

The whole-genome sequence of the novel yeast species *Metschnikowia persimmonesis* isolated from medicinal plant *Diospyros kaki* Thunb

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Abstract

The new yeast *Metschnikowia persimmonesis* KCTC 12991BP (KIOM G15050 strain) exhibits strong antimicrobial activity against some pathogens. This activity may be related to the medicinal profile of secondary metabolites that could be found in the genome of this species. Therefore, to explore its future possibility of producing some beneficial activities, including medicinal ability, we report high-quality whole-genome assembly of *M. persimmonesis* produced by PacBio RS II sequencer. The final draft assembly consisted of 16 scaffolds with GC content of 45.90% and comprised a fairly complete set (82.8%) of BUSCO result using *Saccharomycetales* lineage data set. The total length of the genome was 16.473 Mb, with a scaffold N50 of 1.982 Mb. Annotation of the *M. persimmonesis* genome revealed presence of 7029 genes and 6939 functionally annotated proteins. Based on the analysis of phylogenetic relationship and the average nucleotide identities, *M. persimmonesis* was proved to a novel species within the *Metschnikowia* genus. This finding is expected to significantly contribute to the discovery of high-value natural products from *M. persimmonesis* as well as for genome biology and evolution comparative analysis within *Metschnikowia* species.

Keywords: yeast; *Metschnikowia* sp. ; *de novo* assembly; genome annotation; next-generation sequencing

Introduction

Persimmon (*Diospyros kaki* Thunb.) is an edible medicinal fruit belonging to the Ebenaceae family. It is usually cultivated in countries like Korea, China, Japan, Brazil, Turkey, and Italy (Butt et al. 2015). The calyx of *Diospyros kaki* Thunb. exhibits antimicrobial, anticancer, anti-inflammatory, antioxidant, anticoagulant, antithrombotic, antigenotoxic, diaphragm contraction inhibitory, elastase inhibitory, and tyrosinase inhibitory effects (Jang et al. 2010; Park et al. 2017; Choi et al. 2019). In traditional Korean medicine (TKM), the calyx has a mild nature, a bitter and astringent taste, poison-free (Heo 2001) and it is known to release blocked Qi. A number of microorganisms that could become a new valuable source of medicine (Choi et al. 2018) also inhabited *D. kaki* fruit stalk. Based on microbial community analysis, *Metschnikowia* sp. are the dominant microorganisms associated with the native varieties of *Diospyros kaki* fruits cultivated in Gyeongnam Province, Korea (Choi et al. 2018). *Metschnikowia* is one of the most diverse genera of ascomycetous yeasts, consisting of more than 80 identified species isolated from many types of hosts (Lachance 2016). *Metschnikowia* (Saccharomycetaceae) has needle-shaped ascospores as their sexual forms (Lachance 2011) and some of species in this genus are used as commercial biocontrol agents to kill molds in the fruits (Hershkovitz et al. 2013).

A novel yeast species, *Metschnikowia persimmonesis*, was later discovered in the calyx of Korean *Diospyros kaki* cultivars originating from various parts of South Korea (Kang et al. 2017), and has been registered for the Korean Patent (10-2016-0137873) and International Patent (PCT-KR2017-010681). Based on these patents, *M. persimmonesis* KIOM G15050 possesses antimicrobial activity against some pathogenic microorganisms, like *Botrytis cinerea*, *Fusarium oxysporum*, *Paecilomyces inflatus*, *Colletotrichum higginsianum*, *Sclerotinia sclerotiorum*, and *Alternaria alternata* (Kang et al. 2017). These activities are possibly related to the presence of some medicinal secondary metabolites in *M. persimmonesis* such as cyclodipeptides, benzoic acid, tyrosol, etc. Owing to the mycostatic effects of this strain, it can be used as a natural preservative that is much safer than chemical preservatives.

