Highly Contiguous Genome Resource of *Colletotrichum fruticola* Generated Using Long-Read Sequencing

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Abstract

Colletotrichum fruticola is a plant-pathogenic fungus with a broad host range. It causes significant losses to important crops, including apple, pear, strawberry, and other Rosaceae and non-Rosaceae species. To date, two short read-based *C. fruticola* genomes are publicly available, but both are fragmented. In this study, we re-sequenced the genome of *C. fruticola* using nanopore long-read technology and refined the assembly with Hi-C map data. The resulting high-quality assembly is an important resource for further comparative and experimental studies with *C. fruticola*.

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Keywords

fungal genome, *Glomerella* spp.

Genome Announcement

Colletotrichum fruticola Prihast., L. Cai & K.D. Hyde was first identified from coffee (*Coffea arabica*) berries in 2009 (Prihastuti et al. 2009). It has since been reported from a wide range of plant species, such as tobacco, orchids, cassava, citrus, and tea-oil trees (Bragança et al. 2016; Hu et al. 2019; Li et al. 2016; Silva-Cabral et al. 2019; Wang et al. 2016). *C. fruticola* damages both foliar and fruit tissues and routinely impairs the commercial production of apple, strawberry, and pear. In addition to causing foliar and fruit diseases, *C. fruticola* can also associate with plants as an endophyte (Prihastuti et al. 2009). Such diverse plant associations and distinct plant-interaction lifestyles indicate that this broad host-range fungus may comprise forms with distinct host preferences. Relevant to this hypothesis, isolates derived from diseased apple leaf and apple fruit infect in tissue-specific manners (Rockenbach et al. 2016). Genomic comparisons would be an important approach to revealing factors governing such pathogenic specificity variation. A high-quality reference genome is pivotal to conducting these analyses. To date, two *C. fruticola* genomes (Nara gc5, isolated from strawberry in Japan, and 1104-7, isolated from an apple leaf-spot lesion in China) are publicly available (Gan et al. 2013; Liang et al. 2018a). However, both genomes were generated with Illumina sequencing technology and are, thus, highly fragmented. Combining long-read sequencing with Hi-C assisted scaffolding has been a powerful approach for generating chromosome-scale scaffolds (Ghurye et al. 2017). In this study, we resequenced isolate 1104-7 with a long-read approach and further refined the assembly using Hi-C mapping data.

Young mycelia of 1104-7 (deposited in the Agricultural Culture Collection of China, ACCC39328) was used as the source material for genome sequencing. Mycelia was harvested from a 200-ml 2-day old potato dextrose broth (PDB) shake culture inoculated with

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conidia at a final concentration of 1×10^5 per milliliter. Total genomic DNA was extracted with a cetyltrimethylammonium bromide (CTAB)-based approach and were further gel-purified with the PippinHT DNA size selection system (Sage Science, Beverly, MA, U.S.A.) prior to nanopore sequencing with the PromethION platform (Oxford Nanopore, Oxford). Both genomic DNA extraction and nanopore sequencing were performed at Nextomics Biosciences Sequencing Center, Wuhan, China. A total of 357,984 reads (total bases 7.56 Gb, $N_{50} = 27,858$ bp) were obtained, from which draft assemblies were generated using both Canu v. 1.8 (Koren et al. 2017) and Flye v. 2.4.2 (Kolmogorov et al. 2019), using default parameters and an estimated genome size of 60 Mb (Liang et al. 2018a). Both assemblies were highly contiguous (21 and 27 contigs, respectively), whereas more contigs from the Flye assembly were capped with copies of the telomeric repeat (TTAGGG). This Flye assembly was thus chosen for downstream refinements. The Flye assembly was polished with Pilon v. 1.2.3 (Walker et al. 2014), using previously generated Illumina reads (5.8 Gb 100-bp pair-ended reads [Liang et al. 2018a]), resulting in changes at 231,173 (0.39%) positions. To further refine the genome, we performed Hi-C sequencing using PDB-derived young mycelia as the source material for genomic DNA. Library generation and DNA sequencing were performed at Annoroad Genome Sequencing Center (Beijing). Restriction with *Mbol* was used to fragment the genomic DNA and the generated library was sequenced with the HiSeq2500 platform. The 150-bp pair-end reads (filtered, 77.3 M) were mapped against the Pilon-polished Flye assembly, using HiC-Pro v. 2.11.1 (Servant et al. 2015). For simplicity, 12 short contigs (three shorter than 1 kb, five between 1 and 6 kb, four between 29.6 and 55.5 kb) were omitted from the Hi-C analysis. Two of these short contigs represented the mitochondrial genome, while nine others were repetitive sequences based on BLASTN search against the genome assembly. After Hi-C read mapping, 30.6 M (47.3%) nonredundant valid interactions were obtained. The fractions of *trans*-interaction, *cis* short-range interaction and *cis* long-range interaction were 29, 23, and 48%, respectively, which were in accordance with a high quality Hi-C library (Servant et al. 2015). These Hi-C interaction data were used to guide the assembly refinement by running SALSA (Ghurye et al. 2017) and by manual examination aided by Juicebox v. 1.9.8 (Durand et al. 2016). Two contigs were joined in an end-to-end manner, and two regions showing complex interaction patterns were cut from the original contigs and were treated as independent scaffolds.

