

Role of *Pectobacterium atrosepticum* (*Pba*) effectors in the manipulation of host defences

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Pectobacterium atrosepticum (*Pba*), formally known as *Erwinia carotovora* subsp. *atroseptica*, is an economically significant bacterial pathogen of potato which causes soft rot of tubers and blackleg disease of stems. Discovery of the Type III secretion system (T3SS), effectors such as *DspE* and *HrpW* and *HrpN* within the *Pba* genome, supports the hypothesis that *Pba* may be actively suppressing plant defences in a similar way to hemi-biotrophic relatives such as *Pseudomonas syringae*. We are investigating the role of *Pba* effectors in pathogenicity and manipulation of potato defences using *Pba* Tn5 mutants and analysing the effect on the host potato using our own custom designed 11K microarrays (Agilent).

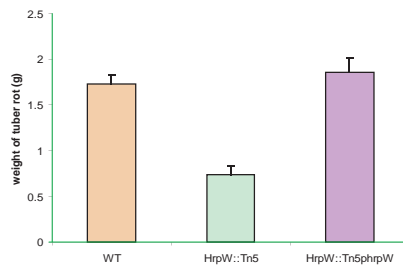


Do *Pba* effector proteins have a role in virulence?

A *hrpW* Tn5 insertion mutant of *Pba* was identified. Pathogenicity tests on both tubers and stems have demonstrated reduced virulence of the *hrpW* mutant.

To restore pathogenicity, *hrpW* mutant was complemented with pGEM T easy plasmid carrying *HrpW* together with its chaperone.

Complementation restored virulence.

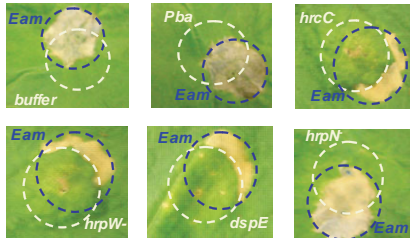


hrpW *Pba* has reduced virulence

Do *Pba* effectors suppress basal resistance?

Challenge HR Assay

White circles represent inoculation sites of *Pba* WT or mutants. PAMPs on surface of *Pba* trigger basal resistance.



6 hr later, HR-inducing *Erwinia amylovora* (*Eam*) was inoculated overlapping with *Pba*

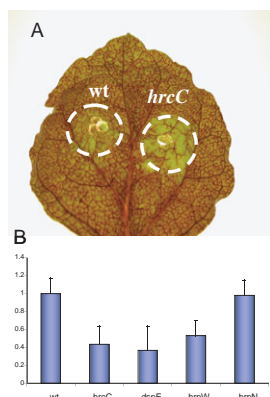
Pba suppresses basal resistance using T3SS delivered effectors. A full (blue) circle of *Eam*-induced HR shows basal resistance has been suppressed in overlap with white circle. A crescent of HR shows *Pba* has been unable to suppress basal resistance within white circle. *hrcC* is disabled in T3SS.

Vascular Stain Assay

White circles reveal inoculation sites of *Pba* and mutants into leaf. 6 hpi, leaves were detached and petiole dipped in neutral red stain.

Where *Pba* suppresses basal resistance, stain uptake will occur freely. If *Pba* mutants are unable to suppress basal resistance, the vascular system cannot uptake dye (A).

Dye uptake can be quantified by spectrophotometer (B)



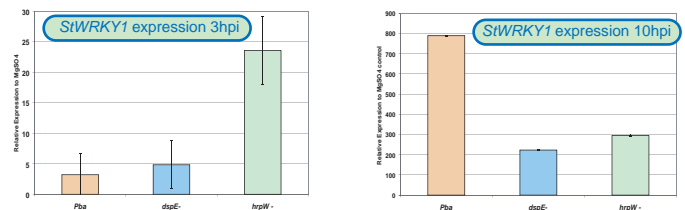
Both assays reveal that *HrpW* and *DspE* required for suppression of basal resistance, but not *HrpN*.

Do *Pba* effectors manipulate defence pathways?

To determine which host response pathways are manipulated by *Pba* at the transcriptional level, a potato 11K defense gene array was designed and hybridised with cDNA comparing WT *Pba* and *hrpW* through a 10 hr timecourse post-inoculation in potato leaves.

StWRKY1 Expression

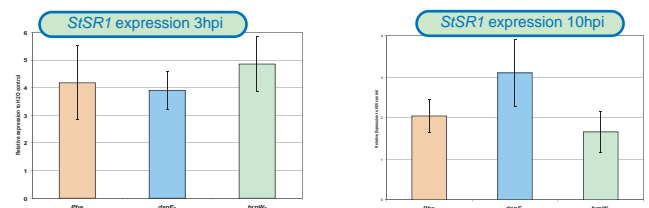
StWRKY1 belongs to a large family of transcription factors. *StWRKY1* was differentially expressed in every microarray throughout the *Pba* vs *hrpW* timecourse. These data were then confirmed using qRT-PCR.



HrpW is required for early suppression of *StWRKY1*

StSR1 Expression

Stress Response (*SR1*) is a control gene known to be induced by both abiotic and biotic stress.



No significant difference in level of induction indicates similar potato responses to each strain

Does *StWRKY1* confer resistance to *Pba*?

Two transgenic lines of 35S::*StWRKY1* were found to be significantly more resistant to *Pba*.

Pictures show lesion development at 17 dpi in cultivar Desiree compared to 35S::*StWRKY1* Desiree.



Conclusions

1. *HrpW* and *DspE* suppress basal resistance
2. *HrpW* and *DspE* suppress a pathway leading to *StWRKY1* expression
3. Overexpression of *StWRKY1* enhances potato resistance