





Chromosome-Level Aspergillus flavus Strain CA14 Genome **Assembly**

Mark A. Weaver, Brian M. Mack, Matthew K. Gilbert

^aBiological Control of Pests Research Unit, National Biological Control Laboratory, USDA ARS, Stoneville, Mississippi, USA ^bFood and Feed Safety Unit, USDA ARS, New Orleans, Louisiana, USA

ABSTRACT We report here a chromosome-level genome assembly of the aflatoxigenic fungus Aspergillus flavus strain CA14. This strain is the basis for numerous studies in fungal physiology and secondary metabolism. This full-length assembly will aid in subsequent genomics research.

he filamentous fungus Aspergillus flavus is an opportunistic pathogen of several crops, including corn, cotton, and peanut, and colonizes many stored grains intended for food and feed. It may be best known as a producer of aflatoxins, the most potent carcinogens found in nature (1). Isolates of A. flavus within a field population vary greatly with regard to aflatoxin production and microsatellite-based haplotype (2, 3). Additionally, numerous species-specific genes and genomic rearrangements have been documented within the A. flavus clade of Aspergillus section Flavi (4). Until recently, the reference genome for A. flavus was the Sanger-sequenced assembly of strain 3357, with $5\times$ coverage and 331 scaffolds (5) (Table 1). Presently, >100 sequenced genomes have been made publicly available for A. flavus, created using various sequencing technologies or combinations of available systems (https://www.ncbi.nlm.nih.gov/genome/browse #%21/overview/aspergillus%20flavus). A pseudomolecule-level assembly of strain 3357 and AF13 (6) and full chromosome-level assembly of strain 3357 (7) were also reported in 2020 (Table 1). We report here the sequence of strain CA14, assembled at the chromosome level. Strain CA14 is a wild-type, large-sclerotia-producing, aflatoxigenic strain isolated from pistachio at the Wolfskill Grant Experimental Farm of University of California, Davis (8), that has been used in numerous knockout and functional studies of A. flavus isolates (9, 10).

Genomic DNA (gDNA) was extracted from $\sim 10^7$ conidia of A. flavus strain CA14 by grinding in liquid nitrogen and extracting in hot cetyltrimethylammonium bromide buffer (11). After gDNA cleanup, creation of a PacBio Express library, and size selection,

TABLE 1 Assembly statistics of some representative *Aspergillus flavus* genomes

			Fold	No. of		No. of
Aspergillus	GenBank assembly	Genome	coverage	contigs/	N ₅₀	genes
flavus strain	accession no.	size (mbp)	(×)	scaffolds	(Mb)	predicted
3357 ^a	GCA_000006275.2	36.89	5	331	2.39	13,485
3357 ^b	GCA_009017415.1	37.75	600	8		
3357 ^c	GCA_014117465.1	37.0	70	8 ^e	2.40	
AF13 ^c	GCA_014117485.1	37.44	70	8 ^e	2.39	
KuPG#1 ^d	GCA_003709025.1	37.7	121	199	1.3	12,846
CA14	GCA_014784225.1	37.81	140	8	6.27	

^a From reference 5.

Citation Weaver MA, Mack BM, Gilbert MK. 2021. Chromosome-level Aspergillus flavus strain CA14 genome assembly. Microbiol Resour Announc 10:e01150-20. https://doi.org/ 10.1128/MRA.01150-20.

Editor Christina A. Cuomo, Broad Institute This is a work of the U.S. Government and is not subject to copyright protection in the United States. Foreign copyrights may apply. Address correspondence to Mark A. Weaver, mark.weaver@usda.gov.

Received 1 October 2020 Accepted 1 December 2020 Published 7 January 2021

^b From reference 7.

^c From reference 6.

d Chang et al. (12), derived from isolate CA14.

^e Pseudochromosomes inferred from alignment to Aspergillus oryzae strain RIB40.



6.75 Gb of sequence ($140 \times$ coverage) was generated from a single 10-hour singlemolecule real-time (SMRT) cell using PacBio Sequel. De novo genome assembly was done using Flye (version 2.5, settings at "genome size 37m -t 14") (https://github .com/fenderglass/Flye) followed by three rounds of polishing with Arrow (version 2.3.3, default settings) (https://github.com/PacificBiosciences/GenomicConsensus) yielding 8 full chromosome-length scaffolds with an N_{50} value of 6.27 Mb. Nine to 11 telomere repeat sequences were present on six of the contig ends.

Data availability. The GenBank accession number is GCA_014784225.1, and the SRA accession number is SRR12683076.

ACKNOWLEDGMENTS

We thank Perng-Kuang Chang for providing strain CA14 and sharing data from strain KuPG#1.

We thank the University of Minnesota Genomic Center for helpful discussion and technical services.

