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RESOURCE ANNOUNCEMENT

Draft Genomic Resources for the Brown Rot Fungal Pathogen *Monilinia laxa*

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Abstract

Monilinia laxa is the causal agent of brown rot on stone fruit, and it can cause heavy yield losses during field production and postharvest storage. This article reports the draft genome assembly of the *M. laxa* Mlax316 strain, obtained using a hybrid genome assembly with both Illumina short-reads and PacBio long-reads sequencing technologies. The complete draft genome consists of 49 scaffolds with total size of 42.81 Mb, and scaffold N_{50} of 2,449.4 kb. Annotation of the *M. laxa* assembly identified 11,163 genes and 12,424 proteins which were functionally annotated. This new genome draft improves current genomic resources available for *M. laxa* and represents a useful tool for further research into its interactions with host plants and into evolution in the *Monilinia* genus.

Brown rot and blossom blight are caused by different species of the genus *Monilinia*, and these are among the most economically important fungal diseases of stone and pome fruit (Mordue 1979). These diseases can occur in the field as well as postharvest, and they can have great impact on fruit yield and quality (Martini and Mari 2014; Petróczy et al. 2012; Romanazzi et al. 2001). Several *Monilinia* spp. are known to be responsible for these diseases, although with different prevalence depending on geographical area and host plant.

Monilinia laxa (Aderh. & Ruhland) Honey and *M. fructigena* (Pers.) Honey cause European brown rot, and these were the only *Monilinia* spp. present in Europe until the turn of the millennium. *M. fructicola* (G. Winter) Honey causes American brown rot, and is spreading worldwide, while *M. polystroma* (G. Leeuwen) L. M. Kohn is restricted to Asiatic and European regions (Abate et al. 2018a; Petróczy et al. 2012; Rungjindamai et al. 2014; Villarino et al. 2013). On stone fruit, *M. laxa* was the prevalent species in California in the first half of the 20th century (Hewitt and Leach 1939), and was first identified as the causal agent of brown rot on stone fruit in Europe (Byrde and Willetts 1977). Today, *M. laxa* is very common on stone fruit, where it coexists with *M. fructicola* in areas where this pathogen is present (Abate et al. 2018a; Villarino et al. 2013).

The genetic mechanisms that underlie the diversity in the populations of each of these pathogens and their fitness and interactions with different host plants remain to be clarified. Genomics approaches are powerful tools to study fungal pathogens but they require the availability of high-quality sequenced and annotated genomes (Möller and Stukenbrock

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Keywords

brown rot, de novo assembly, genomics, metabolomics, next-generation sequencing, proteomics, stone fruit, third-generation sequencing

2017). This article reports on a high-quality genome draft of *M. laxa* obtained through a hybrid and hierarchical de novo assembly strategy. This combines Illumina short-reads next-generation sequencing and Pacific Biosciences (PacBio) long-reads third-generation sequencing, as previously reported for the de novo assembly of the *M. fructigena* (Landi et al. 2018) and *M. fructicola* genomes (De Miccolis Angelini et al. 2019).

The monoconidial strain Mlax316 (CBS 144852) of *M. laxa* (NCBI:txid61186) was isolated from cherry fruit (*Prunus avium* L.) in southern Italy in 2014 and characterized at the phenotypic and molecular levels (Abate et al. 2018a and b; De Miccolis Angelini et al. 2018). The strain was grown in liquid medium (2% malt extract; Oxoid) for 36 h at 25 ± 1°C in darkness and under shaking (150 rpm). Intact genomic DNA was extracted using Gentra Puregene tissue kits (Qiagen, Milan, Italy), according to the manufacturer's recommendations. The genomic DNA quality and quantity was determined using a spectrophotometer (Nanodrop 2000; Thermo Fisher Scientific Inc., Wilmington, DE, U.S.A.) and a fluorimeter (Qubit 2.0; Life Technologies Ltd., Paisley, U.K.). DNA integrity was analyzed using Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, U.S.A.).

