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GENOME SEQUENCES

Draft Genome Sequences of *Pseudomonas syringae* pv. tomato Strains J4 and J6, Isolated in Florida

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ABSTRACT *Pseudomonas syringae* pv. tomato causes bacterial speck in tomato. We report the genome sequences of two *P. syringae* pv. tomato strains, J4 and J6, that are genetically closely related, with >99.9 average nucleotide identity (ANI), but vary in the presence of coronatine-associated genes.

Pseudomonas is a Gram-negative genus of bacteria that belongs to the *Gammaproteobacteria* in the *Pseudomonadaceae* family. This genus contains more than 220 validly published species that inhabit diverse environmental niches and are associated with human and plant diseases (1–3). *Pseudomonas syringae* pv. tomato causes bacterial speck disease in tomato and requires a type III secretion system to infect and colonize the host (4). Additionally, *P. syringae* pv. tomato produces the phytotoxin coronatine, which functions as a defense suppressor (5). Coronatine is reported to mimic methyl jasmonate and promote *P. syringae* pv. tomato virulence and is important for symptom development resulting in chlorotic lesions in host plants (6–8) and inducing stomatal opening to facilitate entry into stomates (9–11).

In April 2010, two *P. syringae* pv. tomato strains, J4 and J6, were isolated from tomato fields in Florida using a standard isolation procedure (12). Pathogenicity was confirmed in tomato, and the *in planta* bacterial populations were quantified by infiltrating strains at $\sim 10^5$ CFU/ml into tomato leaves (11). Populations of both J4 and J6 were similar 6 days postinfiltration (Fig. 1A). Additionally, tomato plants were dip inoculated using suspensions of both strains at $\sim 10^8$ CFU/ml. Interestingly, chlorosis was observed in J4- but not J6-inoculated plants (Fig. 1B). To investigate differences, J4 and J6 were subjected to whole-genome sequencing.

Genomic DNA was extracted from cultures grown in nutrient broth for 24 h using a Wizard genomic DNA purification kit (Promega, Chicago, IL). The genomic library was prepared using a Nextera DNA library preparation kit (Illumina, San Diego, CA). Sequencing was performed at the Interdisciplinary Center for Biotechnology Research, University of Florida, using Illumina MiSeq technology, generating 251-bp paired-end reads for each sample. Raw sequences were assembled using a previously described pipeline (13, 14). Briefly, raw reads were trimmed and paired with Trim Galore (15) and then assembled into contigs with Spades v.3.10.1 (16). Contigs smaller than 500 bp and with k-mer coverage less than 2.0 were removed. Validated reads were mapped to filtered contigs using Bowtie 2 v.2.3.3 (17). SAMtools was used for file conversion, and Pilon v.1.22 was used to polish the draft assembly to generate an improved FASTA file (18, 19). Genome assemblies were annotated using the Prokaryotic Genome

