



Draft Genome Sequence of an Antarctic Isolate of the Black Yeast Fungus *Exophiala mesophila*

Claudia Coleine,^a Laura Selbmann,^{a,b} Sawyer Masonjones,^c Silvano Onofri,^a Laura Zucconi,^a  Jason E. Stajich^c

^aDepartment of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy

^bMycological Section, Italian National Antarctic Museum (MNA), Genoa, Italy

^cDepartment of Microbiology and Plant Pathology, University of California—Riverside, Riverside, California, USA

ABSTRACT A 30.43-Mb draft genome sequence with 10,355 predicted protein-coding genes was produced for the ascomycete fungus *Exophiala mesophila* strain CCFEE 6314, a black yeast isolated from Antarctic cryptoendolithic communities. The sequence will be of importance for identifying differences among extremophiles and mesophiles and cataloguing the global population diversity of this organism.

Black yeasts are a polyphyletic morphoecological group of fungi classified in either the Chaetothyriales order of class Eurotiomycetes or class Dothideomycetes (phylum Ascomycota; subphylum Pezizomycotina). They are distinguished by high melanin content, thick and multilayered cell walls, and an extraordinary ability to survive in extremes and tolerate chemical and physical stresses, such as extreme pH and temperatures, desiccation, UV ionizing radiation, and alpha particles (1–8).

Within the Herpotrichiellaceae family (Chaetothyriales), there are many recognized species in the genus *Exophiala* which are adapted to a multitude of ecological niches, including human environments (9, 10). Isolates from oligotrophic water sources, such as sinks, drainpipes, swimming pools, bathing facilities, and drinking water, have been described (11, 12). Species in this genus have been explored for their potential in bioremediation applications (13, 14), and several species have been isolated from glaciers (15) and microbial ecosystems specialized to extreme temperature and aridity, such as Antarctic endolithic communities (16–18). We assembled a draft genome sequence of an Antarctic strain to provide resources for comparative studies of adaptation and evolution of this intriguing group of fungi.

Exophiala mesophila strain CCFEE 6314 was provided by the Culture Collection of Fungi from Extreme Environments (CCFEE) of the Mycological Section of the Italian Antarctic National Museum. The culture was isolated from a cryptoendolithic community at Mt. Billing (71°15'S, 163°00'E) on continental Antarctica. The rock sample was collected using a sterile chisel and preserved at –20°C until the strain was isolated by directly plating fragments of colonized rock on petri dishes containing 2% malt extract agar (MEA). The pure culture was grown on 2% MEA medium plates for 6 weeks at 10°C and DNA extracted from the total biomass following the cetyltrimethylammonium bromide (CTAB) protocol (19). Melanin was removed through two phenol-chloroform purification steps. Genomic DNA was sheared with Covaris S220 ultrasonicator and sequencing library constructed using a NeoPrep TruSeq Nano DNA sample prep kit (Illumina) in the University of California—Riverside Genomics Core.

A total of 2.9 million 2 × 300-bp paired-end sequence reads were obtained from a multiplexed library from one Illumina MiSeq flow cell. A quality check of reads was performed with FastQC (v0.11.3) (20), followed by genome assembly with MaSuRCA (v2.3.2) (21), using default parameters (cgwErrorRate = 0.15), which included quality-based read trimming and corrections. Trimmed reads averaged 198 bp. Genome scaffolding was performed with SOAPdenovo2 (v2.0.4) (22) and scaffolds were ordered and oriented with SSPACE (v2.0) (23). The draft genome was annotated with Prokka (v1.11.2) (24) and the protein-coding genes were predicted with GeneMark-ES (v3.82) (25). The draft genome sequence and annotation are available in GenBank (SRR1011421) and NCBI BioProject (PRJNA501142).

Citation Coleine C, Selbmann L, Masonjones S, Onofri S, Zucconi L, Stajich JE. 2019. Draft genome sequence of an Antarctic isolate of the black yeast fungus *Exophiala mesophila*. *Microbiol Resour Announc* 8:e00142-19. <https://doi.org/10.1128/MRA.00142-19>.

Editor Antonis Rokas, Vanderbilt University

Copyright © 2019 Coleine 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 Laura Selbmann, selbmann@unitus.it, or Jason E. Stajich, jason.stajich@ucr.edu.

