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Communication

A Chromosome-Level Genome Assembly of *Rhizopus stolonifer* from Passion Fruit

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Abstract

Flower rot of passion fruit, caused by *Rhizopus stolonifer*, is an emerging disease that threatens commercial cultivation of passion fruit by reducing yield and fruit quality. To investigate the molecular basis of pathogenicity and provide genomic resources for disease management, we generated a high-quality, chromosome-level genome assembly of *R. stolonifer* isolate PRFJ02 using PacBio HiFi sequencing combined with Hi-C chromatin conformation capture. The assembled genome spanned 48.2 Mb across 11 chromosomes, with a BUSCO completeness of 95.2%, and encoded 11,885 protein-coding genes. Functional annotation revealed a strong metabolic capacity, including 422 carbohydrate-active enzymes (CAZymes) involved in carbohydrate degradation and modification. Effector prediction identified 274 candidate apoplastic effectors, highlighting potential roles in host–pathogen interactions. Whole-genome phylogenetic analysis across 48 *Rhizopus* genomes confirmed that *R. stolonifer* forms a distinct phylogroup, separate from *R. microsporus*, *R. delemar*, and *R. arrhizus*. Comparative orthologous clustering identified conserved core gene sets alongside clusters unique to PRFJ02 enriched in functions related to carbohydrate binding, DNA recombination, and energy metabolism. This underscores the genetic adaptation and pathogenicity of *R. stolonifer*. Together, these findings provide novel insights into the molecular mechanisms underlying flower rot in passion fruit and establish a valuable genomic framework for developing targeted disease management strategies.

Keywords: flower rot of passion fruit; *Rhizopus stolonifer*; Pacbio HiFi; Hi-C; genome assembly; fungal effectors; pathogenicity; whole-genome phylogenetics

1. Introduction

Passiflora edulis, commonly known as passion fruit, is an economically important fruit crop with yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Deg.) and purple passion fruit (*Passiflora edulis* f. *edulis*) being the two most commercially important types for passion fruit production [1]. Native to South America, passion fruit has been widely introduced and is cultivated throughout tropical and

subtropical regions, including parts of Asia, Africa, and Oceania. In recent years, the cultivation area of passion fruit has expanded significantly in China, particularly in the southern provinces such as Guangxi and Guangdong, driven by increasing consumer demand and governmental initiatives supporting tropical fruit development [2]. However, its production faces several significant challenges, particularly its susceptibility to biotic stresses, including flower rot [3], stem rot [4], Phytophthora blight [5] and various viral infections [6,7]. These diseases not only reduce yield and fruit quality but also threaten the long-term sustainability of cultivation in affected regions.

Flower rot of passion fruit, caused by *Rhizopus stolonifer*, is an emerging and economically significant disease, particularly affecting its reproductive organs [3]. The disease primarily manifests during the flowering and fruit-setting stages, resulting in flower necrosis, premature abscission, and significant yield losses [8]. The development of flower rot is promoted by warm and humid environmental conditions, making it particularly prevalent in tropical and subtropical cultivation regions. Flower rot has been reported to cause yield losses up to 60% under favorable environmental conditions, posing a serious threat to commercial passion fruit production [3]. Despite its growing prevalence and substantial economic burden, comprehensive insights into its etiology, epidemiology and the development of effective, integrated management approaches remain insufficient.

Long-read genome sequencing was performed to gain insights into the pathogenic mechanisms of *R. stolonifer* and to identify genes related to its reproduction, metabolism, and stress responses. To generate a high-quality, chromosome-level genome assembly, we employed PacBio high-fidelity (HiFi) sequencing in combination with Hi-C chromatin conformation capture technology. This genome resource will contribute towards understanding the pathogenicity and aiding the development of improved strategies to manage flower rot of passion fruit in the future.

2. Materials and Methods

2.1. Sample collecting and Fungal Isolate

Passion fruit samples showing symptoms of flower rot were collected from fields in Wuhua, Guangdong (23.81°N, 115.71°E) on yellow passion fruit plants at flowering. Under aseptic conditions, the diseased flower pieces were surfaced sterilized using 1% bleach, rinsed in sterilized water, blotted dry on paper towels, and then placed on potato dextrose agar (PDA) medium at 25°C for 2 days. Resultant mycelia were sub-cultured onto new PDA plates, after which monoconidial cultures were generated. Isolate PRFJ02, was identified as *Rhizopus stolonifer* by morphology and molecular analysis.

