Genome Sequence Data of *Peronophythora litchii*, an Oomycete Pathogen Causing Litchi Downy Blight

Hengyuan Guo,¹ Jiandong Bao,² Lianyu Lin,² Zhixin Wang,¹ Mingyue Shi,³ Yuting Huang,³ Rongbo Wang,³ Benjin Li,³ Peiqing Liu,³ and Qinghe Chen^{1,3,†}

¹ Key Laboratory of Green Prevention and Control of Tropical Plant Diseases and Pests, Ministry of Education, College of Plant Protection, Hainan University, Haikou 570228, China

² Fujian University Key Laboratory for Plant-Microbe Interaction, College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, 350002, China

³ Fujian Key Laboratory for Monitoring and Integrated Management of Crop Pests, Institute of Plant Protection, Fujian Academy of Agricultural Sciences, Fuzhou 350003, China

Abstract

Peronophythora litchii is an oomycete pathogen that exclusively infects litchi, with infection stages affecting a broad range of tissues. In this study, we obtained a near chromosome-level genome assembly of *P. litchii* ZL2018 from China using Oxford Nanopore Technologies long-read sequencing and Illumina short-read sequencing. The genome assembly was 64.15 Mb in size and consisted of 81 contigs with an N₅₀ of 1.43 Mb and a maximum length of 4.74 Mb. Excluding 34.67% of repeat sequences, 14,857 protein-coding genes were identified, among which 14,447 genes were annotated. We also predicted 306 candidate RxLR effectors in the assembly. The high-quality genome assembly and annotation resources reported in this study will provide new insight into the infection mechanisms of *P. litchii*.

Litchi (*Litchi chinensis* Sonn.) is a commercially important subtropical fruit; however, its production and quality are restricted by litchi downy blight, which is caused by the pathogen *Peronophythora litchi* (Chi et al. 1984; Vien et al. 2001). *Peronophythora litchi* is among the major diseases of litchi, with commercial losses of more than 60% reported in Southern China (Ann and Ko 1984; Huang et al. 1983; Wang et al. 2009a). *Peronophythora litchii* is an oomycete pathogen that exclusively infects litchi, with infection stages affecting a broad range of tissues, including leaves, panicles, mature fruit, new shoots, and even extending to postharvest transportation and storage (Kao and Leu 1980; Vien et al. 2001).

Peronophythora litchii infects litchi leaves or fruits through oospores, which subsequently develop into sporangia that release zoospores into the litchi orchard. Oomycete infection initially causes withering and watery brown spots at infection sites on tender leaves, fruit, or panicles, finally producing downy white sporangiophores during late infection, which leads to major pre- and postharvest decay of the fruit, resulting in considerable economic loss (Jing et al. 2013). Efficient control of litchi downy blight is mainly based on the application of agrochemicals, including traditional fungicides, such as mancozeb, cymoxanil, and metalaxyl, as well as the recently registered QoI fungicide azoxystrobin (Wang et al. 2009b; Zhou et al. 2015).

[†]Corresponding author: Q. Chen; qhchen@hainanu.edu.cn

Hengyuan Guo and Jiandong Bao contributed equally to this work.

The author(s) declare no conflict of interest.

Accepted for publication 26 January 2021.

Funding

This work was supported by grants from the National Natural Science Foundation of China (31772141), and the Scientific Research Foundation of Hainan University [KYQD(ZR)-20080], and the Innovation Team of Plant Protection, Fujian Academy of Agricultural Sciences (STIT2017-1-8).

Keywords

genomics, litchi downy blight, pathogenicity, *Peronophythora litchii*

The author(s) have dedicated the work to the public domain under the Creative Commons CC0 "No Rights Reserved" license by waiving all of his or her rights to the work worldwide under copyright law, including all related and neighboring rights, to the extent allowed by law. 2021

Table 1. Genome features of *Peronophythora litchii*

Feature ^a	Strain ZL2018
ONT long reads	10.89 Gb (approximately 170×)
N ₅₀ of ONT Reads	21.37 kb
Mean ONT Read length	15.57 kb
Maximum ONT read length	166 kb
Illumina short reads ^b	5.10 Gb (approximately 73×)
Estimated genome size	57.08 Mb
Estimated genome repeats	34.93%
Estimated genome heterozygosity	0.485%
Assembly size (Mb)	64.15 Mb
Contig number	81
Contig N ₅₀ (Mb)	1.43 Mb
Contig L ₅₀	14
Average contig length	0.79 Mb
Maximum contig length	4.74 Mb
GC content	49.72%
Repeat sequence	34.67%
Illumina reads mapping rate	92.47%
Protein-coding genes ^c	14,857
Candidate RxLR effectors	306
Genes annotated by Pfam	14,385
Genes annotated by GO	4,270
Genes annotated by KEGG	6,629
Genes annotated by KOG	10,307
Genes annotated by CAZy	334

^a ONT = Oxford Nanopore Technologies.