Received 7 February 2019

Accepted 15 April 2019

Published 9 May 2019

folds were filtered of vector contamination with Sequin (v15.10) (<https://www.ncbi.nlm.nih.gov/Sequin/>) and redundant scaffolds eliminated if completely aligned with at least 95% identity to a longer contig using MUMmer (v3.23) (22) as implemented in “funannotate clean” in Funannotate (v0.5.5) (23). The assembly was 30.43 Mb in total length (number of contigs, 207; N_{50} value, 522 kb; maximum contig size, 1.43 Mb; L_{50} value, 20; GC content, 50%; coverage, 54×).

The Funannotate (v0.5.5) (23) pipeline was used to annotate the genome. Briefly, consensus gene models were produced by EvidenceModeler (EVM) (24) using *ab initio* predictions from AUGUSTUS (v3.2.2) (25) and GeneMark.hmm-ES (v4.32) (26) combined with protein-to-genome alignments from Exonerate (v2.2.0) (27). Self-training for GeneMark.hmm-ES was performed using default parameters, AUGUSTUS was trained with alignments of the BUSCO ascomycota_odb9 data set (v9) (28), and prediction parameters were archived (29). Protein annotations were assigned by similarity to Pfam (30) and CAZy domains (31, 32) using HMMER (3.1b2) (33), MEROPS (34), eggNOG (v4.5) (35), InterProScan (v5.20-59.0) (36), and Swiss-Prot (37) by BLASTP (v2.5.0+) (38) searches using Funannotate defaults. A total of 10,355 protein-coding genes were predicted and prepared for GenBank submission by the Genome Annotation Generator (39).

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [NAJM000000000](https://www.ncbi.nlm.nih.gov/nuccore/NAJM000000000). The version described in this paper is the first version, NAJM01000000. The Illumina sequence reads were released under SRA accession number [SRR5223779](https://www.ncbi.nlm.nih.gov/sra/SRR5223779) and associated with BioProject number [PRJNA342238](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA342238).

ACKNOWLEDGMENTS

The Italian Antarctic National Museum (MNA) is kindly acknowledged for financial support to the Mycological Section on the MNA for providing the strains sequenced in this study and stored in the Culture Collection of Fungi from Extreme Environments (CCFEE), University of Tuscia, Italy.

L.S., C.C., and L.Z. kindly acknowledge the Italian National Program for Antarctic Researches (PNRA) for funding sampling campaigns and the research activities in Italy. Sequencing costs were supported through the U.S. Department of Agriculture-National Institute of Food and Agriculture Hatch project CA-R-PPA-5062-H to J.E.S. Data analyses were performed on the High-Performance Computing Cluster at the University of California—Riverside in the Institute of Integrative Genome Biology supported by NSF grant DBI-1429826 and NIH grant S10-OD016290.

REFERENCES

- Dadachova E, Casadevall A. 2008. Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin. *Curr Opin Microbiol* 11: 525–531. <https://doi.org/10.1016/j.mib.2008.09.013>.
- Onofri S, Barreca D, Selbmann L, Isola D, Rabbow E, Horneck G, de Vera JPP, Hatton J, Zucconi L. 2008. Resistance of Antarctic black fungi and cryptoendolithic communities to simulated space and Martian conditions. *Stud Mycol* 61:99–109. <https://doi.org/10.3114/sim.2008.61.10>.
- Onofri S, de la Torre R, de Vera J-P, Ott S, Zucconi L, Selbmann L, Scalzi G, Venkateswaran KJ, Rabbow E, Sánchez Iñigo FJ, Horneck G. 2012. Survival of rock-colonizing organisms after 1.5 years in outer space. *Astrobiology* 12:508–516. <https://doi.org/10.1089/ast.2011.0736>.
- Onofri S, de Vera J-P, Zucconi L, Selbmann L, Scalzi G, Venkateswaran KJ, Rabbow E, de la Torre R, Horneck G. 2015. Survival of Antarctic cryptoendolithic fungi in simulated Martian conditions on board the International Space Station. *Astrobiology* 15:1052–1059. <https://doi.org/10.1089/ast.2015.1324>.
- Selbmann L, Isola D, Zucconi L, Onofri S. 2011. Resistance to UV-B induced DNA damage in extreme-tolerant cryptoendolithic Antarctic fungi: detection by PCR assays. *Fungal Biol* 115:937–944. <https://doi.org/10.1016/j.funbio.2011.02.016>.
- Pacelli C, Selbmann L, Zucconi L, De Vera J-P, Rabbow E, Horneck G, de la Torre R, Onofri S. 2017. BIOMEX experiment: ultrastructural alterations, molecular damage and survival of the fungus *Cryomyces antarcticus* after the experiment verification tests. *Orig Life Evol Biosph* 47:187–202. <https://doi.org/10.1007/s11084-016-9485-2>.
- Pacelli C, Selbmann L, Moeller R, Zucconi L, Fujimori A, Onofri S. 2017. Cryptoendolithic Antarctic black fungus *Cryomyces antarcticus* irradiated with accelerated helium ions: survival and metabolic activity, DNA and ultrastructural damage. *Front Microbiol* 8:2002. <https://doi.org/10.3389/fmicb.2017.02002>.
- Pacelli C, Selbmann L, Zucconi L, Raguse M, Moeller R, Shuryak I, Onofri S. 2017. Survival, DNA integrity, and ultrastructural damage in Antarctic cryptoendolithic eukaryotic microorganisms exposed to ionizing radiation. *Astrobiology* 17:126–135. <https://doi.org/10.1089/ast.2015.1456>.
- Porteous NB, Grooters AM, Redding SW, Thompson EH, Rinaldi MG, De Hoog GS, Sutton DA. 2003. Identification of *Exophiala mesophila* isolated from treated dental unit waterlines. *J Clin Microbiol* 41:3885–3889. <https://doi.org/10.1128/JCM.41.8.3885-3889.2003>.
- Gostiñar C, Zajc J, Lenassi M, Plemenitaš A, de Hoog S, Al-Hatmi AM, Gunde-Cimerman N. 2018. Fungi between extremotolerance and opportunistic pathogenicity on humans. *Fungal Divers* 93:195–213. <https://doi.org/10.1007/s13225-018-0414-8>.
- Göttlich E, van der Lubbe W, Lange B, Fiedler S, Melchert I, Reifenrath M, Flemming H-C, de Hoog S. 2002. Fungal flora in groundwater-derived