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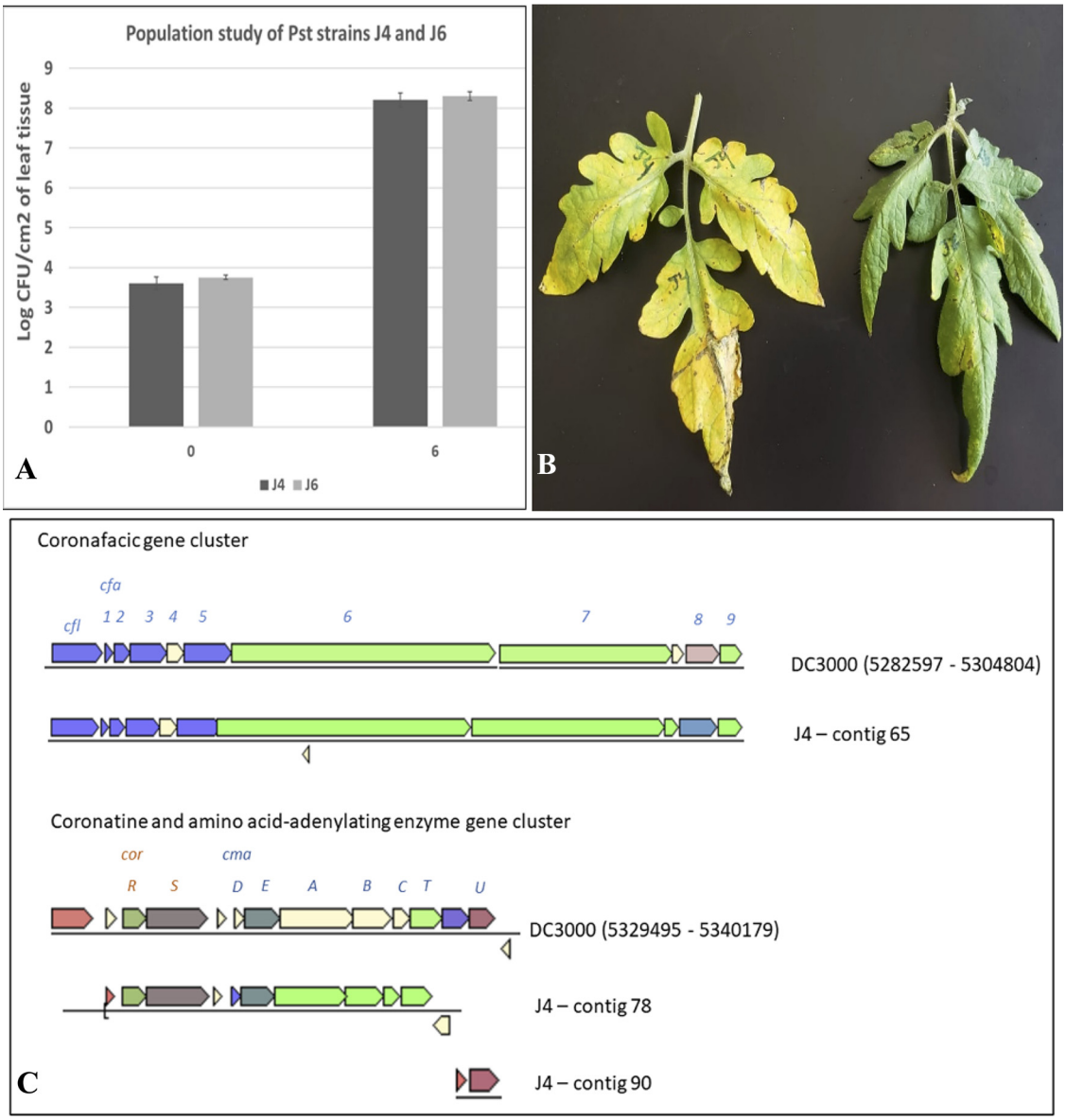

FIG 1 (A) Population study comparison of *Pseudomonas syringae* pv. tomato strains J4 and J6 in tomato. Bacteria multiplied to similar populations 6 days postinoculation. (B) Disease symptom development after inoculation with strains J4 (left) and J6 (right). High yellowing and chlorosis were observed in leaflets inoculated with strain J4 6 days postinoculation. For both experiments, the Bonny Best tomato cultivar was used. Leaves were infiltrated with bacterial suspension at $\sim 10^5$ CFU/ml for the population study, while dip inoculation was done with bacterial suspension at $\sim 10^8$ CFU/ml for monitoring the symptom development. (C) Coronafacic and coronatine gene clusters found in DC3000 and J4 strains of *Pseudomonas syringae* pv. tomato. The genomic cluster is missing in strain J6.

Annotation Pipeline v.4.13 from the National Center for Biotechnology Information (20).

Genome statistics for J4 and J6 are provided in Table 1. Average nucleotide identity (ANI) based on BLAST, computed using JSpecies (21), showed >99.97% sequence identity between J4 and J6. Meanwhile, ANIs with a representative *P. syringae* pv. tomato strain, DC3000 (GenBank accession number GCF_000007815.1) were 98.64% and 98.63% for J4 and J6, respectively. Genome annotations of J4 and J6 were compared with that of DC3000 to identify the coronatine coding cluster using the JGI platform (https://img.jgi.doe.gov). J6 lacks the genomic region that encompasses the coronatine and coronafacic genes involved in coronatine production. However, J4 and DC3000 share similar genomic clusters necessary for coronatine production (Fig. 1C).

TABLE 1 Sequencing and genome statistics for *Pseudomonas syringae* pv. tomato strains J4 and J6

Characteristic	Data for strain: J4	J6
Total no. of reads	603,606	858,404
Genome length (bp)	6,334,619	6,264,625
Genome coverage (×)	23.9	34.4
No. of contigs	151	111
Total no. of genes	5,678	5,591
N_{50} (bp)	112,230	138,773
GC content (%)	58.6	58.6

Comparison of the genomic region encompassing the coronatine-associated genes (~33 kb) indicated more than 97% sequence identity between J4 and DC3000. Previous studies showed that following inoculation with DC3000, stomates closed and later reopened, while with J4, stomates remained open (10, 11). Interestingly, with J6, stomates stayed open but later closed, supporting the possible importance of coronatine for keeping stomates open.

Data availability. The whole-genome sequence assemblies for J4 and J6 are deposited in GenBank under accession numbers JADODR000000000 and JADCNI000000000, respectively. The raw data are available under SRA numbers SRR12817639 for J4 and SRR12817638 for J6.

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