





High-Quality Draft Genome Sequence of the Causal Agent of the Current Panama Disease Epidemic

Rachel J. Warmington,^a William Kay,^b Aaron Jeffries,^b Paul O'Neill,^b Audrey Farbos,^b Karen Moore,^b  Daniel P. Bebber,^b  David J. Studholme^b

^aEden Project, Bodelva, United Kingdom

^bDepartment of Biosciences, University of Exeter, Exeter, United Kingdom

ABSTRACT We present a high-quality draft genome assembly for *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (*Fusarium odoratissimum*), assembled from PacBio reads and consisting of 15 contigs with a total assembly size of 48.59 Mb. This strain appears to belong to vegetative compatibility group complex 01213/16.

A scomycete fungus *Fusarium oxysporum* f. sp. *cubense* is the causal agent of Fusarium wilt (Panama disease), which decimated banana plantations in the 1950s (1–3). The tropical race 4 (TR4) variant of *F. oxysporum* f. sp. *cubense*, also known as *Fusarium odoratissimum* (4), is responsible for a current epidemic spreading through Asia, Africa, and Latin America (4–9). Here, we present a high-quality draft sequence of *F. oxysporum* TR4; previously available TR4 genome assemblies were assembled from short reads (10) and were highly fragmented. The data from a recently reported PacBio-based TR4 assembly are not publicly available (11).

We isolated *F. oxysporum* f. sp. *cubense* TR4 strain UK0001 from a symptomatic banana plant (*Musa* sp.) at the Eden Project (Cornwall, UK). Genomic DNA was extracted from microconidia (that is, asexual spores) cultured in potato dextrose broth, shaken at 140 rpm, and stored at 25°C for 5 days. Lysis buffer (500 μ l; 1% SDS, 100 mM Tris, 10 mM EDTA) was added to a pellet of filtered and washed spores before it was placed on a Vibrax for 30 mins at 2,500 rpm. Five hundred microliters of 25:24:1 phenol-chloroform-isoamyl alcohol was added to tubes before vortexing for 60 s and centrifuging for 10 min at 16,000 $\times g$. The resulting supernatant was mixed with 28 μ l cold 7.5 M ammonium acetate and 204 μ l cold isopropanol and incubated at –20°C overnight. The following day, the pellets were washed twice in 70% ethanol and twice in 96% ethanol before tubes were inverted and air dried for 60 min at room temperature. Pellets were resuspended in 40 μ l MilliQ water and treated with RNase I_f (Biolabs, UK).

The genome was sequenced using one PacBio single-molecule real-time (SMRT) cell with v3.0 chemistry. Libraries were prepared with the SMRTbell Express template preparation kit and size selected with a 15-kb cutoff using BluePippin (Sage Science, MA, USA). Additionally, we used the Illumina MiSeq system to sequence paired-end libraries prepared with the NEXTFLEX 8-barcode kit (Perkin Elmer), generating 2,889,905 pairs of 300-bp reads.

A total of 723,327 filtered PacBio subreads (N_{50} length, 19,851 bp) were assembled using the Hierarchical Genome Assembly Process (HGAP) v4 in SMRT Link v7.0.0.63985 (12). Illumina sequence reads were not used in the assembly. The assembly yielded 15 contigs with a total assembly size of 48,588,396 bp (47.54% G+C content, N_{50} length of 4,494,293 bp). Completeness was estimated using the Sordariomycetes data set in Benchmarking Universal Single-Copy Orthologs (BUSCO) v3.0.2 (13, 14). Of 3,725 target genes, 3,676 (98.6%) occurred as intact and single copies, similar to the 3,673 in a recent PacBio-based assembly of an *F. oxysporum* f. sp. *cubense* race 1 genome (15).

Citation Warmington RJ, Kay W, Jeffries A, O'Neill P, Farbos A, Moore K, Bebber DP, Studholme DJ. 2019. High-quality draft genome sequence of the causal agent of the current Panama disease epidemic. *Microbiol Resour Announc* 8:e00904-19. <https://doi.org/10.1128/MRA.00904-19>.

Editor Antonis Rokas, Vanderbilt University

Copyright © 2019 Warmington et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to David J. Studholme, dj.studholme@exeter.ac.uk.

