Rhizobium leguminosarum</i> bv. trifolii CC278f <i>Rhizobium leguminosarum</i> bv. trifolii WSM2012	+
<i>tsp85</i>	71	54/68(82%)	<i>Rhizobium leguminosarum</i> bv. trifolii WSM597 <i>Neorhizobium galegae</i> bv. officinalis HAMB1 1145	+
<i>tsp86</i>	225	183/224(82%)	<i>Rhizobium leguminosarum</i> bv. trifolii WSM2012 <i>Agrobacterium</i> sp. ATCC 31749 AGT101 <i>Ochrobactrum cytilis</i> strain IPA7.2	+
<i>tsp87</i>	84	70/76(92%)	<i>Rhizobium phaseoli</i> Ch24-10 plasmid d <i>R. leguminosarum</i> bv. trifolii WSM2012	+
<i>tsp88</i>	279	252/279(90%)	<i>R. phaseoli</i> strain R650 <i>R. phaseoli</i> R650 pRphR650d <i>R. leguminosarum</i> bv. trifolii WSM2012	-
<i>tsp89</i>	90	22/35(63%)	<i>Stinella</i> sp. SUS2 scf_44948_8 <i>Rhizobium</i> sp. Kim5	+

## Growth of *E. coli* expressing *tsp78* and/or *tsp79*



## Conclusions

- The T6SS of *R. etli* Mim1 is expressed at high cell density, in the presence of plant exudates and in pea bacteroids
- Mutants in structural genes of T6SS from *R. etli* Mim1 and *Bradyrhizobium* sp. A16 are impaired in symbiosis with different legumes
- tsp78* and *tsp79* have been found in databases only associated to T6SS clusters of few rhizobial strains
- Growth experiments with *E. coli* expressing *tsp78* and *tsp79* are consistent with the possibility of these genes encoding an immunity/effector pair.

## References

- Ho et al. (2013) Cell Host Microbe 15:9-21.
- Wu et al. (2012) PLoS Pathog. 8:1-18

## Acknowledgements

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