

Genomes of three tomato pathogens within the *Ralstonia solanacearum* species complex reveal significant evolutionary divergence

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The *Ralstonia solanacearum* species complex includes thousands of strains pathogenic to an unusually wide range of plants. The genome of *R. solanacearum* complex strains is organized into two replicons, a chromosome and a megaplasmid. Strains within this ancient species complex can be subdivided into four phylotypes, corresponding to strains from Asia (phylotype I), the Americas (phylotype II), Africa (phylotype III) and Indonesia (phylotype IV)¹. Previous phylogenetic studies indicate greater than 30% divergence among strains despite an apparently common mode of pathogenesis. Comparison of genome sequences of *R. solanacearum* strains representative of this phylogenetic diversity can help determine which traits allow this bacterium to be such an aggressive and lethal pathogen of so many different plant species and how the bacteria survive in many different habitats such as soil, water, non-host plant rhizospheres and host xylem vessels.

We used comparative genomic analysis to explore the diversity of three strains of *R. solanacearum* all of which are able to cause bacterial wilt of tomato. *R. solanacearum* strains CFBP2957 (phylotype IIA), CMR15 (phylotype III) and PSI07 (phylotype IV) were isolated in the Caribbean, Cameroon, and Indonesia, respectively. The bipartite genomes of these three strains were manually annotated and the genomes were compared with those of previously sequenced *R. solanacearum* strains GMI1000 (phylotype I)², Molk2 (phylotype IIB) and IPO1609 (phylotype IIB)³. Comparative genomic analyses were completed using a comparative genomic hybridization (CGH) microarray on a larger set of *R. solanacearum* strains.

The major genomic features were conserved across all of the six strains for which genomic sequences were available and the organisation of genomes was highly syntenic. Comparative analysis of genome sequences and gene content confirmed the differentiation of *R. solanacearum* species complex strains into the four phylotypes. The genomic sequences of the three *R. solanacearum* strains revealed the presence of several previously unknown traits within the genomes of *R. solanacearum*, including a Type IV secretion system and a rhi-type anti-mitotic toxin. Small plasmids of 35 and 13kb were also identified in strains CMR15 and PSI07, respectively. Average Nucleotide Identity (ANI) scores between strains in conjunction with CGH microarray analysis of a larger set of diverse strains revealed differences great enough to warrant reclassification of the *R. solanacearum* species complex into three species; one species comprising strains previously classified within phylotypes I and III the second comprising strains previously classified within phylotype II and the third comprising strains previously classified within phylotype IV.

References:

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