EcfE, a master regulator of pea root attachment and colonization of Rhizobium leguminosarum by viciae 3841



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Background

- Legumes tightly regulate attachment and colonization by very specific timedependent signals and the ability to perceive and respond to such signals by rhizobia is a requirement for successful colonization (1).
- Rhizobia respond to host signals by activating a subset of genes, directed by extracytoplasmic function sigma factors (ECF). We found the IppE, ecfE, asfE operon to be up-regulated in the rhizosphere (Panel A)
- Root attachment and colonization requires an orchestrated transcription of a number of genes encoding polysaccharides (exo-, lipopolysaccharides and glucomannan), extracellular proteins (rhicadesin and rhizobium adhering proteins), celluose fibrils and secretion systems (2).
- We used Lux marked bacteria to follow attachment to roots and colonization.

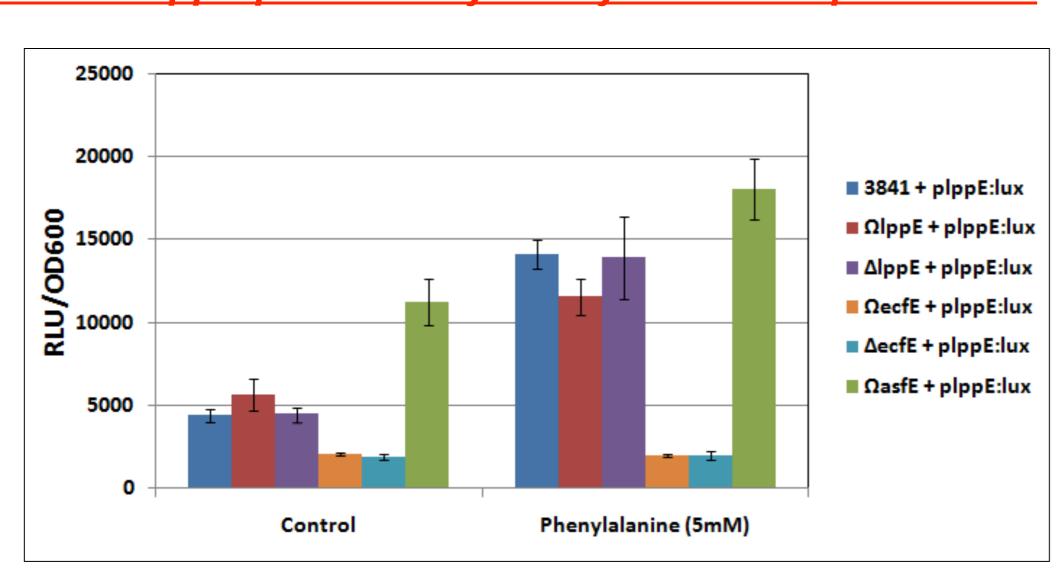
A. IppE operon IppE ecfE asfE RL3234 RL3235 RL3236 3384600 3385600 3384800 3385000 3385200 3385400 Comparative rhizosphere transcriptomics (3) *IppE* – lipoprotein fold up Vs Glucose - lab culture **Growth conditions RL3234 RL3235 RL3236** ecfE – extracytoplasmic sigma factor E ecfE *lppE* asfE pea rhizosphere asfE – anti sigma factor E alfalfa rhizosphere 29 sugar beet rhizosphere lab culture (+ Phenylalanine)

Root attachment assay: total luminescence from 2 cm pea root sections, incubated with

Results

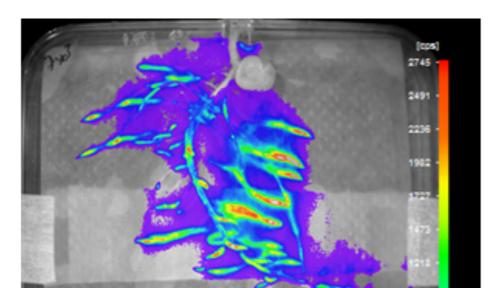
- EcfE is required to activate *lppE* operon (Panel B)
- IppE operon is specifically transcribed on the pea root hairs (Panel C)
- \blacksquare Overexpression of EcfE (Ω asfE), causes hyper-attachment to pea roots (Panel D), but blocks its spread and colonization (Panel E)
- \blacksquare Microarray analysis of Ω as E in the pearhizosphere, identified the EcfE target genes, revealing a complex regulatory cascade (Panel F)

