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Genome Sequences of Three Strains of *Aspergillus flavus* for the Biological Control of Aflatoxin

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ABSTRACT Aflatoxin is a carcinogenic contaminant of many commodities that are infected by *Aspergillus flavus*. Nonaflatoxigenic strains of *A. flavus* have been utilized as biological control agents. Here, we report the genome sequences from three biocontrol strains. This information will be useful in developing markers for postrelease monitoring of these fungi.

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The fungus *Aspergillus flavus* is a common soil saprophyte (1), an entomopathogen (2), an opportunistic human pathogen (3, 4), and a pathogen of corn and several other crops (5). It is perhaps best known as the major producer of aflatoxin, a toxic and carcinogenic secondary metabolite (6). While developed nations screen food and feed to minimize the consumption of aflatoxin, aflatoxin consumption causes a global economic and health burden (7, 8). Presently, the most effective means of preventing aflatoxin contamination of corn, cotton, and peanut is the application of nonaflatoxigenic biological control strains of *A. flavus* (9). Two biocontrol strains are registered and are used for biological control of aflatoxin in the United States, NRRL 21882 (Afla-Guard) and NRRL 118543 (AF36). Strain NRRL 30797 (K49) is another nonaflatoxigenic strain that has been shown to be effective in reducing economic losses due to aflatoxin contamination (10).

We are interested in monitoring the survival, persistence, and spread of applied biocontrol strains in treated soil, crops, and commodities. Various approaches, with various levels of specificity, have been developed for the detection of *A. flavus* and optimized for various applications (11–13). Whole-genome sequencing projects for *A. flavus* have been reported (14, 15). Additional DNA sequence information is needed to develop better strain-specific molecular probes to detect, differentiate, and quantify the biocontrol strains within the matrices that include a large, diverse, indigenous population of *A. flavus*. To facilitate the development of more specific probes for these strains, we report here the genome sequences for three biocontrol strains of *A. flavus*.

Each strain of *A. flavus* was grown in potato dextrose broth. The mycelium was freeze-dried (model 2400 freeze dryer; The Freeze Dry Company, Nisswa, MN) and ground to a fine powder using a tissue pulverizer (Garcia Manufacturing, Visalia, CA) before automated genomic DNA extraction (Maxwell 16; Promega, Madison, WI), according to the manufacturer's protocols. Sequencing libraries from each of the three genomic DNA extracts were prepared using the Nextera DNA sample prep kit (Illumina, San Diego, CA, USA), followed by whole-genome resequencing using the Illumina HiSeq 2000 with high output version 3 chemistry for 2 × 101 cycles to generate 100-bp paired-end reads. The raw yields of high-quality (Illumina quality score greater than or equal to Q30) reads for NRRL 118543, NRRL 21882, and NRRL 30797 were 11.48 Gb, 7.12 Gb, and 16.29 Gb, respectively. A reference-guided assembly was performed with

TABLE 1 Assembly statistics

Aspergillus flavus isolate[a]	NCBI accession no.	Genome size (bp)	Fold coverage (×)	N_{50} (Mb)	No. of genes predicted	No. of genes mapped to isolate 3357	G+C content (%)
3357[b]	EQ963472	36,892,344	5	2.39	13,485		
118543	NWUH00000000	36,647,142	100	2.39	11,371	11,045	48.3
21882	NWUI00000000	36,288,313	100	2.39	11,042	10,731	48.3
30797	NWUG00000000	36,667,244	100	2.39	11,339	11,037	48.3

[a]NRRL isolate numbers. See the text for descriptions.
[b]Nierman et al. (14).

MIRA (16) and annotated with Augustus (17) using *A. flavus* strain 3357 as a reference (14).

Accession number(s). The GenBank accession numbers for the three genomes are listed in Table 1.

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