The genome sequencing yielded both short 92-bp paired-end reads (HiScanSQ platform; Illumina Sequencing Technology; SELGE Network Sequencing Service, Bari, Italy) and long 20-kb reads (RSII platform; PacBio Sequencing Technology; Macrogen Inc., Next-Generation Sequencing Service, Geumcheon-gu, Seoul, South Korea). The Illumina short reads were analyzed for quality using FastX-tools and were trimmed using the Trimmomatic 0.36 software (Bolger et al. 2014). Adaptor-free PacBio subreads were used for the genome assembly. In total, 9,389 Mb of Illumina short reads and 1,007.8 Mb of PacBio long reads were generated, with a mean subread length of 9.24 kb and N_{50} subread length of 13.19 kb, and with an estimated average depth of sequencing coverage of 215× (Illumina) and 24× (PacBio).

The genome of *M. laxa* strain Mlax316 was assembled in scaffolds using all of the reads from both the Illumina and PacBio sequencing, through the DBG2OLC pipeline (Ye et al. 2016). SparseAssembler (Ye et al. 2012) was used to primarily assemble short but accurate contigs from the Illumina reads using the following parameter settings: NodeCovTh, 1; EdgeCovTh, 0; k, 51; g, 15; and PathCovTh, 100. The overlap and layout of the contigs obtained from SparseAssembler and the PacBio long reads were used to construct the assembly backbone from the best overlaps of reads, as performed with the DBG2OLC module (Ye et al. 2016), with the following parameters: AdaptiveTh, 0.02; KmerCovTh, 5; and MiniOverlap, 50. The refinement of the assembly in the consensus step was performed using the Sparc module (Ye and Ma 2016), in which BLASR (Chaisson and Tesler 2012) was used to align all of the raw reads to the assembly backbone, using the default settings. At this stage, a first-draft genome was obtained, as constructed from 54 scaffolds. To improve the contiguity of the assembly, the scaffolds were reordered using the MAUVE aligner (Rissman et al. 2009), with the *M. fructigena* genome as reference (Landi et al. 2018).

The final genome draft of 42.81 Mb was constructed from 49 scaffolds without any gaps and scaffold N_{50} of 2,449.4 kb (Table 1); 40.5 Mb of the genome were included in the 21 largest scaffolds. This new genome assembly has a similar size but is less fragmented than the previously available *M. laxa* genomes of the 8L strain (GenBank assembly accession GCA_002938945.1) (Rivera et al. 2018) and the EBR-Ba11b strain (GenBank assembly accession GCA_002909725.1) (Naranjo-Ortíz et al. 2018) (Table 1).

Simple repeats and low-complexity elements were searched for using Repeat Masking against the Repbase-derived RepeatMasker library of repetitive elements. The assembled genome showed 28,675 elements (3.27% of the genome size) identified as single repeats and 2,920 elements (0.36% of the genome size) recognized as low-complexity regions (Table 1). Benchmarking Universal Single-Copy Ortholog (BUSCO) v3.0.2 (Simão et al. 2015) was used with the default parameters to evaluate the completeness of the assembly, based on the 290 BUSCO groups in the fungi_odb9 lineage dataset, with selection of *Botrytis cinerea* Pers. as the closely related species for the Augustus v3.3.2 gene finding. The results showed that 99.0% of the predicted genes in the assembled genomic draft were complete and single copy, and only a few were missing (0.7%) or fragmented (0.3%). These findings indicate the high degree of completeness of the assembled genome. For quality assessment, RNA sequencing (RNA-Seq) reads from the same *M. laxa* Mlax316 strain (De Miccolis Angelini et al. 2018) were mapped on the assembled genome draft using the CLC Genomics Workbench software (v. 7.0.3; CLC Bio, Aarhus, Denmark), with the default parameters. Approximately 79.9% of the RNA-Seq reads mapped on the final genome draft version.