B. Induction of IppE promoter by Phenylalanine requires EcfE

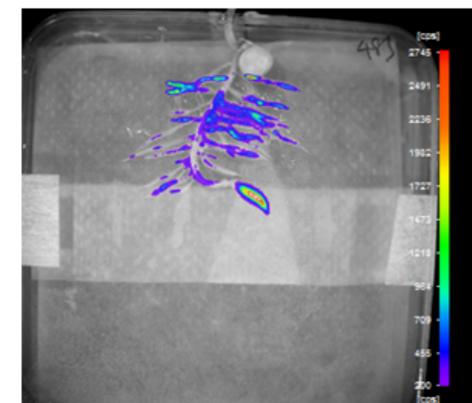


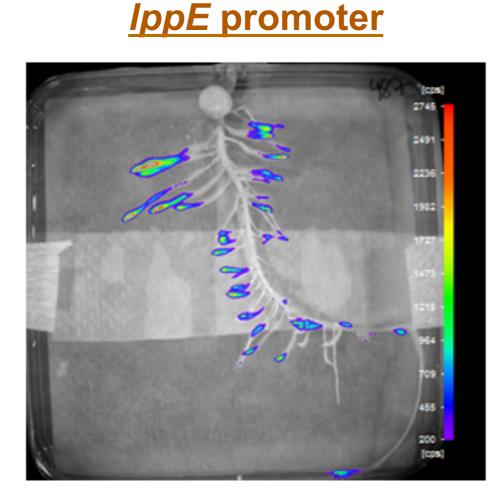
C. Spatial induction of IppE promoter on pea root hairs shown by lux imaging

