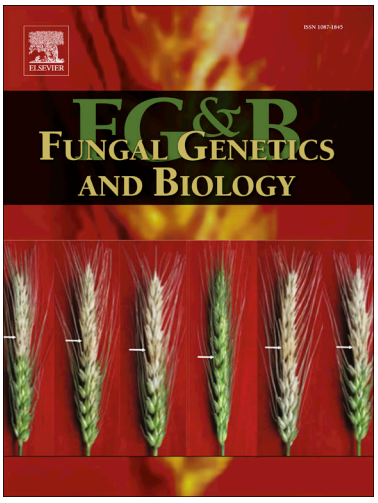

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Identification and functional analysis of the aspergillic acid gene cluster in *Aspergillus flavus*

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Abstract

Aspergillus flavus can colonize important food staples and produce aflatoxins, a group of toxic and carcinogenic secondary metabolites. Previous *in silico* analysis of the *A. flavus* genome revealed 56 gene clusters predicted to be involved in the biosynthesis of secondary metabolites. *A. flavus* secondary metabolites produced during infection of maize seed are of particular interest, especially with respect to their roles in the biology of the fungus. A predicted nonribosomal peptide synthetase-like (NRPS-like) gene, designated *asaC* (AFLA_023020), present in the uncharacterized *A. flavus* secondary metabolite gene cluster 11 was previously shown to be expressed during the earliest stages of maize kernel infection. Cluster 11 is composed of six additional genes encoding a number of putative decorating enzymes as well as a transporter and transcription factor. We generated knock-out mutants of the seven predicted cluster 11 genes. LC-MS analysis of extracts from knockout mutants of these genes showed that they were responsible for the synthesis of the previously characterized antimicrobial mycotoxin aspergillic acid. Extracts of the *asaC* mutant showed no production of aspergillic acid or its precursors. Knockout of the cluster 11 P450 oxidoreductase afforded a pyrazinone metabolite, the aspergillic acid precursor deoxyaspergillic acid. The formation of hydroxyaspergillic acid was abolished in a desaturase/hydroxylase mutant. The hydroxamic acid functional group in aspergillic acid allows the molecule to bind to iron resulting in the production of a red pigment in *A. flavus* identified previously as ferriaspergillin. A reduction of aflatoxin B_1 and cyclopiazonic acid that correlated with reduced fungal growth was observed in maize kernel infection assays when aspergillic acid biosynthesis in *A. flavus* is halted.

Keywords: natural products, *Aspergillus flavus*, gene cluster, pyrazinones, siderophores