2.2. DNA extraction and genome sequencing

A starter culture of the isolate PRFJ02 was placed on PDA and grown for 3 days at 25°C. From the subsequent growth, five mycelia disks (~10 mm in diameter) were transferred to 250 mL Erlenmeyerflasks containing 200 mL Potato Dextrose Broth and incubated on shaker for 3 days at 25°C. The mycelia mass was then filtered through sterile cheesecloth and rinsed with sterile distilled water to remove any remaining media. The mycelia mass was used to extract genomic DNA using a MagAttract HMW DNA kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Quantification of total DNA was performed with the Quant-iTPicoGreen dsDNA Assay Kit (Invitrogen, Thermo Fisher Scientific). The isolate was sequenced on an Illumina NovaSeq 6000 platform (NGS) and a PacBio SMRT flow cell, respectively. Genomic library for paired-end (2 × 400 bp) sequencing on an Illumina sequencing platform was constructed by Personalbio Technology Co. Ltd. (Shanghai, China) using the TruSeq™ DNA Sample Prep Kit (Illumina, San Diego, CA, USA). The required library for PacBio SMRT sequencing was prepared using the PacBio Template Prep Kit 1.0 (Pacific Biosciences, Menlo Park, CA, USA) according to the manufacturer's instructions. Summarily, genomic DNA was sheared to an average fragment size of 10-15 kb by g-TUBE. The fragmented DNA was then ligated with specific adapters and subjected to enzymatic digestion. Size selection for fragments 10-15 kb was performed using the BluePippin system (Sage Science, Beverly,

MA, USA) to construct SMRTbell library. The purified libraries were subsequently assessed for quality and size distribution using the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) prior to sequencing on the PacBio Revio platform. Based on an existing draft genome assembly generated from NGS and PacBio sequencing data, Hi-C sequencing data was performed to cluster the draft genome sequences into chromosomal groups and to determine the order and orientation of sequences within each chromosome, thereby improving the assembly to the chromosome level. The genomic DNA library for Hi-C sequencing was generated with TruSeq DNA PCR-free prep kit (Illumina, San Diego, CA, USA) and sequenced for paired-end (2×150 bp) on the Illumina NovaSeq 6000 platform.

2.3. Genome assembly

Quality control of raw sequencing data was conducted using fastp (v0.20.0) (<https://github.com/OpenGene/fastp>) to trim adapters, remove low-quality reads, and filter out reads with high levels of ambiguous bases. In advance of genome assembly, short-insert libraries (insert size $<1,500$ bp) obtained through NGS sequencing were analyzed to estimate basic genome parameters, such as genome size, heterozygosity, and repeat content by using GenomeScope 2.0 [9]. HiFi reads obtained from PacBio sequencing were initially assembled de novo using Hifiasm (v0.18.5). To elevate the assembly to the chromosome level, Hi-C sequencing data were processed with HiC-Pro (v3.1.0) [10], with the restriction enzyme site set to GATCGATC (EcoRI) and all other parameters kept at default values. Hi-C reads were mapped using Chromap (v0.1.3) [11] to generate alignments, which were used to anchor and scaffold the draft genome assembly to the chromosome level. The T2T (telomere-to-telomere) genome assembly was polished using NextPolish (v1.4.0) with PacBio HiFi reads to enhance the accuracy and quality of the genome assembly. The completeness of the final chromosome-level assembly was assessed using QUAST initially [12] and then BUSCO v5.4.5 (<http://busco.ezlab.org>) based on the lineage specific BUSCO dataset mucorales_odb10 from the order Mucorales [13]. Telomere repeats of 5'-ACAACC-3' were detected on each chromosome sequence using the 'telo' function of the tool 'seqtk' (<https://github.com/lh3/seqtk>).

