




Draft Genome Sequences of Clinical and Environmental Isolates of *Aspergillus tamaraii* from Colombia

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ABSTRACT *Aspergillus* is a very diverse genus of fungi that are common in the environment and can affect human health. Here, we report the draft genome sequences of two Colombian isolates of *Aspergillus tamaraii*, an emerging pathogenic species. One isolate was obtained from an infected patient and the other from the environment in a hospital.

The genus *Aspergillus* is a group of opportunistic fungi that cause infections with high morbimortality in immunocompromised hosts (1). Approximately 350 species have been described in this genus, classified into 7 subgenera and 22 sections (2, 3). In Colombia, *A. fumigatus* is one of the most frequently isolated species causing infection (4). In recent years, the isolation of other *Aspergillus* species in hospital environments has been reported; however, the relationship between environmental and clinical isolates had not been established (5). *Aspergillus tamaraii* is an emerging pathogenic species of the section *Flavi*, which has been associated with a wide spectrum of clinical manifestations (6). Thus, the aim of this work was to characterize the genotypes of *Aspergillus tamaraii* isolates obtained from a Colombian hospital (clinical and environmental sources).

First, we collected two Colombian samples of *Aspergillus tamaraii*, one from a patient with an infected wound and the other from the indoor environment of the same hospital. The isolates were identified according to their macroscopic and microscopic characteristics in peptone-dextrose agar (PDA), malt extract agar (MEA), and Czapek yeast autolysate (CYA) agar culture media, as described by Samson and coworkers (2). The isolates were cultured in brain heart infusion (BHI) medium supplemented with 1% glucose at 20°C and 120 rpm. The biomass was collected during the exponential growth phase after 96 h of incubation. Genomic DNA for sequencing was prepared from mycelium culture using phenol-chloroform extraction (7). Approximately 1 µg of DNA (optical density at 260/289 nm [OD_{260/289}] ratio, 1.8 to 2.0) was used to prepare 170- to 800-bp libraries, and 150-bp paired-end sequencing was performed using the Illumina HiSeq 4000 platform. A total of 13,498,096 raw reads were generated for strain UdeA_At2 and 13,602,390 for strain UdeA_Afl2. The low-quality reads (1.17 and 1.3%, respectively) and adapter sequences were removed after FastQC v0.11.5 analysis with default settings (8). The high-quality (Q score, >30) reads were assembled *de novo* using the SPAdes v3.10 pipeline with the BayesHammer module for error correction, iterative k-mer lengths (21, 33, 55, and 77 bp), and the “careful” option (9). Scaffolds smaller than 500 bp were filtered out. The draft genome assembly quality was analyzed with QUAST v4.5 using default settings (10). The

Citation Gómez OM, Freyle CG, Torres S, Rúa ÁL, Tamayo DP, McEwen JG, Borges CL, Hernández O. 2020. Draft genome sequences of clinical and environmental isolates of *Aspergillus tamaraii* from Colombia. Microbiol Resour Announc 9:e01514-19. <https://doi.org/10.1128/MRA.01514-19>.

Editor Christina A. Cuomo, Broad Institute

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Received 10 December 2019

Accepted 12 March 2020

Published 2 April 2020

TABLE 1 Summary of assembly statistics

Sample	Species	Source	Genome size (Mb)	No. of reads	No. of scaffolds	Scaffold N_{50} (bp)	Largest scaffold (Mb)	Coverage (×)	No. of genes	G+C content (%)	Accession no.
UdeA_At2	<i>Aspergillus tamarii</i>	Hospital environment (air)	38.2	13,498,096	911	101,804	0.53	32	13,771	47.62	VYTV000000000
UdeA_Af2	<i>Aspergillus tamarii</i>	Left foot biopsy	37.7	13,602,390	593	145,682	0.80	33	13,630	47.63	VYTW000000000

assembled scaffolds generated by the isolates were aligned and oriented with *Aspergillus flavus* NRRL3357 (GenBank accession number [GCF_000006275.2](https://doi.org/10.6084/m9.figshare.11862822)) using MAUVE v2.4.0 (10). AUGUSTUS v3.0.1 was used for gene prediction based on gene models from *Aspergillus oryzae* (11). The assembly statistics are shown in Table 1.

