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GENOME SEQUENCES

Draft Genome Sequences of One *Aspergillus parasiticus* Isolate and Nine *Aspergillus flavus* Isolates with Varying Stress Tolerance and Aflatoxin Production

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ABSTRACT *Aspergillus flavus* and *Aspergillus parasiticus* produce carcinogenic aflatoxins during crop infection, with extensive variations in production among isolates, ranging from atoxigenic to highly toxigenic. Here, we report draft genome sequences of one *A. parasiticus* isolate and nine *A. flavus* isolates from field environments for use in comparative, functional, and phylogenetic studies.

Aspergillus flavus and *Aspergillus parasiticus* are fungi that exist as saprophytes in soil environments and can, under favorable environmental conditions such as drought and heat stress, colonize compromised plant tissues. These adverse environmental conditions stimulate production by the fungi of carcinogenic mycotoxins called aflatoxins (1, 2). Aflatoxin contamination of important crop species such as maize and peanut poses a significant threat to global food safety and security, particularly in developing countries (1). Isolates of these fungi vary in their ability to produce aflatoxins, and the mechanisms contributing to exacerbated aflatoxin production under environmental stresses are hypothesized to be related to oxidative stress tolerance and host plant composition (3, 4). To investigate these phenomena, we sequenced the genomes of one *A. parasiticus* isolate and nine *A. flavus* isolates, with varying levels of aflatoxin production and oxidative stress tolerance, from field environments for use in comparative analyses (5, 6).

The *A. flavus* isolates A1, A9, AF36 (NRRL18543), Afla-Guard (NRRL21882-3), Tox4, VCG1, and VCG4 were obtained from the Louisiana State University AgCenter, Department of Plant Pathology and Crop Physiology (Baton Rouge, LA, USA). The *A. flavus* isolates K49 (NRRL30797) and K54A were obtained from the USDA Agricultural Research Service (ARS) Biological Control of Pests Research Unit (Stoneville, MS, USA). The *A. parasiticus* isolate NRRL2999 was obtained from the USDA ARS Northern Regional Research Center (Peoria, IL, USA). The isolates used in this study were isolated from cotton, maize, and peanut plants or fields within the continental United States except for NRRL2999, which originated from Uganda. The isolates were cultured on yeast extract with supplements (YES) liquid medium (2% [wt/vol] yeast extract, 1% [wt/vol] sucrose) for 5 days at 30°C in the dark. Mycelial mats from

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TABLE 1 *Aspergillus flavus* and *Aspergillus parasiticus* isolate genomes

Species	Isolate	Aflatoxin production[a]	Amt of filtered data (Mbp)	Score of >Q30 (%)	GC content (%)	Estimated coverage (×)	No. of contigs	Contig N_{50} (bp)	No. of scaffolds	Scaffold N_{50} (Mbp)	Assembled length (Mbp)	SRA accession no.[b]	GenBank accession no.[c]
A. flavus	A1	−	4,679.8	92.0	47.3	71	1,123	436,753	8	5.043	37.254	SRR11486427	CP051059 (Chr 1), CP051060 (Chr 2), CP051061 (Chr 3), CP051062 (Chr 4), CP051063 (Chr 5), CP051064 (Chr 6), CP051065 (Chr 7), CP051066 (Chr 8)
	A9	+ + +	5,631.6	92.5	47.2	83	1,581	357,152	8	5.377	37.366	SRR11486426	CP051035 (Chr 1), CP051036 (Chr 2), CP051037 (Chr 3), CP051038 (Chr 4), CP051039 (Chr 5), CP051040 (Chr 6), CP051041 (Chr 7), CP051042 (Chr 8)
	AF36	− − −	10,151.0	92.2	47.5	168	609	355,215	8	4.959	37.642	SRR11486425	CP051019 (Chr 1), CP051020 (Chr 2), CP051021 (Chr 3), CP051022 (Chr 4), CP051023 (Chr 5), CP051024 (Chr 6), CP051025 (Chr 7), CP051026 (Chr 8)
	Afla-Guard	− − −	11,128.2	91.8	47.5	171	680	518,829	8	5.091	37.392	SRR11486424	CP051067 (Chr 1), CP051068 (Chr 2), CP051069 (Chr 3), CP051070 (Chr 4), CP051071 (Chr 5), CP051072 (Chr 6), CP051073 (Chr 7), CP051074 (Chr 8)
	K49	− − −	5,514.9	92.5	47.5	68	1,593	424,252	8	5.280	37.271	SRR11486423	CP051075 (Chr 1), CP051076 (Chr 2), CP051077 (Chr 3), CP051078 (Chr 4), CP051079 (Chr 5), CP051080 (Chr 6), CP051081 (Chr 7), CP051082 (Chr 8)
	K54A	−	6,145.6	92.2	47.6	40	805	380,569	8	5.206	37.622	SRR11486422	CP051083 (Chr 1), CP051084 (Chr 2), CP051085 (Chr 3), CP051086 (Chr 4), CP051087 (Chr 5), CP051088 (Chr 6), CP051089 (Chr 7), CP051090 (Chr 8)
	Tox4	+ + +	5,669.2	88.0	47.3	87	895	336,276	8	4.842	37.354	SRR11486421	CP051043 (Chr 1), CP051044 (Chr 2), CP051045 (Chr 3), CP051046 (Chr 4), CP051047 (Chr 5), CP051048 (Chr 6), CP051049 (Chr 7), CP051050 (Chr 8)
	VCG1	−	5,501.2	94.1	48.1	141	589	463,008	8	5.024	36.933	SRR11486420	CP051091 (Chr 1), CP051092 (Chr 2), CP051093 (Chr 3), CP051094 (Chr 4), CP051095 (Chr 5), CP051096 (Chr 6), CP051097 (Chr 7), CP051098 (Chr 8)
	VCG4	+ + +	5,939.2	94.1	48.1	152	658	401,225	8	5.050	37.048	SRR11486419	CP051051 (Chr 1), CP051052 (Chr 2), CP051053 (Chr 3), CP051054 (Chr 4), CP051055 (Chr 5), CP051056 (Chr 6), CP051057 (Chr 7), CP051058 (Chr 8)