2.4. Gene prediction

The assembly was then annotated for functional elements including repeats, non-coding RNAs (ncRNAs) and protein-coding genes. The tandem repeat sequences in the genome were firstly predicted and masked using Tandem Repeats Finder (TRF, v4.10.0) [14]. RepeatModeler (v2.0.4) [15] and RepeatMasker (v4.1.4, <http://www.repeatmasker.org>) [16] were then utilized to detect and generate a genome-wide profile of repetitive elements in PRFJ02. To annotate ncRNAs in the genome, tRNA genes were predicted using tRNAscan-SE (v2.0) [17], and rRNA genes were identified using Barrnap (v0.9) [18]. Protein-coding genes were performed using Augustus (v2.5.5) [19], GlimmerHMM (version 3.0.4) [20], and GeneMark-ES (version 4.71) software [21]. Homology-based gene prediction was conducted using Exonerate (v2.2.0) software by aligning protein sequences from closely related species to the assembled genome. To obtain a high-confidence, non-redundant gene set, gene models derived from de novo prediction and homology-based approaches were integrated using EvidenceModeler (EVM, v2.0.0) [22].

2.5. Functional annotation

Functional annotation of protein-coding genes was carried out using multiple databases. Protein sequences were aligned to the NCBI non-redundant (nr) protein database (release 2017.10.10) using DIAMOND (v2.0.14) [23] with an E-value threshold of $1e-6$, and the best hit was retained for functional assignment. Functional annotation of protein-coding genes based on orthology was performed using eggNOG-mapper (V4.5) [24]. KEGG and COG annotations were obtained by mapping gene sequences to the their respective databases. To obtain more precise annotations, the Swiss-Prot database was queried using BLAST with default parameters. InterPro (v66.0, release

2017.11.23) [25] was used to identify conserved protein domains and motifs. The resulting InterPro entries were processed in InterPro2GO database to obtain GO terms, which were then mapped to a list of GO slims using map2slim tool (<https://github.com/elhumble/map2slim>, accessed on 18 February 2025). Protein domains were identified using HMMER (v3.3.2) against the Pfam database (release 35.0), and functional annotation of protein-coding genes related to pathogen–host interactions was performed by aligning protein sequences against the PHI-base (Pathogen–Host Interactions database, version 4.17) using BLASTP with an E-value cutoff of $1e-5$. The best hit was retained for annotation. The CAZy database (<http://www.cazy.org>, accessed on 7 October 2025) and the Database of Fungal Virulence Factors (DFVF) (<http://sysbio.unl.edu/DFVF/index.php>, accessed on 7 October 2025) was performed using Diamond (v2.0.14) to classify carbohydrate-active enzymes and fungal virulence factors, respectively.

2.6. Subcellular localization analysis

The signal peptide sequences in the predicted protein-coding genes were assessed using SignalP (v5.0) [26] and TargetP (v2.0) [27]. The transmembrane helix structures of the predicted protein-coding genes were predicted using TMHMM (v2.0) [28]. Additionally, the prediction of effector proteins in pathogenic fungi was performed using EffectorP (v3.0) [29], which identifies candidate effectors based on machine learning models trained on known effector sequences.

2.7. Whole-genome phylogenetic analysis

The genomes of 47 *Rhizopus* strains, as well as a single isolate of *Sporodiniella umbellata* (isolate MES1446), were retrieved from NCBI and then used to determine the phylogenetic placement of the *R. stolonifer* strain PRFJ02 sequenced in this study. Custom-made bash scripts were used to incorporate strain names into the FASTA headers of the genome sequences which allowed merging of alignments downstream.

All 48 genomes were loaded into the Galaxy computing environment (GalaxyCommunity 2024) and subject to de novo gene annotation using AUGUSTUS [30–32] (version 3.4.0), with *R. oryzae* splicing models applied to obtain gene models without internal stop codons. The resulting protein sequences were then analyzed using BUSCO (*--mode prot*) [13] (v5.8.0) and the Mucorales lineage, and a custom bash script was used to filter for complete, single-copy amino acid sequences of each genome.

Protein FASTA sequences that were complete and single-copy in at least 36 of the 48 genomes were extracted using the seqkit grep command [33] (version 2.9.0), aligned using MAFFT [34] (version 7.520) using default settings, refined by removing poorly aligned regions with trimAl [35] (version v1.5. rev0) using the *gappyout* setting. The edited alignments were then concatenated using the seqkit concat command. A custom bash script was used to process the workflow from BUSCO and AUGUSTUS outputs to the final alignment [36]. The final alignment consisted of 2,540,600 amino acids with 65% of those being invariant.

Phylogenetic reconstruction was performed using RAxML GUI version 2.0 [37]. The best model, determined to be GTR+FO+I+G4m, was applied for the maximum likelihood tree search, with bootstrapping conducted using 100 replicates. *Sporodiniella umbellata* isolate MES1446 was selected as the outgroup. The resulting phylogeny was imported into the interactive tree of life [38] prior to export to Adobe Illustrator for final figure preparation.

