Genome Sequence of *Rhizoctonia solani* Anastomosis Group 4 Strain Rhs4ca, a Widespread Pathomycete in Field Crops

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Abstract

Rhizoctonia solani is an important soil-borne fungal pathogen that causes serious diseases on many agricultural crops and vegetables. Here, we report a complete genome assembly of *R. solani* AG4 (assembly: 45.47 Mb; contig N₅₀: 1.56 Mb), using a combination of Illumina paired-end and PacBio long-read sequencing data. A total of 267 noncoding RNAs and 11,592 genes were predicted, including 109 genes associated with carbohydrate-active enzymes and 2,488 genes involved in host-pathogen interactions. The complete genome of *R. solani* AG4 represents a valuable base for studying interactions between host plants and pathogenic fungi and to search for potential antimicrobial targets.

Genome Announcement

Rhizoctonia solani is a significant fungal pathogen that demonstrates a broad host range and causes disease on diverse cereal, vegetable, and forest tree plants, including rice, maize, potato, pepper, bean, cotton, sugar beet, lettuce, melon, and ornamental plants, resulting in huge economic losses (Carling et al. 2002; Ghosh et al. 2018; Rao et al. 2019; Zheng et al. 2013). *R. solani* is particularly difficult to control because it survives in soil and fields by forming sclerotia, which are condensed bodies of fungal hyphae that can survive in the soil for as long as 2 years (Long et al. 2019). *R. solani* comprises a species complex of 14 genetic groups called anastomosis groups (AGs), named AG-1 to AG-13 and AG-BI (Carling et al. 2002; Ghosh et al. 2018; González García et al. 2006). *R. solani* AG-4 has a wide host range, infecting crops in the *Chenopodiaceae*, *Fabaceae*, *Solanaceae*, and *Cucurbitaceae* families (Yang and Wu 2013). In the present study, we sequenced strain Rhs4ca of *R. solani* AG4 to provide a preliminary understanding of its genetic information.

Previously, 11 assemblies of *R. solani*, including *R. solani* AG1 (Zheng et al. 2013), *R. solani* AG3 (Cubeta et al. 2014), and *R. solani* AG8 (Hane et al. 2014), have been published. All the published *R. solani* genomes were sequenced using the Illumina platform, which produces short reads of less than 150 bp. Third-generation sequencing technologies, represented by Oxford Nanopore and Pacific Biosciences (PacBio) single-molecule real-time sequencing

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Keywords

genome, PacBio, *Rhizoctonia solani* AG4

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Fig. 1. K-mer analysis of the *Rhizoctonia solani* Rhs4ca genome. **A**, The 21-mer coverage distribution of the Illumina paired-end reads. A total of 1,311,292,933 21-k-mers were counted, with a peak depth of 28. **B**, Partial schematic of the 21-mer coverage distribution. The blue area is the actual observed k-mer distribution. The red line represents k-mers with low frequency, which were considered as sequencing errors. The black lines represent the reliable k-mer data that were used to evaluate the size of the genome. The vertical dashed lines are the k-mer peaks. The yellow line at the bottom represents the size of the repeat region.

technology, offer an effective approach to assemble genomes with high heterozygosity and containing high levels of repetitive sequences (Kyriakidou et al. 2020). For disease-causing fungi, high-quality genome assemblies are crucial because disease-causing genes are often identified among the highly repetitive regions of the genome (Thomma et al. 2016). Considering the high random error rate of PacBio sequencing technology, assembly combining PacBio long reads and error correction using Illumina short reads is a widely used approach to obtain contiguous genomes with a low error rate.

R. solani Rhs4ca was isolated from diseased pepper leaves showing root rot symptoms that were collected from the field of the Economic Plant Research Institute, Jilin Academy of Agricultural Sciences, Gongzhuling, Jilin, China. Pure cultures of isolates were obtained using the hyphal tip technique described by Dhingra and Sinclair (1985). Observation of morphological traits, including mycelium branched at right angles with a septum near the branch and a slight constriction at the branch base, was consistent with the description by Yang and Wu (2013). DNA was extracted from mycelia using a REDExtract-N-Amp plant PCR kit (Roche, Basel, Switzerland). The internal transcribed spacer (ITS)–ribosomal DNA region was amplified using the ITS1F and ITS4 PCR primers following the protocol of Weir et al. (2012), and the amplicon was sequenced on an ABI 3500xl Sanger sequencer (Applied Biosystems, Foster City, CA, U.S.A.). The ITS1 and ITS4 sequences were compared with entries in the GenBank database and were found to be most similar to group AG-4 HG-II at 99.27 and 100% identity, respectively.