The advances in next generation sequencing technologies have rapidly boosted fundamental and applied studies on fungal species (Shendure et al. 2017; Rahmat and Kang 2020). To date, many of the *Metschnikowia* sp. yeast genomes have been successfully analyzed using various next-generation sequencing platforms, such as genome sequence of *Metschnikowia fructicola* (Piombo et al. 2018), draft genome of *Metschnikowia australis* (Batista et al. 2017), draft genome of *Metschnikowia pulcherrima* subclade, UCD127 (Venkatesh et al. 2018), etc. Here, we report the first genome assembly of *M. persimmonesis* KCTC 12991BP (KIOM

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G15050 strain). A hybrid and hierarchical *de novo* assembly approach was applied to predict the genome of *M. persimmonesis* using Pacific Biosciences (PacBio) long reads RS II sequencer.

Materials and methods

DNA sample collection, library construction, and sequencing

The yeast *M. persimmonesis* was isolated from the calyx of local *Diospyros kaki*, acquired from Gyeongnam Province, South Korea (35.141180 N 128.144161 E). The single colony strain was cultured in Potato-Dextrose or Luria-Bertani medium for 48 h at 25°C, with shaking (100 rpm) in dark conditions. The strain was earlier characterized at the phenotypic, physiological, and molecular levels and was deposited in the Korean Collection for Type Cultures (KCTC) (Kang et al. 2017). Genomic DNA was extracted using a DNA extraction kit (MGTM gDNA purification kit, www.macro-gen.com). The genomic DNA (gDNA) quality and integrity were evaluated by 0.8% agarose gel electrophoresis and densitometry and were compared to suitable size standards. The yield and purity of the genomic DNA were verified using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA) and a Qubit 2.0 fluorimeter (Life Technologies Ltd., Paisley, UK).

The library was prepared using 16 µg gDNA, which was trimmed to a length of 20 kb fragments using g-tubes (Covaris, Inc., Woburn, MA, USA). DNA integrity and actual size distribution were determined using Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). The fragmented DNA was purified using 0.45 × AMPure[®] PB beads (Pacific Biosciences, USA). DNA Template Prep Kit 1.0 was used to build the SMRTbell 20 kb template library using a 20 kb insert library protocol (Pacific Biosciences; Menlo Park, CA, USA). The DNA size was selected using BluePippin (Sage Science, Beverly, MA, USA). The P6/C4 chemistry of 24 single-molecule real-time (SMRT) cells (8 with BluePippin and 16 without) was used to sequence the library, and a 240 min collection protocol was applied with the stage start feature to achieve longer reads.

De novo assembly of the genome

Initially, the pre-assembly process was performed, followed by correction and filtering of the reads. The length cutoff for seed reads used for initial mapping was 6000 bp. The pre-assembly option details are as follows: length_cutoff_pr: 6000 bp; falcon_sense_option: -output_multi -min_idt 0.70 -min_cov 4 -local_match_count_threshold 2 -max_n_read 200 -n_core 6; overlap_filtering_setting: -max_diff 240 -max_cov 360 -min_cov 5 -bestn 10. *De novo* genome assembly of PacBio sub-reads was conducted using FALCON (v0.2.1) to acquire primary contigs and haplotigs (Chin et al. 2016). The sequence of the assembled DNA was polished using Quiver (v1). Errors were rectified using SMRT Pipe (v2.3.0.139497) following the outcome evaluation of FALCON diverse parameters during the pre-assembly step. Mapping process of assembled contigs to long reads from PacBio was carried out using BLASR (Chaisson and Tesler 2012).

