

Complete Genome and Plasmid Sequences for *Rhodococcus fascians* D188 and Draft Sequences for *Rhodococcus* Isolates PBTS 1 and PBTS 2

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***Rhodococcus fascians*, a phytopathogen that alters plant development, inflicts significant losses in plant production around the world. We report here the complete genome sequence of *R. fascians* D188, a well-characterized model isolate, and *Rhodococcus* species PBTS (pistachio bushy top syndrome) 1 and 2, which were shown to be responsible for a disease outbreak in pistachios.**

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Rhodococcus fascians, the sole characterized plant pathogen in the genus *Rhodococcus*, is a Gram-positive pleomorphic bacterium that causes disease through the production and modulation of plant growth regulators, such as cytokinins and auxins (1). *R. fascians* infects a large variety of plants, including monocots and dicots, often causing cryptic symptomatology (2). *R. fascians* D188, which has intensively been studied for decades, has revealed a wealth of knowledge about this plant pathogen, including chromosomal and plasmid localized virulence genes necessary for symptomatic host colonization and cytokinin production (3, 4). The virulence plasmid pFiD188 was previously sequenced using Sanger technology, and fragmented draft genome sequences for multiple *R. fascians* isolates, including D188, are available (5, 6). Pistachio bushy top syndrome (PBTS) describes a suite of symptoms affecting clonally propagated UCB-1, an interspecific hybrid rootstock planted in California, Arizona, and New Mexico (7, 8). Two distinct *Rhodococcus* species isolates (PBTS 1 and PBTS 2) were isolated from diseased trees and shown to cause stunting and abnormal root and shoot development in pathogenicity assays. Given the global scale of nursery plant production and the diverse population structure of *R. fascians*, as indicated by initial genome sequences (6), high-quality complete reference sequences are needed to fully characterize newly discovered isolates. The reference *R. fascians* strain D188 (from the private collection of D. Vereecke) and PBTS isolates (7) were stored in glycerol stocks at -80°C. Cultures were grown in 50 ml of LB broth, and DNA was extracted using a Wizard genomic DNA purification kit, according to the manufacturer's instructions (Promega, Madison, WI). DNA was shipped on dry ice to the National Center for Genomic Resources (NCGR, Santa Fe, NM) for single-molecule real-time (SMRT) sequencing. Libraries with a 10-kb insert were prepared, and each isolate was sequenced using one SMRT cell on the PacBio RS II instrument (Pacific Biosciences, Menlo Park, CA). Hierarchical Genome Assembly Process (HGAP) was used for *de novo* assembly (9) (RS_HGAP assembly.2; Pacific Biosciences). For

strain D188, an additional round of polishing was performed using only reads with a quality of ≥ 84 . The genomes were annotated with Prokka version 1.12-beta (10), protein-coding features were predicted using Prodigal version 2.6 (11), tRNA was predicted by ARAGORN version 1.2 (12), and rRNA was predicted by RNAmmer version 1.2 (13). The genome annotations reveal a wealth of predicted secondary metabolism-related genes, including novel antibiotic synthesis, heavy metal resistance, siderophore production, pilus-like assembly mechanisms, secreted proteins, transposons and other mobile elements, and prophage sequences.

Nucleotide sequence accession numbers. The complete genome and plasmid sequences for this whole-genome shotgun project have been deposited at DDBJ/EMBL/GenBank under the following accession numbers: CP015235, CP015236, and CP015237 for D188, CP015219 for PBTS 1, and CP015220 and CP015221 for PBTS 2. The versions described in this paper are CP015235.1, CP015236.1, CP015237.1, CP015219.1, CP015220.1, and CP015221.1, respectively.

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