

Complete Genome Sequence of the Plant Pathogen *Ralstonia solanacearum* Strain Po82[∇]

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***Ralstonia solanacearum* strain Po82, a phylotype IIB/sequevar 4 strain, was found to be pathogenic to both solanaceous plants and banana. Here, we report the complete genome sequence of Po82 and its comparison with seven published *R. solanacearum* genomes.**

Ralstonia solanacearum, the causal agent of bacterial wilt disease, can be divided into four phylotypes on the basis of a newly proposed phylotype subclassification system (3, 9). In our phylogenetic research, the *R. solanacearum* strain Po82, originally isolated from potato in Mexico, was found unexpectedly to fall into phylotype IIB/sequevar 4, in the same group with banana Moko disease-causing strains (10). Normally, Moko disease-causing strains are not pathogenic to potato. To solve the question of how this pathogenic variation arose, the complete genome of Po82 has been sequenced, annotated, and compared to the genomes of seven sequenced *R. solanacearum* strains.

The nucleotide sequence of the Po82 genome was determined by using a massively parallel pyrosequencing technology (Roche 454 GS FLX) (5). One hundred fifteen contigs (>500 bp) with a total size of 5.43 Mb were assembled from 453,135 reads (average length of 327 bp) using Newbler software of the 454 suite package, providing a 27.6-fold coverage. The relationship among contigs was determined by multiplex PCR, and gaps were filled by sequencing PCR products. The final sequence assembly was carried out using the Phred/Phrap/Consed package (<http://www.phrap.org/phredphrapconsed.html>), which produced a circular chromosome sequence of 3,481,091 bp and a megaplasmid of 1,949,172 bp.

Putative protein-coding sequences were predicted by Glimmer (2) and Genemark (1) software. Functional annotation was based on BLASTp with the KEGG and NR databases. tRNA genes were directly predicted with tRNAscan-SE, version 1.23 (4). Protein domain prediction and COG (Clusters of Orthologous Groups of proteins) assignment were performed by RPS-BLAST using the NCBI Conserved Domains Database (CDD) library.

In brief, the 5.43-Mb genome of *R. solanacearum* strain Po82, which is ~250 kb smaller than the average size (5.68

Mb) for the sequenced *R. solanacearum* strains, is composed of two circular replicons. The average GC content of the chromosome is 66.6%, while that of the megaplasmid is 66.7%. The entire genome contains 5,019 coding sequences, 54 tRNA genes, and 3 complete rRNA loci. Among the coding sequences (CDSs), 3,610 proteins can be assigned to COG families (8). Biological functions could be defined for 3,552 (70.8%) of the predicted proteins, while the other 1,094 CDSs (21.8%) are homologous to conserved proteins of unknown function in other organisms. The remaining 373 hypothetical proteins (7.4%) have no match to any known proteins in the databases. Altogether, the CDSs and stable RNA genes represent 90.9% and 0.32% of the genome, respectively.

In comparison to the seven sequenced *R. solanacearum* strains, 390 strain-specific genes were identified within the genome of strain Po82, most of which encoded hypothetical and conserved hypothetical proteins (6). Notably, a type III effector-coding gene which shared 54% amino acid sequence identity with the *P. syringae* type III effector HopAF1 was identified in the Po82 chromosome. Interestingly, HopAF1 was also found in *Xanthomonas campestris* pathovar *musacearum* 4381, pathogen of banana *Xanthomonas* wilt (7). In addition, the megaplasmid of Po82 contained 8 of the 9 *rhi* genes which compose the *rhi* operon in *R. solanacearum* strains CFBP2957 and PSI07.

Nucleotide sequence accession numbers. The *Ralstonia solanacearum* strain Po82 chromosome and megaplasmid sequences have been deposited in GenBank with accession numbers CP002819 and CP002820.

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