Genome Sequence Resource for *Ilyonectria mors-panacis*, Causing Rusty Root Rot of *Panax notoginseng*

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Abstract

Ilyonectria mors-panacis is the cause of a serious disease hampering the production of *Panax notoginseng*, an important Chinese medicinal herb, widely used for its anti-inflammatory, antifatigue, hepato-protective, and coronary heart disease prevention effects. Here, we report the first Illumina-Pacbio hybrid sequenced draft genome assembly of *I. mors-panacis* strain G3B and its annotation. The availability of this genome sequence not only represents an important tool toward understanding the genetics behind the infection mechanism of *I. mors-panacis* strain G3B but also will help illuminate the complexities of the taxonomy of this species.

Genome Announcement

Ilyonectria mors-panacis is the pathogen inducing the severe rusty root rot of *Panax notoginseng*, an important traditional Chinese medicinal herb (Hong et al. 2014; Mi et al. 2017), which is widely used for its anti-inflammatory, antifatigue, hepato-protective, and coronary heart disease prevention effects (Li et al. 2007; Xia et al. 2016). *I. mors-panacis* was grouped in the genus *Cylindrocarpon*, which is commonly associated with rot and decay of woody and herbaceous plants (Rahman and Punja 2005). Mantiri et al. (2001) estimated that there are approximately 125 described species of *Cylindrocarpon* and most of them have been classified into the genus *Neonectria* (Mantiri et al. 2001). Based on phylogenetic and phenotypical characteristics, Chaverri et al. (2011) redefined *Neonectria* into four genera: anamorph *Ilyonectria* (Booth's group 3) with chlamydospores, teleomorph genera *Neonectria* or *Cylindrocarpon sensu stricto* (groups 1 and 4), and *Rugonectria* and *Thelonectria* (group 2) without chlamydospores (Chaverri et al. 2011). Recently, Cabral et al. (2012) renamed the *Nectria* or *Neonectria radicola* (including *Cylindrocarpon destructans*) complex as the *Ilyonectria radicola* complex, and reclassified the fungi in this complex into 15 species under the genus *Ilyonectria*. In particular, highly virulent *I. radicola* isolates of *P. ginseng* (e.g.,

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Keywords

genomics, *Ilyonectria mors-panacis*, metabolomics, Pacbio, *Panax notoginseng*, pathogenesis, proteomics

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*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary figure and one supplementary table are published online.

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IFO31881, IFO31882, NSAC SH1, and NSAC SH2.5) were included in *I. mors-panacis* (Song et al. 2014). Until now, only one strain from *I. radicola* complex was partially sequenced (>3,000 contigs). Due to its impact as a traditional Chinese medicinal herb and its complex taxonomic classification, *I. mors-panacis* is a good candidate to carry out whole-genome sequencing.

I. mors-panacis strain G3B was first isolated from *P. notoginseng* (Burk.) F. H. Chen in 2014 from Wenshan, Yunnan Province, China (Mi et al. 2017). The strain was maintained on potato dextrose agar. It was subcultured in potato dextrose broth at 22°C for 5 days at 180 rpm. High-quality genomic DNA was extracted from 50 ml of culture, using the Plant/Fungi DNA Isolation Kit (Sigma-Aldrich). To confirm the ploidy of the G3B genome, Jellyfish V 2.2.10 was used to calculate the k-mer distribution from the reads (Marçais and Kingsford 2011). The result showed that the k-mer curve of G3B is significantly a single peak without a heterozygous peak (Supplementary Fig. S1). Based on the above plotting, we confirmed that the sequenced genome is a haploid. The quality of the extracted DNA was tested with a Qubit 2.0 fluorometer (Life Technologies) and a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific). For Pacbio sequencing, after genomic DNA shearing, 1.5 µg of the sheared DNA was used to prepare an SMRTbell library with the PacBio SMRTbell Template Prep Kit 1 (Pacific Biosciences). Sequence runs for one single-molecule real-time (SMRT) cell were performed on the PacBio RS II sequencer with a 120-min movie time per SMRT cell. After quality control, 321,858 reads were used for further assembly. For Illumina sequencing, the library was prepared using the TruSeq Nano DNA LT Library Preparation Kit (Illumina) using 250 ng of genomic DNA. The resulting library was 150 bp paired-end sequenced on the Illumina HiSeq 2500. After quality control, 23,449,680 reads were used for further assembly. The *I. mors-panacis* G3B genome was de novo assembled by using the Hierarchical Genome Assembly Process, which can be accessed through the SMRT Analysis Portal. PBJelly v14.1.14 was used to fill and reduce as many captured gaps as possible, and produce a draft genome (English et al. 2012) by using Illumina sequencing reads. Last, Illumina reads were used to repair the sequencing errors generated by SMRT sequencing. The completeness of assembly was evaluated using the software BUSCO v3 with a set of common fungal single-copy orthologs (Waterhouse et al. 2017). This result showed that the G3B genome assembly is estimated to be 96.8% complete. The genome annotation was done with Augustus V3.3.1

