PROKARYOTES



Draft Genome Sequence of *Pseudomonas fluorescens* Strain TR3, a Potential Biocontrol Agent against the Rice Blast Fungus *Magnaporthe oryzae*

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ABSTRACT We present the draft genome sequence of the potential biocontrol agent *Pseudomonas fluorescens* TR3, which was isolated from rice leaves infected with *Magnaporthe oryzae* in a greenhouse. The genome of TR3 was assembled into 26 scaffolds (~6 Mbp) and includes genes potentially involved in bacterial interactions with fungi.

Pseudomonas fluorescens is a Gram-negative rod-shaped bacterium that commonly inhabits water, soil, and plant rhizospheres. *P. fluorescens* strains are of interest to agricultural and environmental scientists because of their ability to produce a variety of antimicrobial secondary metabolites to suppress pathogens (1). Currently, the whole genomes of several *P. fluorescens* strains that exhibit antifungal activity, such as *P. fluorescens* strains Pf-5, CHA0, F113, SS101, KD, and LBUM223 (2–5), have been sequenced. *P. fluorescens* strains Pf-5, CHA0, and F113 produce a spectrum of antibiotics, such as 2,4-diacetylphloroglucinol (DAPG) (4, 6, 7), hydrogen cyanide (HCN) (8), and pyoluteorin (2, 9), which are active against various phytopathogenic fungi (10). The type III secretion system (T3SS) is involved in inhibition of *Pythium ultimum* by strain KD (3). To date, no strain of this species has been tested for antifungal activity against *Magnaporthe oryzae*.

The genomic DNA was isolated according to a basic phenol-chloroform purification protocol for *Pseudomonas* spp. (11). Whole-genome shotgun sequencing of *P. fluorescens* strain TR3 was performed by using a HiSeq 2500 (Illumina, Inc., San Diego, CA, USA) instrument and a 100-bp paired-end library (DNA PCR-free sample prep kit [Illumina]), Genomic contigs were *de novo* assembled using ABySS (12). A total of 42,852,340 reads (4.1×10^9 total bases) were assembled *de novo* into 26 scaffolds totaling 6,158,893 bp with a G+C content of 59.9%. To order the contigs, the *P. fluorescens* strain TR3 draft genome was matched to a reference genome, *P. fluorescens* strain Pf0-1 (GenBank accession number NC_007492). We predicted 5,625 genes with Rapid Annotation using Subsystem Technology (RAST) (13) and COG (14) in the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) tools, 12 rRNA operons with RNAmmer (15), and 44 tRNA genes with tRNAscan-SE (16).

Multilocus sequence-based phylogenetic analysis (MLSA) was performed using three housekeeping genes, *gyrB* (2,418 bp), *rpoD* (1,848 bp), and 16S rRNA (1,139 bp). There was >98% sequence similarity to *P. fluorescens* A506 (17), confirming the phylogenetic position of the species. Additional phylogenetic analysis was performed using whole-genome-based average nucleotide identity (ANI) analysis (18, 19). The ANI calculator computed 95.70% ANI shared between strain TR3 and *P. fluorescens* A506.

P. fluorescens TR3 was isolated from rice leaves infected with the fungal plant pathogen *Magnaporthe oryzae* in the greenhouse. Strain TR3 inhibits fungal growth in

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a contact-based manner. Genome sequence analysis revealed various genes that code for proteins potentially related to bacterial and fungal interaction, including gene clusters for antibiotics (e.g., pyoverdine phenazine), chitinase, metabolites for plantbacterium communications (e.g., catabolism of acetoin and 2,3-butanediol), and potential mechanisms for bacterial and fungal contact, including T3SS and the type II (T2SS), type IV (T4SS), and type VI (T6SS) secretion systems and the widespread colonizing island (WCI) (20). The draft genome sequence of *P. fluorescens strain* TR3 presented here is a source of information for bacterial and fungal interactions.

Accession number(s). The genome sequence of strain TR3 was deposited in GenBank under the accession number MKHA00000000. The version described in this paper is the first version.

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