### University of Nebraska - Lincoln

### DigitalCommons@University of Nebraska - Lincoln

Publications from USDA-ARS / UNL Faculty

U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska

2016

# Genome Sequences of Eight *Aspergillus flavus* spp. and One *A. parasiticus* sp., Isolated from Peanut Seeds in Georgia

Paola C. Faustinelli National Peanut Research Laboratory, USDA-ARS, Paola.Faustinelli@ars.usda.gov

Xinye Monica Wang National Peanut Research Laboratory, USDA-ARS

Edwin R. Palencia National Peanut Research Laboratory, USDA-ARS

Renee S. Arias
National Peanut Research Laboratory, USDA-ARS, renee.arias@usda.gov

Follow this and additional works at: https://digitalcommons.unl.edu/usdaarsfacpub

Faustinelli, Paola C.; Wang, Xinye Monica; Palencia, Edwin R.; and Arias, Renee S., "Genome Sequences of Eight *Aspergillus flavus* spp. and One *A. parasiticus* sp., Isolated from Peanut Seeds in Georgia" (2016). *Publications from USDA-ARS / UNL Faculty.* 2132.

https://digitalcommons.unl.edu/usdaarsfacpub/2132

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Publications from USDA-ARS / UNL Faculty by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.







## Genome Sequences of Eight Aspergillus flavus spp. and One A. parasiticus sp., Isolated from Peanut Seeds in Georgia

Paola C. Faustinelli, Xinye Monica Wang, Edwin R. Palencia, Renée S. Arias

National Peanut Research Laboratory, Dawson, Georgia, USA

Aspergillus flavus and A. parasiticus fungi produce carcinogenic mycotoxins in peanut seeds, causing considerable impact on both human health and the economy. Here, we report nine genome sequences of Aspergillus spp., isolated from Georgia peanut seeds in 2014. The information obtained will lead to further biodiversity studies that are essential for developing control strategies.

Received 25 February 2016 Accepted 29 February 2016 Published 14 April 2016

**Citation** 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(2):e00278-16. doi:10.1128/genomeA.00278-16.

Copyright © 2016 Faustinelli et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license. Address correspondence to Paola C. Faustinelli, Paola, Faustinelli@ars.usda.gov, or Renée S. Arias, Renee, Arias, @ars.usda.gov.

he United States is the third largest peanut producer in the world, with Georgia yielding 49% of the national total (1). People in the United States consume an annual average of 6 lb of peanut products per capita (2). The fungal Aspergillus flavus and A. parasiticus spp. typically produce aflatoxins, which are considered the most powerful mycotoxins associated with liver cancer, child growth impairment, and acute toxicoses (3–5). These two members of the section Flavi contaminate agricultural products and, consequently, foodstuffs, including peanut products (6). Stringent regulations have been imposed on the level of aflatoxins allowed for marketed peanut products (7). The impact of aflatoxins costs the peanut industry between \$25 and \$58 million annually in the United States (8, 9). Despite all efforts, more than 5 billion people worldwide are at risk of exposure to aflatoxin (10). Currently, there is a need to develop systemic and effective approaches to manage aflatoxin contamination of susceptible crops.

The natural populations of Aspergillus flavus and A. parasiticus are diverse (11), and since evidence of sexual reproduction was found for these species (12, 13), genetic variability is expected, especially in the aflatoxin biosynthesis gene cluster (AB cluster) (4). Although the sequences of the genes and the intergenic distances in the AB cluster are considered well conserved (14), deletions are common and seem to have different patterns that may be due to processes of adaptation/evolution in nature (15, 16). Here,

we present the genome sequences of eight isolates of *A. flavus*, three of which are nonaflatoxigenic, and one isolate of *A. parasiticus*. The DNA sequences obtained will provide valuable molecular information to determine genetic diversity in the section *Flavi*—data necessary to select appropriate target genes to control aflatoxin accumulation in crops.

