



Genome Sequences of 20 Georeferenced *Aspergillus flavus* Isolates

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ABSTRACT Several agricultural commodities can be infected by *Aspergillus flavus*, a fungus that can produce the carcinogen aflatoxin. Here, we report the whole-genome sequences for 20 georeferenced isolates collected from soil and corn under field conditions. This information contributes to an understanding of *A. flavus* population structure and dynamics in a field environment.

Aspergillus flavus is a plant pathogen that can infect corn, especially under conditions of heat and/or drought stress (1). This infection can result in contamination with aflatoxins (AF), a group of potent toxins and carcinogens. A biocontrol approach for AF has been developed but involves significant expense that may not always be justified (2). A risk assessment for predicting the necessity of *A. flavus* biocontrol application was developed for corn that incorporated environmental factors (3), but fungal diversity was not taken into consideration. It has been demonstrated that from a relatively diverse *A. flavus* field population, a small number of genetic lineages participate in the infection of maize (4). Knowledge of the pathogen population structure might allow differentiation of *A. flavus* biotypes and more accurate measurement of the AF risk for a given field.

There have been several approaches to characterizing the *A. flavus* population based on morphotype, chemotype (5–7), vegetative compatibility (4, 8, 9), or the presence of a few unique genetic markers (10, 11). Multilocus DNA-based systems have been developed that better characterize isolates and detect genetic recombination (12). These approaches might be improved with greater knowledge of *A. flavus* genetic diversity. Here, we make available the genome sequences for 20 isolates of *A. flavus* from the agroecosystem of the Mississippi Delta that add to the number of whole-genome sequences for *A. flavus* (13–16).

Twenty corn fields in the Mississippi Delta were examined for this project. Soil and corn samples were collected from four georeferenced points in each field in June and August, respectively, in 2017. Putative *A. flavus* isolates were obtained from all samples by plating samples onto semiselective medium and isolating colonies with *A. flavus*-like morphology (15). For this initial survey of genetic diversity, isolates from three fields were subject to whole-genome sequencing. Each isolate was grown in potato dextrose broth. The mycelium was freeze-dried (model 2400; Freeze Dry Company, Nisswa, MN) and ground to a powder using a tissue pulverizer (Garcia Manufacturing, Visalia, CA) before automated genomic DNA extraction (Maxwell 16; Promega, Madison, WI), following the manufacturer's protocols. Sequencing libraries from each genomic DNA extract were prepared using the plexWell-384 library kit (product PW384; SeqWell, Inc., Beverly, MA), followed by whole-genome resequencing using the NextSeq500 platform (Illumina, Inc., San Diego, CA) with high-output version 2.5 chemistry to generate 150-bp paired-end reads. The raw yield of high-quality (>Q30 [17]) reads ranged from 300 Mb to 4.1 Gb. Adapters were trimmed from the reads using BBDuk (version

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TABLE 1 Assembly statistics for the *A. flavus* genomes

<i>Aspergillus flavus</i> isolate	Source, location, coordinates	GenBank accession no.	SRA accession no.	Coverage (fold)	Qual score (Q)	No. of contigs >1,000 bp	N_{50} (bp)	Genome size (Mb)	G+C content (%)
MW1701	Soil, Coahoma Co., MS, 34.33, -90.48	RAXR00000000	SRR8556699	98	33.4	215	426,509	36.8	48.2
MW1703		RAXQ00000000	SRR8556698	60	33.7	295	304,327	36.6	48.2
MW1706	Corn, Coahoma Co., MS, 34.33, -90.48	RAXP00000000	SRR8556562	113	33.8	219	497,957	36.7	48.1
MW1707		RAXO00000000	SRR8556561	29	33.9	870	72,545	36.5	48.2
MW1708		RAXN00000000	SRR8556560	182	33.7	286	587,181	36.9	48.1
MW1709	Soil, Washington Co., MS, 33.40, -90.84	RAXM00000000	SRR8556559	43	33.5	346	212,837	37.0	48.3
MW1710		RAXL00000000	SRR8556566	107	33.8	176	469,209	37.0	48.3
MW1711		RAXK00000000	SRR8556565	93	33.5	207	442,413	37.0	48.2
MW1712		RAXJ00000000	SRR8556564	102	33.8	189	501,954	36.9	48.2
MW1713	Corn, Washington Co., MS, 33.40, -90.84	RAXI00000000	SRR8556563	70	33.6	169	543,609	37.0	48.3
MW1714		RAXH00000000	SRR8556568	106	33.6	288	454,228	36.8	48.1
MW1715		RAXG00000000	SRR8556567	41	33.7	318	312,329	36.6	48.2
MW1716		RAXF00000000	SRR8556558	162	33.6	255	517,865	36.8	48.1
MW1717	Soil, Yazoo Co., MS, 32.78, -90.48	RAXE00000000	SRR8554744	76	33.9	189	539,618	37.0	48.1
MW1718		RAXD00000000	SRR8554745	74	33.8	199	679,380	36.7	48.1
MW1719		RAXC00000000	SRR8554742	104	33.9	163	644,999	37.0	48.2
MW1720		RAXB00000000	SRR8554743	68	33.9	212	589,377	37.0	48.1
MW1722	Corn, Yazoo Co., MS, 32.78, -90.48	RAXA00000000	SRR8554026	77	33.9	934	261,065	38.8	48.1
MW1723		RAWZ00000000	SRR8554025	79	33.9	261	398,115	36.6	48.2
MW1724		RAWY00000000	SRR8554027	127	33.9	222	492,627	36.7	48.1

3/30/17). Genome assembly was conducted using SPAdes (version 3.11.1) with the "careful" option and k-mer sizes of 21, 33, 55, 77, and 99.

