Mitochondrial Genome Resource of a Grapevine Strain of *Trichoderma harzianum*, a Potential Biological Control Agent for Fungal Canker Diseases

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Resource Announcement

Trichoderma spp. are commonly used as bioremediation agents, as biological controls, and for making biofuels. Herein, a *Trichoderma harzianum* strain PAR3 was isolated from grapevine roots in central California, U.S.A. As part of a larger whole genome sequencing effort, the mitochondrial genome (mitogenome) sequence was obtained for the PAR3 strain. The mitogenome is 27,631 bp, containing genes of 14 core mitochondrial proteins, 25 transfer RNAs, and two ribosomal RNAs, with a GC% content of 27.55%. A BLAST search using the PAR3 mitogenome as a query against the GenBank sequence database showed that the mitogenome MUT3171 (29,791 bp) of *Trichoderma lixii* was the most similar (query coverage = 99%; percentage identity = 100%) with two major deletions, 1,339 bp and 821 bp. The PAR3 mitogenome sequence will provide a useful reference for comparing different *Trichoderma* strains from the United States and around the world.

Fungal strain PAR3 was isolated from roots of a Scarlet Royal grapevine on Freedom rootstock growing in a vineyard in Parlier, CA, U.S.A., in the summer of 2020. Morphological identification of PAR3 suggested it was *T. harzianum* Rifai (which has the teleomorph *Hypocrea lixii* Pat. according to Chaverri and Samuels 2002), following a dichotomous key by Chaverri and Samuels (2003) and Chaverri et al. (2016). As a strain of *T. harzianum*, PAR3 exhibits putative activity against various fungal pathogens, including those that could cause grapevine trunk diseases such as *Diplodia seriata*, *Eutypa lata*, and *Neofusicoccum parvum*. All of these pathogens cause cankers that develop over time, ultimately resulting in the need to replant vineyards every 10 to 12 years where prevalent (Baumgartner et al. 2019; Kaplan et al. 2016). Thus, there exists a need to further characterize PAR3.

Mitochondrial genomes are important in species identification and marker development due to small sizes, high copy numbers, and presence of both conserved and variable regions (Medina et al. 2020). Previously, a variety of *Trichoderma* spp. have had mitochondrial genomes reported and analyzed (Kwak 2020). However, only a handful of species, consistently mostly of industry-useful isolates, have been analyzed by Kwak (2020). Therefore, the PAR3 mitochondrial genome as a resource is an important addition to expand our knowledge of *Trichoderma* species.

Total genomic DNA was extracted using a Macherey-Nagel (Bethlehem, PA, U.S.A.) Plant Mini Kit from 1-week-old PAR3 cultures grown in potato dextrose broth (Difco Laboratories,

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Data availability: The PAR3 mitogenome sequence has been deposited at DDBJ/EMBL/GenBank under accession number MZ713368.

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Table 1. Nucleotide variations of mitogenome sequences between *Trichoderma lixii* strain MUT3171 (NC_052832.1/MT495248.1, 29,791 bp) and *T. harzianum* PAR3 (MZ713368.1, 27,631 bp)

Position (NC_052832.1)	MUT3171	PAR3
188	—	A
794	—	A
795	—	T
1,544–2,883	1,339 bp	—
13,417–14,238	821 bp	—
13,413	G	A
13,414	T	C
13,415	C	T
13,416	C	T
15,406	T	C
17,536	A	C
18,743	G	A
19,127	T	A
20,330	C	T
28,305	A	—

Detroit, MI, U.S.A.) on a shaker under ambient light and at 26°C. An Invitrogen (Carlsbad, CA, U.S.A.) Qubit fluorometer was used to assess DNA quantity and quality. Afterward, the genomic DNA was sequenced using an Illumina HiSeq 2 × 100 paired-end format (Illumina, San Diego, CA, U.S.A.) through a commercial source after the amount of DNA was increased using Illustra GenomiPhi version 2 DNA amplification kits (GE Healthcare, Waukesha, WI, U.S.A.).