More than 240 Aspergillus spp. were isolated on MDRB medium (17) from peanut seeds from the state of Georgia, United States, during 2014, and the isolates were fingerprinted with 25 InDel markers (unpublished). Genomes of nine representative isolates from various clades in the cluster analysis were sequenced using next-generation sequencing (Illumina HiSeq2500) at the University of Washington, United States. Prior to assembly, the sequence reads were processed using CLC Genomics tools (CLC Genomics Workbench version 8.5.1, Qiagen, Denmark) to remove sequence adapters and trim off any ambiguous nucleotides. Using Geneious version 8.1.7 (18), the A. flavus processed reads were mapped to the published A. flavus NRRL3357 genome (19), and the A. parasiticus read was mapped to its corresponding published genome (20). The total length of each draft A. flavus genome ranged from 35.8 to 36.5 Mbp, slightly smaller than the A. flavus NRRL3357 genome (37 Mbp) (19), and the G+C average was 48.3% (Table 1). Alignments of the AB Cluster with the published A. flavus NRRL3357 cluster (19) using the Clone Manager

TABLE 1 Listing of Aspergillus sp. isolate genomes released to NCBI

Isolate	Accession no.	Aspergillus sp.	Aflatoxin producer	Genome size (bp)	Fold coverage $(\times)$	% G+C
26-3	LOAN00000000	flavus	Nonaflatoxigenic	36,329,774	38.9	48.2
54-2	LLET00000000	flavus	Nonaflatoxigenic	36,578,699	65.6	48.3
78-6	LOAO00000000	flavus	Nonaflatoxigenic	36,203,959	39.6	48.3
206-4	LOAM00000000	flavus	Aflatoxigenic	36,084,636	33.8	48.3
40-5	LIZI00000000	flavus	Aflatoxigenic	36,412,503	45.7	48.3
61-4	LIZJ00000000	flavus	Aflatoxigenic	36,322,355	39.3	48.3
72-5	LOAK00000000	flavus	Aflatoxigenic	35,993,818	29.8	48.3
79-2	LOAL00000000	flavus	Aflatoxigenic	35,835,174	32.9	48.3
68-5	LOAP00000000	parasiticus	Aflatoxigenic	30,136,366	36.9	48.3

tool (Clone Manager version, 9 Professional Edition, USA) indicated a 98 to 99% homology, except in *A. parasiticus*, which indicated an expected 81%.

**Nucleotide sequence accession numbers.** GenBank accession numbers for the nine genomes are listed in Table 1.

### **ACKNOWLEDGMENTS**

This work was supported by USDA-ARS, the Peanut Mycotoxin Innovation Laboratory at the University of Georgia, and USAID—Feed the Future grants. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

We thank Imana Power and Valerie Orner for critical suggestions.

### **FUNDING INFORMATION**

This work, including the efforts of Renee S. Arias, was funded by United States Agency for International Development (USAID) (6044-21000-004-02). This work, including the efforts of Renee S. Arias, was funded by USDA | Agricultural Research Service (ARS) (6044-21000-004-00D). This work, including the efforts of Renee S. Arias, was funded by University of Georgia (UGA) (PMIL 6044-21000-004-01).

### **REFERENCES**

- USDA NASS 2016. Quick stats. Crop production 2015 summary. http://www.nass.usda.gov. Accessed 23 January 2016.
- 2. National Peanut Board. 2016. Peanut country, U.S.A. http://nationalpeanutboard.org. Accessed 27 January 2016.
- Horn BW. 2007. Biodiversity of Aspergillus section Flavi in the United States: a review. Food Addit Contam 24:1088–1101. http://dx.doi.org/ 10.1080/02652030701510012.
- 4. Moore GG, Elliott JL, Singh R, Horn BW, Dorner JW, Stone EA, Chulze SN, Barros GG, Naik MK, Wright GC, Hell K, Carbone I. 2013. Sexuality generates diversity in the aflatoxin gene cluster: evidence on a global scale. PLoS Pathog. 9:e1003574. http://dx.doi.org/10.1371/journal.ppat.1003574.
- Wu F, Groopman JD, Pestka JJ. 2014. Public health impacts of foodborne mycotoxins. Annu Rev Food Sci Technol 5:351–372. http://dx.doi.org/ 10.1146/annurev-food-030713-092431.
- 6. Perrone G, Susca A, Cozzi G, Ehrlich K, Varga J, Frisvad JC, Meijer M, Noonim P, Mahakarnchanakul W, Samson RA. 2007. Biodiversity of *Aspergillus* species in some important agricultural products. Stud Mycol 59:53–66. http://dx.doi.org/10.3114/sim.2007.59.07.
- 7. Wu F. 2006. Economic impact of fumonisin and aflatoxin regulations on global corn and peanut markers, p. 83–93. *In* Barug D, Bhatnagar D, Egmond HPv, Kamp JWvd, Osenbruggen Wav, Visconti A (ed.), The mycotoxin factbook: food and feed topics. Wageningen Academic Publishers, The Netherlands.
- 8. Lamb MC, Sternitzke DA. 2001. Cost of aflatoxin to the farmer, buying