^b Genome heterozygosity, repeat content, and size were estimated by kmer-based method GenomeScope V2.0 using Illumina short reads.

^c Protein Family (Pfam) was annotated by InterProScan, while gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), EuKaryotic Orthologous Groups (KOG), and carbohydrate-active enzyme (CAZy) were annotated by eggNOG-mapper.

The first genome assembly of *P. litchi* SHS3 was recently performed using Illumina short-read sequencing technology (Ye et al. 2016). However, the reported genome had an assembly size of only 38 Mb, which was much smaller than the estimated genome size (58 Mb), and had fragmented into 2,543 scaffolds, potentially losing key genetic information such as interspersed repeats, copy number variation, and gene clusters.

To address this problem and generate a high-quality genome assembly resource for *P. litchi*, strain ZL2018 was isolated from Fujian Province in China. High-quality genomic DNA was extracted from mycelia and was sequenced using both third-generation sequencing (Oxford Nanopore Technologies [ONT]) and next-generation sequencing (NGS) (Illumina) technology at Biomarker Technologies Corp. (Beijing). For ONT long-read sequencing, a 20-kb genomic DNA library was constructed and sequenced using the ONT PromethION platform. We obtained 10.89 Gb (approximately 170× coverage) of long reads, with an average length of 15.57 kb, N₅₀ of 21.37 kb, and maximum length of 166 kb (Table 1). For Illumina short-read sequencing, a 350-bp genomic DNA library was prepared and 150-bp paired-end reads (PE150) were sequenced using the Illumina HiSeq 3000 platform, generating 5.10 Gb of short reads (approximately 73× coverage) (Table 1).

We performed k-mer-based genome size estimation of the Illumina short reads, using GenomeScope ver. 2.0 and KMC ver. 3.1.1. We identified the highest peak in the k-mer distribution histogram ($k = 25$) at the average k-mer depth of 63, a smaller peak at twice the depth of the main peak (123, representing a repeat sequence), and another peak at half of the main peak depth (31, representing a heterozygous sequence). The estimated genome size was 57.14 Mb, which is consistent with the genome size of 58 Mb estimated in a previous study (Ye et al. 2016). The estimated proportion of repeat sequences was 34.93% and the estimated heterozygosity was extremely low at 0.485% (Table 1).

High-continuity *de novo* genome assembly of strain ZL2018 was assembled using Next-Genome ver. 2.3.1 with ONT long reads, followed by correction using NextPolish ver. 1.3.0 with both ONT long reads and Illumina short reads. The final polished assembly was 64.15 Mb in size and consisted of 81 contigs with an N₅₀ of 1.43 Mb, average length of 0.79 Mb,

BUSCO Assessment Results

Fig. 1. Genome completeness evaluated by BUSCO v4.1.4 with Alveolata and Stramenopiles datasets. NGS means previous reported genome assembly of strain SHS3 generated by next generation sequencing, while TGS means our genome assembly of strain ZL2018 generated by third generation sequencing. TGS_raw indicates the unpolished draft genome assembly and TGS_polished indicates final error-corrected genome assembly.

and maximum length of 3.93 Mb (Table 1). The genome assembly size was consistent with the estimated genome size (57.14 Mb). Compared with the previously reported genome assembly, our genome was about 70% larger, with approximately 97% fewer contigs (81 vs. 2,543) and a 43-fold higher contig N₅₀ (1.429 Mb vs. 33 kb).

Genome completeness was assessed using Benchmarking Universal Single-Copy Orthologs (BUSCOs) in Alveolata ($n = 171$) and Stramenopiles ($n = 100$) levels and BUSCO ver. 4.14 software. The final polished assembly contained 168 (98.25%) complete and one fragmented orthologs in the Alveolata dataset and 100 (100%) complete orthologs in the Stramenopiles dataset. It has been greatly improved from the unpolished draft genome assembly (87.13% in the Alveolata dataset and 94% in the Stramenopiles dataset) (Fig. 1). In comparison with the previous reported genome assembly generated by NGS, the final polished assembly had the same number of complete orthologs in both datasets but contained more duplicated orthologs, which revealed higher genome completeness (Fig. 1). The completeness of the genome assembly was also evaluated by mapping Illumina short reads, which showed that a total of 92.47% reads were properly mapped (Table 1). These results represent solid evidence supporting the high continuity and completeness of our assembly.

