Complete Genome Sequence of the Biocontrol Agent Yeast *Rhodotorula kratochvilovae* Strain LS11

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ABSTRACT *Rhodotorula kratochvilovae* strain LS11 is a biocontrol agent (BCA) selected for its antagonistic activity against several plant pathogens both in the field and postharvest. Genome assembly includes 62 contigs for a total of 22.56 Mbp and a G+C content of 66.6%. Genome annotation predicts 7,642 protein-encoding genes.

*R*hodotorula kratochvilovae (formerly known as *Rhodosporidium kratochvilovae* and subjected to reclassification [1]) strain LS11 is a biocontrol agent red yeast isolated from olives of a local southern Italian cultivar, Gentile di Larino (2). This strain was selected among many environmental isolates for its high antagonistic activity against the postharvest pathogens *Botrytis cinerea* and *Penicillium expansum* (3). Due to toxicological, ethical, and technical concerns related to the use of chemical fungicides, biological and integrated control of plant pathogens both in the field and on stored fruit has been an active field of research over the past two decades (4–6). Our studies revealed that *R. kratochvilovae* LS11 exerts its antagonistic activity through competition for nutrients and space, a primary mechanism based on resistance to oxidative stress and timely colonization of fruit tissue wounds, the main sites of penetration of fungal pathogens into the host, accompanied by the production of lytic enzymes that degrade pathogen cell walls (3, 7, 8). *R. kratochvilovae* LS11 is compatible with food grade compounds that enhance its antagonistic activity (9, 10), and it is tolerant to fungicides used in postharvest, thus being suitable for integrated control (11–13). Moreover, *R. kratochvilovae* LS11 is able to resist and degrade patulin (14), a hazardous mycotoxin produced by *P. expansum* (15, 16), through two independent pathways that form the breakdown products ascladiol and desoxypatulinic acid (14, 17–21).

Whole-genome sequencing was performed by Macrogen using PacBio sequencing technology, with a starting data set of ~150,000 reads ranging from 35 bp to 43,052 bp for a total of 837 million sequenced bases. Trimmed reads were subjected to k-mer analysis (22) that revealed high homozygosity and an estimated genome size of 22.56 Mbp. *De novo* genome assembly of PacBio-generated reads was performed using Canu (23), which generated 62 contigs covering 22.10 Mbp (97.96% of the predicted genome size). The largest contig measured 1.7 Mbp, and the N_{50} was 704 kbp. G+C content was 66.6%. The high quality of the assembly generated was confirmed using the software QUAST (24), by mapping back the reads to the assembly with the Burrows-Wheeler Aligner (BWA) (25), and with BUSCO (26). Genome annotation was performed using the software Augustus trained with model genes found by BUSCO in the assembly, and this first prediction was used as input for MAKER together with 8,294 proteins of *Rhodosporidium toruloides* available in GenBank (27–29). The output produced was used to retrain the software Augustus, and the procedure was repeated three times until the best sensitivity and specificity were achieved (0.97 and 0.99, respectively). The final gene prediction generated 7,642 gene models that were searched against the Uniprot (description found for 5,046 genes), KEGG (pathway annotation for 4,475 genes), PFAM (functional domain assigned to 5,319 proteins), and gene ontology (GO) (GO class

assigned to 5,234 sequences) databases. The availability of the genome sequence of *R. kratochvilovae* LS11 coupled with tools for genetic manipulation that we developed (30) represents a crucial step toward the understanding of the molecular mechanisms behind the biocontrol activity of this yeast and its ability to degrade the mycotoxin patulin.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number PQDI00000000. The version described in this paper is version PQDI01000000.

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