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Nitrogen fixation by native *Bradyrhizobia* in symbiosis with *Lupinus mariae-josephae* requires a T3SS encoding a NopE-like effector

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Several bradyrhizobial isolates from *L. mariae-josephae* root nodules [1] contain a type III secretion system (T3SS) within a cluster of about 30 genes. Among those genes, *ttsI* codes for the transcriptional activator of the system. Mutation of *ttsI* resulted in the formation of white, non-fixing nodules with the natural legume host, *L. mariae-josephae*. The T3SS cluster also contains a gene coding for a NopE-like protein. NopE proteins have been demonstrated to be effectors in the *Bradyrhizobium*-soybean symbiosis [2] and belong to a small group of poorly characterized proteins from plant-associated bacteria that contain one or two autocleavage motifs known as DUF1521 (Schirrmeister *et al*. 2011). The amino acid sequence of a NopE-like protein in the *L. mariae-josephae* strain LmjC contains just one autocatalytic motif. This is unlike NopE1 and NopE2 proteins secreted by the T3SS of *B. japonicum,* that contain two motifs [3]. The autocleavage of LmjC NopE protein was analyzed after expression in *E. coli* and purification. Two protein fragments of the predicted sizes appeared in the presence of Ca^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+} and Mn^{2+} cations. In contrast, autocleavage did not take place in the presence of Ni^{2+}, Co^{2+} or Mg^{2+}. Site-directed mutagenesis of the DUF1521 motif in LmjC NopE abolished self-cleavage in vitro. Symbiotic competence of a $NopE^{-}$ mutant with the *L. mariae-josephae* host was not affected. Possible roles of NopE are discussed.

References

[1] Duran et al. (2013) Syst Appl Microbiol 36:128-136.
[2] Wenzel et al. (2010) MPMI 23:124–129.
[3] Schirrmeister et al. (2011). J. Bacteriol. 193:124-129.

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