2.8. Comparative analysis

To obtain orthologous gene clusters specific to *R. stolonifer* isolate PRFJ02 versus those shared among multiple species of this genus, gene-encoding protein clustering in the genomes of *R. stolonifer* (LSU 92-RS-03), *R. microsporus* (ATCC11559), *R. delemar* RA (99-880), *R. arrhizus* (GL30), and *R. stolonifer* (PRFJ02) were performed using the OrthoMCL algorithm and a e-value threshold of 1×10^{-2} in the software OrthoVenn3 (<https://orthovenn3.bioinfotoolkits.net>, accessed on 27 August 2025).

GO term enrichment was performed by comparing a selected set of single-copy gene clusters (genes unique to each genome) with these of the background set (all single-copy gene clusters). Statistical testing (Fisher's Exact Test) was then used to determine significant overrepresentation of GO terms at $p < 0.01$ with multiple test corrections applied.

3. Results and Discussion

3.1. Pathogen isolation

Flower rot of passion fruit caused by *R. stolonifer* has led to significant losses to the local passion fruit industry, with severe field infections observed (Figure 1A). *R. stolonifera* isolate PRFJ02 was isolated from flowers in a local passion fruit plantation in Guangdong, which exhibited severe rot symptoms, such as a mold layer covering the floral tissues and fruits and subsequent abscission of the pedicel (Figure 1B-D). The fungal colonies on PDA exhibit diffuse, cottony mycelia that are hyaline and aseptate (Figure 1E). The rhizoids, sporangia, and sporangiospores of *Rhizopus stolonifera* PRFJ02 were clearly observed and their morphology was visualized and confirmed under a microscope (Figure 1F-H).



Figure 1. Symptoms of flower rot on passion fruit and morphological characteristics of *Rhizopus stolonifera*. (A) Symptomatic passion fruit plants infected with flower rot in Guangdong, China. (B) and (C) The mold layer covered in the flowers and fruits, respectively. (D) The rotted pedicel abscised easily. (E) *Rhizopus stolonifera* isolate PRFJ02 grown on potato dextrose agar medium. (F), (G) and (H) Morphology of rhizoids, sporangia and sporangiospores of *Rhizopus stolonifera* PRFJ02, respectively. Bars correspond to a scale of 20 μm in F and H and 50 μm in G.

3.2. Genome assembly

Kmer analysis using $k = 19$ in GenomeScope (v 1.0) and with Pac-bio short insert libraries (insert size $< 1,500$ bp) as input predicted a maximum genome size of 49 Mb, a genome repeat length of 28.4 Mb and a heterozygosity of 0.18% (Figure S1).

Based on an approximately 138-fold coverage, a 48.2 Mb genome assembly containing 21 contigs was produced for *Rhizopus stolonifera* isolate PRFJ02 using Hifiasm (Table 1). Read pairs from Illumina Hi-C were filtered to obtain 49.7% of read pairs that were mapped as either singletons or to multiple locations (Figure S2). Out of these, 70% of the read pairs have valid 3C interactions ($p < 1 \times 10^{-5}$), based on alignment to the Restriction enzyme-digested fragments (Figure S3). Hi-C contact map for *R. stolonifera* isolate PRFJ02 shows that fungus carries 11 chromosomes (Figure 2A). Each axis represents the 11 assembled chromosomes, ordered and oriented based on Hi-C contact density. The strong diagonal signals present intra-chromosomal contacts, whereas enriched off-diagonal interaction hotspots correspond to inter-chromosomal contacts, including centromere clustering that can be used to infer centromere positions. A total of 20 telomere regions containing the 5'-ACAACC-3' repeats

were identified on 10 out of 11 chromosomes of PRFJ02 (Table S1). This visualization supports the correct chromosome-scale structure for each chromosome and confirms the near-completeness of the *R. stolonifer* PRFJ02 assembly.

