High-Quality Genome Resource of *Clonostachys rosea* Strain CanS41 by Oxford Nanopore Long-Read Sequencing

Haoming Wang,¹ Yongrong Dong,¹ Weixue Liao,¹ Xin Zhang,¹ Qinhu Wang,¹ Guoqing Li,² Jin-Rong Xu,³ and Huiquan Liu^{1,†}

¹ State Key Laboratory of Crop Stress Biology of Arid Areas and NWAU-Purdue Joint Research Center, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, China

² College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, 430070, China

³ Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, U.S.A.

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Abstract

Clonostachys rosea is a necrotrophic mycoparasitic fungus with excellent biological control ability against numerous fungal plant pathogens. Here, we performed genomic sequencing of *C. rosea* strain CanS41 using Oxford Nanopore sequencing technology. We generated a high-quality genome assembly (>99.99% accuracy), which comprised 26 contigs containing 60.68 Mb sequences with a GC content of 48.55% and a repeat content of 8.38%. The N₅₀ contig length is 3.02 Mb. In total, 20,818 protein-coding genes were identified and functionally annotated. Genes encoding secreted proteins and carbohydrate-active enzymes as well as secondary metabolic gene clusters were also identified and analyzed. In summary, the high-quality genome assembly and gene annotation provided here will allow further exploration of biological functions and enhance biological control ability of *C. rosea*.

Keywords

Clonostachys rosea, Oxford Nanopore Technology, fungal genome, long-read sequencing

Genome Announcement

Plant pathogens cause destructive crop diseases leading to significant yield and quality decline. To counteract this, chemical pesticides are extensively used throughout the world, which has led to concerns about environmental pollution and human health. Biological control is an environmentally friendly alternative. Therefore, the use of biological control agents against plant pathogens can be expected to increase in future agricultural and horticultural production.

The necrotrophic mycotrophic fungus *Clonostachys rosea*, a member of the order Hypocreales in the class Sordariomycetes, is a highly versatile biological control agent that is reported to control diseases caused by a wide range of plant pathogenic fungi (Sun et al. 2020), such as *Botrytis cinerea* (Wang et al. 2019), *Fusarium graminearum* (Xue et al. 2008), and *Sclerotinia sclerotiorum* (Rodríguez et al. 2011). The biocontrol mechanisms of *C. rosea* against plant pathogenic fungi are primarily attributed to direct parasitism, secretion of fungal cell wall degrading enzymes, production of secondary metabolites such as antibiotics and toxins, and induction of plant resistance (Sun et al. 2020). Recently, the genome assembly of three *C. rosea* strains has been published (Broberg et al. 2018; Karlsson et al. 2015; Liu et al. 2016; Sun et al. 2015). The available genome resources are valuable for identifying biocontrol-related genes to improve the biological control ability of *C. rosea*. Nevertheless, these genome assemblies are fragmented and contain hundreds of contigs, thus losing value as reference genomes.

The *C. rosea* strain CanS41 reported here was initially isolated from healthy oilseed rape (*Brassica napus*) as an endophytic fungus in Hongshan District, Wuhan, China. Here, we de novo sequenced and assembled the *C. rosea* CanS41 genome using long-read sequencing by

[†]Corresponding author: H. Q. Liu; liuhuiquan@nwsuaf.edu.cn

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Table 1. Summary of genome assembly statistics of different *C. rosea* strains

	CanS41	67-1 ^a	YKD0085 ^b	IK726 ^c
Sequencing platform	Oxford Nanopore	Illumina HiSeq	Illumina MiSeq	Illumina + PacBio
Assembly size (Mb)	60.68	55.42	55.18	70.74
Read coverage depth	230	150	31	NA
% GC content	48.55	49.88	49.27	48.74
# of contigs	26	1,552	1,082	2,321
N50 contig (Mb)	3.02	0.08	0.38	0.07
# of gene models	20,818	20,747	16,120	21,246
% repetitive DNA	8.38	2.43	2.85	NA ^d
% BUSCO completeness	100	99.6	100	NA
% k-mer completeness	97.9	NA	NA	NA
Consensus QV score ^e	42.45	NA	NA	NA

^a Reported by Sun et al. 2015.^b Reported by Liu et al. 2016.^c Reported by Broberg et al. 2018.^d NA, values unavailable due to the raw sequencing data or genome sequences (for IK726) not available at public databases.^e Consensus quality (QV) = $-10 \log_{10} E$ for a probability of error E at each base in the assembly (Rhie et al. 2020). Q40 corresponds to 99.99% accuracy.

