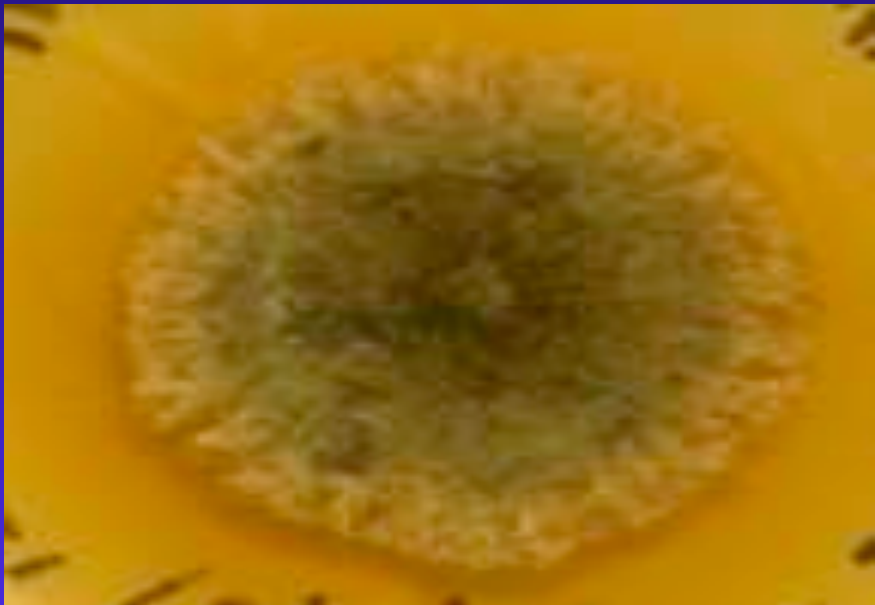
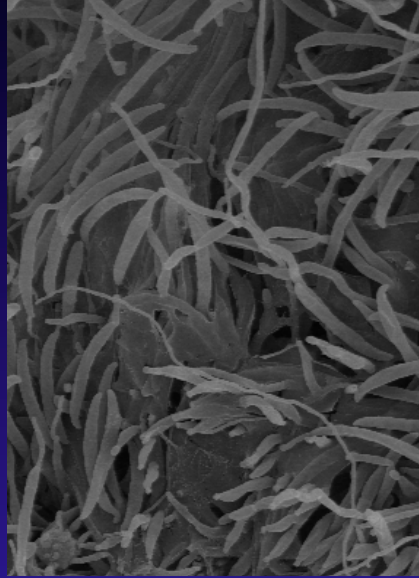
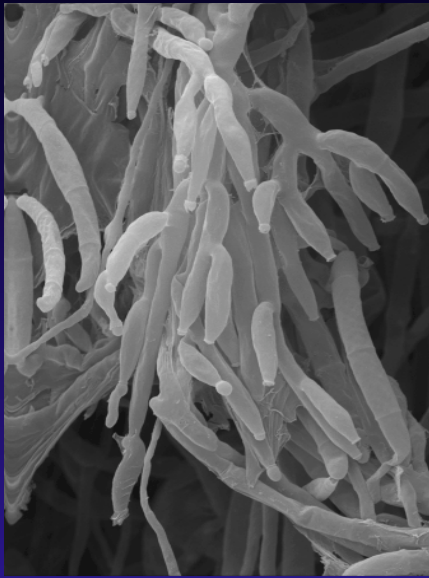


**Identifying fungal factors affecting *Fusarium*
virguliforme aggressiveness and
SDS development**



Fusarium virguliforme



Objectives

- Identification of fungal genes involved in the development of SDS
- Identification and characterization of pathways involved in virulence and pathogenesis
- Detection of the karyotypic variation among *Fusarium virguliforme* isolates

Limitations

- Lack of genetic material (mutants, sequences...)
- Lack of tools to manipulate genes in the pathogen

Approach

- Lack of genetic material
 - Generate REMI mutants
 - Generate and collaborate to generate sequence material
- Lack of tools to manipulate genes in the pathogen
 - Optimize transformation system
 - Optimize site directed mutagenesis