Protein-coding gene prediction and functional annotation

Using the assembled draft sequence of *M. persimmonesis* and the estimated transcriptome and protein sequence data (GCF_001664035.1) on NCBI, a gene prediction pipeline was followed. This pipeline builds up a gene prediction model [SNAP (2012/05/17)]. These prediction results were then incorporated

with MAKER annotation software (v2.28) (Cantarel et al. 2008). For extra annotation, the consensus sequences were blasted against the GenBank non-redundant and Swiss-Prot database using blastx (v2.4.0+) (E value threshold 1e-3) (Camacho et al. 2009). Annotation and classification of the orthologous groups (COGs) correlated with *M. persimmonesis* genes were performed using the EggNog tool (<http://eggnogdb.embl.de/>) (Huerta-Cepas et al. 2015).

Genome quality assessment

The completeness of the assembled and annotated draft genome was evaluated using the BUSCO program (v5) (Seppey et al. 2019) with the help of which we confirmed the number of core genes of the species (*Saccharomycetales*) present in our analysis results. Furthermore, the contiguity and completeness of the draft genome was validated by mapping the RNA sequences from the same *M. persimmonesis* strain using CLC Genomics Workbench ver. 7.0.3, with the default parameters.

Genome comparison, phylogenetic analysis, and ANI calculation

A genome comparison analysis was performed with closely related species (*Metschnikowia bicuspidata* and *M. fructicola*). We used the cross-match program (v1.080812) to get information on the aligned positions and the Circos program (v0.69-3) to draw circular maps (Krzywinski et al. 2009).

To identify phylogenetic relationships, we used orthologous genes from 17 available *Metschnikowia* genomes. Of these, 16 genome sequences were downloaded from NCBI GenBank (Supplementary Figure S1). First, we obtained single-copy genes from BUSCO (v5) (Seppey et al. 2019) with default parameters. Each FASTA file was generated for each coding sequence (CDS) ID and aligned using MAFFT program (v7) (Katoh and Standley 2013) with default parameters. A total of 17 alignment files (299 total CDSs of analyzed *Metschnikowia* species) were merged using in-house scripts. The alignment files were filtered to remove ambiguously aligned regions using Gblocks (v5) (Castresana 2000). Phylogenetic tree was constructed using RAXML (v8.2.12) (Stamatakis 2014) with GTRGAMMA model. The constructed tree was visualized using iTOL (v6) (<https://itol.embl.de/>). The average nucleotide identity (ANI) value between *M. persimmonesis* and *M. fructicola* was calculated using pairwise genome comparisons of JSpeciesWS webpage (<http://jspecies.ribohost.com/jspeciesws/>). ANIb algorithm was used for the analysis.

Results and discussion

Assembly, gene prediction, and functional annotation of the genome of *Metschnikowia persimmonesis*