Phe – induced promoter

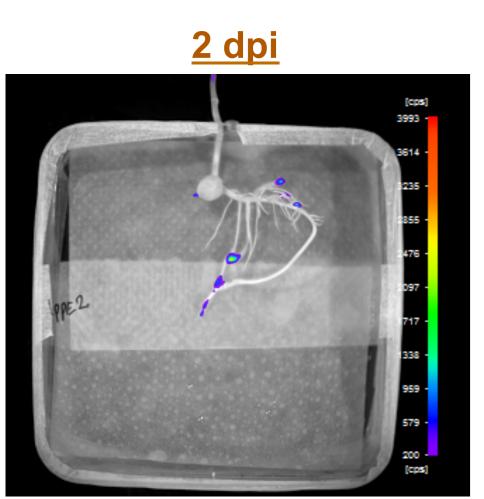


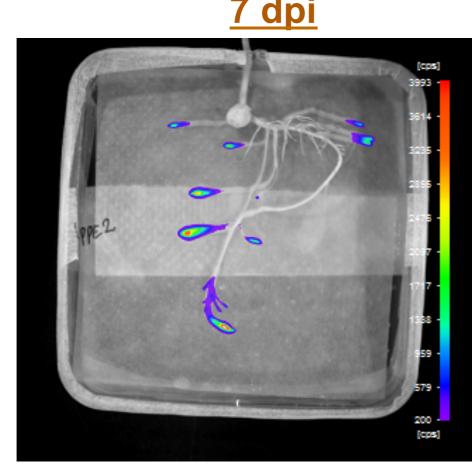
constitutive promoter

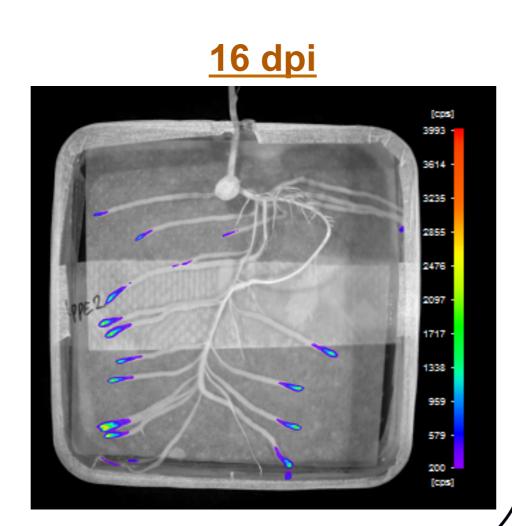




Induction of IppE promoter on pea root hairs over time





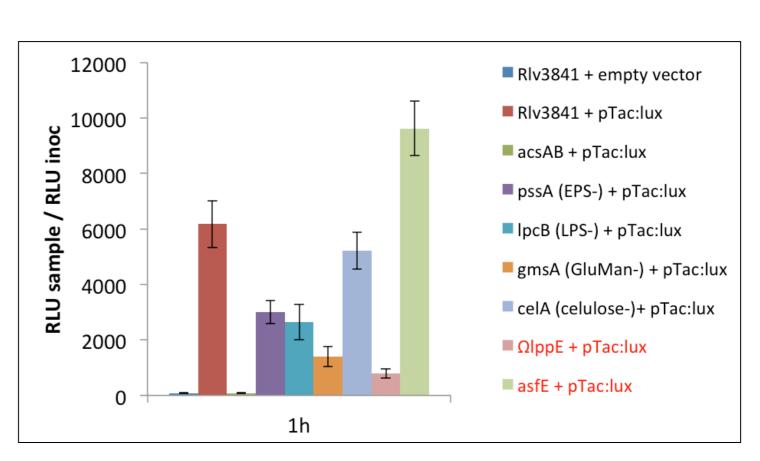


Strain **Root attachment**

D. ΩasfE shows hyper-attachment phenotype

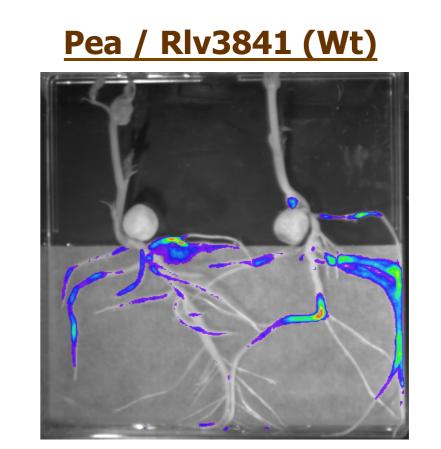
rhizobia for 1 hr

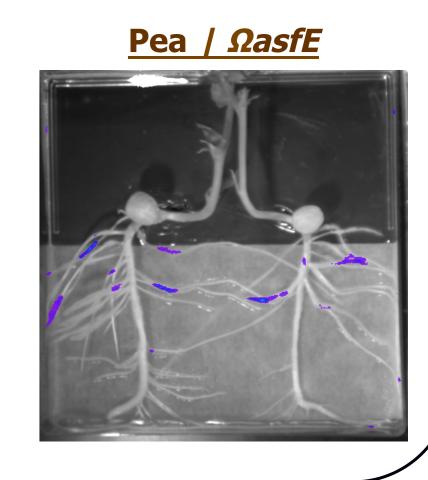
Rlv3841 (Wt) $\Omega lppE$ ΔlppE ΩecfE ΔecfE ΩasfE ++



