Quorum sensing and pectin catabolism regulate phytotoxin production in *Pectobacterium atrosepticum*

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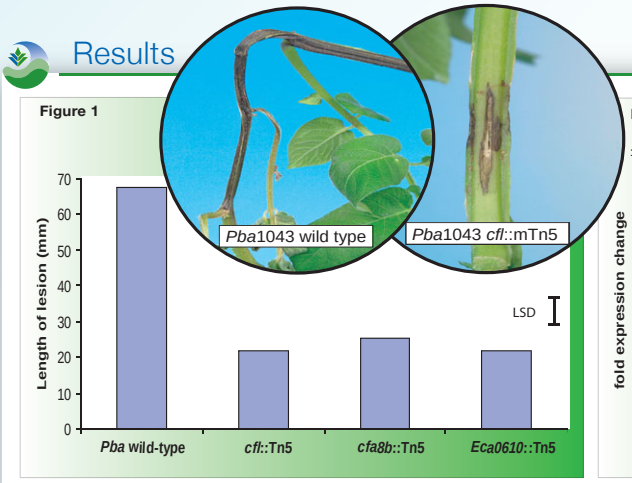
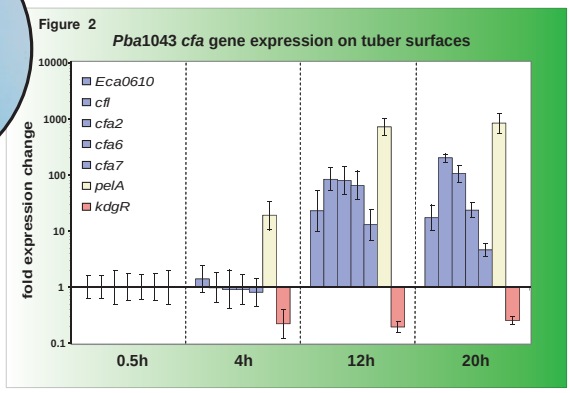
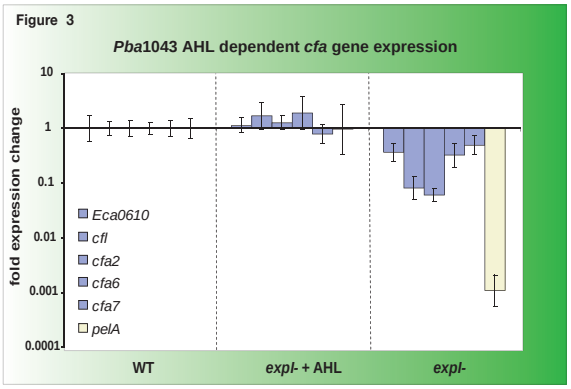
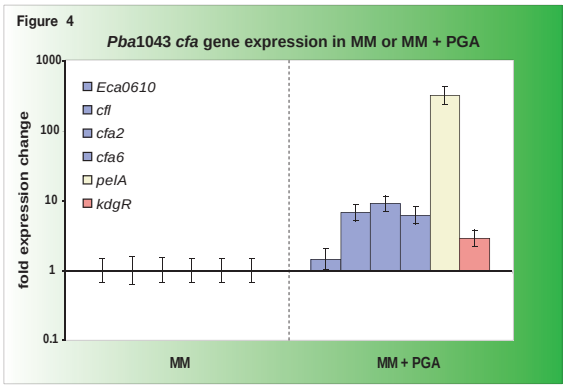
The potato pathogen *Pectobacterium atrosepticum* (*Pba*) causes disease through the prolific production of a wide variety of plant cell wall degrading enzymes (PCWDEs) regulated, at least in part, by the quorum sensing (QS) regulatory system. Quorum sensing is a cell density-dependant process involved in the regulation of PCWDEs and other virulence factors in *Pectobacterium* spp., and involves the synthesis of a hormone acyl-homoserine lactone (AHL) from the *expI* gene. Because of this coordinated physical attack on the plant cell wall *Pba* has been termed a "brute force" pathogen. However, through sequencing and annotation of the complete genome of *Pba* strain SCRI1043 (*Pba*1043), 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. *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, LC/MS analysis has identified the chemical structure of the phytotoxin isolated from the supernatant of *Pba* cell cultures.

Results









*Pba*1043 strains carrying mutations in the *cfa* biosynthetic genes exhibited a significant reducion in virulence on Estima potato stems (Fig. 1); virulence was restored to wild-type levels by complementation of the mutants with *cfa* genes (data not shown). 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. 2). During potato infection and growth in liquid culture, the expression of *cfa* biosynthetic genes was significantly reduced in a *Pba*1043 strain carrying a mutation in the QS regulator *expI*; wild-type expression was restored via exogenous application of AHL (Fig 3). Additionally, the expression of *cfa* biosynthetic genes was significantly increased by the addition of polygalacturonic acid (PGA) to the growth media (Fig 4). LC/MS analysis indicated the presence of two peaks corresponding to the compounds: coronofacoyl valine and coronafacoyl isoleucine (data not shown).

Conclusions

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

cfa biosynthetic genes are significantly up-regulated during the later stages of tuber infection. This induction is dependent on the QS signaling hormone AHL, and the genes are thus regulated in a QS-dependent manner. The in vitro expression of these genes is increased by the addition of PGA to the media.

The two main coronafacoyl conjugates produced by *Pba*1043 are coronofacoyl 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.