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Genome Sequence of the Biocontrol Agent *Coniothyrium minitans* Conio (IMI 134523)

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Abstract

Coniothyrium minitans (synonym, *Paraphaeosphaeria minitans*) is a highly specific mycoparasite of the wide host range crop pathogen *Sclerotinia sclerotiorum*. The capability of *C. minitans* to destroy the sclerotia of *S. sclerotiorum* has been well recognized and it is available as a widely used biocontrol product Contans WG. We present the draft genome sequence of *C. minitans* Conio (IMI 134523), which has previously been used in extensive studies that formed part of a registration package of the commercial product. This work provides a distinctive resource for further research into the molecular basis of mycoparasitism to harness the biocontrol potential of *C. minitans*.

Genome Announcement

Coniothyrium minitans (synonym: *Paraphaeosphaeria minitans* [Verkley et al. 2004]) is an effective biocontrol agent of one of the most destructive soilborne plant pathogens, *Sclerotinia sclerotiorum* (Lib.) De Bary (Budge et al. 1995; Jones and Stewart 2000; Zeng et al. 2012). *C. minitans* is an ecologically obligate mycoparasite that is highly efficient in colonizing *S. sclerotiorum* sclerotia with little effect on the surrounding microbial populations (Whipps and Gerlagh 1992; Whipps et al. 2008). *C. minitans* can significantly reduce sclerotial inoculum and, also, inhibit the production of apothecia (Jones et al. 2004). The strain *C. minitans* Conio (IMI 134523) was originally isolated from *S. sclerotiorum* sclerotia colonizing potato stems in Scotland in the United Kingdom, and *C. minitans* has been used extensively to control sclerotinia diseases of several vegetable crops (Ashraf and Zuhaib 2013; Sun et al. 2017).

C. minitans Conio (IMI 134523) was grown in potato dextrose broth for 10 days at 20°C. The mycelium was harvested using sterile filter paper, was air dried at room temperature for 15 min, and was ground into a fine powder with liquid nitrogen, using a sterile pestle and mortar. The genomic DNA was extracted from the mycelial powder using a modified CTAB method (Shittu 2018). The genome sequence of *C. minitans* Conio was generated using a combination of Illumina MiSeq-600 and PacBio-Sequel platforms, utilizing the services provided by the University of Cambridge, United Kingdom, and McGill University, Quebec, Canada. For Illumina sequencing, the paired-end (PE) and mate pair (MP) libraries were

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Table 1. Genome assembly statistics of *Coniothyrium minitans* Conio (IMI 134523)

Features	Value
Number of scaffolds	34
Genome assembly size (bp)	47,922,910
Largest scaffold (bp)	5,154,056
N50 (bp)	2,642,187
L50	7
BUSCO completeness (%)	97.7
Total number of predicted genes	13,677

prepared with the TruSeq PCR-free and Nextera MP kits, using 550 bp and 2.5 to 4 kb DNA fragments, respectively. For the ultralong read PacBio sequencing, a single molecule real-time (SMRT) bell library of 20-kb fragments was prepared. A total of approximately 16.53 million reads were generated from the PE (4,493,194), MP (11,458,494), and SMRT bell (593,557) libraries, together yielding approximately 12.38 billion bases. FastQC v0.11.5 was used to assess the quality of the Illumina reads. Low-quality bases, with a Phred score of less than Q20, and adaptor sequences, where present, were filtered using Trimmomatic v0.36 (Bolger et al. 2014) in the PE reads and BBDuk plugin within Geneious v9.1.5 in the MP reads (Kearse et al. 2012). Curated Illumina reads (PE and MP) comprising approximately 3.92 billion bases and PacBio reads comprising approximately 7.78 billion bases were assembled using SPAdes v3.5.0 (Bankevich et al. 2012) and CANU v1.6 (Koren et al. 2017), respectively. The assembled genome from the PacBio reads was polished with the high-quality reads from Illumina PE sequencing, using the program Pilon (Walker et al. 2014). The polished assembly was then integrated into the genome assembled from the Illumina reads using the Quickmerge program (Chakraborty et al. 2016).