In order to classify the species of the *Aspergillus* isolates by barcoding, we identified the sequences of the internal transcribed spacer (ITS), *cmdA*, and *benA* markers in the assemblies using BLAST v2.2.25 with default settings (12); only one copy of these genes was found in each of the two assemblies. These sequences were aligned against RefSeq entries for *Aspergillus* spp. from DDBJ/EMBL/GenBank (<https://doi.org/10.6084/m9.figshare.11862822>) using MAFFT (<https://www.ebi.ac.uk/Tools/msa/mafft/>). The retrieved alignments were manually checked and concatenated. IQ-TREE v1.4.4 software was used for phylogenetic reconstruction with the maximum likelihood (ML) method with the options “-m TEST” and “-sp partitioned matrix” (13). TreeGraph 2 v1.4.4 was used for tree visualization (14). In the phylogeny, the two isolates sequenced were grouped with reference strains of *Aspergillus tamarii* (Fig. 1).

Data availability. These whole-genome sequences were deposited at DDBJ/ENA/GenBank under the accession numbers [VYTV000000000](https://doi.org/10.6084/m9.figshare.11862822) and [VYTW000000000](https://doi.org/10.6084/m9.figshare.11862648). The raw sequence reads have been deposited in the NCBI Sequence Read Archive under BioProject number [PRJNA529233](https://doi.org/10.6084/m9.figshare.11862648). The aggregated data are available on Figshare at <https://doi.org/10.6084/m9.figshare.11862648>.

ACKNOWLEDGMENTS

This research was supported by CODI-Universidad de Antioquia via a grant (Mi Primer Proyecto 2015) titled “Caracterización fenotípica y tipificación molecular de aislamientos clínicos y ambientales de *Aspergillus* spp.” (2015).

We thank Diana Marcela González Gil for providing the isolates.

REFERENCES

- Latgé J-P, Chamilo G. 2019. *Aspergillus fumigatus* and aspergillosis in 2019. Clin Microbiol Rev 33:e00140-18. <https://doi.org/10.1128/CMR.00140-18>.
- Houbraken J, de Vries RP, Samson RA. 2014. Modern taxonomy of biotechnologically important *Aspergillus* and *Penicillium* species. Adv Appl Microbiol 86:199–249. <https://doi.org/10.1016/B978-0-12-800262-9.00004-4>.
- Samson RA, Visagie CM, Houbraken J, Hong S-B, Hubka V, Klaassen CHW, Perrone G, Seifert KA, Susca A, Tanney JB, Varga J, Kocsubé S, Szigeti G, Yaguchi T, Frisvad JC. 2014. Phylogeny, identification and nomenclature of the genus *Aspergillus*. Stud Mycol 78:141–173. <https://doi.org/10.1016/j.simyco.2014.07.004>.
- Alvarez-Moreno CA, Cortes JA, Denning DW. 2018. Burden of fungal infections in Colombia. J Fungi (Basel) 4:E41. <https://doi.org/10.3390/jof4020041>.
- Cárdenas MX, Cortes JA, Parra CM. 2008. *Aspergillus* spp. in risk areas of [sic] transplant patients in a university hospital. Rev Iberoam Micol 25:232–236. (In Spanish.) [https://doi.org/10.1016/s1130-1406\(08\)70055-x](https://doi.org/10.1016/s1130-1406(08)70055-x).
- Homa M, Manikandan P, Szekeres A, Kiss N, Kocsubé S, Kredics L, Alshehri B, Dukhyil AAB, Revathi R, Narendran V, Vágvölgyi C, Shobana CS, Papp T. 2019. Characterization of *Aspergillus tamarii* strains from human keratomycoses: molecular identification, antifungal susceptibility patterns and cyclopiazonic acid producing abilities. Front Microbiol 10:2249. <https://doi.org/10.3389/fmicb.2019.02249>.
- Sambrook J, Fritsch EF, Maniatis T. 2001. Molecular cloning: a laboratory manual, 2nd ed, vol 1. Cold Spring Harbor Laboratory Press, New York, NY.
- Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski 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. <https://doi.org/10.1089/cmb.2012.0021>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- 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>.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274. <https://doi.org/10.1093/molbev/msu300>.
- Stöver BC, Müller KF. 2010. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. BMC Bioinformatics 11:7. <https://doi.org/10.1186/1471-2105-11-7>.