Table 1. Core metrics of the *Ilyonectria mors-panacis* G3B genome draft and ANI comparison between *I. mors-panacis* G3B and other closely related fungi genomes

Metrics and comparisons ^a	Value	Strain	Accession number	ANI-NUCmer values	ANI-Blast values
General genome characteristic					
Assembly size (bp)	61,489,895	...	...	...	...
Contig number	145	...	...	...	...
Maximum contig size (bp)	2,825,696	...	...	...	...
Minimum scaffold size (bp)	12,370	...	...	...	...
Average scaffold size (bp)	424,068	...	...	...	...
N ₅₀ length (bp)	1,010,205	...	...	...	...
N ₉₀ length (bp)	290,231	...	...	...	...
G+C (%)	48.7	...	...	...	...
Intron number	45,382	...	...	...	...
Intron density	1,355	...	...	...	...
Gene contains intron	13,594	...	...	...	...
PCG	18,321	...	...	...	...
PCG > 100 aa	17,585	...	...	...	...
Predicted PCG > 100 aa					
Gene total length (bp)	26,931,632	...	...	...	...
Gene density	3,496	...	...	...	...
Median gene length (bp)	1,239	...	...	...	...
Mean gene length (bp)	1,531	...	...	...	...
G+C (%)	51.9	...	...	...	...
ANI comparison (species)					
<i>Ilyonectria mors-panacis</i>	...	G3B	PPHJ000000000	1.000	1.000
<i>Dactylonectria macrodidyma</i>	...	JAC 15-245	JYGD000000000	0.852	0.787
<i>Neonectria ditissima</i>	...	RS 324p	LDPL01000040.1	0.846	0.780
<i>Calonectria pseudonaviculata</i>	...	CBS 139394	JYJY000000000	0.842	0.747
<i>Fusarium solani</i>	...	JS-169	NGZQ000000000	0.745	0.732
<i>Ilyonectria destructans</i>	...	C1	MPHF000000000	0.915	0.906

^a PCG = protein coding genes, aa = amino acid, and intron and gene density = 1 gene every *n* bp.

(Stanke and Morgenstern 2005) and WebAUGUSTUS, a web interface, was used for training Augustus (Hoff and Stanke 2013). The training was performed by using it on its own genome sequence and the gene model of *Fusarium oxysporum* f. sp. *lycopersici* 4287 (Ma et al. 2010) obtained from the Joint Genome Institute. Additional analysis was carried out using NCBI's UniProt database, Kyoto Encyclopedia of Genes and Genomes (Kanehisa and Goto 2000), and Gene Ontology terms (Ashburner et al. 2000). Virulence genes were annotated by using the Database of Virulence Factors in Fungal Pathogens (DFVF) (Lu et al. 2012). Genome comparison among G3B and other closely related genomes was carried out by using average nucleotide identity (ANI), which was calculated by ANIcalculator (Varghese et al. 2015).

After assembly, this genome contained 145 contigs with N_{50} of 1,010,205 bp. The total size of the genome is 61,489,751 bp and it has a GC content of 48.72%. In total, 18,321 coding sequences were predicted. Additional features of this genome are shown in Table 1. To access the similarity among G3B and other closely related organisms, all available sequenced fungal genomes from the family Nectriaceae were downloaded from NCBI and compared against G3B. Strikingly, we found low ANI between G3B and the other closely related organisms (Table 1). Even the ANI between *I. mors-panacis* G3B and *I. destructans* C1 is only 90.27% (Table 1).

Overall, the annotation analysis predicted 120 genes defined by DFVF with involvement in pathogenesis (Supplementary Table S1): 28 as cell-wall-degrading enzymes, which include cellulases, β -glucosidase, endo- β -1,4-xylanase, pectinases, pectin methylesterase, and laccase; 18 as antioxidant systems, which include superoxide dismutase and carotenoid; and, finally, 7 defined as effectors.

The draft genome presented here is the first available genome sequence of *I. mors-panacis*, and only the second from the genus *Ilyonectria*. Because these are causal organisms leading to *P. notoginseng* replanting problems, understanding the whole genome of *I. mors-panacis* G3B will be important for developing suitable root rot disease management strategies in the future. This Whole-Genome Shotgun project has been deposited at GenBank under the accession number PPHJ000000000.1 (BioProject: PRJNA431033). The version described in this article is version PPHJ000000000.1.

Author-Recommended Internet Resources

Joint Genome Institute: <https://genome.jgi.doe.gov/Fusox2/Fusox2.home.html>

NCBI's UniProt database: <https://www.ncbi.nlm.nih.gov/>

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