A Genome Sequence Resource for the Geographically Widespread Anthracnose Pathogen *Colletotrichum asianum*

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Abstract

Colletotrichum asianum is a worldwide plant pathogen causing serious fruit or leaf anthracnose diseases on a variety of plant hosts such as mango, coffee berry, chili, and other potential hosts, and it is distributed widely in Asia, America, Africa, and Oceania. This is the first genome resource available for *C. asianum*. The draft genome assembly will allow further analysis of species diversity and evolutionary mechanisms, and may serve as a foundation for genetic analysis that leads to greater understanding of interactions between plants and fungal pathogens.

The genus *Colletotrichum* includes a number of important plant pathogens that cause diseases on a variety of plants worldwide (Cannon et al. 2012). *Colletotrichum asianum* Prihast., L. Cai & K. D. Hyde is a species in the *C. gloeosporioides* species complex (Prihastuti et al. 2009) and causes anthracnose diseases that may result in significant losses on several tropical fruit crops such as mango. The species was originally described as a coffee berry (*Coffea arabica* L.) pathogen from Thailand (Prihastuti et al. 2009). Later, the pathogen was also reported causing chili (*Capsicum annuum* L.) anthracnose (Phoulivong et al. 2010) and fruit or leaf anthracnose on mango (*Mangifera indica* L.) (Mo et al. 2018). Furthermore, potential hosts included papaya (*Carica papaya* L.), banana (*Musa acuminata* Colla), guava (*Psidium guajava* L.), bell pepper (*Capsicum annuum* L.), avocado (*Persea americana* Mill.), and tree tomato (*Solanum betaceum* Cav.) (Cabrera et al. 2018; Giblin et al. 2018; Lima et al. 2015). *Colletotrichum asianum* is geographically widespread, with a distribution that includes Asia, America, Africa, and Oceania (Krishnapillai and Wilson Wijeratnam 2014; Lima et al. 2013; Mo et al. 2018; Phoulivong et al. 2010; Sharma et al. 2013; Tovar-Pedraza et al. 2020; Weir et al. 2012; Zakaria et al. 2015). These studies indicate that *C. asianum* can adapt to diverse geographic environments and hosts. Sequencing the genome of the fungus may advance genetic understanding of the nature of host–pathogen interactions, assisting in the development of more effective disease control measures.

Colletotrichum asianum ICMP 18580 was deposited at the International Collection of Microorganisms from Plants (ICMP) in New Zealand as the ex-type strain (Weir et al. 2012). The fungus was originally isolated from asymptomatic coffee berry (*Coffea arabica* L.) at Chiang Mai, Thailand, and identified as a pathogen on mature fruit, causing typical anthracnose (Prihastuti et al. 2009). In the present study, this isolate was repurified using the single-spore isolation method. Highly purified total genomic DNA was extracted from fungal mycelia collected from 5-day-old single-spore cultures grown on potato dextrose agar at room temperature in darkness based on a modified cetyltrimethylammonium bromide procedure (Murray and Thompson 1980). Genomic library construction was accomplished by the

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Keywords

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Table 1. Complete genome summary of *Colletotrichum asianum* strain ICMP 18580

Variables	Statistics
Sequence coverage	167x
Assembly size	64.73 Mb
Number of scaffolds	486
Scaffold N ₅₀	389,703 bp
GC content	49.37%
Number of predicted genes	17,966
Candidate effectors	351
Repeat rate	4.86%
BUSCO estimates	99.65%

