Characterization of the *Aspergillus flavus rdiA* gene with a *rdi1Δ* mutant of *C. neoformans*

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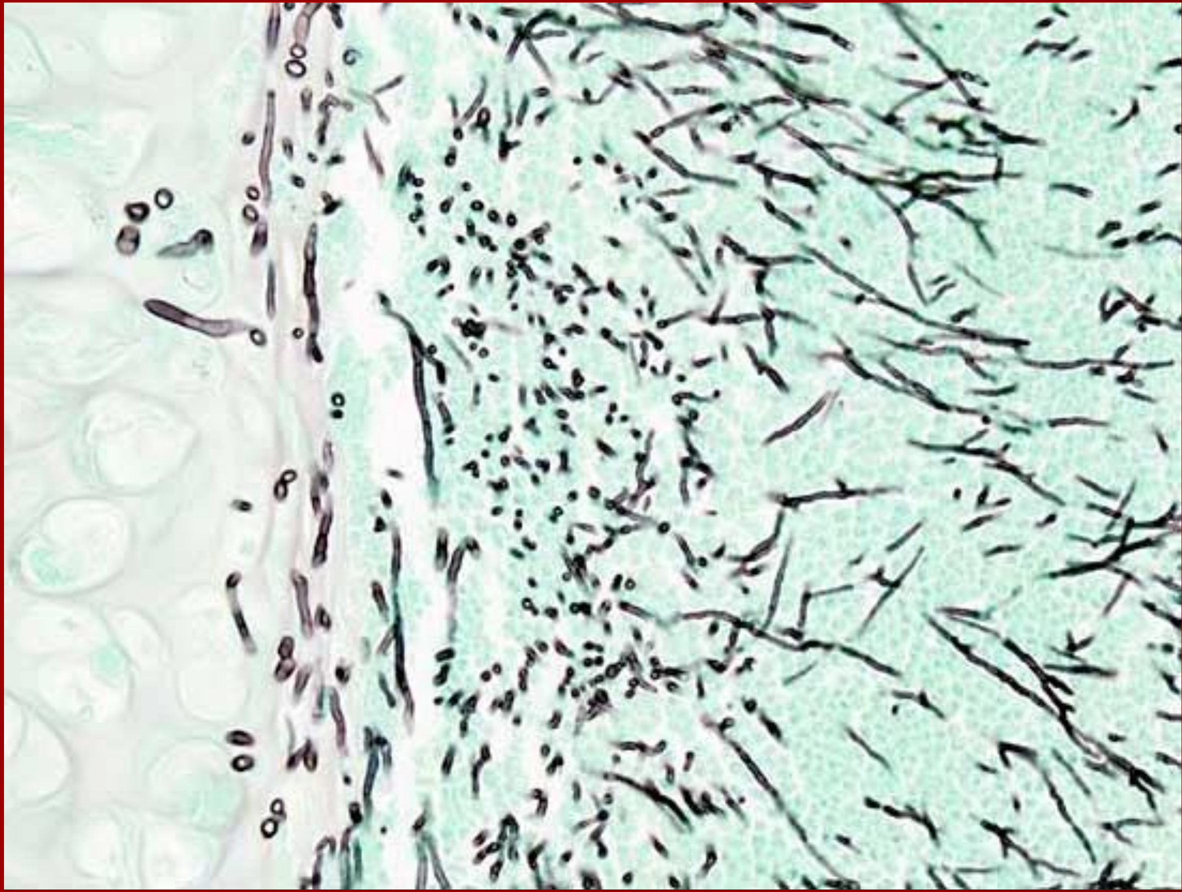
Aspergillus flavus

- Very common soil fungus
- *Aspergillus* spp. produce the carcinogenic mycotoxin, aflatoxin (AF)
- Mycotoxins contaminate 60-80% of crops worldwide
- In total 4.5 billion people have been exposed to aflatoxin worldwide

https://phil.cdc.gov/details.aspx?pid=19546

Aflatoxin Pathology

- *A. flavus* produces B type AFs (AFB1 & AFB2)
- Aflatoxicosis
  - Caused by excessive consumption of AF
  - Either acute or chronic
  - AFB1 considered a Group 1 carcinogen
- Aspergillosis
  - Lung infection present in immunocompromised individuals
  - Ranks top 4 in diseases that cause death in the immunocompromised



https://www.britannica.com/science/aspergillosis#/media/1/38934/126528

Cryptococcus neoformans

Epidemiology

- Fungal pathogen primarily affecting immunocompromised patients
- Causes cryptococcal meningitis (CM)
- Leading cause of death in AIDS patients

Pathogenesis

- Characterized by its cell-wall bound capsule
- Virulence factors:
  - Capsule
  - Melanin production
  - Mannitol production

Why *rdiA*?