Approach

- Lack of genetic material
 - Generate REMI mutants
 - ~800 mutants have been generated so far
 - Mutants screened for conidiation and growth pattern
 - One mutant with impaired ability to grow on lignin and to infect soybean has been selected for further studies

Approach

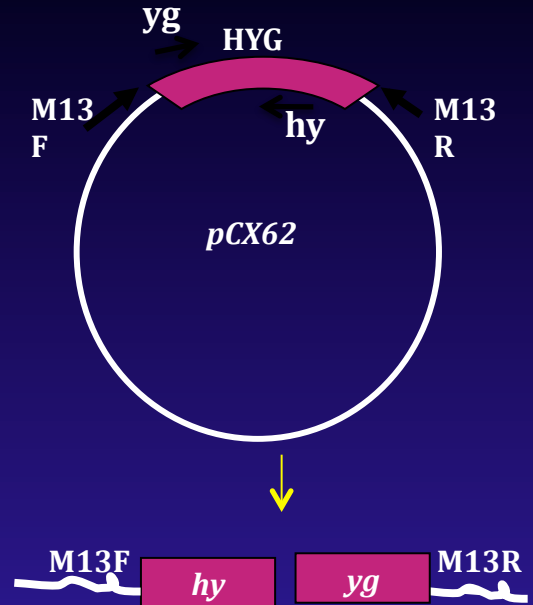
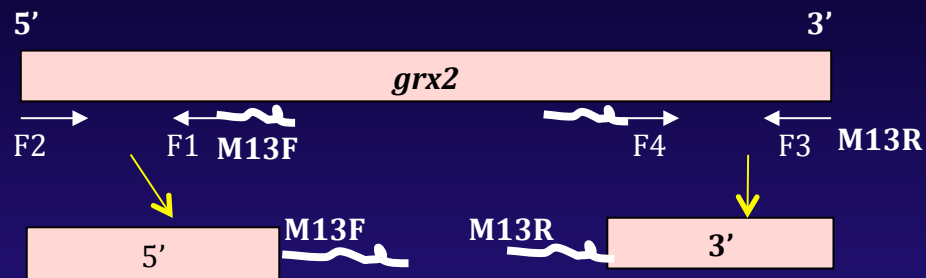
- Lack of genetic material
 - Generate and collaborate to generate sequence material
 - Necessary in identifying targets for disruption
 - Expedite gene disruption
 - Permits genomic and comparative genomic studies (complements karyotyping, population studies...)

Approach

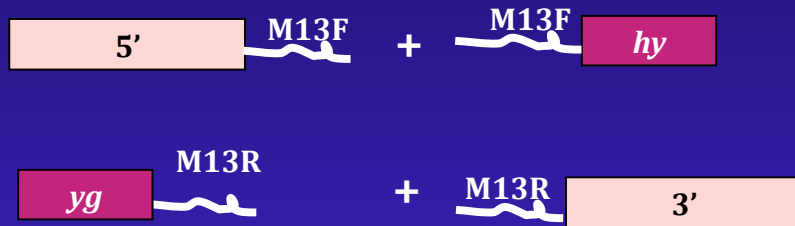
- Lack of tools to manipulate genes in the pathogen
 - Optimize transformation system
 - A split-maker approach is being tested to disrupt genes
 - Developing auxotrophs
 - Three genes are being targeted at this point (cloned from *virguliforme*)
 - *Snf1*, *grx*, and *fsr1*