Nuclear genome of PRFJ02 was assembled into primary contigs with N50 of 4.2 Mb (Table 1, Additional File 1). Genome completeness analysis showed a 95.2% BUSCO coverage (Table 1). The PRFJ02 genome encoded 11,885 genes across 11 chromosomes, with an average gene length of 1530 bp (Figure 2B). GC content averaged 35.2% across the genome. A total of 36,917 tandem repeats mostly micro- and minisatellite DNAs were masked using TRF. A total of 19,111,983 bp sequence was masked using RepeatMasker which occupied 39.64% of the total genome sequence (Figure 2B, Table S2). Retroelements, LTR elements, and DNA transposons occupied 3.34%, 2.92% and 2.46% of the total repeat sequence, respectively. Eighty-seven rRNA genes and 398 tRNA genes were detected, which occupied 0.25% and 0.06% of the PRFJ02 genome (Table S3).

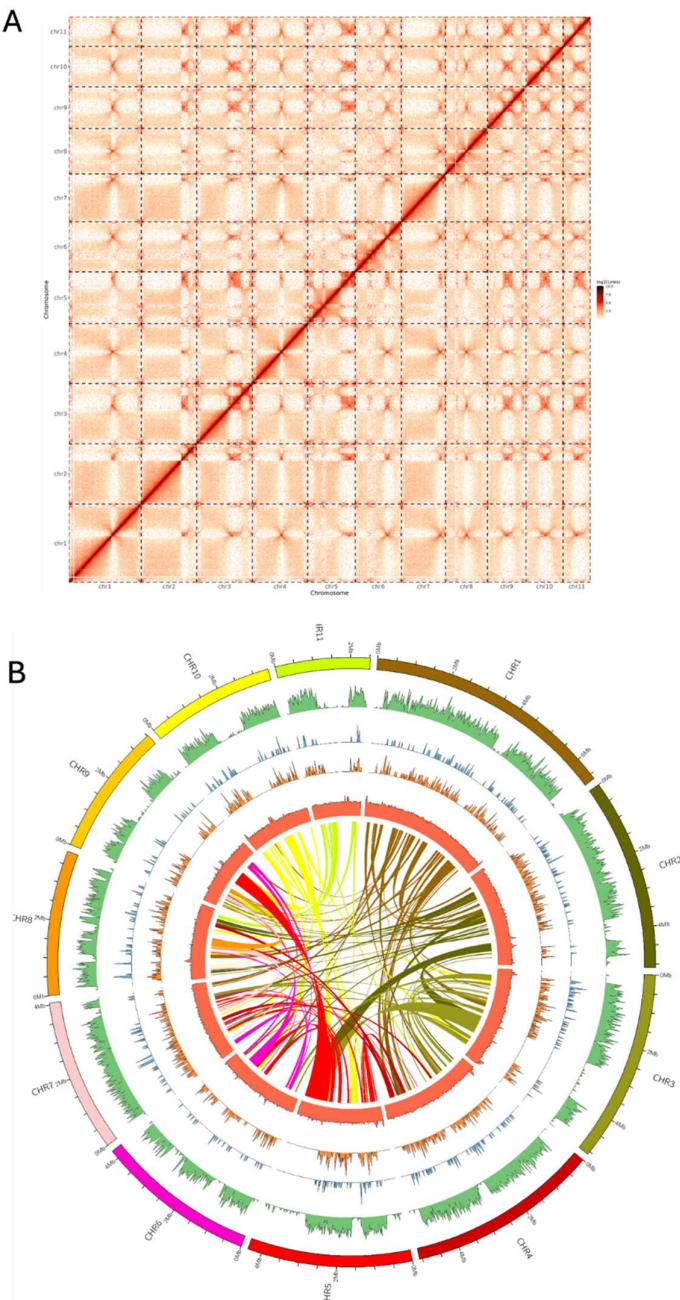


Figure 2. Overview of a gap-free genome assembly and annotation of *Rhizopus stolonifer* strain PRFJ02. (A) Hi-C contact maps showing the interaction matrices between the 11 chromosomes of *Rhizopus stolonifer* isolate PRFJ02.

Color scale indicates contact intensity between two genomic regions in a log2 scale. Red indicates a high contact intensity while white or yellow indicates a low contact intensity as in chromatin interaction frequency. (B) Ideograms of the 11 chromosomes of PRFJ02 with chromosome length displayed using a 0.5 Mb interval; COG categories of each CDS on the forward strand; COG categories of each CDS on the minus strand; DFVF; CAZY; GC content; block illustration of syntenic relationships between chromosome pairs.

