UC Riverside UC Riverside Previously Published Works

Title

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

Permalink https://escholarship.org/uc/item/22k8w90p

Journal Microbiology Resource Announcements, 8(19)

ISSN 2169-8287

Authors

Coleine, Claudia Selbmann, Laura Masonjones, Sawyer <u>et al.</u>

Publication Date

2019-05-09

DOI

10.1128/mra.00142-19

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <u>https://creativecommons.org/licenses/by/4.0/</u>

Peer reviewed





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 D 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 proteincoding 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 scaf-

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

Citation Coleine C, Selbmann L, Masonjones S,

Copyright © 2019 Coleine et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

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 NAJM00000000. The version described in this paper is the first version, NAJM01000000. The Illumina sequence reads were released under SRA accession number SRR5223779 and associated with BioProject number 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.
- Gostinčar 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.
- 11. 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.

- Matos T, de Hoog GS, de Boer AG, de Crom I, Haase G. 2002. High prevalence of the neurotrope *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.
- 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.
- 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.
- 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.
- 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.
- Coleine C, Stajich J, Zucconi L, Onofri S, Pombubpa N, Egidi 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.
- 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.
- 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.
- Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. https://www.bioinformatics.babraham.ac.uk/projects/ fastqc/.
- Zimin AV, Marçais G, Puiu D, Roberts M, Salzberg SL, Yorke JA. 2013. The MaSuRCA genome assembler. Bioinformatics 29:2669–2677. https://doi .org/10.1093/bioinformatics/btt476.
- 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/.
- 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.

- Ter-Hovhannisyan 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.
- 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.
- 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.
- Stajich JE. 2018. Fungi gene prediction parameters. https://github.com/ hyphaltip/fungi-gene-prediction-params.
- 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.
- 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.
- 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.
- Eddy SR. 2011. Accelerated profile HMM searches. PLoS Comput Biol 7:e1002195. https://doi.org/10.1371/journal.pcbi.1002195.
- 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.
- 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.
- 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.
- 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.
- 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/.