Site directed mutagenesis

1. PCR on Mont-1 and *pCX62* using *grx2* and HYG primers respectively



2. Fusion PCR on *HYG* and *grx2*

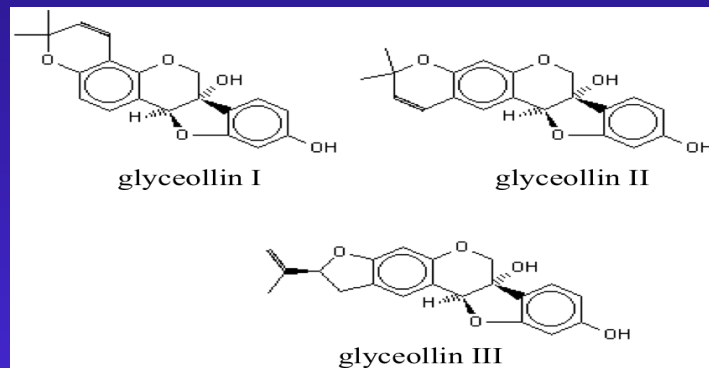


3. *F. virguliforme* (Mont-1) transformation using 3'*grx2-hyg* and 5'*grx2-hyg*

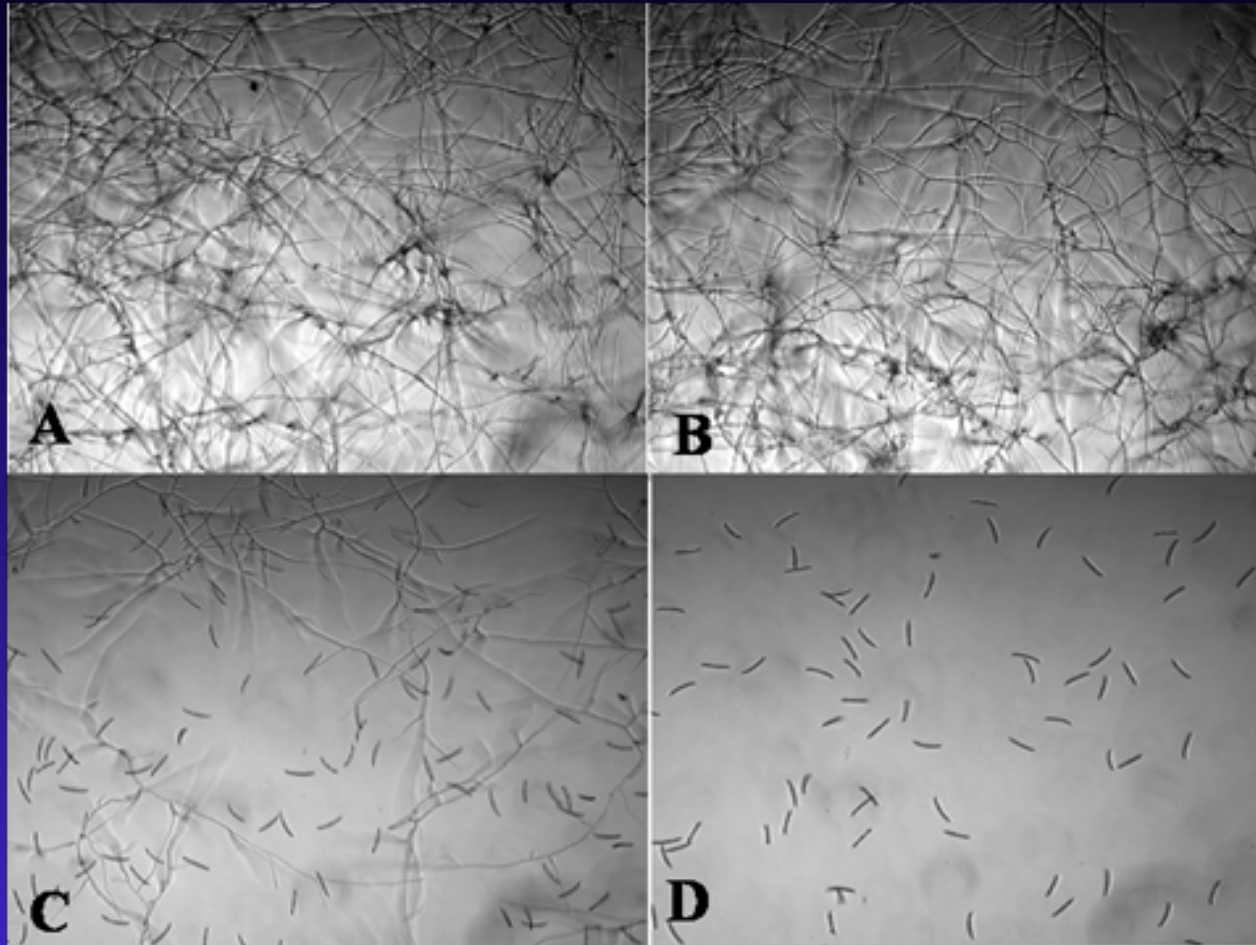


Glyceollin

- Genes differentially expressed upon exposure to glyceollin
- Glyceollins are the major phytoalexins in soybean