3.3. Functional annotations

A total of 11,737 protein-coding genes were annotated in PRFJ02 (Table 1, Additional File 1). GO term classification for PRFJ02 revealed that the most enriched Biological Process terms were cellular nitrogen compound metabolic process (1934), biosynthetic process (1849), small molecule metabolic process (1201), transport (1121), and catabolic process (959) (Figure S4). For the Cellular Component category, the top five terms included cell (3556), intercellular (3642), organelle (2681), cytoplasm (2314), and protein complex (811). In the Molecular Function category, the most abundant terms were ion binding (2415), DNA binding (647), oxidoreductase activity (597), kinase activity (566), and RNA binding (465).

KEGG annotation of the *R. stolonifer* PRFJ02 genome showed protein-coding genes commonly associated with metabolism, particularly carbohydrate, amino acid, lipid, and energy metabolism, as well as secondary metabolite biosynthesis (Figure S5). Genes related to Genetic Information Processing were also abundant, spanning translation, replication/repair, and protein and folding. Signal transduction formed the largest component of Environmental Information Processing, while additional genes were linked to Cellular Processes, including transport and catabolism. Overall, the KEGG classification showed that PRFJ02 possesses a strong metabolic capacity and regulatory flexibility. The three most abundant KOG categories are ‘Translation, ribosomal structure and biogenesis’, ‘Post-translational modification, protein turnover, chaperones’, and ‘Signal transduction mechanisms’ (Table S4).

Carbohydrate-active enzymes (CAZymes) represent a diverse group of enzymes that are essential for the degradation, modification, and synthesis of carbohydrates [39]. A subset of these enzymes function as pathogen-secreted proteins that contribute to infection processes. Classification into the six CAZyme subfamilies [39] revealed that *R. stolonifer* isolate PRFJ02 contained 422 carbohydrate-active enzymes, with glycosyl transferases (146) and glycoside hydrolases (125) being the two most abundant classes (Table S5, Additional File 2).

Table 1. Assembly statistics for *Rhizopus stolonifer* isolate PRFJ02.

Assembly statistics	Primary assembly	Hi-C scaffolded assembly
Total length (Gbp)	6.6 ^a , 6.8 ^b , 4.1 ^c	6.6 ^a , 6.8 ^b , 4.1 ^c
Coverage (fold)	138 ^d	138 ^d
Assembly size (bp)	48,208,123	48,208,381
No. of contigs (or scaffolds)	21	18
Maximum contig length (bp)	6,159,681	6,651,178
N50 contig (or scaffold) length (bp)	4,233,866	4,366,636
Contig L50	5	5
Contig L90	10	10
GC content (%)	35.9	35.9
BUSCO coverage (%)	95.2	95.2
Total no. of BUSCOs	2,449	2,449
No. of duplicate BUSCOs	104	100
No. of fragmented BUSCOs	3	5
No. of missing BUSCOs	116	112

Gene model		
Total no. of genes	NA	11,885
Total no. of proteins	NA	11,737

^a Pac-bio HIFI reads, ^b Illumina reads, ^c Hi-C Illumina reads. ^d This coverage refers to the Pac-bio sequence data relating to the de novo Pac-bio assembly.

3.4. Effector Annotation

Fungal effectors are small, secreted proteins that aid host infection by suppressing plant host immune responses [40]. These effectors are typically characterised by their small size, and a high cysteine content, which contributes to stability. Beyond that, fungal effectors can also be pivotal in broader microbial dynamics, including roles in establishing ecological niches and interaction with other microbes [41]. To define the secretome of *R. stolonifer* isolate PRFJ02, 635 proteins carrying signal peptides were identified (Additional File 3), of which, 448, through Tmhmm analysis, contained no transmembrane domains, are therefore classified as secretory proteins (Additional file 4). These numbers are lower than the predictions previously reported for other fungal species [42,43]. Effector profiling using EffectorP revealed a total of 274 apoplastic effectors within the genome of PRFJ02 (Additional file 5). Characterising these effectors will offer insights into the molecular mechanisms underlying their pathogenicity in passion fruit.