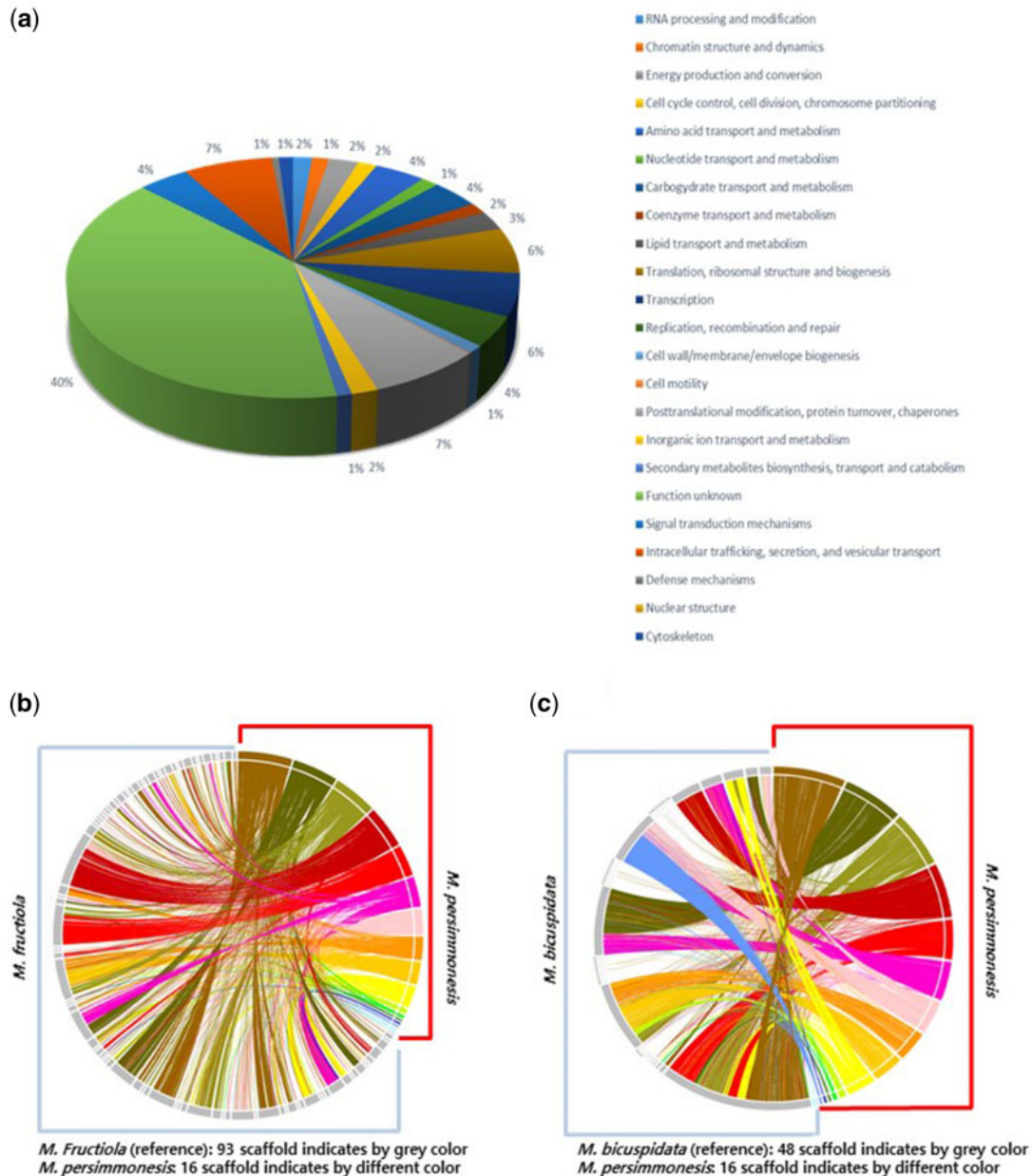
The genome assembly (accession number JACBPP000000000) of *M. persimmonesis* (type strain KIOM G15050 = KCTC 12991BP) was created using sequence data generated from the PacBio RS II Sequencer. The results of the filtered subreads were as follows: Mean subread length—5921 bp, total number of bases—1,272,475,916 bp, number of reads—214,881 bp, and N50—9372 bp (Supplementary Figure S2). The genomic data sequences were assembled using the FALCON (v0.2.1) program and a draft genome comprising 17 contigs with an N50 of 1,981,760 bp was produced (Supplementary Figure S3). After the process of eukaryotic annotation pipeline, we removed mitochondria DNA sequence (contig15), thus leaving 16 contigs in the final draft genome. The size of the genome estimated was approximately 16.473 Mb. Moreover, histogram analysis of biallelic single-

Table 1 Statistics of *M. persimmones* final genome assembly compared to *M. fructicola* and *M. bicuspidata*