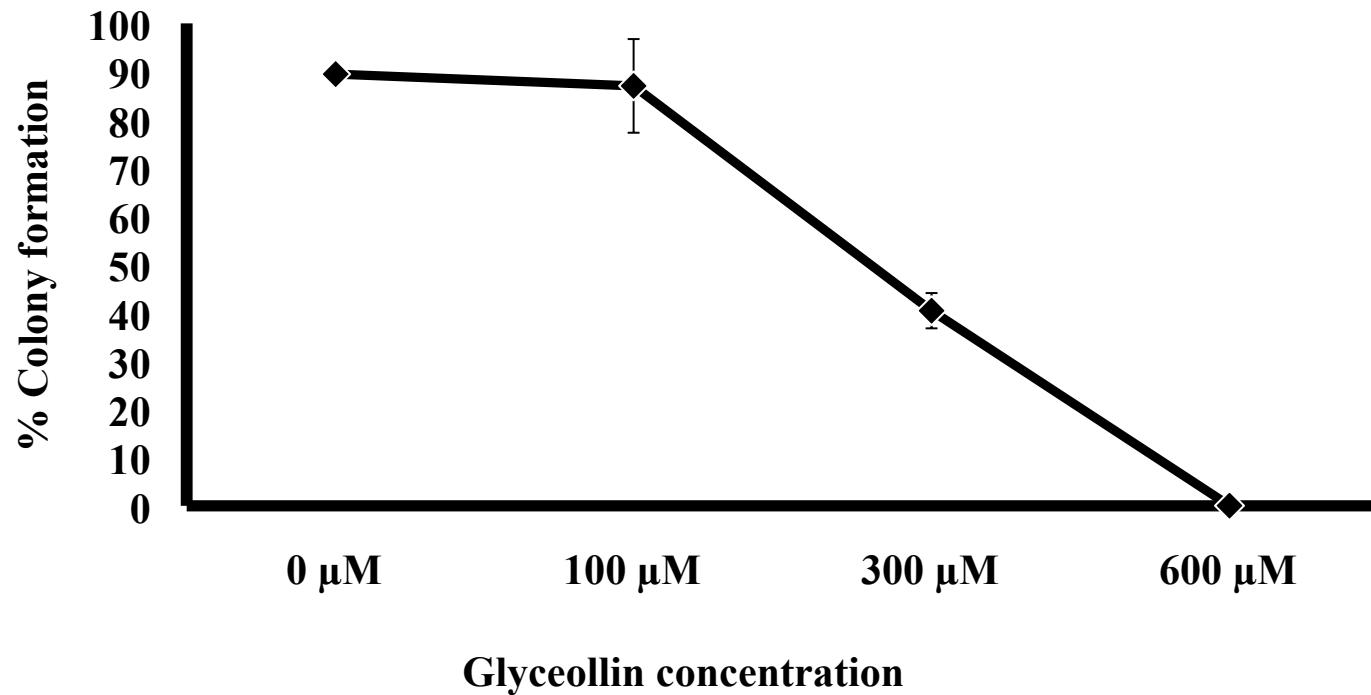


Effect of glyceollin on *F. virguliforme*



A) no glyceollin; B) 100 μ M glyceollin; C) 300 μ M glyceollin; D) 600 μ M glyceollin