Oxford Nanopore Technologies (ONT) aiming to present a high-quality reference genome. Genomic DNA of CanS41 was extracted from pure cultured hyphae by the CTAB method. In total, 971,501 long-reads (14.13 Gb, ~230× coverage) were generated from ONT platform. The mean length of reads is 14,547 bp and the read length N_{50} is 17,955 bp. We performed read corrections, trimming, and genome assembly of CanS41 using Canu v. 1.7 (Koren et al. 2017). Redundant contigs in the primary assembly were removed using purge_dups v. 1.0.1 (Guan et al. 2020). The assembled genome was further polished by ONT reads using Racon v. 1.4.13 (Vaser et al. 2017) through three iterations. The final genome assembly of *C. rosea* strain CanS41 is 60.68 Mb containing 26 contigs with an N_{50} of 3.02 Mb (Table 1, Fig. 1). We estimated the base-level accuracy and completeness of the genome assembly using Merqury software (Rhie et al. 2020) based on efficient k-mer set operations. The results revealed a consensus quality (QV) score of 42.45 (Q40 corresponds to 99.99% accuracy) and a k-mer completeness score of 97.9% (Table 1), suggesting that our genome assembly is highly accurate and complete.

De novo identification and modeling of repeat families in CanS41 were performed with RepeatModeler v. 1.0.11 (<http://www.repeatmasker.org>). Repeat elements were then annotated and classified by RepeatMasker v. 4.0.9 (Tarailo-Graovac and Chen 2009). As a result, 8.38% of the CanS41 genome are repetitive DNA (Table 1) and 3.48% of the repeats are long terminal repeat retrotransposons (LTR-RT).

To call gene models from the genome sequences of *C. rosea* strain CanS41, we downloaded RNA-seq data of *C. rosea* during growth, development, and parasitic interaction with different plant pathogenic fungi under accession number PRJEB4200, PRJNA272346, PRJNA420967, and PRJNA540080. The RNA-seq reads were mapped to the CanS41 genome by HISAT2 v. 2.1.0 (Kim et al. 2015). We used BRAKER2 pipeline (Hoff et al. 2019) to perform gene model prediction with parameters ‘-fungus’ and ‘-etpmode’ by integrating the Augustus v. 3.3.3 (Stanke et al. 2008) and GeneMark-ES suite v. 4.48_3.60 (Ter-Hovhannisyan et al. 2008) software. RNA-Seq alignments and the fungi OrthoDB v. 9 database (Zdobnov et al. 2017) were supplied as transcript and protein evidence. In total, we identified 20,818 protein-coding genes (>50 aa) for CanS41. Assessing the gene annotation completeness using BUSCO (Simão et al. 2015) with the ‘fungi_odb9’ library followed by manual correction showed that all of the BUSCO genes were present in our predicted protein-coding gene set (Table 1). We then performed gene ontology (GO) functional annotation using InterproScan v. 5.45-80.0 (Jones et al. 2014) and assigned GO terms to 10,631 genes.

Carbohydrate-active enzymes (CAZymes) and secondary metabolites (SMs) fulfill the important functions for parasitizing, acquiring nutrition, and communicating between different species (Macheleidt et al. 2016; Walton 1994; Zhao et al. 2014). In total, 694 genes encoding CAZymes were identified by dbCAN (Yin et al. 2012). Among them, glycoside hydrolases (GHs) contribute more than half (387). We identified 66 SM gene clusters using AntiSMASH (Medema et al. 2011), including 36 nonribosomal peptide synthetases (NRPS), 32 polyketide synthases (PKS),

Fig. 1. Circos-plot of genomic features of *C. rosea* strain CanS41. **a**, Ideograms of all contigs of *C. rosea* CanS41; **b**, gene density heatmap; **c**, repetitive DNA density heatmap; **d**, distribution of CAZyme genes; **e**, distribution of secondary metabolic (SM) backbone genes; **f**, nanopore reads coverage histogram; **g**, GC content plotted as the deviation from the average GC content of the entire genome.

15 NRPS-like, 10 terpenes, two indoles, and two betalactones. In addition, 2,099 proteins were predicted to be secretory proteins by SignalP-5.0 (Almagro Armenteros et al. 2019) and DeepLoc v. 1.0 (Almagro Armenteros et al. 2017). Among them, 469 genes were predicted to be putative effectors by EffectorP v. 2.0 (Sperschneider et al. 2018).

The genome assembly and gene annotation of *C. rosea* strain CanS41 were deposited at GenBank under the accession JADCTT000000000. The version described in this paper is JADCTT010000000. Nanopore long reads are available in the NCBI Sequenced Read Archive database under the accession number SRR12847398.

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