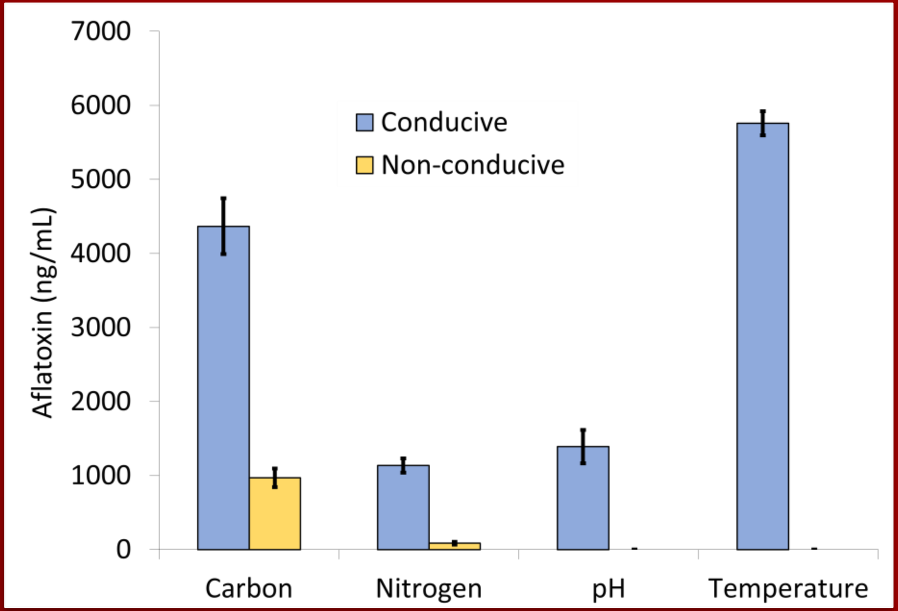
Genotype

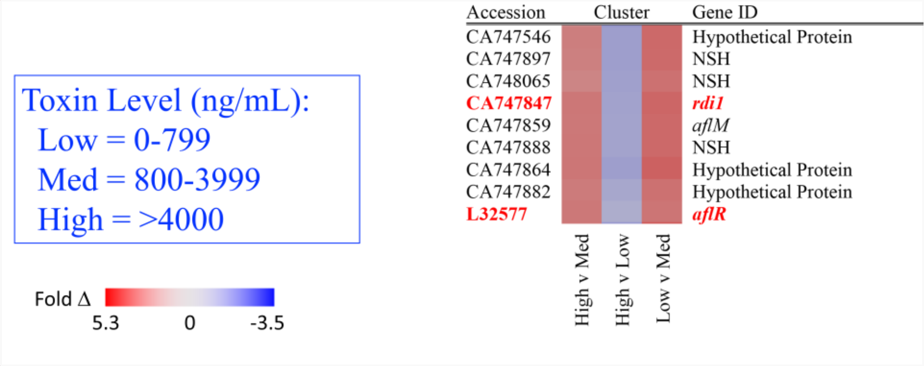
- Has a similar transcription profie to *aflR*
- Previously characterized in *A. flavus* to regulate AF production
- Is a putative Rho-GDP dissociation inhibitor

Phenotype

- *ΔrdiA* mutant is similar to *rdi1Δ* mutant of *C. neoformans* and *bem4Δ* mutant of *S. cerevisiae*
- *A. flavus rdiA* expression partially rescues temperature phenotype of *S. cerevisiae bem4Δ*

Why *rdiA*?