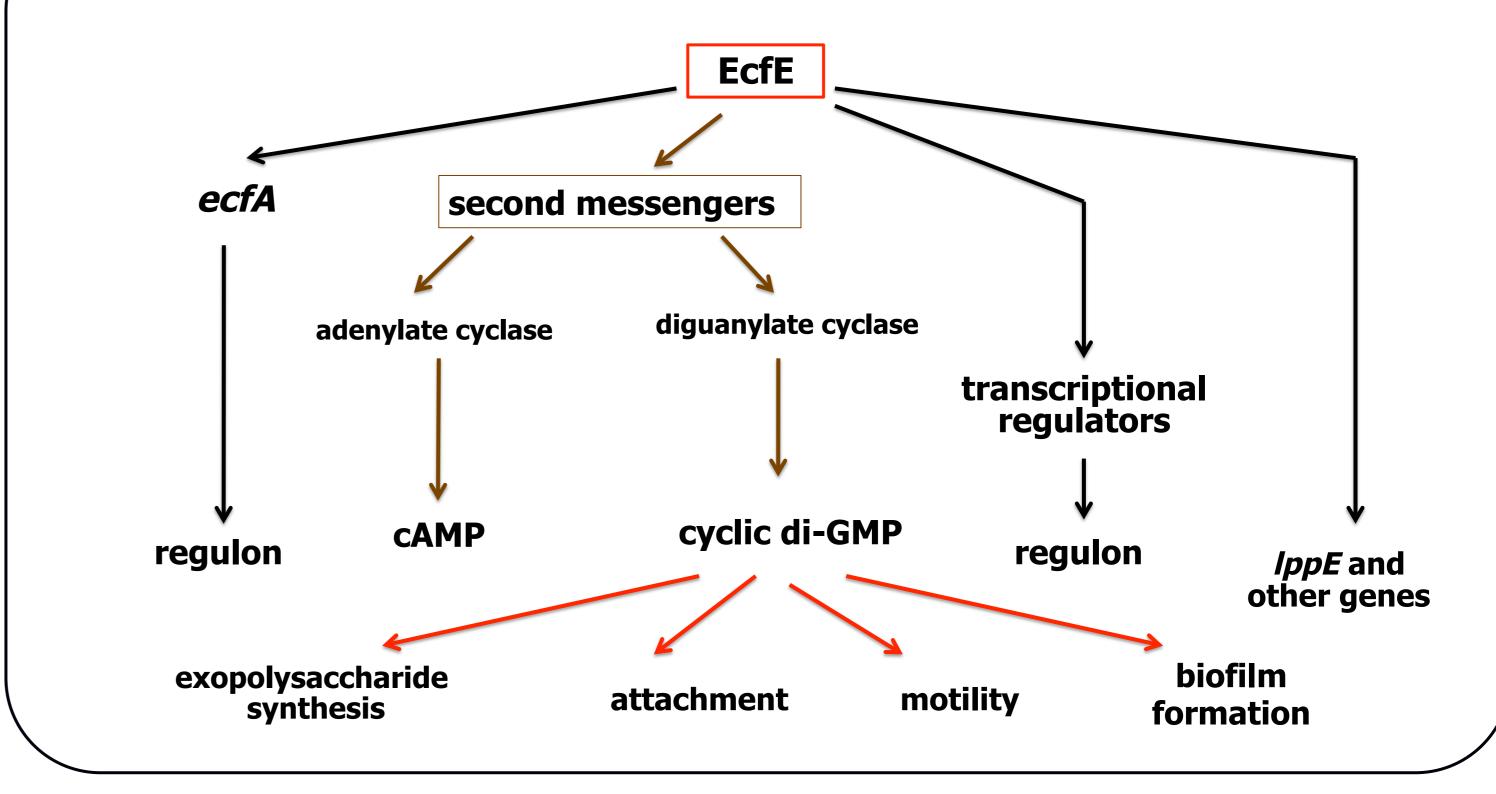
E. ΩasfE compromised for pea rhizosphere colonization

Strain	Pea rhizosphere colonization
	COIOIIIZATIOII
Rlv3841 (Wt)	+
ΩΙρρΕ	++
ΩecfE	+
ΩasfE	-





F. EcfE, a master regulator of attachment on pea roots



Summary

- ♣ EcfE is the master switch controlling rhizobial pea root attachment, in response to a pea root hair derived signal.
- ♣ Temporal switching off of EcfE is critical, to enable rhizobia to spread and colonize, rather than just attaching to roots.

Future work

- ♣ Identification and characterization of the ecfE & ecfA binding sites and target genes
- Confocal imaging to precisely localize the induction of *lppE* operon on pea root hairs
- ♣ Elucidate the participation of NodD in the activation of *lppE* operon
- Identification of the plant derived signal which activates lppE operon
- Surface localization and characterization of lipoprotein (*lppE*)

References

- 1. G. Oldroyd *et al.*, (2011) *Annual Review of Genetics* 45:119-144.
- 2. J.A. Downie (2010) *FEMS Microbiol Rev.* Mar;34(2):150-70.
- 3. V.K. Ramachandran *et al.*, (2011) *Genome Biology* 2011, **12**:R106.