3.5. Whole-genome phylogenetic analysis

The final alignment included 48 isolates with genomes available from the genus *Rhizopus* and comprised 2,540,600 sites, of which 278,101 were variable. The dataset contained 64.72% invariant sites and 22.68% gaps. In total, 1,421 genes were included in the alignment, selected on the basis of being complete, single-copy, and present in at least 36 of the 48 isolates. The phylogeny revealed that *R. stolonifer* forms a distinct phylogroup that is separate from a larger clade containing other *Rhizopus* species, namely *R. microsporus*, *R. delemar*, and *R. arrhizus* (Figure 3). Within this broader clade, *R. delemar* is closely related to but clearly distinct from *R. arrhizus*. *R. microspores* and *R. azygosporus* clustered together as an independent subclade, separate from the *R. delemar* and *R. arrhizus* phylogroup. Two isolates, highlighted in blue, displayed incongruence between their taxonomic classification and phylogenetic placement (Figure 3). The phylogenetic position of PRFJ02 within the *R. stolonifer* clade confirmed its identity.

3.6. Comparative study

Protein clustering analysis using OrthoVenn3 revealed 12,269 conserved orthologous clusters in *R. stolonifer* isolate LSU 92-RS-03, *R. microsporus* isolate ATCC11559, *R. delemar* RA isolate 99-880, *R. arrhizus* isolate GL30, and *R. stolonifer* isolate PRFJ02 (Table S6). A core orthologous gene set of 5,568 was evident, of which 3,526 appeared to be single-copy orthologues amongst the five genomes.

Orthologous clustering showed that *R. stolonifer* isolates PRFJ02 and LSU 92-R03 possessed 85 (322) and 63 (153) unique clusters (genes), respectively (Figure S6). Significant GO terms ($p < 0.01$) unique to PRFJ02 included carbohydrate binding, nuclease activity, response to cytokinin, proton transport, NADH dehydrogenase activity, DNA recombination, and electron transport, while LSU 92-R03 was associated with cAMP-mediated signaling and maltose alpha-glucosidase activity (Table S7). Both isolates had significant GO terms for translation and transmembrane transport in their unique clusters.

In PRFJ02, GO enrichment analysis revealed significant overrepresentation of terms related to carbohydrate binding, suggesting a role in plant host cell wall recognition and interaction. Additional enrichment was observed in processes linked to translation and energy metabolism, including electron transport and ATP synthesis, indicative of elevated metabolic activity. Enrichment of nuclease activity and DNA recombination points to potential genomic adaptability, while the response to cytokinin suggests possible involvement in host hormone signaling pathways.

Collectively, these enriched functions highlight key molecular features associated with the pathogenic potential of PRFJ02. Overall, this analysis reveals the extent of conservation and divergence in orthologous gene content among the *Rhizopus* isolates, highlighting both species-specific and shared gene clusters.

