Genome Sequence Resource of *Phytophthora colocasiae* from China Using Nanopore Sequencing Technology

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Abstract

Phytophthora colocasiae is a destructive oomycete pathogen of taro (*Colocasia esculenta*), which causes taro leaf blight. To date, only one highly fragmented Illumina short-read-based genome assembly is available for this species. To address this problem, we sequenced strain Lyd2019 from China using Oxford Nanopore Technologies long-read sequencing and Illumina short-read sequencing. We generated a 92.51-Mb genome assembly consisting of 105 contigs with an N₅₀ of 1.70 Mb and a maximum length of 4.17 Mb. In the genome assembly, we identified 52.78% repeats and 18,322 protein-coding genes, of which 12,782 genes were annotated. We also identified 191 candidate RXLR effectors and 1 candidate crinkling and necrosis effector. The updated near-chromosome genome assembly and annotation resources will provide a better understanding of the infection mechanisms of *P. colocasiae*.

Genome Announcement

Taro (*Colocasia esculenta* (L.) Schott) is an increasingly important crop used as a vegetable, staple food, and traditional medicine in Asia, Africa, South America, and the Caribbean and Pacific islands (Kreike et al. 2004).

Taro leaf blight, caused by *Phytophthora colocasiae* Racib., is the most destructive disease of taro (Raciborski 1900); it has become a major constraint on taro production in all taro-growing countries, causing yield losses of 25 to 30% (Jackson et al. 1980; Mbong et al. 2013). The pathogen also causes serious postharvest corm decay, which leads to heavy storage losses (Mbong et al. 2015). Taro leaf blight symptoms appear as small, water-soaked spots that increase in size and number over time. Under cloudy weather conditions, with intermittent rain and temperatures of approximately 28°C, the disease quickly spreads across entire fields, giving them a blighted appearance (Mishra et al. 2008). Heavily infected taro fields in locations such as Southeast Asia have experienced crop losses of more than 30% (Singh et al. 2012; Vasquez 1990). Therefore, appropriate and effective control strategies are urgently needed.

Genome data represent a valuable resource for the identification and elucidation of pathogenesis mechanisms. Several genome sequences for plant-pathogenic oomycetes are now available, and much of the current understanding of the mechanisms and evolution of the virulence in oomycetes has been based on the genome and transcriptome sequencing efforts

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Keywords

genomics, pathogenicity, *Phytophthora colocasiae*, taro leaf blight

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Table 1. Genome characteristics

Features	Strain Lyd2019
ONT long reads ^a	11.78 Gb (approximately 127×)
Average read length	13.15 kb
Reads N ₅₀	16.85 kb
Maximum read length	176 kb
Illumina short reads	4.56 Gb (approximately 49×)
Estimated genome size ^b	92.30 Mb
Estimated genome repeats ^b	56.71%
Estimated genome heterozygosity ^b	0.28%
Assembly size	92.51 Mb
Contig number	105
Contig N ₅₀	1.70 Mb
Contig L ₅₀	20
Average contig length	0.88 Mb
Maximum contig length	4.17 Mb
GC content	51.05%
Repeat sequence	52.78%
Illumina reads mapping rate	96.67%
Protein-coding genes	18,322
Candidate RXLR effectors	191
Candidate CRN effectors ^c	1
Genes annotated by Pfam ^c	11,538
Genes annotated by GO ^c	4,375
Genes annotated by KEGG ^c	6,857
Genes annotated by KOG ^c	11,382
Genes annotated by CAZy ^c	335
Total of protein-coding genes annotated	12,782

^a ONT = Oxford Nanopore Technology.

^b Genome size heterozygosity, repeat content, and size were estimated by GenomeScope ver. 2.0.

^c CRN effector = crinkling and necrosis effectors. Protein Family (Pfam) annotation was conducted by InterProScan ver. 5.47.82 while Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Eukaryotic Orthologous Groups (KOG), and carbohydrate-active enzyme (CAZy) annotations were conducted by eggNOG mapper ver. 2 online service (<http://eggno-mapper.embl.de/>).

(Lee et al. 2020; Srivastava et al. 2020). A recent study reported an Illumina short-read-based *P. colocasiae* genome assembly of strain 7290 from Vietnam (Vetukuri et al. 2018). The genome assembly was 56.6 Mb in size and consisted of 19,853 scaffolds with an N₅₀ of 11.26 kb and maximum length of 136.77 kb (Vetukuri et al. 2018). The genome assembly contained 19,984 predicted genes, among which 11,200 were annotated using the Protein Family (Pfam) database; 337 genes were found to encode candidate RXLR effectors (Vetukuri et al. 2018). These resources provide fundamental *P. colocasiae* genomic information; however, the reported genome assembly was highly fragmented and the scaffold N₅₀ was low, indicating poor quality of the genome assembly and limiting its downstream applications such as gene synteny analysis.

To address this limitation, high-quality genome assembly of *P. colocasiae* is urgently needed. We collected *P. colocasiae* strain Lyd2019 from China and its mycelia were sent to Biomarker Technologies Corp. (Beijing, China) for DNA extraction, genomic library construction, and whole-genome sequencing. We obtained 11.78 Gb (approximately 127×; N₅₀ = 16.85 kb) of long reads using the Oxford Nanopore Technologies (ONT) PromethION sequencing platform, and 4.56 Gb (approximately 49×) of 150-bp paired-end short reads (PE150) using the Illumina HiSeq 3000 sequencing platform (Table 1).

