Chromosome-Scale Genome Assembly of *Talaromyces rugulosus* W13939, a Mycoparasitic Fungus and Promising Biocontrol Agent

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Abstract

Here, we report a chromosome-level genome assembly of *Talaromyces rugulosus* (syn. *Penicillium rugulosum*) W13939 (six chromosomes; contig N50: 5.90 Mb), generated using a combination of PacBio long-read and Illumina paired-end data. *T. rugulosus* is not only a potent enzyme producer, but also a mycoparasite of *Aspergillus flavus*, which is a notorious plant pathogen and mycotoxin producer, making it a promising biocontrol agent. The *T. rugulosus* genome is rich in genes encoding proteases, carbohydrate-active enzymes, fungal cell wall-degrading enzymes, lectin, and secondary metabolite biosynthetic enzymes, reflecting its mycoparasitic life style and mycotoxigenic capability. This high-quality assembly of the *T. rugulosus* genome will be a valuable resource to assist us in the understanding of the molecular basis of mycoparasitism and facilitate the agricultural and biotechnological applications of *Talaromyces* spp.

Genome Announcement

Biological control agents significantly reduce the incidence of plant diseases caused by pathogenic fungi (Abdel-Rahim and Abo-Elyousr 2018; Abo-Elyousr et al. 2014; Freimoser et al. 2019). Various mechanisms such as mycoparasitism, niche and nutrient competition, antibiotics production, or priming plant defense responses contributed to biocontrol effects (Benítez et al. 2004; Guzmán-Guzmán et al. 2019; Harman 2006). Since *Trichoderma* spp. were recognized as mycoparasitic fungi by Weindling (1932), several mycoparasites, such as *Talaromyces flavus* (Madi et al. 1997), *Chaetomium globosum* (Moya et al. 2016), *Alternaria alternata* (Zheng et al. 2017), *Pythium periplocum* (Kushwaha et al. 2017), and *T. pinophilus* (Abdel-Rahim and Abo-Elyousr 2018), have been reported as promising biocontrol agents against phytopathogenic fungi for crop disease management. As a member of *Talaromyces* section *Islandici*, *T. rugulosus* W13939, isolated from air in Beijing, China, grows slowly on artificial culturing media, but when parasitizing *Aspergillus flavus* colonies, it creeps fast to overgrow entire colonies of *A. flavus* in 7 days (Fig. 1A and B). In addition, *T. rugulosus* is a potent producer of industrial enzymes such as beta-rutinosidase, phosphatase (Reyes et al.

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Keywords

biocontrol, chromosome, filamentous ascomycetes, genome assembly, mycoparasitism

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Fig. 1. The mycoparasitic morphology and genome features of *Talaromyces rugulosus* W13939. **A**, *T. rugulosus* parasitizing the colony of *Aspergillus flavus* on malt extract agar (MEA) at 25°C after 7 days. **B**, *T. rugulosus* parasitizing the colony of *A. flavus* on MEA at 25°C after 14 days. **C**, The six chromosomes of *T. rugulosus* genome features. Tracks *a* to *g* represent the distribution of karyotype, carbohydrate-active enzymes, proteases, secondary metabolite biosynthetic genes, density of transposable elements, gene density, and GC density, respectively, with densities calculated in 100-Kb windows (*e* to *f*) and 10-Kb windows (*g*).

1999; Narikawa et al. 2000), and secondary metabolites, including skyrin and rugulosin, with antibioticism against *Staphylococcus aureus* (Breen et al. 1955; Yamazaki et al. 2010). Furthermore, *T. rugulosus* strains colonize the maize rhizosphere and stimulate plant growth, increasing dry matter yields by 3.6 to 28.6% (Reyes et al. 2002). Genome sequencing and assembly have been reported for several mycoparasitic species (Karlsson et al. 2015, 2017; Kubicek et al. 2011; Li et al. 2017; Zhao et al. 2020). However, a genomic resource of *T. rugulosus* remains lacking, impeding our exploitation of its mycoparasitic and plant growth stimulation effects. Thus, decoding the *T. rugulosus* genome will provide a valuable resource not only in facilitating dissection of mycoparasitic and biocontrol mechanisms but, also, contributing to comparative genomic studies toward understanding the genome evolution of mycoparasitic fungi. Seven-day-old *T. rugulosus* mycelia were collected to extract high molecular weight genomic DNA using the CTAB (cetyl trimethyl ammonium bromide) method. A total of 4.88 Gb of paired-end short sequence reads (2×150 bp; average fragment size: 400 bp; Illumina NovaSeq 6000 platform) and 18.42 Gb of single-molecule long-read sequence reads (read length N50: 23,169 bp; PacBio SMRT RS II platform) were generated. A Jellyfish-GenomeScope pipeline using Illumina reads (133× coverage) with ‘-m 17’ (Marçais and Kingsford 2011; Vurture et al. 2017) revealed an estimated genome size of 35.94 Mb for *T. rugulosus* (haploid). We then assembled a *T. rugulosus* genome from the PacBio reads (526× coverage), using the long-read assembler NextDenovo v2.0 with parameters

Table 1. Summary of the genome assembly and annotation statistics^a

Strains	<i>T. rugulosus</i>	<i>T. pinophilus</i>	<i>Trichoderma atroviride</i>
Number of contigs	6	8	29
Number of chromosomes	6	8	NA
Genome size (Mb)	36.76	36.48	36.14
GC content (%)	46.92	46.25	49.75
Contig N50 (bp)	5,896,210	4,804,168	2,007,903
Number of protein-coding genes	11,907	12,402	11,816
Repeat content (%) ^b	2.52	1.35	1.29
Proteases	391	390	378
Secondary metabolite biosynthetic genes	67	59	30
Carbohydrate-active enzymes	741	761	663
Fungal cell wall-degrading enzymes	107	81	101
Lectin	8	5	8

^a *Talaromyces rugulosus* W13939 compared with mycoparasitic *T. pinophilus* (GenBank accession: GCA_001571465.2) (Abdel-Rahim and Abo-Elyousr 2018; Li et al. 2017) and mycoparasitic *Trichoderma atroviride* (GenBank accession: GCA_000171015.2) (Kubicek et al. 2011). NA = unavailable.