Received 13 August 2019

Accepted 16 August 2019

Published 5 September 2019

We used the MAKER pipeline (16, 17) v2.31.10, including AUGUSTUS (18) v3.1 for *ab initio* gene prediction with the “*Fusarium*” species option and homology evidence from a set of 53,031 *F. oxysporum* f. sp. *cubense* proteins from the NCBI Proteins database (19) after soft masking by RepeatMasker v4.0.7 against the “Ascomycota” section of RepBase (20–22). This predicted 14,472 protein-coding genes. BLASTN searches against discriminative sequences (23) suggest that UK0001 belongs to vegetative compatibility group complex 01213/16.

Data availability. These data are deposited at DDBJ/ENA/GenBank under the accession number [VMNF000000000](https://www.ncbi.nlm.nih.gov/nuclseq/VMN000000000) and the Sequence Read Archive (24) under Bio-Project accession number [PRJNA556111](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA556111).

ACKNOWLEDGMENTS

This work was supported by a grant from the Gatsby Charitable Foundation, titled “Banana genetic resources at Eden Project.” Sequencing infrastructure was supported by a Medical Research Council Clinical Infrastructure award (MR/M008924/1), the Wellcome Trust Institutional Strategic Support Fund (WT097835MF), a Wellcome Trust Multi-user Equipment Grant (WT101650MA), and a BBSRC Longer and Larger (LOLA) award (BB/K003240/1).

We acknowledge the University of Exeter Sequencing Service and high-performance computing facilities (Isca) at the University of Exeter.