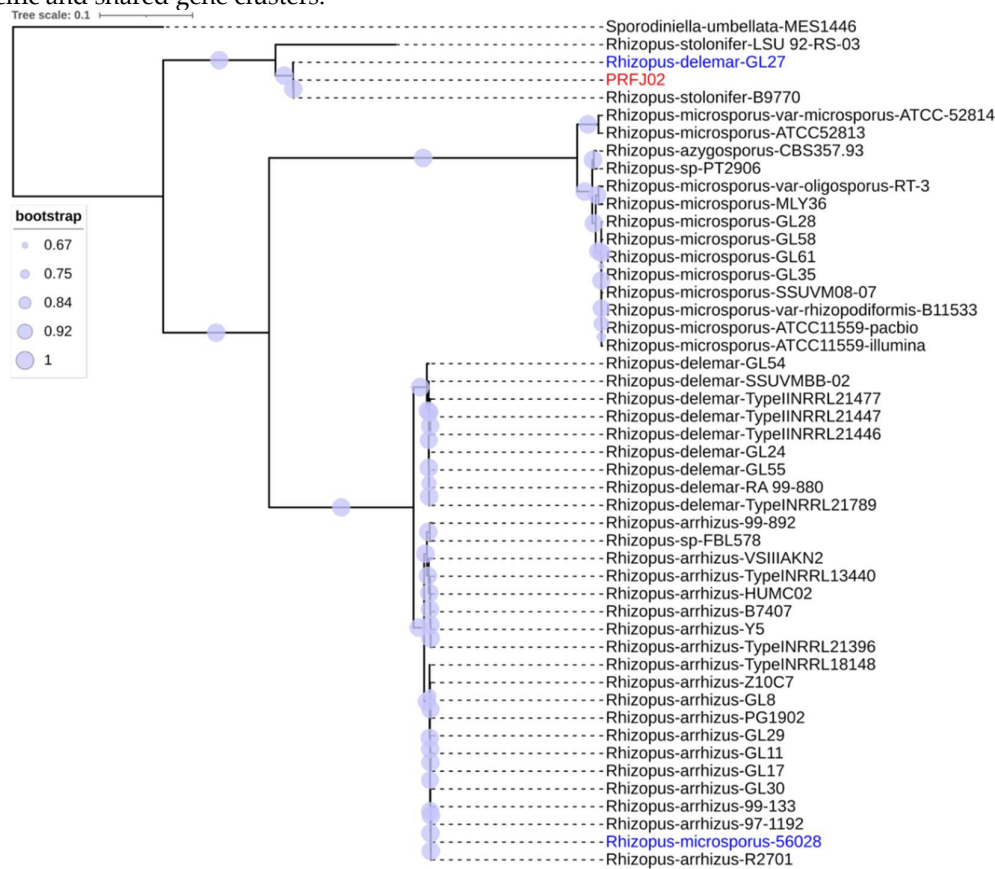


Figure 3. Whole genome phylogeny of *Rhizopus* species using a set of conserved proteins. The isolate PRFJ02 is highlighted in red. Isolate names are shown according to their NCBI records. Blue highlight indicates accessions whose species names are inconsistent with their phylogenetic placement. The phylogeny was based on the protein alignment of 1421 conserved genes. The outgroup was set to *Sporodiniella umbellata*. Branch support from bootstrap analysis is expressed as a proportion and indicated by circles.

4. Conclusion

This study presents the first chromosome-level genome assembly of *R. stolonifer*, the causal agent of flower rot in passion fruit. The high-quality assembly allowed a high-confidence set of annotations to be produced. Whole-genome phylogenetic reconstruction confirmed the distinct evolutionary placement of *R. stolonifer* within this genus, while comparative analyses revealed both conserved orthologs and isolate-specific gene clusters linked to host interaction and adaptation. In particular, the enrichment of functions associated with carbohydrate binding, translation, and energy metabolism in PRFJ02 underscores potential molecular drivers of pathogenicity. These genomic resources and insights not only improve our understanding of the pathogenicity of *R. stolonifer* but also provide a foundation for developing resistant passion fruit cultivars and sustainable management strategies to mitigate the impact of flower rot disease.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: Kmer analysis using k = 19; Figure S2: Hi-C Illumina read pair data filtering; Figure S3: Read pairs alignment on Restriction Fragments; Figure S4: GO term classification for the genome of *Rhizopus stolonifer* isolate PRFJ02; Figure S5: KEGG classification for the genome of *Rhizopus stolonifer*

isolate PRFJ02; Figure S6: Comparative analysis of orthologous gene clusters among five *Rhizopus* isolates; Table S1: Telomeric repeats ('ACAACC') were identified at the chromosomal ends of *Rhizopus stolonifer* isolate PRFJ02 using seqtk_telo; Table S2: Identification of repetitive elements in the genome of *Rhizopus stolonifer* PRFJ02 using RepeatMasker; Table S3: rRNA and tRNA genes identified in the genome of PRFJ02; Table 4S: Clustering of *Rhizopus stolonifer* isolate PRFJ02 proteins based on the functional classification of KOG; Table S5: Classification of *Rhizopus stolonifer* isolate PRFJ02 proteins into the six CAZyme subfamilies; Table S6. Overall results of the protein orthologous clustering analysis using Orthovenn3 and *R. stolonifer* isolate LSU 92-RS-03, *R. microspor* isolate ATCC11559, *R. delemar* RA isolate 99-880, *R. arrhizus* isolate GL30, and *R. stolonifer* isolate PRFJ02. Table S7: GO enrichment analysis showing GO terms ($p < 0.01$) detected in the orthologous gene clusters unique to each of the *Rhizopus stolonifer* isolates PRFJ02 and LSU 92-RS-03.

Author Contributions: Conceptualization, J.S. and A.C.; methodology, J.S. and A.C.; software, X.Z., D.M.G and G.C.; validation, J.S., G.C. and A.C.; formal analysis, D.M.G., X.Z. and L.Y.; investigation, G.C. and L.Y.; resources, G.C.; data curation, X.Z. and D.M.G.; writing—original draft preparation, J.S. and A.C; writing—review and editing, J.S., D.M.G., E.A.B.A. and A.C; visualization, X.X.; supervision, A.C., E.A.B.A.; project administration, A.C. and J.S.; funding acquisition, L.Y. All authors have read and agreed to the published version of the manuscript.

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