

# Quorum sensing regulates novel phytotoxin production in the plant pathogen *Pectobacterium atrosepticum*

The potato pathogen *Pectobacterium atrosepticum* (*Pba*) causes disease through the production of large numbers and levels of plant cell wall degrading enzymes (PCWDEs). Because of this physical attack on the plant cell wall *Pba* has been termed a “brute force” pathogen. However, through sequencing and annotation of the complete *Pba* genome, a new virulence factor has been identified. This factor is similar to the phytotoxin coronatine that has been well

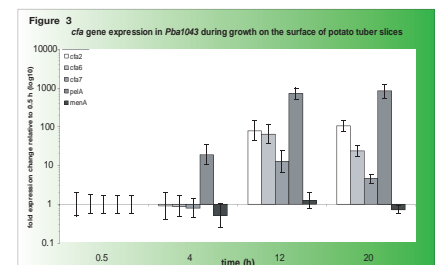
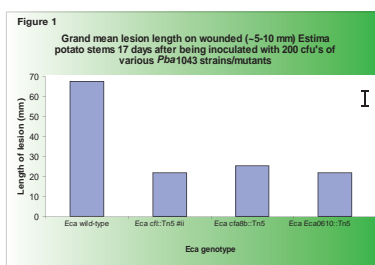
characterized in *Pseudomonas syringae*, where it appears to suppress host resistance during the infection process. Quorum sensing (QS) is a cell density-dependant process involved in the regulation of virulence factors (particularly PCWDEs) in *Pectobacterium* spp., and involves the production of the hormone acyl-homoserine lactone (AHL) encoded by the *expI* gene. *Pba* strains containing mutations within the

phytotoxin biosynthetic genes (*cfl* and *cfa*) have been isolated and assayed for virulence on *Estima* potato stems. Gene expression studies have determined the transcriptional profiles of these genes throughout the course of tuber infection, and during *in vitro* culture. Finally, preliminary LC/MS analysis has identified the chemical structure of the phytotoxin isolated from the supernatant of *Pba* cell cultures.

## Results

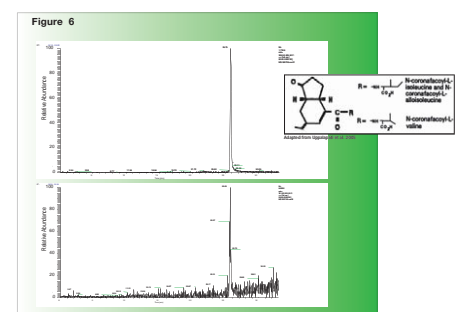
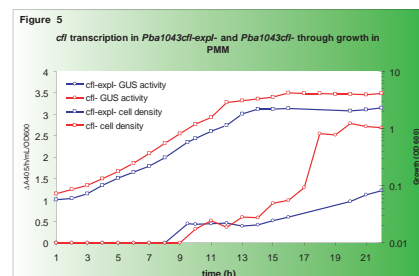
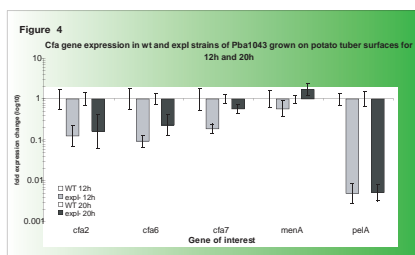
*Pba1043* strains carrying mutations in the *cfa* biosynthetic genes exhibited significantly reduced virulence when inoculated into *Estima* potato stems (Fig. 1 and 2).

Transcriptional profiling using real-time PCR revealed that *cfa* biosynthetic genes were significantly upregulated at 12 h and 20 h during potato infection (when AHL was at its maximum level) (Fig. 3).



During potato infection and growth in liquid culture, the expression of *cfa* biosynthetic genes were significantly reduced in a *Pba1043* strain carrying a mutation in the QS regulator *expI* (Fig 4 and 5).

LC/MS analysis indicated the presence of two peaks (Fig 6) corresponding to the compounds: coronafacoyl valine and coronafacoyl isoleucine.



## Conclusions

The production of coronafacoyl phytotoxins is essential for the full virulence of *Pba*.

*cfa* biosynthetic genes are significantly upregulated during the later stages of tuber infection, and in stationary phase during *in vitro* culture; upregulation of these genes is dependent upon the QS signaling hormone and are thus regulated in a QS-dependent manner.

The two main coronafacoyl conjugates produced by *Pba* are coronafacoyl valine and coronafacoyl isoleucine; these compounds are structurally analogous to the plant defense-signaling hormone methyl jasmonate, indicating that plant defenses may be suppressed at the latter stages of infection during the production of PCWDEs.