Genome size estimation was conducted using GenomeScope ver. 2.0 (Ranallo-Benavidez et al. 2020) based on the k-mer distribution of the Illumina short reads ($k = 25$; diploid model). The results showed a main peak at a depth of 37, which was the average k-mer depth. The estimated genome size was 92.30 Mb, the estimated proportion of repeats was 56.71%, and the estimated heterozygosity was 0.28% (Table 1; Fig. 1A).

We used NextDenovo ver. 2.3.1 (<https://github.com/Nextomics>), a string-graph-based de novo assembler, to generate a draft genome assembly using only ONT long reads (seed reads were cut off at 21,827 bp). Then, we used NextPolish ver. 1.3.0 (<https://github.com/Nextomics>) to correct base errors in the draft genome assembly using both ONT long reads and Illumina short reads. Finally, we obtained a 92.51-Mb polished genome assembly consisting of 105

Fig. 1. Genome features of *Phytophthora colocasiae* strain Lyd2019. **A**, Genome size, repeat, and heterozygosity were estimated by GenomeScope ver. 2.0 using Illumina short reads. **B**, Completeness of genome assembly and predicted genes evaluated by BUSCO ver. 5.0.0 with alveolata and stramenopiles datasets. Note: Completeness of genome predicted genes was evaluated with translated proteins using BUSCO under protein model.

contigs with a contig N_{50} of 1.70 Mb ($L_{50} = 20$), average contig length of 0.79 Mb, and maximum contig length of 3.93 Mb (Table 1). The assembly size was very similar to the estimated genome size (92.51 versus 92.30 Mb). Compared with the previously reported genome assembly of strain 7290 (Vetukuri et al. 2018), the genome size of our assembly was 63.45% larger (92.51 versus 56.60 Mb), with 99.47% fewer contigs (105 versus 19,853) and an approximately 1,382-fold larger contig N_{50} value (1.70 Mb versus 1.23 kb). These findings indicate that high-level repeats may result in high fragmentation in short-read-based genome assembly, which can be corrected through long-read sequencing.

Genome completeness was assessed using BUSCO ver. 5.0.0 software (Seppey et al. 2019) (<https://busco.ezlab.org/>) with alveolata ($n = 171$) and stramenopiles datasets ($n = 100$). The assembly was found to contain 95.32% (163 of 171) and 100% (100 of 100) complete single-copy orthologs at alveolata and stramenopiles level, respectively (Fig. 1B). The Illumina short reads were mapped to the genome assembly, and the mapping rate was used to evaluate genome completeness; we found that 96.67% of the reads were properly mapped (Table 1). These results provide solid evidence that our assembly had high continuity and completeness.

We further generated a library of de novo repeats using RepeatModeler ver. 2.01 (<http://www.repeatmasker.org/RepeatModeler/>), and used it to perform repeat masking with RepeatMasker ver. 4.1.1 (<http://www.repeatmasker.org/>). We identified 52.78% of our genome assembly as repetitive sequences (Table 1), consistent with the estimated repeat content (56.71%). BRAKER2 (Hoff et al. 2019) and Fgenesh (Solovyev et al. 2006) were then applied for protein-coding gene (PCG) prediction using the repeat-masked genome assembly; this process integrated ab initio and homology-based gene prediction using proteins from *Peronophythora sojae* as a training dataset. In total, 18,322 PCGs were identified, among which 12,782 were annotated by InterProScan ver. 5.47.82 (Jones et al. 2014) or eggNOG mapper ver. 2 (Huerta-Cepas et al. 2019), using the Pfam (11,538; 63.22%), Gene Ontology (GO; 4,375; 23.88%), Kyoto Encyclopedia of Genes and Genomes (KEGG; 6,857; 37.42%), Eukaryotic Orthologous Groups (KOG; 11,382; 62.12%), and carbohydrate-active enzymes (CAZys; 335; 1.8%) databases (Table 1). Proteins with signal peptide (signalP ver. 5) and without transmembrane helices (TMHMM ver. 2.0) in mature protein were defined as candidate effectors in this study. We found 191 candidate RXLR effectors (with RxLR motif) and only 1 candidate crinkling and necrosis (CRN) effector (with LxLFLAK-HVLVxxP motif) (Table 1). We also try to find more candidate CRN effectors with similarity search by Pfam (no one hit with crinkler effector protein N-terminal domain PF20147) and eggNOG mapper (93 annotated with CRN family protein). However, only three of those with signal peptide but without CRN motif were defined as candidate CRN-like effectors.

Completeness of predicted genes were also evaluated by BUSCO with a protein model, and predicted genes contained 170 complete single-copy orthologs (including 7 duplicated) in the alveolata dataset and 100 complete single-copy orthologs (including 1 duplicated) in the stramenopiles dataset (Fig. 1B).

The whole-genome sequence data as well as the gene prediction and annotation information reported in this article has been deposited in the Genome Warehouse (<https://ngdc.cn.ac.cn/gwh/>) in the National Genomics Data Center, Beijing Institute of Genomics (China National Center for Bioinformatics), Chinese Academy of Sciences, under accession number GWHAO7V00000000 (BioProject: PRJCA003784). This Whole Genome Shotgun project has also been deposited at NCBI under the accession JAGKQI000000000.

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