The final Hi-C validated 1104-7 assembly had a total size of 58.69 Mb, an overall GC content of 53.11%, and was comprised of 16 scaffolds (S1 to S16) with sizes ranging from 165 kb to 7.12 Mb. Three scaffolds contained telomeric repeats on both ends and nine scaffolds contained telomeric repeats on one end. The 3' end of scaffold 3 (S3) corresponded to ribosomal DNA repeats. In the Hi-C contact map, the strongest interaction signal was diagonal, supporting the integrity of the assembly. In addition, based on the Hi-C interaction signal pattern, putative centromeres could be identified from 12 scaffolds (S1 to S10, S12, S13), all of which contained extremely AT-rich DNA. The assembly completeness was evaluated by searching for conserved genes in the fungi_odb9 library (1,438 total genes), using BUSCO v1.2 (Simão et al. 2015). From this analysis 1,404 had hits as 'complete' genes and 1,311 had hits as 'complete and single-copy' genes. Both these statistics were higher than those for the previous Illumina assembly (1,392 and 1,301, respectively). Moreover, 34 fungal core genes had fragmented hits or lacked a hit in the nanopore assembly using Flye, whereas 46 genes were in this category in the Illumina assembly.

To predict gene structures, GeneMark-ET (Lomsadze et al. 2014) was trained with mapped RNA-seq reads derived from representative biological samples (conidia, cellophane-induced appressoria, cellophane infectious hyphae, developing perithecia, and infected apple leaves). Perithecial samples were collected from a 'mating' line formed between 1104-7 and its sibling strain 1104-6 on oatmeal agar. Procedures to collect samples for other cell types have been described previously (Liang et al. 2018b). Augustus v. 3.1 (Stanke et al. 2006) was trained with gene models of the Joint Genome Institute (JGI) *Glomerella cingulata* 23 strain (from the JGI MycoCosm database). *G. cingulata* was the name previously used for the sexual stage of *C. fructicola* and other members of the *C. gloeosporioides* species complex clade. RECON v. 1.08 (Bao and Eddy 2002) and RepeatScout v. 1.0.5 (Price et al. 2005) were used for the de novo identification of repetitive sequences. Finally, integrative gene prediction was performed with Maker2 v. 2.31.8 (Holt and Yandell 2011), using input files including the generated GeneMark-ET hmm model file, Augustus hmm model file, de novo assembled transcripts, repeat consensus fasta file, and predicted proteins of the *G. cingulata* 23 strain. De novo assembled transcripts were obtained with Stringtie v. 1.3.6 (Pertea et al. 2015) and Transdecoder v. 5.4.0.

Table 1. Comparison of the assembly statistics of *Colletotrichum fructicola* isolate 1104-7 (new nanopore assembly and previous Illumina assembly) and isolate Nara gc5

Features	Nara gc5	1104-7 (Illumina)	1104-7 (nanopore)
Total length (Mb)	55.61	57.08	58.69
Scaffold number	1,241	684	16
N ₅₀ (Mb)	0.11	0.34	4.99
L ₅₀	152	50	5
Putative centromeres	Not determined	Not determined	12
BUSCO complete (%)	94.58	96.80	97.64
Genes ^a	15,381	17,827	18,121
Secondary metabolism key enzymes	83	88	88
Cytochrome P450s	272	276	283
Carbohydrate-active enzymes	1,112	1,211	1,114
Proteases	593	617	607
Transcription factors	432	586	615
Transporters	1,067	1,109	1,106
Secreted proteins	1,225	1,440	1,470
Candidate effectors	292	372	378

^a Predictions were based on a previously described pipeline (Liang et al. 2018a), except for the candidate effectors, which were predicted based on EffectorP 2.0 (Sperschneider et al. 2018) with default parameters.

The integrated pipeline predicted 18,121 protein-coding genes, similar to the number predicted in the previous Illumina assembly annotation (17,827). BLASTP search (E-value cutoff = 1e-05) revealed that the new annotation included 1,850 (10.2%) new genes lacking a match in the previous annotation, whereas the old annotation included 1,116 (6.27%) unique genes. Among the genes that were unique to the Illumina annotation, 902 (80.8%) lacked a significant (E-value cutoff = 1e-05) BLASTP hit in the nonredundant National Center for Biotechnology Information (NCBI) protein database, and 447 (40.1%) lacked any RNA-seq reads mapping from three in-vitro samples and one in-planta sample. Conversely, among genes newly identified from annotation of the nanopore Flye assembly, 1,462 (79.0%) lacked a significant BLASTP hit and 311 (16.81%) lacked RNA-seq mapped reads. Based on the new gene annotation, we re-predicted virulence-related genes including secondary metabolite key enzymes, cytochrome P450s, carbohydrate-active enzymes, proteases, transcription factors, transporters, small secreted proteins, and candidate effectors (Table 1). Gene numbers in each functional category were similar between the two gene annotation versions. Among sequenced *Colletotrichum* genomes, *C. fructicola* and *C. gloeosporioides* encoded the largest repertoire of putative transporters and cytochrome P450s (unpublished data).

The long-read sequencing-based assembly of *C. fructicola* 1104-7 is by far the most complete genome assembly of *C. fructicola* and serves as an important resource for further genomic studies with this important phytopathogen. The assembled genome has been deposited at GenBank under the accession number MVNS000000000, and the gene annotations have been deposited in figshare. Nanopore and Hi-C Illumina data are available in the NCBI Sequenced Read Archive under accession numbers SRR10423968 and SRR10416202, respectively.

Author-Recommended Internet Resources

figshare: <https://figshare.com/s/76d0c0bc1e53fc9f3a5e>

JGI MycoCosm database: <https://genome.jgi.doe.gov/programs/fungi/index.jsf>

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