	<i>M. persimmones</i>	<i>M. fructicola</i>	<i>M. bicuspidata</i>
Total length	16,473,584	26,126,100	16,055,203
Scaffolds	16	93	48
N50	1,981,760	957,836	2,544,904
MaxLength	2,532,630	2,548,689	5,390,915
MinLength	17,888	14,113	2018
AvgLength	1,029,599	280,925	334,483
GC (%)	45.90	45.90	48
Number of Gap	0	0	546
N's	0	0	377,479

nucleotide polymorphisms frequencies showed that the genome is diploid. This assembled genome of *M. persimmones* has 16 final scaffolds, which are relatively fewer than those in *M. fructicola* and *M. bicuspidata* genomes (Table 1). Long length reads of PacBio sequencing technology can result in a more complete genome. A summary of the complete draft genome without gaps is presented in Table 1. The total predicted transcripts by MAKER annotation software were 7,029 and the total annotated proteins with blastP (v2.4.0+) were 6,939 (Supplementary Table S1). COGs correlated with *M. persimmones* genes are shown in Figure 1A.

Analysis of genome completeness using BUSCO software showed that 82.8% of the detected genes of *M. persimmones*



assembled data were complete and single copy, and only a few were fragmented (7.7%) or missing (9.5%) BUSCO orthologs (Table 2). This value is fairly enough and not very different with the result of BUSCO analysis of other *Metschnikowia* genome such as *M. fructicola* (86.8%), *M. pulcherrima* (85%), and *M. reukaufii* (87%) (Supplementary Figure S4). Further validation using RNA-Seq reads of *M. persimmonesis* KIOM G15050 showed that about 93.8% of them mapped in pairs on the final genome draft version, indicating its high quality in terms of contiguity and completeness (Supplementary Table S2).

Genome comparison

A comparison of genome sizes between *M. persimmonesis* and two closely related species (Table 1) showed that the genome size of *M. persimmonesis* (16.473 Mb) strain used in this study was similar to that of *M. bicuspidata* (16.055 Mb), unlike *M. fructicola* (genome size of 26.126 Mb), with 16, 93, and 48 scaffolds, respectively. The comparatively larger genome size of *M. fructicola* is probably due to a whole-genome duplication event (Piombo et al. 2018). However, final genome assembly of *M. persimmonesis* and *M. fructicola* showing no gap compared to *M. bicuspidata* with relatively high number of gap (546). This happens because both *M. persimmonesis* and *M. fructicola* genomes were assembled from long reads data, unlike *M. bicuspidata* which used a short read sequencer. Short-read sequence data more difficult to assembled because of sequencing biases, repetitive genomic features, genomic polymorphism, many gaps, and other complicating factors (English et al. 2012).

Whole-genome alignment between *M. persimmonesis* (query) and *M. fructicola* (subject) showed that 99.01% of *M. persimmonesis* sequences were aligned to 64.02% *M. fructicola* sequences with substitution, deletion, and insertion ratios of 6.54, 0.71, and 0.62,

Table 2 Statistics of the genome completeness using BUSCO

C: 82.8% [S: 79.3%, D: 3.5%], F: 7.7%, M: 9.5%, n: 1711

1416	Complete BUSCOs (C)
1356	Complete and single-copy BUSCOs (S)
60	Complete and duplicated BUSCOs (D)
132	Fragmented BUSCOs (F)
163	Missing BUSCOs (M)
1711	Total BUSCO groups searched

respectively (Supplementary Table S3). Meanwhile, genome alignment of *M. persimmonesis* with *M. bicuspidata* (subject) had 33.64% query coverage and a 0.04% query overlap region compared to 32.44% subject coverage and 2.27% subject overlap region with substitution, deletion, and insertion ratios of 20.58, 0.38, and 0.33, respectively (Supplementary Table S4). The relationship and genomic interval between *M. persimmonesis*, *M. fructicola*, and *M. bicuspidata* are shown in Figure 1, B and C.