- public drinking water. *Int J Hyg Environ Health* 205:269–279. <https://doi.org/10.1078/1438-4639-00158>.
12. Matos T, de Hoog GS, de Boer AG, de Crom I, Haase G. 2002. High prevalence of the neurotropic *Exophiala dermatitidis* and related oligotrophic black yeasts in sauna facilities. *Mycoses* 45:373–377. <https://doi.org/10.1046/j.1439-0507.2002.00779.x>.
 13. Isola D, Selbmann L, de Hoog GS, Fenice M, Onofri S, Prenafeta-Boldú FX, Zucconi L. 2013. Isolation and screening of black fungi as degraders of volatile aromatic hydrocarbons. *Mycopathologia* 175:369–379. <https://doi.org/10.1007/s11046-013-9635-2>.
 14. Blasi B, Tafer H, Tesei D, Sterflinger K. 2015. From glacier to sauna: RNA-seq of the human pathogen black fungus *Exophiala dermatitidis* under varying temperature conditions exhibits common and novel fungal response. *PLoS One* 10:e0127103. <https://doi.org/10.1371/journal.pone.0127103>.
 15. Branda E, Turchetti B, Diolaiuti G, Pecci M, Smiraglia C, Buzzini P. 2010. Yeast and yeast-like diversity in the southernmost glacier of Europe (Calderone Glacier, Apennines, Italy). *FEMS Microbiol Ecol* 72:354–369. <https://doi.org/10.1111/j.1574-6941.2010.00864.x>.
 16. Selbmann L, Onofri S, Coleine C, Buzzini P, Canini F, Zucconi L. 2017. Effect of environmental parameters on biodiversity of the fungal component in lithic Antarctic communities. *Extremophiles* 21:1069–1080. <https://doi.org/10.1007/s00792-017-0967-6>.
 17. Coleine C, Stajich J, Zucconi L, Onofri S, Pombubpa N, Egidio E, Franks A, Buzzini P, Selbmann L. 2018. Antarctic cryptoendolithic fungal communities are highly adapted and dominated by Lecanoromycetes and Dothideomycetes. *Front Microbiol* 9:1392. <https://doi.org/10.3389/fmicb.2018.01392>.
 18. Coleine C, Zucconi L, Onofri S, Pombubpa N, Stajich JE, Selbmann L. 2018. Sun exposure shapes functional grouping of fungi in cryptoendolithic Antarctic communities. *Life* 8:19. <https://doi.org/10.3390/life8020019>.
 19. Fulton TM, Chunwongse J, Tanksley SD. 1995. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. *Plant Mol Biol Rep* 13:207–209. <https://doi.org/10.1007/BF02670897>.
 20. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
 21. Zimin AV, Marçais G, Pui D, Roberts M, Salzberg SL, Yorke JA. 2013. The MaSuRCA genome assembler. *Bioinformatics* 29:2669–2677. <https://doi.org/10.1093/bioinformatics/btt476>.
 22. Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL. 2004. Versatile and open software for comparing large genomes. *Genome Biol* 5:R12. <https://doi.org/10.1186/gb-2004-5-2-r12>.
 23. Palmer J, Stajich JE. 2017. Funannotate: eukaryotic genome annotation pipeline. <https://funannotate.readthedocs.io/en/latest/>.
 24. Haas BJ, Salzberg SL, Zhu W, Pertea M, Allen JE, Orvis J, White O, Buell CR, Wortman JR. 2008. Automated eukaryotic gene structure annotation using EVIDENCEModeler and the program to assemble spliced alignments. *Genome Biol* 9:R7. <https://doi.org/10.1186/gb-2008-9-1-r7>.