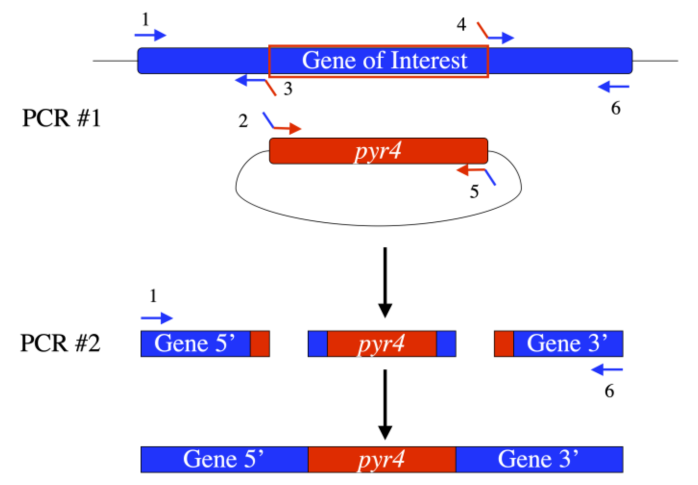



Toxin Level (ng/mL):
Low = 0-799
Med = 800-3999
High = >4000

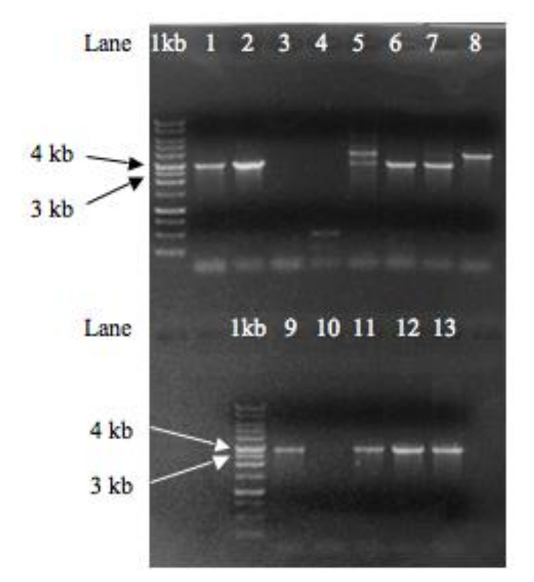
Accession	Gene ID
CA747546	Hypothetical Protein
CA747897	NSH
CA748065	NSH
CA747847	***rdi1***
CA747859	*aflM*
CA747888	NSH
CA747864	Hypothetical Protein
CA747882	Hypothetical Protein
L32577	***aflR***

Characterizing *rdiA*

- Gene-deletion construct



- Confirmation of gene deletion



Characterizing rdiA

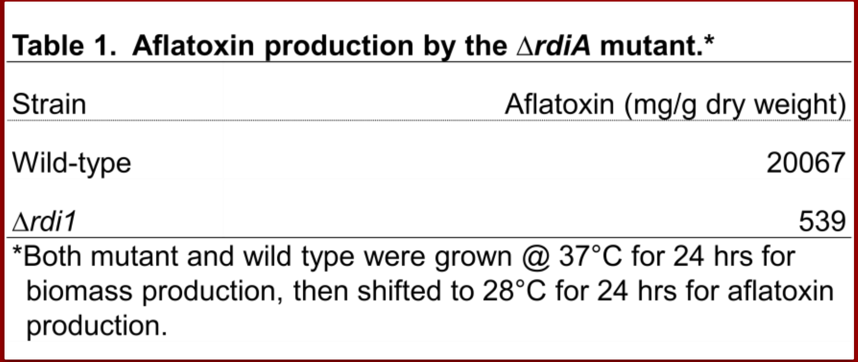
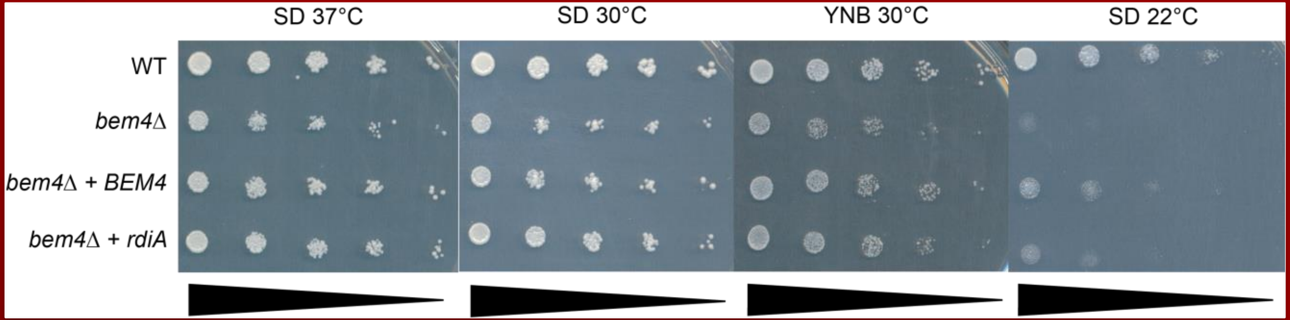


Table 1. Aflatoxin production by the Δ*rdiA* mutant.*

Strain	Aflatoxin (mg/g dry weight)
Wild-type	20067
Δ*rdi1*	539

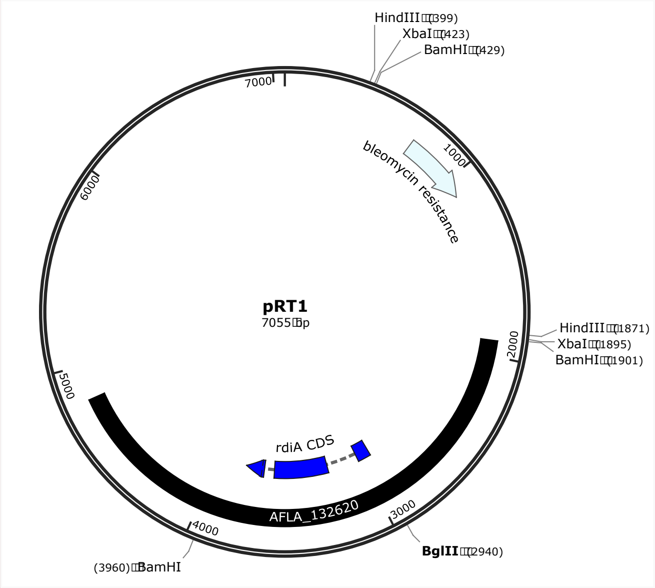
*Both mutant and wild type were grown @ 37°C for 24 hrs for biomass production, then shifted to 28°C for 24 hrs for aflatoxin production.

