A whole genome transcriptomics approach to determine the quorum sensing regulon of *Pectobacterium atrosepticum* during infection

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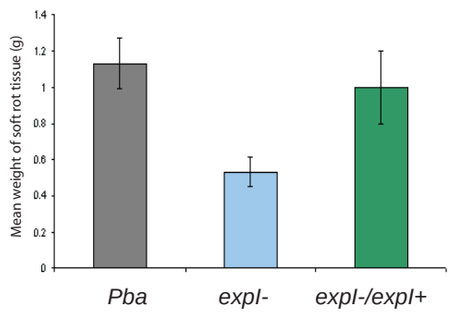
INTRODUCTION:

Pectobacterium atrosepticum (*Pba* - formerly *Erwinia carotovora* subsp. *atroseptica*), is the causal agent of the economically-important potato disease blackleg. In *Pba*, plant cell wall degrading enzymes (PCWDEs) and other pathogenesis-related factors are controlled, at least in part, by quorum sensing (QS). QS is a population density-dependant regulatory mechanism controlled by the production of *N*-acyl homoserine lactone (AHL) – the product of ExpI. Using whole-genome *Pba* microarrays (Agilent Technologies Inc.), we have generated global gene expression data based on a comparison between the fully sequenced *Pba* strain SCRI1043 (*Pba*1043) verses an *expI*::*Tn5* strain in vivo, during a potato tuber infection time course (Bell *et al*. 2004; Corbett *et al*. 2005). This is now being used to build a model of the QS regulon to gain a better understanding of the role of QS in the *Pba*-plant interaction, and to provide candidates for functional analysis that may offer new insights into pathogenesis, colonisation and disease control. Microarray data were analysed using GeneSpring software 7.3 (Agilent Technologies, Inc.).

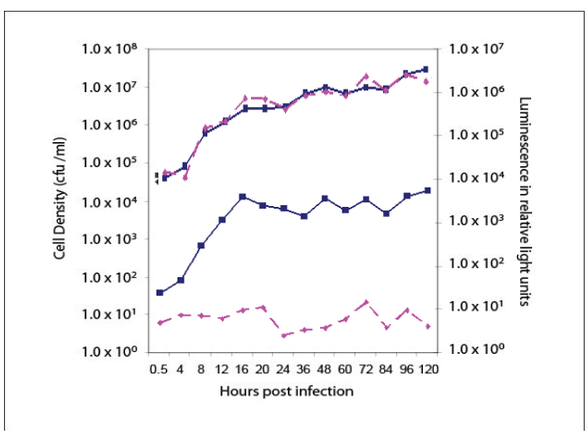
RESULTS:

Pathogenicity and complementation assay



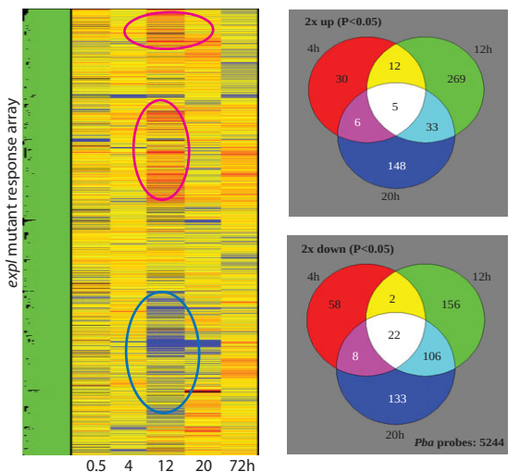
In *Pba*, the *expI* mutant strain is significantly reduced its pathogenesis on potato and its virulence has been restored by the addition of plasmid based *expI+*

Growth and AHLs level assay



The *expI* mutant strain was reduced in pathogenicity and AHL production compared to the wild type (WT) strain. However, cell growth was not affected. The top lines indicate cell growth, blue line = WT *Pba*; pink line = *expI* mutant; the centre line is AHL level in WT *Pba* and the bottom is AHL in the *expI* mutant strain. Potato variety - Maris piper was used for the assay

Profiling the transcriptomes of the *expI* mutant



The results show a total of 605 differentially expressed genes (P<0.05, 319 up-regulated and 286 down-regulated genes) in the *expI* mutant strain compared to the WT in potato tubers at 12 hours post inoculation (hpi). Red bar = up-regulated genes; blue bar = down-regulated genes.

CONCLUSION:

The *expI* mutant strain was significantly reduced in virulence and AHL production compared to the WT strain, as has previously been reported. In our microarray study, we confirmed that the production of many PCWDEs, and other virulence-associated genes, eg *nip* and *svx,* were QS regulated as they were down-regulated in the *expI* mutant strain (Corbett*et al*. 2005, Pemberton *et al*. 2005). Over 70 genes associated with regulation, and those associated with phytotoxins production, type II secretion and many other functions were also differentially regulated in the *expI* mutant compared to the WT strain at both 12 and 20 hpi. Our data demonstrates that the QS system has a far wider influence on the interaction between *Pba* and its host, potato, than has previously been shown, uncovering many genes previously unassociated with the QS regulon. We are now further investigating the roles of some of these genes in both the QS regulon and in virulence.

REFERENCES:

Bell et al. (2004) Proc Natl Acad Sci USA, Jul 27; 101: 11105-10.
Corbett et al. (2005) Mol Plant Microbe Interact, Apr 18 (4): 334-42
Pemberton et al (2005) Mol Plant Microbe Interact, Apr 18 (4): 343-53