Complete Genome Sequence Resource for the Necrotrophic Plant-Pathogenic Bacterium *Dickeya dianthicola* 67-19 Isolated From New Guinea Impatiens

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Abstract

New Guinea impatiens (NGI, *Impatiens hawkeri*) are popular bedding plants that can be affected by a number of pathogens. Using 16S rDNA sequencing and genus-specific PCR, we identified the first *Dickeya dianthicola* strain isolated from NGI presented with blackleg symptoms, herein designated as *D. dianthicola* 67-19. Here, we report a high-quality complete and annotated genome sequence of *D. dianthicola* 67-19. The 4,851,809 bp genome was assembled with Nanopore reads and polished with Illumina reads, yielding 422× and 105× coverage, respectively. This closed genome provides a resource for future research on comparative genomics and biology of *D. dianthicola*, which could translate to improved detection and disease management.

Announcement

Dickeya spp. are necrotrophic bacterial pathogens that can cause blackleg and soft rot disease on a wide range of host plants including field crops and ornamentals (Ma et al. 2007). Recent genomic analysis of *Dickeya* spp. isolated from Europe found little sequence diversity between *D. dianthicola* strains isolated from ornamentals and field crops, which suggests that *D. dianthicola* may have spread between greenhouse ornamentals and field crops (Toth et al. 2011). *D. dianthicola* was reported to cause blackleg disease of potatoes in Maine in 2015 (Jiang et al. 2016), and has since been reported in Morocco, Pakistan, Australia, and other parts of the United States (Boluk and Arif 2019; Nasaruddin et al. 2019; Oulghazi et al. 2017; Patel et al. 2019; Sarfraz et al. 2018; Wright et al. 2018). Here, we report the complete and annotated genome sequence of *D. dianthicola* 67-19, which was originally isolated from New Guinea impatiens (*Impatiens hawkeri*) in Long Island, NY, U.S.A. (Liu et al. 2020).

D. dianthicola 67-19 was cultured in Luria-Bertani (LB) medium at 28°C (per one liter of medium: 10 g of tryptone, 5 g of yeast extract, 10 g NaCl) (Bertani 1951). High molecular weight DNA was extracted from overnight bacterial cultures using a previously described method (Miller et al. 1988). DNA quantity was assessed using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, U.S.A.) and a Qubit 3.0 fluorometer (Thermo Fisher

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Keywords

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Table 1. Genome summary and average nucleotide comparisons of *Dickeya dianthicola* 67-19 to other *Dickeya* species type strains.

<i>Dickeya</i> strains	NCBI Accession	ANI (%) ^a	Genome size (bp)	Contig(s)
<i>D. dianthicola</i> 67-19 (this work)	CP051429	NA	4,851,809	1
<i>D. dianthicola</i> NCPPB 453	GCA_000365305.1	97.43	4,676,060	52
<i>D. solani</i> IPO 2222	GCA_001644705.1	92.40	4,919,833	1
<i>D. dadantii</i> NCPPB 898	GCA_000406145.1	92.35	4,937,637	52
<i>D. fangzhongdai</i> DSM 101947	GCA_002812485.1	92.10	5,032,447	1 chromosome, 1 plasmid
<i>D. undicola</i> 2B12	GCA_000784735.1	89.13	4,349,790	77
<i>D. chrysanthemi</i> NCPPB 516	GCA_000406065.1	87.33	4,617,243	34
<i>D. zeae</i> NCPPB 2538	GCA_000406165.1	85.38	4,563,790	46
<i>D. aquatica</i> 174/2	GCA_900095885.1	82.23	4,501,560	1
<i>D. paradisiaca</i> NCPPB 2511	GCA_000400505.1	81.70	4,631,867	45

^a The average nucleotide identity (%) between the *D. dianthicola* 67-19 strain sequenced in this study compared with other type strains of *Dickeya* species. Average nucleotide identity (ANI) was calculated using FastANI (Jain et al. 2018).

Scientific, U.S.A.). DNA integrity was assessed using the Agilent 2100 BioAnalyzer system (Agilent, U.S.A.).

Whole-genome sequencing technology capable of generating long reads (Nanopore) was used to obtain a complete scaffold of the genome and short reads (Illumina) were used to polish the assembled genome. All software were run with their default settings unless otherwise noted. For long-read sequencing, the DNA library was prepared using the Rapid Sequencing Kit SQK-RAD004 on a R9.4.1 SpotON flow cell with a MinION Mk1B device from Oxford Nanopore Technologies (United Kingdom). This generated 217,262 reads with a total length of 2,049,947,687 bp, and a genome coverage of 422×. The reads were basecalled using GUPPY v3.5.1 (Wick et al. 2019) and assembled using Flye v2.5 (Kolmogorov et al. 2019). For short-read sequencing, the DNA library was prepared using the NEBNext Ultra II FS DNA Library Prep Kit for Illumina with the NEBNext Multiplex Oligos (New England Biolabs, U.S.A.). AMPure XP beads (Beckman Coulter, U.S.A.) were used for DNA size-selection and purification. The DNA library was sequenced on a MiSeq instrument (Illumina, U.S.A.) with the 2×250 bp mode, yielding 1,760,199 paired-end reads. Raw reads were processed to remove the adaptor and low-quality sequences using Trimmomatic v0.39 (reads were removed if quality value [QV] ≤20 or length ≤50) (Bolger et al. 2014). Duplicate read pairs were removed using a customized Perl script (https://github.com/Sunhh/NGS_data_processing/blob/master/drop_dup_both_end.pl) (Sun et al. 2017). BWA v0.7.17 (Li and Durbin 2009) and Pilon v1.22 (Walker et al. 2014) were used with default parameters to align the resulting 1,532,757 high-quality trimmed Illumina read pairs to polish the draft genome for three rounds, with a genome coverage of 105×. Circlator v1.5.5 (Hunt et al. 2015) was used with the “fix start” argument to set the start of the genome at the *dnaA* gene.

The final polished *D. dianthicola* 67-19 genome is 4,851,809 bp long with 55.93% GC content. It was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP v4.11) (Tatusova et al. 2016). The resulting genome contains 4,352 genes in total, with 4,116 protein-coding genes, 132 pseudogenes, 8, 7, and 7 rRNA-coding sequences (5S, 16S, and 23S rRNAs), 75 tRNAs, and 7 noncoding RNAs, and 1 clustered regularly interspaced short palindromic repeat (CRISPR) array. Whole-genome comparison of *D. dianthicola* 67-19 and other *Dickeya* type strains (Oulghazi et al. 2019; Parkinson et al. 2014) based on average nucleotide identity can be found in Table 1, including their genome sizes and number of contigs. Genome sequences were retrieved from publicly available sources (NCBI Resource Coordinators 2016). Average nucleotide identity (ANI) was calculated using FastANI (Jain et al. 2018). For prokaryotes, an ANI score of 95% or higher suggests that the two strains come from the same species (Konstantinidis et al. 2006). Based on ANI, *D. dianthicola* 67-19 showed the closest similarity to the *D. dianthicola* type strain NCPPB 453. This novel genome resource of *D. dianthicola* could aid in population studies of *Dickeya*, monitor future epidemiology, and assist the development of diagnostics tools for these destructive plant pathogens.

Data availability. The *Dickeya dianthicola* 67-19 genome sequence has been deposited at NCBI GenBank under the accession number CP051429. The Nanopore and Illumina raw reads have been deposited in the Sequence Read Archive (SRA) under the accession numbers SRR11531517 and SRR11531516, respectively.

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