^b Repeat contents were identified by the same pipeline (this study) to ensure a fair comparison.

'read_cutoff = 1k' and 'seed_cutoff = 45,821'. Pilon v. 1.22 with parameters '-changes-vcf-frags' (Walker et al. 2014) was used to polish the PacBio assembly, and a total of 2,734 (0.008%) erroneous positions were corrected. The contig N50 before correction and after correction was 5,896,623 and 5,896,210 bp, respectively. BUSCO v. 1.22 with parameters '-m all -l fungi_odb9' (benchmarking universal single copy ortholog) (Simão et al. 2015) was used to assess the *T. rugulosus* genome assembly completeness. The BUSCO scores for the genome before and after correction were 95 and 96%, respectively. These results showed that the error correction by using Illumina reads improved the assembly completeness. The final assembly of the *T. rugulosus* genome was 36.76 Mb, consisting of six contigs (N50: 5.90 Mb), close to its estimated genome size. A telomeric repeat unit (TTAGGG)_(n>3) was present on both ends of five contigs (chromosomes) and only on the 3' end of chromosome 4, whose 5' end was filled with rDNA units. Each chromosome is represented as a single contig, demonstrating that the *T. rugulosus* genome assembly is high-quality at the chromosome level (Fig. 1C). The regions with low GC content (AT-rich blocks) are rich in transposable elements, frequently found in intergenic, putative centromeric and telomeric regions (Fig. 1C). In model fungi, such as *Neurospora crassa* and *Saccharomyces cerevisiae*, AT-rich blocks play an important role in mediating chromatin structure and chromosomal contacts (Aramayo and Selker 2013; Blat and Kleckner 1999; Galazka et al. 2016). These findings provide a valuable resource for future centromere and chromosome architecture studies in the mycoparasitic fungus *T. rugulosus*.

For *T. rugulosus* genome annotation, de novo gene structure was predicted by GeneMark-ES with parameters '-ES-fungus' (Borodovsky and Lomsadze 2011). A *T. marneffei* gene model (GenBank assembly accession GCA_009650675.1) (Cuomo et al. 2020) was then used to train Augustus v. 3.1 (Stanke and Morgenstern 2005). MAKER2 pipeline (Cantarel et al. 2008), with the RepeatMasker v. 4.0.7 (Tarailo-Graovac and Chen 2009) option on to find and mask repetitive elements, was used to find protein-coding genes, integrating gene models predicted from GeneMark-ES and Augustus, and protein sequences of *T. islandicus* (GenBank assembly accession GCA_000985935.1), a close relative of *T. rugulosus* (Schafhauser et al. 2015). In total, the *T. rugulosus* genome has 11,907 protein-coding genes and 2.52% repeat content (Table 1). The final BUSCO score of 100% based on the 'fungi_odb9' library suggests a complete *T. rugulosus* genome assembly (Fig. 1C).

Carbohydrate-active enzymes (CAZymes) and fungal cell wall-degrading enzymes (FCWDEs) were classified using the dbCAN classification system with an E-value threshold of 1e-5 (Amselem et al. 2011; Yin et al. 2012). Putative secondary metabolite key biosynthetic genes were identified by antiSMASH v. 5.0 (Blin et al. 2019). Proteases were identified by the MEROPS database (Rawlings et al. 2018). Candidate lectins were identified by Hmmscan with PFAM domains PF00652, PF01419, PF03388, PF09458, and PF14200. We found that *T. rugulosus* was particularly well-equipped with genes encoding proteases, CAZymes, FCWDEs, lectin, and secondary metabolite biosynthetic enzymes. The CAZymes content in *T. rugulosus* (741) was smaller than that of the mycoparasitic fungus *T. pinophilus* (761) but

larger than that of *Trichoderma atroviride* (663) (Table 1). The proteases (391) and FCWDEs (107) content in *T. rugulosus* was larger than those of the other two species (Table 1), suggesting *T. rugulosus* may have stronger mycoparasitic ability. This high-quality genome assembly and annotation of *T. rugulosus* will be a useful resource for fungal comparative genomics to elucidate the evolution of mycoparasitism and functional genomic studies of *T. rugulosus*, for its industrial use and biocontrol effects against plant pathogens. The genome project has been deposited at GenBank under the BioProject ID PRJNA639236. The six chromosomal sequences and gene annotations of *T. rugulosus* are indexed at GenBank with accession numbers CP055898 through CP055903. PacBio reads and Illumina reads are available in the National Center for Biotechnology Information Sequenced Read Archive under accession numbers SRR12007734 and SRR12007532, respectively.

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Author-Recommended Internet Resource

NextDenovo v2.0: <https://github.com/Nextomics/NextDenovo>

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