Genome Resources for *Thelonectria rubi*, the Causal Agent of Nectria Canker of Caneberry

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Abstract

Thelonectria rubi is the causal agent of Nectria canker of *Rubus* spp. Here, we report a high-quality draft genome sequence for this pathogen, which also represents the first genome sequence for a *Thelonectria* species. The genome assembly was 44.6 Mb in size, assembled into 669 scaffolds and consisting of 12,973 predicted protein-coding genes. The availability of genome data for *T. rubi* provides a critical additional resource for an important plant pathogen and will be useful for fungal biology, comparative genomic, taxonomic and population studies of this and related species.

Thelonectria rubi (Osterw.) C. Salgado & P. Chaverri is a fungal pathogen in the Nectriaceae family (Salgado-Salazar et al. 2015, 2016) and causal agent of Nectria canker of caneberry. This fungus was first observed in Switzerland causing root cankers of raspberry and was described by A. Osterwalder as *Nectria rubi* (Osterwalder 1911). Since this first observation, disease outbreaks have been reported in several countries, including Australia, England, Ireland, New Zealand, Scotland, and Venezuela (Farr and Rossman 2019; Martin et al. 2017), on a variety of hosts; namely, *Rubus glaucus*, *R. idaeus*, and *R. fruticosus* (Farr and Rossman 2019). Disease symptoms include dark-brown or purplish vascular lesions located on roots and crowns. These lesions generally produce wilting of lateral shoots, which can lead to cane death or reduction or complete absence of production of primocanes during the growing season (Cedeño et al. 2004; Martin et al. 2017). Clusters of reddish perithecia representing the sexual state of the fungus can be found on the transition region between the roots and canes of dead plants or plants in advanced stages of the disease (Booth 1966; Martin et al. 2017). Management of this pathogen is restricted to cultural practices that protect cane bases and crowns from environmental stresses such as high winds, humidity, and waterlogging (Martin et al. 2017). Because *T. rubi* has been reported to affect stressed plants, it has been regarded as a secondary or weak pathogen (Martin et al. 2017). The genomic resource generated in this study provides a reference not only for genetic and comparative genome analyses but also for taxonomic studies in the genus *Thelonectria* and the family Nectriaceae, which harbor a variety of widespread and destructive plant pathogens (Lombard et al. 2015).

Mycelial tissue of *T. rubi* CBS 177.27 (= IMI 113917) isolated from *R. idaeus* roots in England was grown on potato dextrose agar at 20°C for 7 days, after which mycelial tissue along

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Keywords

analytical and theoretical plant pathology, Nectriaceae, Nectria canker, *Rubus*

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Table 1. Summary statistics of the draft genome assembly of *Thelonectria rubi*

Isolate	CBS 177.27 (= IMI 113917)
Total length (Mb)	44.6
Number of scaffolds	669
Maximum scaffold length (kb) ^a	1,453.5
Average scaffold size (kb)	66.6
Average coverage	208.6×
N ₅₀ (kb) ^b	303.6
GC content (%)	50.8
Eukaryote orthologs ^c	96.8%
Fungal orthologs ^d	99.1%
Predicted gene models	12,973
Transposable elements ^e	11.396%

^a Minimum scaffold length = 500 bp.

^b Scaffold or contig length at which 50% of the total assembly length is covered.

^c Percentage of complete BUSCOs found, 429 total BUSCO groups searched.

^d Percentage of complete BUSCOs found, 1,438 total BUSCO groups searched.

^e Percentage of the nuclear genome containing transposable elements.

with media were excised from plates and blended with Difco potato dextrose broth medium at 50% concentration. Mycelial tissue was then grown with constant shaking (125 rpm) at 20°C for 3 days. Genomic DNA was extracted using the OmniPrep DNA kit (G-Biosciences, St. Louis, MO, U.S.A.). Genomic DNA libraries were constructed using the TruSeq DNA Nano kit and sequenced on an Illumina MiSeq as 2 × 300 paired-end reads using a MiSeq 600-cycle reagent kit (version 3.0; Illumina, San Diego, CA, U.S.A.). After trimming for quality and adapter removal, 49.2 million reads were obtained. A de novo genome assembly was performed in CLC Genomics Workbench (version 12; CLC Bio, Boston, MA, U.S.A.) using kmer = 50 and bubble = 36. A summary of the genome assembly statistics is provided in Table 1. The 44.6-Mb *T. rubi* genome assembly size is consistent with the known range of genome sizes of species in closely related genera in the Nectriaceae family such as *Calonectria* (53 to 65 Mb) (Malapi-Wight et al. 2019), *Corinectria* (39 Mb) (Salgado-Salazar and Crouch 2019), *Dactylonectria* (58 Mb) (Malapi-Wight et al. 2015), and *Neonectria* (40 to 44 Mb) (Deng et al. 2015; Salgado-Salazar and Crouch 2019). Gene model predictions using the AUGUSTUS web server (Hoff and Stanke 2013) using *Fusarium graminearum* gene models identified a total of 12,973 genes for *T. rubi*. Analysis of putative enzymes associated with the biosynthesis of secondary metabolites using antiSMASH fungal version (<https://fungismash.secondarymetabolites.org/>) (Medema et al. 2011) identified 27 secondary metabolite clusters, including 8 type I polyketide synthases and 14 nonribosomal peptide synthetase clusters. The presence of the *MAT1-1* and *MAT1-2* idiomorphs in the genome assembly of *T. rubi* was determined by performing a local BLASTn search against a *MAT* gene database (Rivera et al. 2018). Only the conserved α domain, indicative of the *MAT1-1* mating type, was identified in the contig 28 of this isolate, indicating that *T. rubi* is likely a heterothallic species. Transposable element (TE) analysis using REPET version 2.5 (Flutre et al. 2011), following the protocol detailed by Rivera et al. (2018), indicated that TEs comprised 11.4% of the *T. rubi* draft genome. Evidence of repeat-induced point (RIP) mutations was detected in 379 dictyostelium repetitive sequences (DIRS) and 16 Helitron TEs.

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