Table 1. Comparisons among the de novo genome assembly results of the *Monilinia laxa* strains Mlax316 (this study), 8L (GCA_002938945.1) (Rivera et al. 2018), and EBR-Ba11b (GCA_002909725.1) (Naranjo-Ortíz et al. 2018)[a]

	Assembly		
Feature	**Mlax316**	**8L**	**EBR-Ba11b**
Total sequence length (bp)	42,814,844	40,799,424	41,843,177
Total ungapped length (bp)	42,814,844	40,727,581	41,788,958
GC content (%)	41.26	40.47	40.22
Number of scaffolds	49	618	548
Maximum scaffold length (bp)	3,307,454	405,692	673,002
Minimum scaffold length (bp)	5,295	1,003	441
Mean scaffold size (bp)	873,772	66,018	76,356
Scaffold N_{50}	2,449,422	124,845	159,077
Scaffold N_{90}	743,087	41,366	51,423
Scaffold L_{50}	8	104	84
Number of simple repeats (% sequence)	28,675 (3.27)	27,226 (3.22)	27,400 (3.11)
Number of low complexity repeats (% sequence)	2,920 (0.36)	2,664 (0.35)	2,759 (0.36)
Number of genes	11,163	NA	NA
Number of CDS	12,424	NA	NA
Complete and single-copy BUSCOs (%)	99.0	97.2	99.7
Fragmented BUSCOs (%)	0.3	0.0	0.0
Missing BUSCOs (%)	0.7	2.8	0.3
Proteins with no BLAST hits	1,679	NA	NA
Proteins with Blast hits	2,078	NA	NA
Proteins with mapping	655	NA	NA
Proteins with GO annotation	8,012	NA	NA
Total proteins with significant hits (%)	10,745 (86.0)	NA	NA

[a] NA = not available, CDS = coding sequences, BUSCO = Benchmarking Universal Single-Copy Ortholog, and GO = gene ontology.

Gene prediction was performed with Augustus implemented in the Blast2GO PRO package (v.5.2.5) using *B. cinerea* as the model species and the RNA-Seq reads as a guide, with the default settings. In total, 11,164 genes and 12,424 proteins were predicted and functionally annotated. In detail, 10,745 proteins (86.0%) had significant BLASTp hits, and 8,012 (64.0%) had gene ontology (GO) annotations. Blast2GO PRO was used to obtain the gene functional classification based on the GO terms in the "biological process", "molecular function", and "cellular component" categories. Based on the distribution of the generic terms at level 3, 14 GO terms were detected as biological process. The most represented terms were organic substance metabolic process (16%), cellular metabolic process (15%), primary metabolic process (15%), and nitrogen compound metabolic process (13%). Among the 11 GO terms identified in molecular function, the most represented were organic cyclic compound binding (15%), heterocyclic compound binding (15%), ion binding (14%), and hydrolase activity (11%). For cellular component, 9 GO terms were identified, among which the most represented were intracellular (19%), intracellular part (19%), intracellular organelle (15%), membrane-bound organelle (14%), and intrinsic component of membrane (13%).

This Whole-Genome Shotgun project has been deposited at DNA Data Bank of Japan/European Nucleotide Archive/GenBank under the accession VIGI00000000 (Bioproject accession PRJNA523196, Biosample SAMN10965822). The version described in this article is version VIGI01000000. Raw reads from both the Illumina and the PacBio sequencing have been deposited in the Sequence Read Archive under the accession numbers SRR8595055 and SRR8607539, respectively. The repeat-masked and unmasked genomic sequences, including the gene, mRNA and coding sequence annotations, protein sequences, and functional annotations of the *M. laxa* Mlax316 strain genome generated in this study have been deposited in the figshare repository (Landi et al. 2019).

This new genome draft improves the genomic resources available for *M. laxa* and represents a useful tool for further research into its mechanisms of pathogenicity and its interactions with different host plants, as well as into the evolution in the *Monilinia* genus and the Sclerotiniaceae family.

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Author-Recommended Internet Resources

DBG2OLC pipeline: https://github.com/yechengxi/DBG2OLC
SparseAssembler: https://github.com/yechengxi/SparseAssembler
Sparc module: https://github.com/yechengxi/Sparc
BLASR: https://github.com/PacificBiosciences/blasr
MAUVE aligner: http://darlinglab.org/mauve/user-guide/mauvealigner.html
Repeat Masking: http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker
BUSCO v3.0.2: https://gitlab.com/ezlab/busco
Augustus v3.3.2: http://bioinf.uni-greifswald.de/augustus

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