**Draft Genome Sequence of the Ant-Associated Fungus Phialophora attae (CBS 131958)**

Leandro F. Moreno, J. Benjamin Stielow, Michel de Vries, Vinicius A. Weiss, Vania A. Vicente, Sybren de Hoog

CBS, KNWA, Fungal Biodiversity Centre, Utrecht, The Netherlands; Department of Pathology, Federal University of Paraná, Curitiba, Paraná, Brazil; Laboratory of Bioinformatics, Federal University of Paraná, Professional and Technological Education Sector, Curitiba, Paraná, Brazil

The black yeast *Phialophora attae* was isolated from the cuticle of tropical ant gynes. The ant-fungus association is sustained due to symbiotic evolutionary adaptations that allow fungal assimilation and tolerance of toxic compounds produced by the ant. The genome sequence of the first ant-associated fungus, *P. attae*, is presented here.

**B**lack yeast-like fungi, which are members of the order *Chaetothyriales*, are commonly found to colonize hostile environments. They have a competitive advantage in habitats with a scarcity of nutrients or that are contaminated with toxic hydrocarbons (1); adaptations enhancing the tolerance of extreme conditions allow these fungi to thrive in habitats where eutrophic saprobes are not regularly present (2). For example, black yeasts have been isolated from deserted mine shafts, from industrial biofilters, and repeatedly from ant nests or their exoskeletons (3, 4). Aromatic compounds produced by ants are toxic for most life forms and have antifungal and antibacterial properties (5). Cytolic lipids are involved in chemical communication and colony-mate recognition (6). The ant-fungus symbiosis probably originated 50 million years ago (7, 8). Interestingly, isolates of black yeasts similar to *Phialophora* located in basal and derived ant genera suggest this association might have been started early in the evolutionary history of this symbiosis (9).

*Phialophora attae* CBS 131958 was isolated from the cuticle of gynes of *Atta capigaua* (10). Phylogenetic analyses showed this ant-associated species is affiliated to the eupean clade (*Cyphellophoraceae*), which comprises members involved in human cutaneous and superficial infections and plant debris-inhabiting species (11).

The strain *P. attae* CBS 131958 was cultured in malt extract broth (MEB), with shaking at 150 rpm at 25°C for 7 days. DNA was extracted via a cetyltrimethylammonium bromide (CTAB)-based method and phenol-chloroform/isoamyl alcohol. Total DNA was purified with the DNeasy blood and tissue kit (Qiagen). Two hundred-base-read libraries were constructed using NEBNext fast DNA fragmentation and library prep kit for Ion Torrent (Thermo Fisher Scientific). Genomic sequence reads were generated on the Ion Torrent PGM platform (Template OT2 200 kit, Ion Sequencing 200 kit, and Ion Chip kit 318 V2; Thermo Fisher Scientific). The reads were assembled *de novo* using SPAdes version 3.5.0 (12) and Newbler version 2.6 (Roche). The draft comprises 139 contigs, with an N₅₀ of 959,784 bp. The genome size was estimated to be 30.4 Mb, with a G+C content of 53.56%. Repetitive elements were identified in the assembly using RepeatMasker (http://www.repeatmasker.org) and RepeatModeler (http://www.repeatmasker.org/RepeatModeler.html). Protein-coding genes were predicted with GeneMark-ES (13). Gene product names for 11,853 predicted genes were assigned based on top blast hits against the UniProt Knowledgebase (14) and InterProScan (15) searches. The genome contains 53 tRNAs identified using tRNAscan-SE (16). The prediction of 15 secondary metabolite gene clusters were carried out by means of the antiSMASH Web server (http://antismash.secondarymetabolites.org/).

*P. attae* is the first ant-associated fully sequenced black yeast member. Information about its genome sequence might provide a better understanding of the genomic adaptations made to colonize environments rich in aromatic hydrocarbons.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession no. LFJN00000000. The version described in this paper is the first version, LFJN01000000.

**ACKNOWLEDGMENTS**

The NGS work at CBS-KNAW was funded by the European Community Research Infrastructures Program under FP7 called Synthesis of Systematic Resources, grant no. 226506-CP-CSA-Infra and partially supported by Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Ion Torrent sequencing was completed at CBS-KNAW Fungal Biodiversity Centre. We thank The Department of Biochemistry at the University of Paraná for the technical support.

**REFERENCES**

1. Prenafeta-Boldú FX, Summerbell R, Sybren de Hoog G. 2006. Fungi growing on aromatic hydrocarbons: biotechnology’s unexpected encounter with biohazard? FEMS Microbiol Rev 30:109–130. http://dx.doi.org/10.1111/j.1574-6976.2005.00007.x.

2. Vicente VA, Attili-Angelis D, Pie MR, Queiroz-Telles F, Cruz LM, Najafzadeh MJ, de Hoog GS, Zhao J, Pizzirani-Kleiner A. 2008. Environmental isolation of black yeast-like fungi involved in human infection. Stud Mycol 61:137–144. http://dx.doi.org/10.3114/sim.2008.61.14.
3. Voglmayr H, Mayer V, Maschwitz U, Moog J, Djieto-Lordon C, Blatrix R. 2011. The diversity of ant-associated black yeasts: insights into a newly discovered world of symbiotic interactions. Fungal Biol 115:1077–1091. http://dx.doi.org/10.1016/j.funbio.2010.11.006.
4. Pagnocca FC, Rodrigues A, Nagamoto NS, Bacci M. 2008. Yeasts and filamentous fungi carried by the gynes of leaf-cutting ants. Antonie Van Leeuwenhoek 94:517–526. http://dx.doi.org/10.1007/s10482-008-9268-5.
5. Schluns H, Crozier RH. 2009. Molecular and chemical immune defenses in ants (Hymenoptera: Formicidae). Myrmecological News 12:237–249.
6. Sigal Lahav VS, Hefetz A, Vander Meer RK. 1999. Direct behavioral evidence for hydrocarbons as ant recognition discriminators. Naturwissenschaften 86:246–249.
7. Chapela IH, Rehner SA, Schultz TR, Mueller UG. 1994. Evolutionary history of the symbiosis between fungus-growing ants and their fungi. Science 266:1691–1694. http://dx.doi.org/10.1126/science.266.5191.1691.
8. Mueller UG, Rehner SA, Schultz TR. 1998. The evolution of agriculture in ants. Science 281:2034–2038. http://dx.doi.org/10.1126/science.281.5385.2034.
9. Little AEF, Currie CR. 2007. Symbiotic complexity: discovery of a fifth symbiont in the attine ant-microbe symbiosis. Biol Lett 3:501–504. http://dx.doi.org/10.1098/rsbl.2007.0253.
10. Attili-Angelis D, Duarte APM, Pagnocca FC, Nagamoto NS, Vries Md, Stielow JB, Hoog G. 2014. Novel Phialophora species from leaf of cutting ants (tribe Attini). Fungal Divers 65:65–75.
11. De Hoog GS, Vicente VA, Najafzadeh MJ, Harrak MJ, Badali H, Seyedmousavi S. 2011. Waterborne Exophiala species causing disease in cold-blooded animals. Persoonia 27:46–72.
12. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. http://dx.doi.org/10.1089/cmb.2012.0021.
13. 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. http://dx.doi.org/10.1101/gr.081612.108.
14. UniProt Consortium. 2015. UniProt: a hub for protein information. Nucleic Acids Res 43:D204–D212.
15. Jones P, Binns D, Chang H-, 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. http://dx.doi.org/10.1093/bioinformatics/btu031.
16. Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25:955–964. http://dx.doi.org/10.1093/nar/25.5.955.