Repeats in the genome assembly were *de novo*-annotated and masked using Repeat-Modeler ver. 2.01 and RepeatMasker ver. 4.1.1, respectively. Notably, 34.67% of the genomic sequences were repetitive (Table 1). Soft repeat-masked genome assembly was applied for protein-coding gene (PCG) prediction, using BRAKER2 software, which integrated *ab initio* and homology-based gene prediction, using proteins from *Phytophthora sojae* as a training dataset. A total of 14,857 PCGs were identified and 306 candidate RxLR effectors were identified using an open reading frame (ORF)-based method (Ye et al. 2016) (ORFs contain a signal peptide, RxLR motif in a 20- to 120-amino acid region and without transmembrane helices). Functional annotation analysis conducted using InterProScan ver. 5.47.82 and eggNOG-mapper ver. 2 resulted in a total of 14,447 (97.24%) genes annotated using the Pfam 14,385; 96.87%), gene ontology (4,270; 28.74%), Kyoto Encyclopedia of Genes and Genomes (6,629; 44.62%), EuKaryotic Orthologous Groups (10,307; 69.37%), and carbohydrate-active enzyme (334; 2.25%) databases (Table 1).

The whole-genome sequence data and raw sequence reads reported in this paper have been deposited in the Genome Warehouse (GWAOTU00000000) and Genome Sequence Archive (CRA003810), respectively, in the National Genomics Data Center, Beijing Institute of Genomics (China National Center for Bioinformation), Chinese Academy of Sciences (Bio-Project PRJCA003784).

Author-Recommended Internet Resources

BRAKER2 software: <https://github.com/Gaius-Augustus/BRAKER>
BUSCO ver. 4.14 software: <https://busco.ezlab.org>
eggNOG-mapper ver. 2: <http://eggno-mapper.embl.de>
Genome Warehouse: <http://bigd.big.ac.cn/gwh>
Genome Sequence Archive: <http://bigd.big.ac.cn/gsa>
GenomeScope ver. 2.0: <https://github.com/tbenavi1/genomescope2.0>
InterProScan ver. 5.47.82: <https://github.com/ebi-pf-team/interproscan>
KMC ver. 3.1.1: <https://github.com/refresh-bio/KMC>
NextDenovo ver. 2.3.1 and NextPolish ver. 1.3.0: <https://github.com/Nextomics>
RepeatMasker ver. 4.1.1: <http://www.repeatmasker.org>
RepeatModeler ver. 2.01: <http://www.repeatmasker.org/RepeatModeler>

Literature Cited

- Ann, P. J., and Ko, W. H. 1984. Blossom blight of litchi in Taiwan caused by *Peronophythora litchii*. Plant Dis. 68:826.
- Chi, P. K., Pang, X. P., and Liu, R. 1984. On downy blight of *Litchi chinensis* Sonn. The pathogen and its infection process. Acta Phytopathol. Sin. 2:113-119.
- Huang, H., Wang, C. P., and Xu, D. Y. 1983. On *Peronophythora litchii*. Acta Mycologica Sin. 2:201-206.
- Jing, G. X., Huang, H., Yang, B., Li, J. R., Zheng, X. L., and Jiang, Y. M. 2013. Effect of pyrogallol on the physiology and biochemistry of litchi fruit during storage. Chem. Cent. J. 7:19-29.
- Kao, C. W., and Leu, L. S. 1980. Sporangium germination of *Peronophythora litchii*, the causal of litchi downy blight. Mycologia 72:737-748.
- Vien, N., Benyon, F. H. L., Trung, H., Summerell, B. A., Van, N., and Burgess, L. W. 2001. First record of *Peronophythora litchii* on litchi fruit in Vietnam. Australas. Plant Pathol. 30:287-288.
- Wang, H. C., Sun, H. Y., Stammler, G., Ma, J. X., and Zhou, M. G. 2009a. Baseline and differential sensitivity of *Peronophythora litchii* (lychee downy blight) to three carboxylic acid amide fungicides. Plant Pathol. 58:571-576.
- Wang, H. C., Sun, H. Y., Ma, J. X., Stammler, G., and Zhou, M. G. 2009b. Fungicide effectiveness during the various developmental stages of *Peronophythora litchii* in vitro. J. Phytopathol. 157:407-412.
- Ye, W., Wang, Y., Shen, D., Li, D., Pu, T., Jiang, Z., Zhang, Z., Zheng, X., Tyler, B., and Wang, Y. 2016. Sequencing of the litchi downy blight pathogen reveals it is a *Phytophthora* species with downy mildew-like characteristics. Mol. Plant-Microbe Interact 29:573-583.
- Zhou, Y., Chen, L., Hu, J., Duan, H., Lin, D., Liu, P., Meng, Q., Li, B., Si, N., Liu, C., and Liu, X. 2015. Resistance mechanisms and molecular docking studies of four novel QoI fungicides in *Peronophythora litchii*. Sci. Rep. 5:17466.