REFERENCES

- Dita M, Barquero M, Heck D, Mizubuti ESG, Staver CP. 2018. Fusarium wilt of banana: current knowledge on epidemiology and research needs toward sustainable disease management. *Front Plant Sci* 9:1468. <https://doi.org/10.3389/fpls.2018.01468>.
- Ordóñez N, Seidl MF, Waalwijk C, Drenth A, Kilian A, Thomma BPHJ, Ploetz RC, Kema GHJ. 2015. Worse comes to worst: bananas and Panama disease—when plant and pathogen clones meet. *PLoS Pathog* 11: e1005197. <https://doi.org/10.1371/journal.ppat.1005197>.
- Ploetz RC. 2015. Fusarium wilt of banana. *Phytopathology* 105: 1512–1521. <https://doi.org/10.1094/PHYTO-04-15-0101-RVW>.
- Maryani N, Lombard L, Poerba YS, Subandiyah S, Crous PW, Kema GHJ. 2019. Phylogeny and genetic diversity of the banana Fusarium wilt pathogen *Fusarium oxysporum* f. sp. *cubense* in the Indonesian centre of origin. *Stud Mycol* 92:155–194. <https://doi.org/10.1016/j.simyco.2018.06.003>.
- Butler D. 2013. Fungus threatens top banana. *Nature* 504:195–196. <https://doi.org/10.1038/504195a>.
- Stokstad E. 2019. Banana fungus puts Latin America on alert. *Science* 365:207–208. <https://doi.org/10.1126/science.365.6450.207>.
- Zheng S-J, García-Bastidas FA, Li X, Zeng L, Bai T, Xu S, Yin K, Li H, Fu G, Yu Y, Yang L, Nguyen HC, Douangboupha B, Khaing AA, Drenth A, Seidl MF, Meijer HJG, Kema GHJ. 2018. New geographical insights of the latest expansion of *Fusarium oxysporum* f.sp. *cubense* tropical race 4 into the greater Mekong subregion. *Front Plant Sci* 9:1–9. <https://doi.org/10.3389/fpls.2018.00457>.
- Warman NM, Aitken EAB. 2018. The movement of *Fusarium oxysporum* f. sp. *cubense* (sub-tropical race 4) in susceptible cultivars of banana. *Front Plant Sci* 9:1748. <https://doi.org/10.3389/fpls.2018.01748>.
- Karangwa P, Mostert D, Ndayihanzamaso P, Dubois T, Niere B, zum Felde A, Schouten A, Blomme G, Beed F, Viljoen A. 2018. Genetic diversity of *Fusarium oxysporum* f. sp. *cubense* in East and Central Africa. *Plant Dis* 102:552–560. <https://doi.org/10.1094/PDIS-02-17-0282-RE>.
- Guo L, Han L, Yang L, Zeng H, Fan D, Zhu Y, Feng Y, Wang G, Peng C, Jiang X, Zhou D, Ni P, Liang C, Liu L, Wang J, Mao C, Fang X, Peng M, Huang J. 2014. Genome and transcriptome analysis of the fungal pathogen *Fusarium oxysporum* f. sp. *cubense* causing banana vascular wilt disease. *PLoS One* 9:e95543. <https://doi.org/10.1371/journal.pone.0095543>.
- Yun Y, Song A, Bao J, Chen S, Lu S, Cheng C, Zheng W, Wang Z, Zhang L. 2019. Genome data of *Fusarium oxysporum* f. sp. *cubense* race 1 and tropical race 4 isolates using long-read sequencing. *Mol Plant Microbe Interact* MPMI03190063A. <https://doi.org/10.1094/MPMI-03-19-0063-A>.
- Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <https://doi.org/10.1038/nmeth.2474>.
- Waterhouse RM, Seppey M, Simão FA, Manni M, Ioannidis P, Klioutchnikov G, Kriventseva EV, Zdobnov EM. 2017. BUSCO applications from quality assessments to gene prediction and phylogenomics. *Mol Biol Evol* 35:543–548. <https://doi.org/10.1093/molbev/msx319>.
- Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31:3210–3212. <https://doi.org/10.1093/bioinformatics/btv351>.
- Asai S, Ayukawa Y, Gan P, Masuda S, Komatsu K, Shirasu K, Arie T. 2019. High-quality draft genome sequence of *Fusarium oxysporum* f. sp. *cubense* strain 160527, a causal agent of Panama disease. *Microbiol Resour Announc* 8:27–29. <https://doi.org/10.1128/MRA.00654-19>.
- Cantarel BL, Korf I, Robb SMC, Parra G, Ross E, Moore B, Holt C, Sánchez Alvarado A, Yandell M. 2008. MAKER: an easy-to-use annotation pipeline designed for emerging model organism genomes. *Genome Res* 18: 188–196. <https://doi.org/10.1101/gr.6743907>.
- Campbell MS, Holt C, Moore B, Yandell M. 2014. Genome annotation and curation using MAKER and MAKER-P. *Curr Protoc Bioinformatics* 48: 4.11.1–4.11.39. <https://doi.org/10.1002/0471250953.bi0411s48>.
- Stanke M, Schöffmann O, Morgenstern B, Waack S. 2006. Gene prediction in eukaryotes with a generalized hidden Markov model that uses hints from external sources. *BMC Bioinformatics* 7:62. <https://doi.org/10.1186/1471-2105-7-62>.
- NCBI Resource Coordinators. 2016. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* 44:D7–D19. <https://doi.org/10.1093/nar/gkv1290>.
- Tarailo-Graovac M, Chen N. 2009. Using RepeatMasker to identify repetitive elements in genomic sequences. *Curr Protoc Bioinformatics* Chapter 4:Unit 4.10. <https://doi.org/10.1002/0471250953.bi0410s25>.
- Jurka J. 2000. Repbase Update: a database and an electronic journal of repetitive elements. *Trends Genet* 16:418–420. [https://doi.org/10.1016/S0168-9525\(00\)02093-X](https://doi.org/10.1016/S0168-9525(00)02093-X).
- Kapitonov VV, Jurka J. 2008. A universal classification of eukaryotic transposable elements implemented in Repbase. *Nat Rev Genet* 9:411–412. <https://doi.org/10.1038/nrg2165-c1>.
- Czislowski E, Fraser-Smith S, Zander M, O’Neill WT, Meldrum RA, Tran-Nguyen LTT, Batley J, Aitken EAB. 2018. Investigation of the diversity of effector genes in the banana pathogen, *Fusarium oxysporum* f. sp. *cubense*, reveals evidence of horizontal gene transfer. *Mol Plant Pathol* 19:1155–1171. <https://doi.org/10.1111/mpp.12594>.
- Leinonen R, Sugawara H, Shumway M. 2011. The Sequence Read Archive. *Nucleic Acids Res* 39:D19–D21. <https://doi.org/10.1093/nar/gkq1019>.