Characterization of functional subnetwork modules associated with ear rot and fumonisin biosynthesis in maize pathogen *Fusarium verticillioides*

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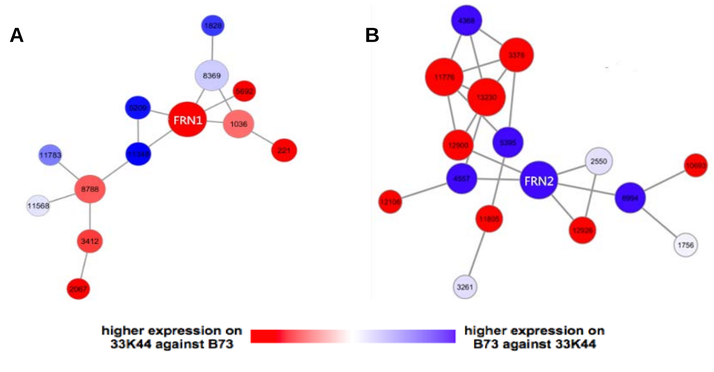
ABSTRACT

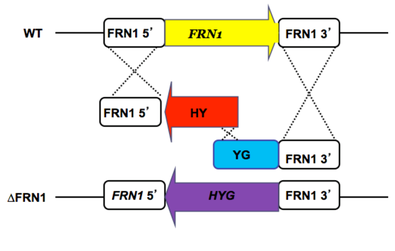
Fusarium verticillioides is a fungal pathogen causing maize ear rot and fumonisin contamination. While certain genes regulating virulence and fumonisin biosynthesis have been characterized, our knowledge of cellular and genetic networks underpinning these mechanisms is still limited. Here, we present a systematic network-based analysis of large-scale *F. verticillioides* RNA-seq datasets to identify potential genetic modules that are associated with virulence and fumonisin regulation. Specifically, our key aims were to identify subnetwork modules in *F. verticillioides* co-expression network that are significantly deferentially expressed in two different maize lines (moderately resistant vs susceptible), then to characterize predicted hub genes in these functional subnetwork modules. Here, we identified two putative hub genes, *FRN1* and *FRN2*, in two independent subnetwork modules; Frn1 is a hypothetical protein devoid of any known domain while Frn2 harbors a peptidoglycan-binding domain. We hypothesized that these genes operate collectively with other linked genes in each subnetwork to regulate fumonisin biosynthesis and pathogenesis. Our gene-deletion study revealed that *FRN1* has little impact in vegetative growth but plays an essential role in fumonisin production, sensitivity to cell wall degrading enzymes and stress response. We hypothesize that Frn2 is a secreted protein and performs a role in host-pathogen interaction and fumonisin biosynthesis.

Introduction

Despite the dramatic recent increase in the number of sequenced fungal genomes, functional characterization of novel fungal genes has not experienced similar success. A large number of uncharacterized genes in plant pathogenic fungi could reveal novel virulence mechanisms. Therefore, predicting putative virulence genes and characterizing the functional role of these unknown genes are critical for elucidating the genetic basis of maize ear rot pathogenesis and fumonisin regulation in *Fusarium verticillioides*. Identification of novel virulence genes can lead to innovative control strategies, such as HIGS-mediated host resistance. In this study, we used transcriptional subnetwork module analysis to identify two novel hypothetical proteins in *F. verticillioides*: Frn1 and Frn2. We hypothesize that these genes play critical role in ear rot and fumonisin regulation. Furthermore, genes associated with two genes in each subnetwork are being investigated to better understand the mechanisms associated with fumonisin production and virulence.