NEBNext Ultra II DNA Library Prep Kit for Illumina following instructions from the supplier, and genome sequencing was performed on the Illumina HiSeq2500 platform (Biomarker, Beijing, China). The mean insertion size of the sequencing library was 270 bp and the sequencing strategy was 150-bp paired-ends reads. Trimmomatic v0.32 (Bolger et al. 2014) was used to remove adaptors and trim ends of the raw Illumina paired-end data, and the quality of trimmed Illumina reads was checked by FastQC v0.11.8 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc>). In total, 73,136,620 reads were generated, with 167-fold coverage. Trimmed Illumina reads were assembled into scaffolds with ABySS assembler v1.3.5 (Simpson et al. 2009). To get the best assembly, the optimal kmer value 63 was empirically determined from 26 different kmer values (51 to 101 with step 2). Trimmed Illumina reads produced a final assembly comprising 1,105 contigs assembled into 486 scaffolds. The total length of all scaffolds was 64.73 Mb (N₅₀ = 389,703 bp); summary statistics are provided in Table 1. The assembly size is the largest of the published genomes in the *Colletotrichum gloeosporioides* species complex (Cannon et al. 2012), including *C. gloeosporioides* Cg-14 (53.21 Mb) (Alkan et al. 2013), *C. fructicola* 1104-7 (57.08 Mb) (Liang et al. 2018), and *C. siamense* ICMP 18578 (55.96 Mb) (Meng et al. 2019). The completeness of the assembly was assessed using BUSCO v1.2 with the lineage-specific profile library fungi_odb9 (Simão et al. 2015), which showed that the genome assembly contained 99.65% of the 1,438 conserved fungal orthologs. In order to predict the proportion of repeat sequences in the genome, a protocol combining two different strategies was applied. First, homology-based repeat element prediction was performed using Repeat Masker v4.0.5 (<http://www.repeatmasker.org>) with the RepBase fungal library (build on 20170127). Second, de novo repeats detection was performed using the Repeat Modeler v1.0.7 pipeline with default settings (Saha et al. 2008). A filtered custom RepeatModeler library was generated after removing repetitive gene sequences from the original RepeatModeler repeat library; then, repeat sequence prediction and categorizing were operated by RepeatMasker based on the filtered custom RepeatModeler library. Finally, the results of the above two strategies were combined and analyzed. About 4.86% of sequences were identified as repeat sequences. For contrast, we subjected the genomes of *C. fructicola* 1104-7 (Liang et al. 2018), *C. gloeosporioides* Cg-14 (Alkan et al. 2013), and *C. siamense* ICMP 18578 (Meng et al. 2019) to detect repeat sequences using the same protocol. The genomes of these species encompassed 2.09, 1.26, and 1.56% repeat sequences, respectively. The percentage of *C. asianum* ICMP 18580 genome repeat sequence was significantly higher than those of the other three species (**, $P = 0.006$, t test). MAKER v2.31.8 (Holt and Yandell 2011) was utilized for gene prediction to integrate annotations from different ab initio gene prediction tools GeneMark-ES (Ter-Hovhannisyan et al. 2008) and Augustus v3.1 (Stanke and Morgenstern 2005) together with all protein sequences of homologous species, including *C. graminicola* M1.001 v1.0 (Cannon et al. 2012), *C. gloeosporioides* Cg-14 v1.0 (Alkan et al. 2013), *C. fructicola* Nara gc5 v1.0 (Gan et al. 2013), and *C. fiorinae* PJ7 v1.0 (Baroncelli et al. 2014), which belong to the same genus with *C. asianum*. GeneMark-ES was self-trained and the gene models of the *Glomerella cingulata* 23 strain (Weir et al. 2012) obtained from the Joint Genome Institute (<https://genome.jgi.doe.gov/programs/fungi/index.jsf>) were used to train Augustus. Overall, 17,966 protein-coding gene models were predicted from the *C. asianum* genome assembly. Of the protein-coding gene models, 60.14% (10,805) contained PFAM domains annotated by InterProScan 5.2-45.0 (threshold e-value of 1E-05) (Jones et al. 2014). Meanwhile 2,353 proteins were predicted with signal peptide by using SignalP v4.1 (Petersen et al. 2011).

Among the 2,353 proteins, 1,718 were computationally predicted to localize in the extra-cellular space as secreted proteins based on results from WoLF PSORT v0.2 (Horton et al. 2007). Within the predicted secretome, in total, 712 small secreted proteins (SSPs) were predicted. Candidate SSPs were defined here as secreted proteins that were smaller than 300 amino acids (Mirzadi Gohari et al. 2015). Among the predicted SSPs, 351 cysteine-rich small secreted proteins (the percentage of cysteine residues in the protein was at least twice as high as the average percentage of cysteine residues in all predicted proteins of this species) were identified, which is generally considered as evidence that a protein is a candidate-secreted effector (Ohm et al. 2012). In all, 1,106 carbohydrate active enzymes (CAZymes) were predicted from the predicted proteomes by dbCAN database 2.0 (threshold e-value of $1E-3$) (Yin et al. 2012). In fungi, plant cell-wall-degrading enzymes (PCWDEs) are known to play important roles in plant pathogenesis (Ospina-Giraldo et al. 2003; Walton 1994). Cellulases, hemicellulases, and pectinases together formed PCWDEs that we considered in present study. These three types of PCWDEs are distributed in different CAZyme families and belong to three functional classes (i.e., carbohydrate esterases, glycoside hydrolases, and polysaccharide lyases). Thus, we could pick out these PCWDE-involved families from the predicted CAZymes and regard them as putative PCWDEs. Finally, of the 1,106 predicted CAZymes, 368 were predicted to be putative PCWDEs (Gan et al. 2013). The draft assembly will allow further analysis of the pathogen species and population diversity and evolutionary mechanism, and may serve as a foundation for genetic analysis that leads to greater understanding of interactions between plants and fungal pathogens.

The genome assembly of *C. asianum* ICMP 18580 was deposited at GenBank with the accession number W000000000 (BioSample: SAMN13475008). The version described in this article is version W000000000.

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