



Genome Sequence of *Ralstonia pseudosolanacearum* SL1931, a Causal Phytopathogen of Bacterial Wilt Disease in *Capsicum annuum* and *Nicotiana benthamiana*

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ABSTRACT Here, we report the genome sequence of *Ralstonia pseudosolanacearum* (*R. solanacearum* phylotype I) strain SL1931 (KACC10711), isolated from pepper (*Capsicum annuum* L.) stems; *R. solanacearum* is the causal pathogen of bacterial wilt. Strain SL1931 had a different type III effector profile than that of the reference genome strain GMI1000.

R *alstonia solanacearum*, which is the causal pathogen of bacterial wilt disease, infects more than 250 plant species, most of which belong to the Solanaceae family, including pepper, tobacco, tomato, and potato (1, 2). *R. solanacearum* invades the xylem vessels through plant root hairs and disseminates into the stem, where it multiplies and causes wilting by blocking water transport and producing excessive exopolysaccharides (3). Virulence and pathogenicity factors of *R. solanacearum* are known from previous studies (4, 5). Here, the genome sequence of *Ralstonia pseudosolanacearum* (phylotype I) race 1, biovar 3 strain SL1931 (KACC10711), is presented. *R. pseudosolanacearum* phylotype I strain GMI1000 was the first strain subjected to whole-genome analysis and therefore serves a reference genome. Strain GMI1000 is pathogenic to a model dicot plant, *Arabidopsis thaliana* (6, 7). On the other hand, *R. pseudosolanacearum* strain SL1931 is not virulent to *A. thaliana* but causes severe disease symptoms in *Nicotiana benthamiana*.

Strain SL1931 was originally isolated from pepper stems in Seosan, Chungcheongnam-do, South Korea, in 1998. The strain was preserved as freeze-dried cultures in the Korean Agricultural Culture Collection (KACC), Wanju, South Korea. The freeze-dried cultures were rehydrated under laboratory conditions in KRIBB, South Korea. The cultures were stored in a 20% glycerol suspension at -80°C for long-term preservation and routine work. Strain SL1931 was cultured on Casamino Acids-peptone-glucose (CPG) agar medium at 30°C for 24 h for purity testing. A single colony was picked and cultured in CPG broth for 8 h. The 1% volume of seed culture was inoculated in CPG broth and cultured for 18 h. Then, genomic DNA (gDNA) of SL1931 was extracted using the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA) according to the manufacturer's protocol.

Library preparation and sequencing were carried using a PacBio RS II system (P6-C4 chemistry) at the Chun Lab (Seoul, South Korea). The sequencing library was constructed according to the manufacturer's instructions for 20-kb template preparation using the PacBio DNA template prep kit 1.0 and BluePippin size selection system. A total of 111,636 reads (999.42 Mb) were produced with an average length of 8,952 bp and N_{50} of 12,217 bp, with an average read quality of 0.857. Default parameters were used for all software unless otherwise specified. *De novo* assembly and two additional rounds of polishing were performed

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with RS_HGAP_Assembly.2 and RS_Resequencing.1 protocols, respectively, using SMRT Analysis v2.3.0 (<https://smrt-analysis.readthedocs.io/en/latest/SMRT-Analysis-Software-Installation-v2.3.0/>). Polished sequences were circularized with Circlator v1.1.5 (8).

A chromosome (3,744,397 bp, 66.85% G+C) and a megaplasmid (2,075,606 bp, 66.85% G+C), along with two additional plasmids (50,417 bp, 61.76% G+C; 34,545 bp, 60.5% G+C), were obtained. Circular topologies for all four replicons were confirmed by Unicycler v0.4.8 (9). Genome completeness and contamination were 100.0% and 0%, respectively, based on quality assessment analysis by CheckM v1.1.3 (10). Two-way average nucleotide identity (ANI) with strain LMG 9673 (RefSeq GCF_919586305.1) was 95.95% based on calculations from the Kostas lab website (<http://enve-omics.ce.gatech.edu/ani/>). Among all *Ralstonia* strain genome sequences available to date (490 GenBank entries as of 14 March 2022), strain Pe_1 (GCA_011420365.1, accession no. SAMN13503698), which is one of the 30 South Korean isolates studied under project no. PRJNA593908, was found to be closest to that of SL1931 (99.98% ANI) as calculated by fastANI v1.32 (11).

The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (12) was used for automatic sequence annotation. Type III effector genes were predicted using the Ralsto T3E database (13). The results showed that the type III effectors RipAL and RipBP were only present in the SL1931 strain and absent in GMI1000. Twenty-three effectors in strain GMI1000 were lacking in strain SL1931, which indicated different host specificity between the two strains, although both were classified within the same phylotype I.

Data availability. The complete sequences, including the chromosome and three plasmids, were deposited in the DDBJ/ENA/GenBank databases under accession no. CP093535 for the chromosome and CP093536, CP093537, and CP093538 for the plasmids. Project data are available under BioProject accession no. PRJNA814415. The PacBio raw sequencing reads were submitted to the NCBI Sequence Read Archive under accession no. SRR18296424.

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