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TABLE 1 (Continued)

Species	Isolate	Aflatoxin production[a]	Amt of filtered data (Mbp)	Score of >Q30 (%)	GC content (%)	Estimated coverage (×)	No. of contigs	Contig N_{50} (bp)	No. of scaffolds	Scaffold N_{50} (Mbp)	Assembled length (Mbp)	SRA accession no.[b]	GenBank accession no.[c]
A. parasiticus	NRRL2999	+ (BG)	5,699.0	92.2	47.0	45	2,321	434,276	8	5.243	37.049	SRR11486418	CP051027 (Chr 1), CP051028 (Chr 2), CP051029 (Chr 3), CP051030 (Chr 4), CP051031 (Chr 5), CP051032 (Chr 6), CP051033 (Chr 7), CP051034 (Chr 8)

[a] Aflatoxin production in each isolate: −, atoxigenic; − − −, atoxigenic biological control isolate; + + +, highly toxigenic; + (BG), produces B and G aflatoxins.
[b] Sequence Read Archive (SRA) accession numbers for raw data.
[c] GenBank accession numbers for scaffolded chromosomes (Chr).

each culture were collected, ground in liquid nitrogen with a chilled mortar and pestle, and used for DNA isolation with cetyltrimethylammonium bromide (CTAB). Following DNA extraction with chloroform-isoamyl alcohol (24:1), the phases were separated by centrifugation. The DNA was then precipitated using isopropanol and pelleted by centrifugation before being suspended in TE buffer (10 mM Tris [pH 8.0], 1 mM EDTA [pH 8.0]). Isolated DNA was then submitted to the Novogene Corp. (Sacramento, CA, USA) for quality checking, sequencing, and initial data filtering. Libraries (350-bp insert size) were generated using a NEB Ultra II DNA library preparation kit (New England Biolabs, Ipswich, MA, USA) and then were used for short-read paired-end (150 bp) sequencing on a HiSeq 4000 platform (Illumina, San Diego, CA, USA). Generated optical sequencing data were transformed into raw sequencing reads using Casava (v 1.8; Illumina) base calling. Adapters were then trimmed, and low-quality sequencing reads (with a Q score of <20) were filtered and removed by discarding paired-end reads if one read contained >10 bases aligned to adapters (<10% mismatches allowed), >10% N bases, or >50% low-quality bases (with a Phred quality score of <5).

Clean reads were then used for *de novo* contig assembly using SPAdes (v 3.13.1) with default k values (7). Contigs of <500 bp were removed from each assembly. Contigs were scaffolded using RaGOO (v 1.1) with the *Aspergillus flavus* AF13 genome as a reference (GenBank accession numbers CP059858 to CP059865) (8). Assembly statistics for each isolate genome are shown in Table 1. For the *A. parasiticus* NRRL2999 isolate, previous studies showed conserved gene contents, similar chromosome structures, and a high degree of genetic relatedness with respect to *A. flavus*, allowing *A. flavus* genomes to be useful references for assembly (8, 9). These new assemblies will be useful for comparative genomic analyses to investigate the root causes of variations in aflatoxin production capability and stress tolerance among these fungi.

Data availability. The genome sequences for these *A. flavus* and *A. parasiticus* isolates have been deposited in the NCBI GenBank under BioProject PRJNA607981. Accession numbers for each isolate genome are listed in Table 1.

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Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

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