The HiSeq sequencing yielded a total of 52,337,510,850 bp with a read size of 100 bp and a Q value greater than 30. The short reads were assembled de novo using the CLC genomics workbench (version 10.0). This resulted in 44,766,176 bp in 1,723 contigs (>1,000 bp). With the help of BLAST searches, a 645-bp internal transcribed spacer (ITS) sequence was identified using the sequences of primer ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCGTAACAAGG) (Chaverri et al. 2016). A 339-bp translation elongation factor 1-alpha (TEF1) sequence was identified using the sequences of primers EF1-728F (CATCGAGAAGTTCGAGAAGG) and EF1-986R (TACTTGAAGGAACCCCTTACC) (Chaverri et al. 2016). A BLAST search against the GenBank database using both ITS and TEF1 sequences confirmed that PAR3 was a strain of *T. harzianum*.

Referenced to MT495248.1, a contig of 27,813 bp was identified through a BLASTn search. After manually editing overlaps at the 5' and 3' ends, a sequence of 27,631 bp was collected and designated as the HiSeq version of the PAR3 mitogenome. For further confirmation of the mitogenome structure, the PAR3 DNA was also sequenced using a minION system from Oxford Nanopore (Alameda, CA, U.S.A.). Nanopore sequencing yielded a total of 1,057,994,148 bp sequences with 516,174,469 bp (140,849 reads) ranging from 1,000 to 50,000 bp with a Q value greater than 20. Sequence reads were assembled with Canu software (version 2.1) (Koren et al. 2017). A total of 7,636,456 bp sequences were assembled with 446 contigs. A circular 43,401-bp contig was identified and trimmed to 27,549 bp after overlap removal; it was designated as the Nanopore version of the PAR3 mitogenome. The HiSeq version (27,631 bp) and Nanopore version (27,549 bp) were compared through BLASTn (word size = 7). The two versions were identical in sequence order and over 99% similar in nucleotides. The difference of 82 bp (27,631 – 27,549 = 82) was likely due to nucleotide sequence errors mostly in the Nanopore versions. Because HiSeq sequences have higher Q values than Nanopore sequences (30 versus 20, respectively), the HiSeq version was selected as *T. harzianum* strain PAR3 mitogenome version 1. Annotation of the mitogenome sequence was performed using GeSeq (Tillich et al. 2017).

The finalized PAR3 mitogenome is 27,631 bp, consisting of 14 core mitochondrial protein genes coding for adenosine triphosphate synthases (*apt*), apocytochrome b (*cob*), cytochrome c oxidases (*cox*), and nicotinamide adenine dinucleotide hydrogen (NADH) dehydrogenases (*nad*); a ribosomal protein (*rps*); 25 transfer RNAs; and two ribosomal RNAs (Fig. 1A). There was one additional open reading frame (*orf40*) annotated as an unknown putative protein. All genes were located on the heavy strand (H-strand). The overall composition of the complete mitochondrial genome had a GC% content of 27.55%.

A BLASTn search using the PAR3 mitogenome sequence as a query against the GenBank nr database showed extensive matches (>99% query coverage and 99.72% identity) to the mitogenome sequence of *T. lixii* strain MUT3171 (MT495248.1, 29,791 bp) reported from Italy (Venice et al. 2020). Table 1 shows the nucleotide variations including two major deletion events between the two mitogenomes identified using the CLUSTAL program (McWilliam et al. 2013).

For phylogenetic analysis, the mitogenome sequences of six *Trichoderma* species and three related fungal species identified through the BLAST search were downloaded from the GenBank database. Together with the PAR3 mitogenome, a phylogenetic tree was constructed using MEGA (version 6) with the maximum likelihood method and a bootstrap replication number of 500 (Fig. 1B).

The mitochondrial genome of the *T. harzianum* PAR3 strain will enable further comparisons with those of other *Trichoderma* spp., facilitating future identification of useful members of that species for use in biological control and bioremediation, as well as for other purposes.

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