REFERENCES

- 1. Coppock RW, Christian RG, Jacobsen BJ. 2018. Aflatoxins. In Gupta RC (ed), Veterinary toxicology, 3rd ed. Academic Press, Cambridge, MA.
- 2. Olarte RA, Horn BW, Dorner JW, Monacell JT, Singh R, Stone EA, Carbone I. 2012. Effect of sexual recombination on population diversity in aflatoxin production by Aspergillus flavus and evidence for cryptic heterokaryosis. Mol Ecol 21:1453-1476. https://doi.org/10.1111/j.1365-294X.2011.05398.x.
- 3. Sweany RR, Damann KE, Jr, Kaller MD. 2011. Comparison of soil and corn kernel Aspergillus flavus populations: evidence for niche specialization. Phytopathology 101:952–959. https://doi.org/10.1094/PHYTO-09-10-0243.
- 4. Kjærbølling I, Vesth T, Frisvad JC, Nybo JL, Theobald S, Kildgaard S, Petersen TI, Kuo A, Sato A, Lyhne EK, Kogle ME, Wiebenga A, Kun RS, Lubbers RJM, Mäkelä MR, Barry K, Chovatia M, Clum A, Daum C, Haridas S, He G, LaButti K, Lipzen A, Mondo S, Pangilinan J, Riley R, Salamov A, Simmons BA, Magnuson JK, Henrissat B, Mortensen UH, Larsen TO, de Vries RP, Grigoriev IV, Machida M, Baker SE, Andersen MR. 2020. A comparative genomics study of 23 Aspergillus species from section Flavi. Nat Commun 11:1106. https://doi.org/10.1038/s41467-019-14051-y.
- 5. Nierman WC, Yu J, Fedorova-Abrams ND, Losada L, Cleveland TE, Bhatnagar D, Bennett JW, Dean R, Payne GA. 2015. Genome sequence of Aspergillus flavus NRRL₃₃₅₇, a strain that causes aflatoxin contamination of food and feed. Genome Announc 3:e00168-15. https://doi.org/10.1128/genomeA.00168-15.
- 6. Fountain JC, Clevenger JP, Nadon B, Youngblood RC, Korani W, Chang P-K, Starr D, Wang H, Isett B, Johnston HR, Wiggins R, Agarwal G, Chu Y, Kemerait RC, Pandey MK, Bhatnagar D, Ozias-Akins P, Varshney RK, Scheffler BE, Vaughn JN, Guo B. 2020. Two new Aspergillus flavus reference genomes reveal a large insertion potentially contributing to isolate stress

- tolerance and aflatoxin production. G3 (Bethesda) 10:3515-3531. https:// doi.org/10.1534/g3.120.401405.
- 7. Drott MT, Satterlee TR, Skerker JM, Pfannenstiel BT, Glass NL, Keller NP, Milgroom MG. 2020. The frequency of sex: population genomics reveals the differences in recombination and population structure of the aflatoxin-producing fungus Aspergillus flavus. mBio 11:e00963-20. https://doi .org/10.1128/mBio.00963-20.
- 8. Hua SS, McAlpin CE, Chang P-K, Sarreal SB. 2012. Characterization of aflatoxigenic and non-aflatoxigenic Aspergillus flavus isolates from pistachio. Mycotoxin Res 28:67-75. https://doi.org/10.1007/s12550-011-0117-4.
- 9. Cary JW, Harris-Coward P, Scharfenstein L, Mack BM, Chang P-K, Wei Q, Lebar M, Carter-Wientjes C, Majumdar R, Mitra C, Banerjee S, Chanda A. 2017. The Aspergillus flavus homeobox gene, hbx1, is required for development and aflatoxin production. Toxins 9:315. https://doi.org/10.3390/ toxins9100315.
- 10. Cary JW, Harris-Coward P, Ehrlich KC, Mack BM, Kale SP, Larey C, Calvo AM. 2012. NsdC and NsdD affect Aspergillus flavus morphogenesis and aflatoxin production. Eukaryot Cell 11:1104-1111. https://doi.org/10.1128/ EC.00069-12.
- 11. Schwessinger B. 2019. High quality DNA from Fungi for long read sequencing, e.g. PacBio V.11. protocols.io. https://www.protocols.io/view/highquality-dna-from-fungi-for-long-read-sequenci-2yfgftn?step=1. Accessed 14 September 2020. https://doi.org/10.17504/protocols.io.2yfgftn.
- 12. Chang P-K, Scharfenstein L, Mack BM, Wei Q, Gilbert M, Lebar M, Cary JW. 2019. Identification of a copper-transporting ATPase involved in biosynthesis of A. flavus conidial pigment. Appl Microbiol Biotechnol 103:4889-4897. https://doi.org/10.1007/s00253-019-09820-0.