Phylogenetic relationship

To identify the phylogenetic relationships between *M. persimmonesis* and other *Metschnikowia* species, we aligned 299 conserved single-copy protein-coding sequences shared by the 17 *Metschnikowia* species (Figure 2). We used yeast model species *Saccharomyces cerevisiae* as an outgroup. This whole-genome-based phylogeny tree revealed that *M. persimmonesis* is grouped with *M. fructicola* and *M. pulcherrima* and shared the closest phylogenetic relationship with *M. fructicola*. The result is consistent with our previous report based on 16S rRNA gene sequences which showed that *M. persimmonesis* is a sister clade of *M. fructicola* (Kang et al. 2017). Furthermore, we performed ANI analysis to ascertain whether *M. persimmonesis* is really a new and distinct species from *M. fructicola*. As a result, ANI value between *M. fructicola* and *M. persimmonesis* was 94.19%, which is lower than the threshold of 95-96% of the boundary for species circumscription. Therefore, the combination of ANI value, whole-genome-based phylogenetic tree, and previous result of 16S rRNA sequence (Kang et al. 2017) confirmed that *M. persimmonesis* belonged to novel species within the genera of *Metschnikowia*.

Conclusion

Using Pacific Biosciences (PacBio) long reads RS II sequencer and high-coverage RNA-seq data we have generated a high-quality draft genome assembly and annotation of the novel species *M. persimmonesis*, providing an important genomic resource for the future study of this important endophytic yeast. Comprehensive study of this *M. persimmonesis* genomic assets could open more insight in the area of evolutionary biology within *Metschnikowia* genus, genetic adaptations to highly distinct environments, and discovery of highly valuable natural product.

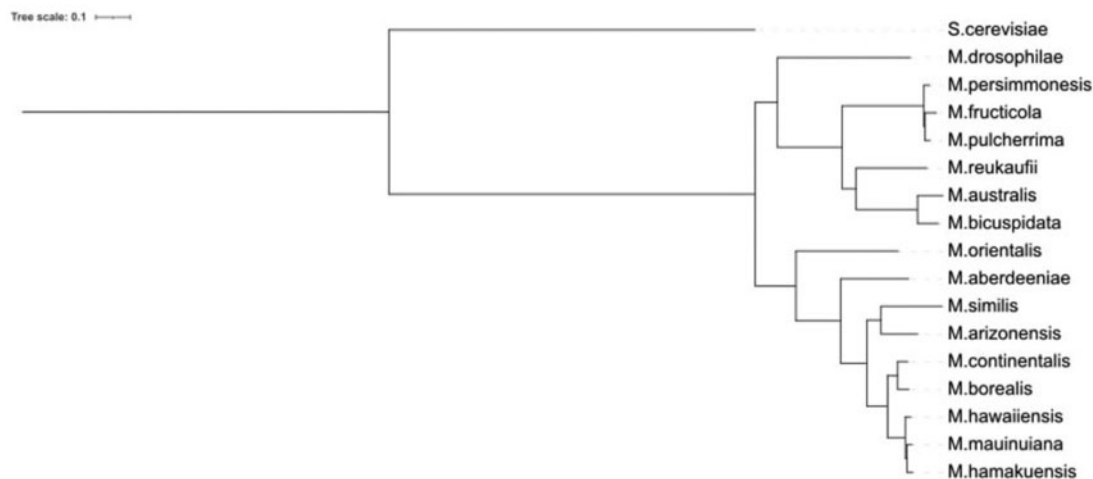


Figure 2 Phylogenetic relationship of 17 *Metschnikowia* strains. The whole genome-based phylogeny tree was constructed using 299 CDSs of available *Metschnikowia* species genome. Yeast model species *Saccharomyces cerevisiae* was used as an outgroup.

Data availability

The *M. persimmonesis* BioSample and BioProject are available at DDBJ/ENA/GenBank under the accession numbers SAMN14558164 and PRJNA623702, respectively. The final genome assembly, including the annotated genes, was deposited at DDBJ/ENA/GenBank under the accession number JACBPP000000000. The version described in this paper is version JACBPP010000000. Supplemental material available at figshare: <https://doi.org/10.25387/g3.14804679>.

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Conflicts of interest

The authors have declared there is no conflict of interest.

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