The nuclear genome of *C. minitans* Conio was assembled into 34 scaffolds, with a total assembly size of 47.9 Mb (48.06% GC content), based on an approximate coverage of 226x. BUSCO v4.0.0 analysis (Waterhouse et al. 2018) utilizing the Ascomycota_odb10 set (1,706 genes) revealed 97.7% completeness of the genome assembly and additional genome statistics are provided in Table 1. The gene set in the nuclear genome was predicted using MAKER3 v3.01.02 (Baroncelli et al. 2016; Holt and Yandell 2011). The *ab initio* gene predictor Augustus was trained using RNAseq data of *C. minitans* ZS-1, available in GenBank (Zhao et al. 2020). Overall, 13,677 protein-coding gene models were predicted. Using antiSMASH v5.12 (Medema et al. 2011), 15 putative secondary metabolite gene clusters were identified, including those encoding nonribosomal peptides, polyketides, and terpenoids. A 30-kb region within scaffold 10 showed similarity to gene clusters encoding epipolythiodioxopiperazine (ETP)-type secondary metabolites known to have diverse biological roles (Muthumeenakshi et al. 2007; Vargas et al. 2014). Of the 15 clusters, seven showed no similarity to any known secondary metabolite gene clusters in the database and therefore could be specific to *C. minitans* Conio. At present, only one *C. minitans* genome sequence (isolate ZS-1) is available in the public domain (Zhao et al. 2020) and an initial comparison of the genome sequence of Conio has suggested a large structural variation in terms of the genome size and gene content.

Despite the successful development of *C. minitans* as a biocontrol agent for commercial application, various biological and environmental factors are known to affect its efficacy and consistency (Nicot et al. 2019; Whipps et al. 2008; Zhao et al. 2020). Abiotic factors such as temperature, pH, light, and water potential are known to affect key biological attributes such as spore germination, hyphal extension, and pycnidial production in *C. minitans* isolates. This can impact the efficacy of *C. minitans* to successfully colonize and degrade the sclerotia of the host *S. sclerotiorum* (Jones et al. 2011; McQuilken et al. 1997). Qualitative or quantitative differences, or both, in secondary metabolite production exist in *C. minitans*. For example, strain Conio is known to produce macrosphelide A, which inhibits *S. sclerotiorum* and various other microbes (McQuilken et al. 2003). However, among 13 *C. minitans* isolates representing a worldwide collection, the inhibitory effect varied considerably (Tomprefa et al. 2009). Notable variation in the mycoparasitic ability of *C. minitans* was observed among 39 isolates from different geographic locations and strain Conio from the United Kingdom was ranked as

a high-performing isolate (Jones and Stewart 2000). Among 75 *S. sclerotiorum* isolates from different regions of France, wide differences were reported in their susceptibility to a monoconidial isolate from Contans (Nicot et al. 2019). Genes such as *CmpacC*, encoding a transcription factor (Lou et al. 2015), and *Cmoxdc1*, encoding oxalate decarboxylase (Ren et al. 2010), have been shown to regulate key functions associated with the mycoparasitic processes of *C. minitans*. The ETP-type gene cluster in *C. minitans* Conio differs in gene number, order, and orientation from the corresponding gene clusters in *Aspergillus* and *Trichoderma* species but contains the core gene *CmgliP*, involved in gliotoxin biosynthesis (Muthumeenakshi et al. 2007). Disruption of this gene in *Trichoderma virens* halted the production of gliotoxin and significantly reduced the mycoparasitic ability (Vargas et al. 2014). Along with Conio, the genome sequences of a collection of *C. minitans* isolates representing diverse geographic locations have been generated, which would enable further analysis of the ETP-type gene cluster and one or more of the putative metabolites.

The genome sequence of strain Conio (IMI 134523) represents a distinctive resource for further research into the mechanisms underpinning the ecological competence of *C. minitans* as a biocontrol agent, including the discovery of novel genes and their products. Investigations integrating comparative and functional genomics with appropriate biological and pathological experimentation offer the scope to identify genome-wide similarities and differences in *C. minitans* related to differing levels of mycoparasitic and biocontrol capabilities. This will also assist in gaining new insights new insights into the associated sensing, signaling, and gene regulation processes. Improved molecular level understanding of how *C. minitans* works as a biocontrol agent and the various factors that influence its efficacy would pave the way to develop refined formulations and effective disease management strategies. The genome sequence of strain Conio (IMI 134523) has been deposited in GenBank under the accession number WJXW00000000 (BioProject PRJNA588476, BioSample SAMN13245827) and the version described in this paper is WJXW01000000.

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