- point, and sheller segments of the southeast United States Peanut Industry. Peanut Sci 28:59–63. http://dx.doi.org/10.3146/i0095-3679-28-2-4.
- 9. Leidner J. 2012. The peanut genomics initiative. Southeastern Peanut Farmer 50:15.
- 10. Strosnider H, Wilson D, Williams JT, Wild C, Stroka J, Shephard G, Schaafsma A, Sabino M, Rubin C, Rogers HS, Pronczuk J, Pineiro M, Phillips T, Patel M, Park D, Page SW, Onsongo MTK, Ong C-N, Njapau H, Misore A, Miraglia M, Mensah P, McCoy L, Luber G, Liu X, Lewis L, Kibata GN, Jolly P, Jolly C, Jeffers D, Henry SH, Hell K, Groopman J, Dilley A, DeCock K, Brune M-N, Breiman R, Bhat RV, Banziger M, Azziz-Baumgartner E. 2006. Workgroup report: public health strategies for reducing aflatoxin exposure in developing countries. Environ Health Perspect 114:1898–1903 http://dx.doi.org/10.1289/ehp.9302.
- 11. Bayman P, Cotty PJ. 1993. Genetic diversity in *Aspergillus flavus*: association with aflatoxin production and morphology. Can J Bot 71:23–31. http://dx.doi.org/10.1139/b93-003.
- 12. Horn BW, Moore GG, Carbone I. 2009. Sexual reproduction in Aspergillus flavus. Mycologia 101:423–429. http://dx.doi.org/10.3852/09-011.
- Horn BW, Ramirez-Prado JH, Carbone I. 2009. The sexual state of Aspergillus parasiticus. Mycologia 101:275–280. http://dx.doi.org/ 10.3852/08-205.
- 14. Ehrlich KC, Yu J, Cotty PJ. 2005. Aflatoxin biosynthesis gene clusters and flanking regions. J Appl Microbiol 99:518–527. http://dx.doi.org/10.1111/j.1365-2672.2005.02637.x.
- 15. Peterson SW, Ito Y, Horn BW, Goto T. 2001. *Aspergillus bombycis*, a new aflatoxigenic species and genetic variation in its sibling species, *A. Nomius*. Mycologia 93:689–703. http://dx.doi.org/10.2307/3761823.
- Chang PK, Horn BW, Dorner JW. 2005. Sequence breakpoints in the aflatoxin biosynthesis gene cluster and flanking regions in nonaflatoxigenic *Aspergillus flavus* isolates. Fungal Genet Biol 42:914–923. http:// dx.doi.org/10.1016/j.fgb.2005.07.004.
- 17. Horn BW, Greene RL, Sobolev VS, Dorner JW, Powell JH, Layton RC. 1996. Association of morphology and mycotoxin production with vegetative compatibility groups in *Aspergillus flavus*, *A. parasiticus*, and *A. Tamarii*. Mycologia 88:574–587. http://dx.doi.org/10.2307/3761151.
- 18. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. BioInformatics 28:1647–1649. http://dx.doi.org/10.1093/bioinformatics/bts199.
- 19. 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* NRRL 3357, a strain that causes aflatoxin contamination of food and feed. Genome Announc 3(2):e00168-15. http://dx.doi.org/10.1128/genomeA.00168-15.
- Linz JE, Wee J, Roze LV. 2014. Aspergillus parasiticus SU-1 genome sequence, predicted chromosome structure, and comparative gene expression under aflatoxin-inducing conditions: evidence that differential expression contributes to species phenotype. Eukaryot Cell 13:1113–1123. http://dx.doi.org/10.1128/EC.00108-14.