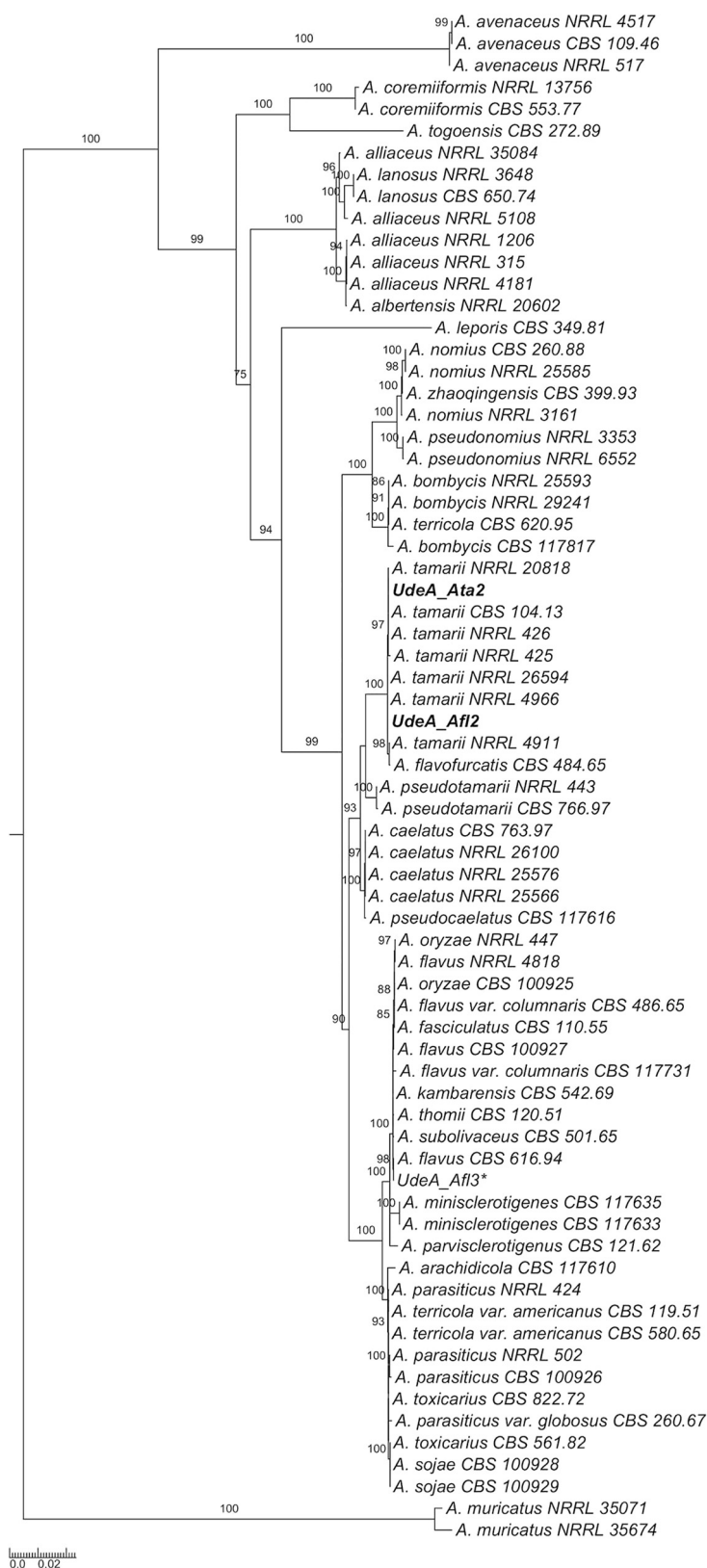


FIG 1 Phylogenetic reconstruction of the *Aspergillus* section *Flavi* using the maximum likelihood (ML) method with IQ-TREE software based on concatenated sequences of the markers ITS, *cmdA*, and *benA*. The numbers near each branch show indices of support that are equal to or greater than 70 based on 1,000 ultrafast bootstrap replications. The Colombian *A. tamarii* isolate is shown in bold. *Aspergillus muricatus* (section *Circumdati*) was used as an outgroup. *, unpublished data.