- Assessment of *rdiA* AF production and discovery of *rdiA* rescuing cold temperature phenotype in *S. cerevisiae bem4Δ* mutant

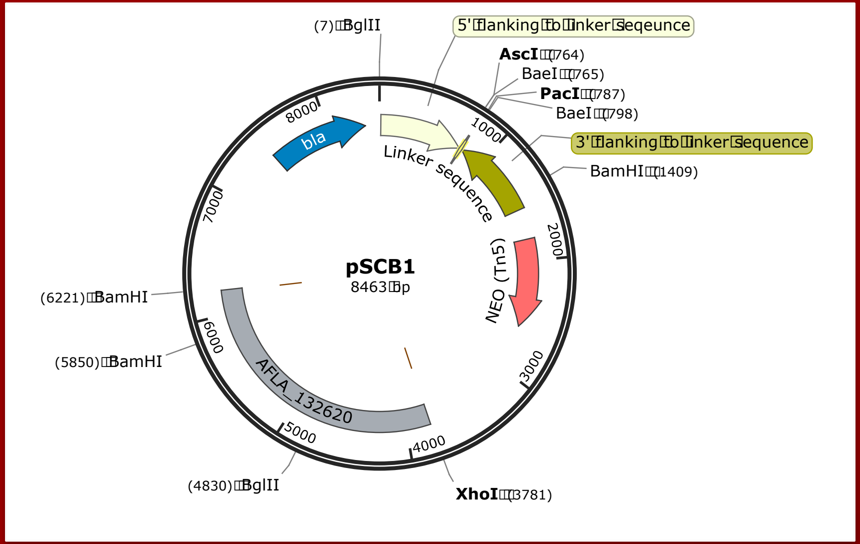


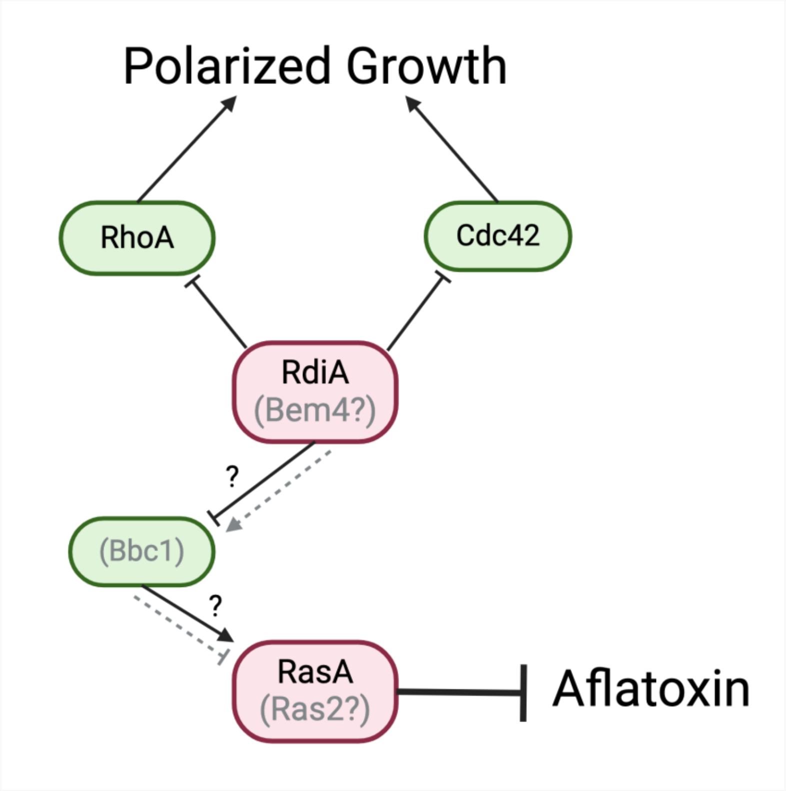
Characterizing *rdiA*

- Plasmid map for complementation of *A. flavus ΔrdiA*



- Plasmid map for complementation of *C. neoformans rdi1Δ*





Proposed Gene Interaction Network

- AF production could be controlled by rdiA

References and Acknowledgements

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Questions?