Potential *F. verticillioides* subnetwork modules of (A) *FRN1* & (B) *FRN2* associated with 33K44 against B73

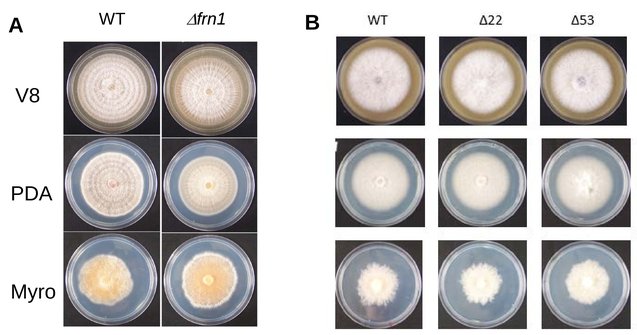




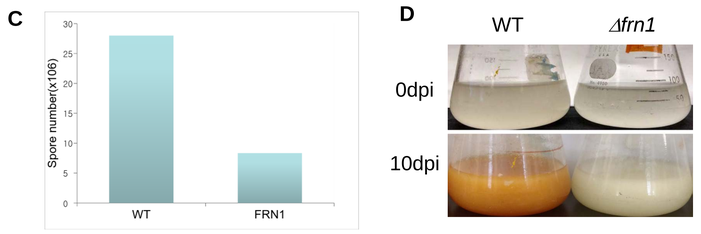
***F. verticillioides* gene knockout strategy**

Schematic depiction of *FRN1* locus in the wild-type genome. The disruption vector and insertion sites are shown as HY and YG, Schematic depiction of disrupted *ΔFRN1*. HYG = hygromycin phosphotransferase

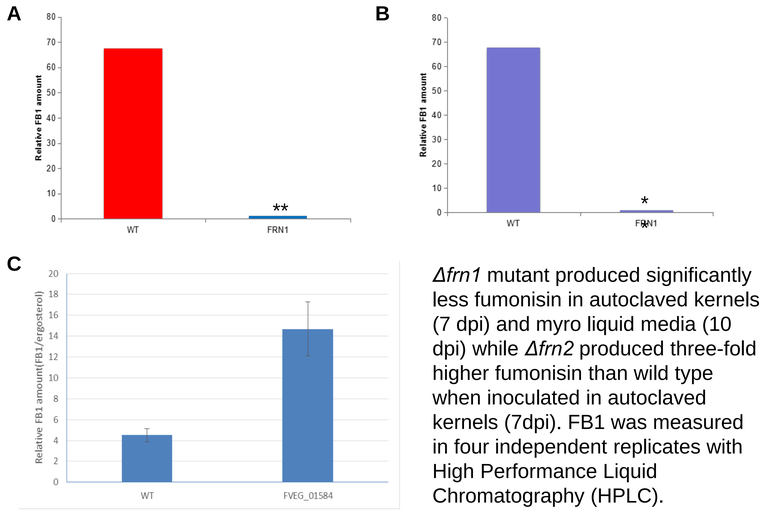
Deletion of (A) *FRN1* and (B) *FRN2* did not impact vegetative growth



***FRN1* deletion mutant produces (C) less spores in living kernels and (D) shows loss of pigmentation in myro liquid medium**

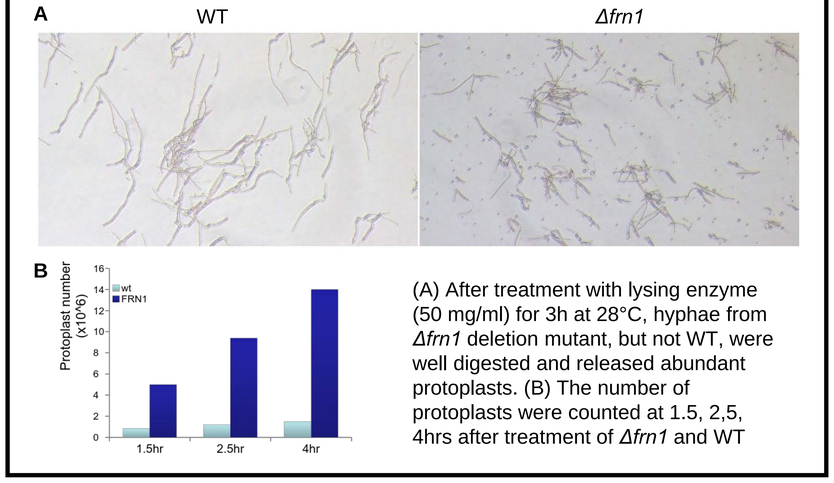


Drastic reduction of fumonisin levels in *Δfrn1* mutant when (A) grown in cracked maize kernels and (B) Myro liquid medium, while (C) *Δfrn2* showed three-fold higher fumonisin production



