

NADH oxidase *nadA*, a link between aflatoxin and oxidative stress in *Aspergillus flavus*.

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nadA, which encodes a predicted NADH oxidase, was identified as part of a sugar utilization cluster that lies adjacent to the aflatoxin biosynthetic cluster in several species of *Aspergillus*. NADH oxidases convert NADH to NAD⁺, which is a possible coenzyme needed for reactions in the aflatoxin biochemical pathway. In a microarray experiment comparing gene expression between a wild type strain of *A. parasiticus* and a deletion mutant for the pathway regulatory gene *aflR*, *nadA* expression was significantly decreased in the mutant background. Although *nadA* is transcriptionally controlled by *AflR*, aflatoxin levels were unaffected in *A. flavus nadA* deletion strains under several conditions. NADH oxidases can also be a source of reactive oxygen species formation. Previous reports have shown a relationship between oxidative stress and aflatoxin production in *Aspergillus sp.* The activity of several antioxidant enzymes were examined in *nadA* deletion strains and peak activity was delayed when compared to wild type. Altered levels of lipoperoxide accumulation were also seen in *nadA* deletion strains. These data suggest that *nadA* plays a role in the oxidative stress response in *A. flavus*. To further understand the function of *nadA*, *A. flavus* was transformed with an overexpression construct containing a *gpdA::nadA* cassette. Constitutive overexpression of *nadA* alters the color of the mycelia in liquid culture and inhibits aflatoxin production. Investigations are underway to characterize additional phenotypes of *nadA* deletion mutants and overexpression mutants.