The isolated DNA of *R. solani* was sequenced by Beijing Novogene Technology Co., Ltd. (Beijing), using the PacBio Sequel platform (Menlo Park, CA, U.S.A.) and the Illumina HiSeq2500 platform (San Diego, CA, U.S.A.) according to manufacturer protocol. Finally, 7.68 Gb of long-read data (approximately 169× coverage) was obtained. The Illumina 350-bp library was PE150 (2 × 150 bp paired-end reads) sequenced and produced 1.51 Gb of short reads (33× coverage). Based on the Illumina paired-end reads, Jellyfish v2.2.10 (Marçais and Kingsford 2011) and Genomescope v2.0 (Ranallo-Benavidez et al. 2020) were used to perform a genome survey of *R. solani*. As shown in Figure 1A and B, *R. solani* Rhs4ca had an estimated size of 43.60 Mb with 0.51% heterozygosity and 17.04% repeats. De novo assembly was performed using SMARTdenovo v1.0 from the pure PacBio reads (Liu et al. 2020). To improve the assembly, three rounds of corrections using Racon v1.4.10 (Vaser et al. 2017) with the PacBio data and one round of correction using Pilon v1.23 (Walker et al. 2014) with the Illumina data were completed based on the draft genome. The draft genome was 45.47 Mb, comprising 82 contigs and a 48% GC content. The N_{50} of the genome (the sequence length of the shortest contig at 50% of the total genome length) was 1.56 Mb. The

Table 1. Summary statistics of the *Rhizoctonia solani* genome

Parameters ^a	<i>R. solani</i> AG4	<i>R. solani</i> AG1 ^b	<i>R. solani</i> AG3 ^c	<i>R. solani</i> AG8 ^d
Isolate name	Rhs4ca	IA	Rhs1AP	WAC10335
Sequencing technology	PacBio, Illumina	Illumina	Illumina	Illumina
Number of contigs	82	6,452	6,040	7,606
Assembly length (bp)	45,474,565	36,938,120	51,705,945	39,822,884
N ₅₀ (bp)	1,560,204	20,319	25,869	7,182
GC content (%)	48.0	47.3	48.4	48.4
Number of predicted genes	11,592	10,593	12,738	13,952
BUSCO completeness (%)	92.0	—	—	—
Repeat rate (%)	19.79	—	—	—
NR database	11,616	—	—	—
Swiss Prot database	6,230	—	—	—
CAZyme database	109	—	—	—

^a BUSCO = benchmarking universal single-copy orthologs, N₅₀ = the sequence length of the shortest contig at 50% of the total genome length, NR = nonredundant, and CAZyme = carbohydrate-active enzyme.

^b Zheng et al. 2013.

^c Cubeta et al. 2014.

^d Hane et al. 2014.

length of the 82 contigs ranged from 22.68 Kb to 3.44 Mb. The final genome was assessed using BUSCO v4.1.4 (Simão et al. 2015), using the lineage-specific profile library fungal_odb10 with 758 markers, which suggested that 92.0% of the fungal genes are present in *R. solani*, 89.8% of which are complete and single-copy. RepeatModeler v1.0.8 (Smit and Hubley 2008) and RepeatMasker v4.0.9 (Tarailo-Graovac and Chen 2009) were used to identify and mask the repeats, resulting in 9.00 Mb (19.79%) of interspersed repeats. Gene prediction was performed using three different *ab initio* gene annotation programs, namely, Augustus v3.3.3 (Stanke et al. 2006), GeneMark-ES v3.0.4 (Borodovsky et al. 2003), and SNAP v2013.11.29 (Johnson et al. 2008). The gene model of *Fusarium graminearum* was used to train Augustus and SNAP. Evidence Modeler v1.1.1 (Haas et al. 2008) was used to integrate the consensus gene structures. Finally, a total of 11,859 protein-coding genes of *R. solani* Rhs4ca were predicted. Gene annotation based on the best alignment (E-value < 10⁻⁵, *k* = 1) match by BLAST searching in the nonredundant protein (NR) database and Swiss Prot database (Bairoch and Apweiler 2000) was performed using Diamond v0.8.22 (Buchfink et al. 2015). Among the annotated genes, 6,230 (52.53%) were mapped to Swiss Prot and 11,616 (97.95%) of their encoded proteins had similarity to proteins in the NR database. A total of 109 genes were mapped to the CAZyme database (Huang et al. 2018) and encoded carbohydrate-active enzymes; 1,531 genes were related to transport, as confirmed by analysis at the Transporter Classification Database (Saier et al. 2006); and 2,488 genes were involved in interactions between the pathogen and the host, as evidenced by analysis at the Pathogen-Host Interactions database (Winnenburg et al. 2006). Infernal v1.1 (Nawrocki and Eddy 2013) and tRNAscan-SE v2.0.6 (Lowe and Eddy 1997) were used to identify 267 noncoding RNAs, including 197 transfer RNAs and 70 ribosomal RNAs. Nine secondary metabolite biosynthesis gene clusters were identified, based on analysis using the antiSMASH fungal v4.0.0 server (Blin et al. 2017) with strict parameters, including two terpene and one nonribosomal peptide synthetase clusters.

Here, the recently published sequences of three *R. solani* isolates from three anastomosis groups were compared with that of strain Rhs4ca (Table 1). The comparison showed that the parameters of assembly length, GC (%), and the number of predicted genes were consistent among the four sequenced strains. In addition, for the number of contigs parameter, our result of 82 contigs was markedly lower than that of the other three isolates, which were 6,452, 6,040, and 7,606, respectively. The N₅₀ value was 1.56 Mb for isolate Rhs4ca, indicating a marked improvement in the integrity and continuity of the *R. solani* genome.

In summary, we reported the draft genome of *Rhizoctonia solani* AG4, a fungal pathogen that causes stem and root rot of many crops and vegetables. A total of 11,592 genes were predicted, including 109 genes related to carbohydrate-active enzymes, 1,531 genes

related to transport, and 2,488 genes involved in host-pathogen interactions. This draft genome sequence will provide a valuable basis for future research into the pathogenesis of *R. solani*. The genome assembly has been deposited at the National Center for Biotechnology Information under accession number PRJNA673572.

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