The assembled genome sequences were subjected to a BLASTn search using the *A. flavus* strain ATCC 200026 internal transcribed spacer 1 (ITS1) sequence (GenBank accession number [JX535495](#)) as the query (17), resulting in 99.8 to 100% identity with [JX535495](#). *Aspergillus clavatus* (GenBank assembly number [GCA_000002715](#)), *Aspergillus parasiticus* (GenBank assembly number [GCA_000956085](#)), and *A. flavus* strains AF70 (GenBank assembly number [GCA_000952835](#)) and 3357 (GenBank assembly number [GCA_000006275](#)) were queried as reference sequences for comparison, resulting in 88.1%, 87.5%, 100%, and 98.5% identity with [JX535495](#), respectively.

Data availability. The GenBank and SRA accession numbers for the genome sequences are listed in Table 1.

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REFERENCES

- Ojiambo PS, Battilani P, Cary JW, Blum BH, Carbone I. 2018. Cultural and genetic approaches to manage aflatoxin contamination: recent insights provide opportunities for improved control. *Phytopathology* 108: 1024–1037. <https://doi.org/10.1094/PHYTO-04-18-0134-RVW>.
- Weaver MA, Abbas HK, Falconer LL, Allen TW, Pringle HL, III, Sciombato GL. 2015. Biological control of aflatoxin is effective and economical in Mississippi field trials. *Crop Prot* 69:52–55. <https://doi.org/10.1016/j.cropro.2014.12.009>.
- Battilani P, Camardo Leggieri M, Rossi V, Giorni P. 2013. AFLA-maize, a mechanistic model for *Aspergillus flavus* infection and aflatoxin B₁ contamination in maize. *Comput Electron Agric* 94:38–46. <https://doi.org/10.1016/j.compag.2013.03.005>.
- 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>.
- Perrone G, Gallo A, Logrieco AF. 2014. Biodiversity of *Aspergillus* section *Flavi* in Europe in relation to the management of aflatoxin risk. *Front Microbiol* 5:377. <https://doi.org/10.3389/fmicb.2014.00377>.
- Horn BW, Dorner JW. 1999. Regional differences in production of aflatoxin B₁ and cyclopiazonic acid by soil isolates of *Aspergillus flavus* along a transect within the United States. *Appl Environ Microbiol* 65: 1444–1449.
- Probst C, Callicott KA, Cotty PJ. 2012. Deadly strains of Kenyan *Aspergillus* are distinct from other aflatoxin producers. *Eur J Plant Pathol* 132: 419–429. <https://doi.org/10.1007/s10658-011-9887-y>.
- Grubisha LC, Cotty PJ. 2010. Genetic isolation among sympatric vegetative compatibility groups of the aflatoxin-producing fungus *Aspergillus flavus*. *Mol Ecol* 19:269–280. <https://doi.org/10.1111/j.1365-294X.2009.04467.x>.
- Barros GG, Torres AM, Rodriguez MI, Chulze SN. 2006. Genetic diversity within *Aspergillus flavus* strains isolated from peanut-cropped soils in Argentina. *Soil Biol Biochem* 38:145–152. <https://doi.org/10.1016/j.soilbio.2005.04.028>.
- Ramirez-Prado JH, Moore GG, Horn BW, Carbone I. 2008. Characterization and population analysis of the mating-type genes in *Aspergillus flavus* and *Aspergillus parasiticus*. *Fungal Genet Biol* 45:1292–1299. <https://doi.org/10.1016/j.fgb.2008.06.007>.
- Jiang J, Yan L, Ma Z. 2009. Molecular characterization of an atoxigenic *Aspergillus flavus* strain AF051. *Appl Microbiol Biotechnol* 83:501–505. <https://doi.org/10.1007/s00253-009-1921-z>.

12. Grubisha LC, Cotty PJ. 2009. Twenty-four microsatellite markers for the aflatoxin-producing fungus *Aspergillus flavus*. *Mol Ecol Resour* 9:264–267. <https://doi.org/10.1111/j.1755-0998.2008.02378.x>.
13. Nierman WC, Yu J, Fedorova-Abrams ND, Losada L, Cleveland TE, Bhatnager D, Bennett JW, Dean R, Payne GA. 2015. Genome sequence of *Aspergillus flavus* NRRL 3357, a strain that causes aflatoxin contamination of food and feed. *Genome Announc* 3:e00168-15. <https://doi.org/10.1128/genomeA.00168-15>.
14. Faustinelli PC, Wang XM, Palencia ER, Arias RS. 2016. Genome sequences of eight *Aspergillus flavus* spp. and one *A. parasiticus* sp., isolated from peanut seeds in Georgia. *Genome Announc* 4:e00278-16. <https://doi.org/10.1128/genomeA.00278-16>.
15. Weaver MA, Scheffler BE, Duke M, Ballard L, Abbas HK, Grodowitz MJ. 2017. Genome sequences of three strains of *Aspergillus flavus* for the biological control of aflatoxin. *Genome Announc* 5:e01204-17. <https://doi.org/10.1128/genomeA.01204-17>.
16. Gilbert MK, Mack BM, Moore GG, Downey DL, Lebar MD, Joardar V, Loasada L, Yu J, Nierman WC, Bhatnagar D. 2018. Whole genome comparison of *Aspergillus flavus* L-morphotype strain NRRL 3357 (type) and S-morphotype strain AF70. *PLoS One* 13:0199169. <https://doi.org/10.1371/journal.pone.0199169>.
17. Richterich P. 1998. Estimation of errors in “raw” DNA sequences: a validation study. *Genome Res* 8:251–259. <https://doi.org/10.1101/gr.8.3.251>.