POST-GENOMIC ANALYSIS OF *ERWINIA CAROTOVORA* VIRULENCE RESPONSES IN *IN VITRO* AND *IN PLANTA* ENVIRONMENTS

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INTRODUCTION:

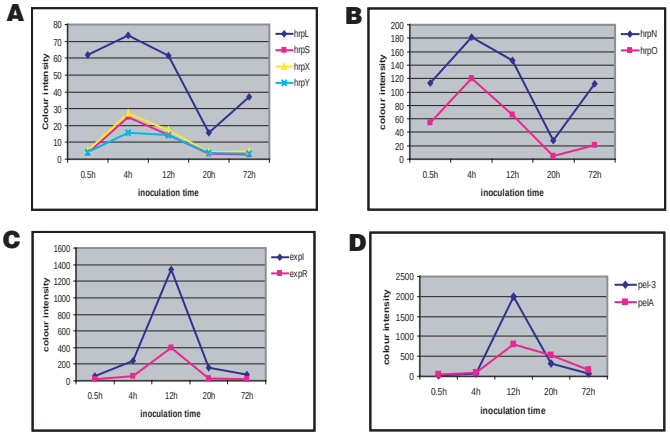
Three erwinias, *Erwinia carotovora* subsp. *carotovora* (*Ecc*), *E. carotovora* subsp.*atroseptica* (*Eca*) and *E. chrysanthemi* (*Ech*), cause potato tuber soft rot and blackleg (stem rot). The genomes of *Eca* SCRI 1043 and *Ech* 3937 strains have recently been sequenced, revealing many 'novel' potential pathogenicity genes. In the post-genomic era, microarray approaches have allowed high-throughput experiments that have improved our understanding of microbial environmental responses and associated global gene expression. We have designed and utilised oligonucleotide microarray chips (the entire ORFs of *Eca* SCRI 1043) to identify differentially expressed genes in mutants compared with wild type strains. Since the *Ecc* genome is not sequenced at present, we therefore compared genomic DNA from *Ecc* SCRI193 with the *Eca* chip to analyse differential and common genes between the two species.

METHODS:

Each gene in the microarray was represented by 60-mer oligonucleotides. In total about 4500 *Eca* genes were spotted onto Agilent chips. Total RNA (12µg) from mutant and wild type strains were isolated from inoculated potato tuber at five time points. cDNAs were labelled by Cy3 or Cy5, and hybridized to the *Eca* chip according to the manufacturer's protocols. Genomic DNA (2µg) from *Eca* 1043 and *Ecc* 193 were labelled and hybridized to the *Eca* chip for comparative analysis of the two genomes. MolecularWare analyzerDG and GeneSpring programs were used to analyse the microarray data.

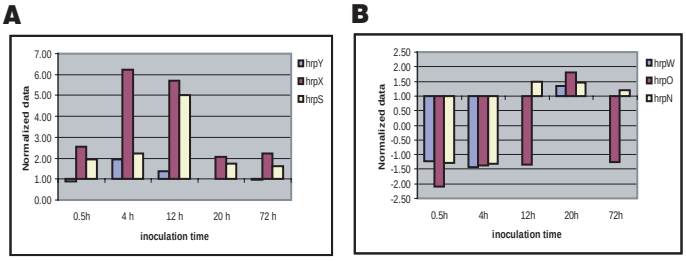
RESULTS:

1. genes expressed in WT 1043 at five time points



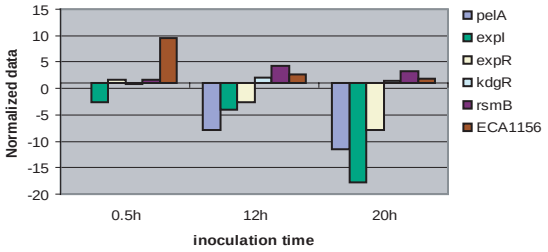
A: The expression patterns of a regulatory cascade (*hrpL*, *hrpXY and hrpS*), that activates *hrp/hrc* type III secretion and effector genes. B: *hrpN* and *hrpO* are regulated by *hrpL*, as shown by the expression patterns similar to *hrpL* in A. C: *expI* and *expR* are associated with quorum sensing. It is believed that they regulate the production of secreted plant cell wall-degrading enzymes. D: The expression patterns of pectate lyase genes are similar to their regulators in C.

2. *hrpL* mutant vs WT *Eca* 1043



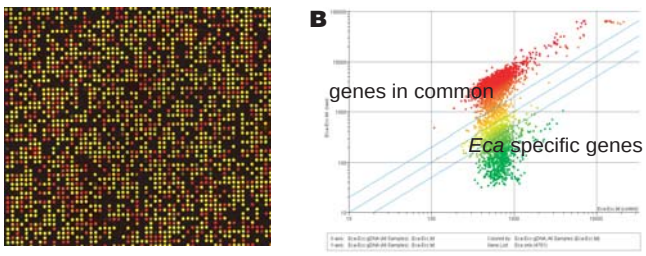
hrpL encodes an alternative sigma factor and activates genes containing a '*hrp* box' promoter. Five time point microarray datasets identified three regulators (*hrpS*, *hrpX* and *hrpY*) that were upregulated in *hrpL* mutant strain (A). This demonstrates that *hrpL* may have a negative regulatory effect on the genes directly involved in its own regulation. B: This figure shows that the genes with a 'hrp box' require *hrpL* for their expression.

3. *expI* mutant vs WT *Eca* 1043



Many Gram-negative bacteria use N-acylhomoserine lactones (AHLs) to monitor their cell density and meanwhile regulate virulence gene sets as part of a quorum-sensing (QS) system. In *Eca*, the AHLs are synthesised via the *expI/carI* gene, which is responsible for regulating the production of secreted plant cell wall-degrading enzymes. The *expI* mutant strain had significantly reduced pathogenicity in potato. The microarry data revealed that *expI* and *expR* (quorum-sensing transcriptional regulator) are down-regulated in the *expI* mutant, and all the pectate lyase genes, e.g. *pel-3*, *pelA, pelC* and *pelZ* are down-regulated. Interestingly, a pectin degradation repressor, *kdgR*, is up-regulated in the *expI* mutant.

4. gDNA microarray of *Ecc* 193 vs *Eca* 1043



A: Cy3 labeled *Ecc* 193 and Cy5 labeled *Eca* 1043. Red spots represent *Eca*-specific genes and yellow spots represent common genes. B: The genomic DNA microarray data revealed that 70% of genes are in common between *Eca* 1043 and *Ecc* 193, and 30% of genes are *Eca*-specific.

CONCLUSION:

Microarrays have allowed us to look at gene expression at the whole genome level. Our microarray datasets demonstrate that *hrpL* regulated TTSS and effector genes expression at an early stage of infection, prior to the expression of 'quorum-sensing' regulator genes. The 'quorum-sensing' regulators activate genes from 12 hours post-inoculation, leading to the high expression of plant cell wall-degrading enzymes that cause host cell maceration. In the genomic DNA microarray data, *Eca* specific clusters of genes were identified. For instance, a cluster that produces coronafic acid, a non-host-specific chlorosis-inducing phytotoxin, is present in *Eca* but not *Ecc* and is required for virulence. This observation was verified by Southern blotting.

ACKNOWLEDGEMENTS

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