 25. Stanke M, Keller O, Gunduz I, Hayes A, Waack S, Morgenstern B. 2006. AUGUSTUS: ab initio prediction of alternative transcripts. *Nucleic Acids Res* 34:W435–W439. <https://doi.org/10.1093/nar/gkl200>.
 26. Ter-Hovhannisyanyan V, Lomsadze A, Chernoff YO, Borodovsky M. 2008. Gene prediction in novel fungal genomes using an ab initio algorithm with unsupervised training. *Genome Res* 18:1979–1990. <https://doi.org/10.1101/gr.081612.108>.
 27. Slater GS, Birney E. 2005. Automated generation of heuristics for biological sequence comparison. *BMC Bioinformatics* 6:31. <https://doi.org/10.1186/1471-2105-6-31>.
 28. 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>.
 29. Stajich JE. 2018. Fungi gene prediction parameters. <https://github.com/hyphal-tip/fungi-gene-prediction-params>.
 30. Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J, Sonnhammer ELL, Tate J, Punta M. 2014. Pfam: the protein families database. *Nucleic Acids Res* 42:D222–D230. <https://doi.org/10.1093/nar/gkt1223>.
 31. Huang L, Zhang H, Wu P, Entwistle S, Li X, Yohe T, Yi H, Yang Z, Yin Y. 2018. dbCAN-seq: a database of carbohydrate-active enzyme (CAZyme) sequence and annotation. *Nucleic Acids Res* 46:D516–D521. <https://doi.org/10.1093/nar/gkx894>.
 32. Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. 2014. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res* 42:D490–D495. <https://doi.org/10.1093/nar/gkt1178>.
 33. Eddy SR. 2011. Accelerated profile HMM searches. *PLoS Comput Biol* 7:e1002195. <https://doi.org/10.1371/journal.pcbi.1002195>.
 34. Rawlings ND, Barrett AJ, Bateman A. 2014. Using the MEROPS database for proteolytic enzymes and their inhibitors and substrates. *Curr Protoc Bioinformatics* 48:1.25–1.33. <https://doi.org/10.1002/0471250953.bi0125s48>.
 35. Huerta-Cepas J, Szklarczyk D, Forslund K, Cook H, Heller D, Walter MC, Rattei T, Mende DR, Sunagawa S, Kuhn M, Jensen LJ, von Mering C, Bork P. 2016. eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. *Nucleic Acids Res* 44:D286–D293. <https://doi.org/10.1093/nar/gkv1248>.
 36. Jones P, Binns D, Chang H-Y, Fraser M, Li W, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G, Pesseat S, Quinn AF, Sangrador-Vegas A, Scheremetjew M, Yong S-Y, Lopez R, Hunter S. 2014. InterProScan 5: genome-scale protein function classification. *Bioinformatics* 30:1236–1240. <https://doi.org/10.1093/bioinformatics/btu031>.
 37. Boutet E, Lieberherr D, Tognolli M, Schneider M, Bansal P, Bridge AJ, Poux S, Bougueleret L, Xenarios I. 2016. UniProtKB/Swiss-Prot, the manually annotated section of the UniProt KnowledgeBase: how to use the entry view. *Methods Mol Biol* 1374:23–54. https://doi.org/10.1007/978-1-4939-3167-5_2.
 38. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402. <https://doi.org/10.1093/nar/25.17.3389>.
 39. Hall B, DeRego T, Geib S. 2014. GAG: the Genome Annotation Generator. <https://genomeannotation.github.io/GAG/>.