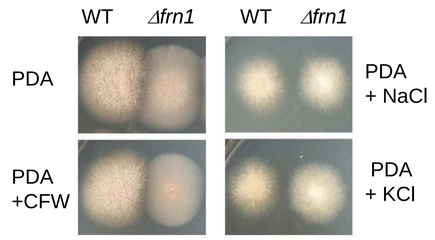
Δfrn1 mutant produced significantly less fumonisin in autoclaved kernels (7 dpi) and myro liquid media (10 dpi) while *Δfrn2* produced three-fold higher fumonisin than wild type when inoculated in autoclaved kernels (7dpi). FB1 was measured in four independent replicates with High Performance Liquid Chromatography (HPLC).

***Δfrn1* strain showed increased sensitivity to lysing enzyme**



(A) After treatment with lysing enzyme (50 mg/ml) for 3h at 28°C, hyphae from *Δfrn1* deletion mutant, but not WT, were well digested and released abundant protoplasts. (B) The number of protoplasts were counted at 1.5, 2,5, 4hrs after treatment of *Δfrn1* and WT

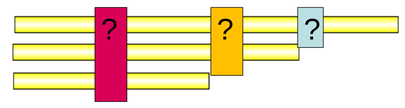
Osmotic stress rescues growth defect in *Δfrn1* mutant on PDA



Effect of CFW (25mg/ml), 1M NaCl, 1M KCl on *F. verticillioides* wild-type and mutant strains. Spore suspension (2μl) containing 2x10^4 was inoculated on plates and incubated at room temperature. The plates were photographed 4 days after inoculation.

Future research plans

A. Testing whether a uncharacterized functional motif exists in Frn1 protein

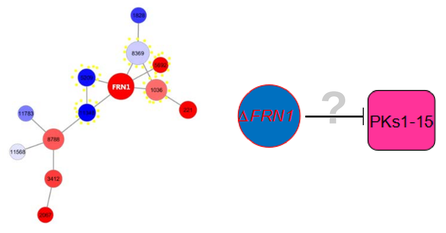


Constructs with complete *FRN1*, 75% *FRN1*, 50%*FRN1* will be used to complement *FRN1* deletion mutant.

B. Testing whether localization of Frn1 protein in the cell can help us predict function

We observed 7 putative TM domains in Frn1. GFP will be fused to *FRN1* and transformed into *FRN1* deletion strain to study how Frn1 plays a role in virulence.

C. qPCR analyses of *FRN1*-neighboring genes in the subnetwork and *PKS* genes



Total RNA samples will be prepared from fungal strains grown in defined medium. Expression levels of neighboring genes in the subnetwork and 15 *F. verticillioides PKS* (polyketide synthases) genes will be analyzed by qPCR in *Δfrn1* mutant.

Summary

This study is focused on characterizing the role of *FRN1* and *FRN2* genes in maize ear rot and fumonisin regulation. These genes were discovered through our network-based gene association analysis. However, these genes encode hypothetical proteins with unknown function. For *FRN1,* our immediate aim is to test how this gene regulates secondary metabolism in *F. verticillioides*, by investigating novel functional motifs, association between cellular localization and function, and transcriptional regulatory network. For *FRN2*, we are focusing on studying putative functional motifs and cellular secretion.

Reference

Ridenour JB, and Bluhm BH. 2016. The novel fungal-specific gene *FUG1* has a role in pathogenicity and fumonisin biosynthesis in *Fusarium verticillioides*. Mol. Plant Pathol. (In Press - doi: 10.1111/mpp.12414)

Kim M, Zhang H, Woloshuk C, Shim WB, Yoon BJ. 2015. Computational identification of genetic subnetwork modules associated with maize defense response to *Fusarium verticillioides*. BMC Bioinformatics 16 Suppl 13:S12

Acknowledgments

This research was supported by the USDA NIFA Agriculture and Food Research Initiative Competitive Grants Program (2013-68004-20359).