Effect of glyceollin on *F. virguliforme*



cDNA-AFLP

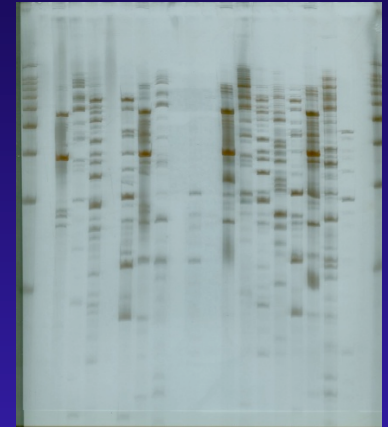
Fungal tissue preparation

Total RNA extraction from fungal tissue

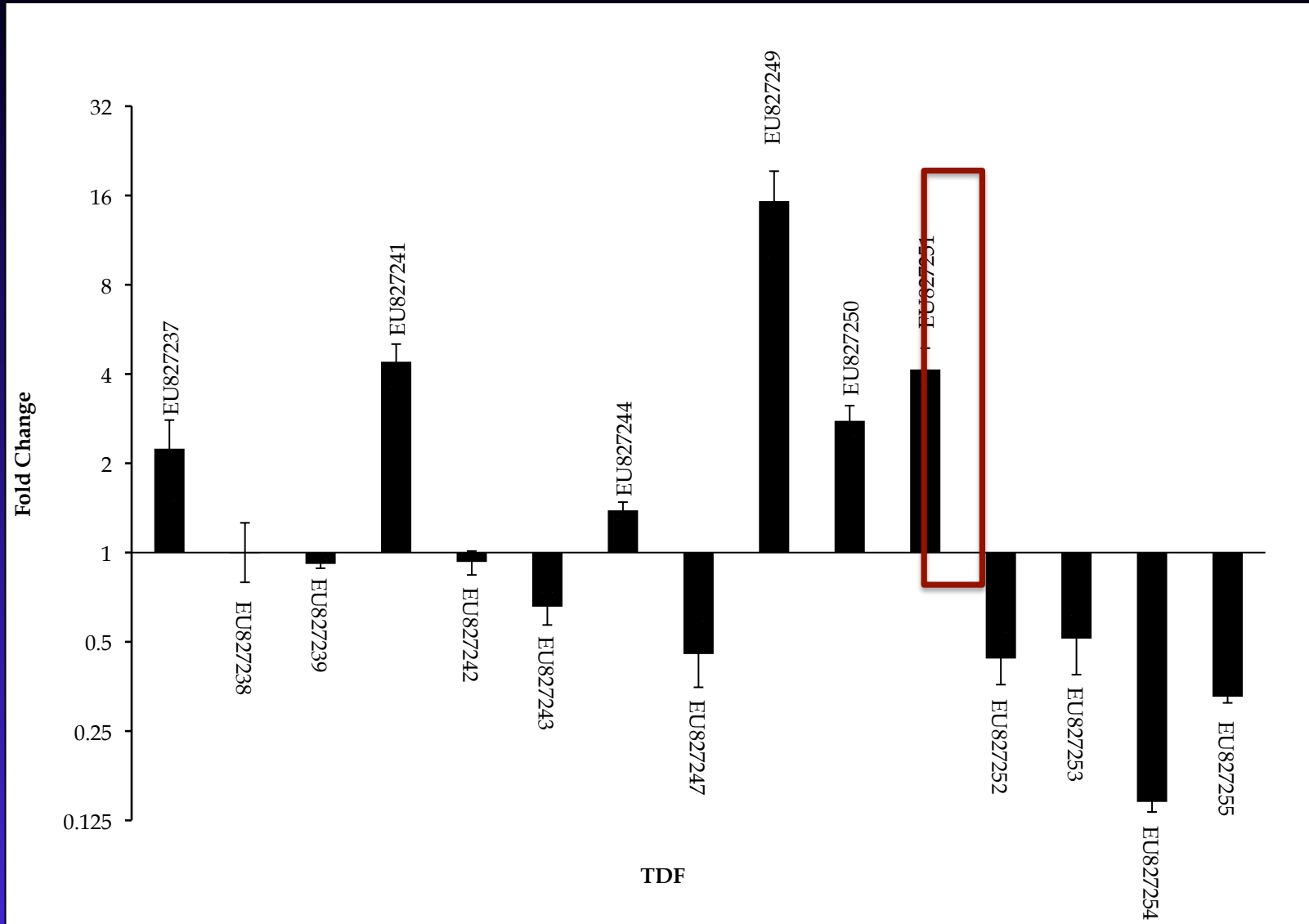
double stranded cDNA preparation

cDNA-AFLP

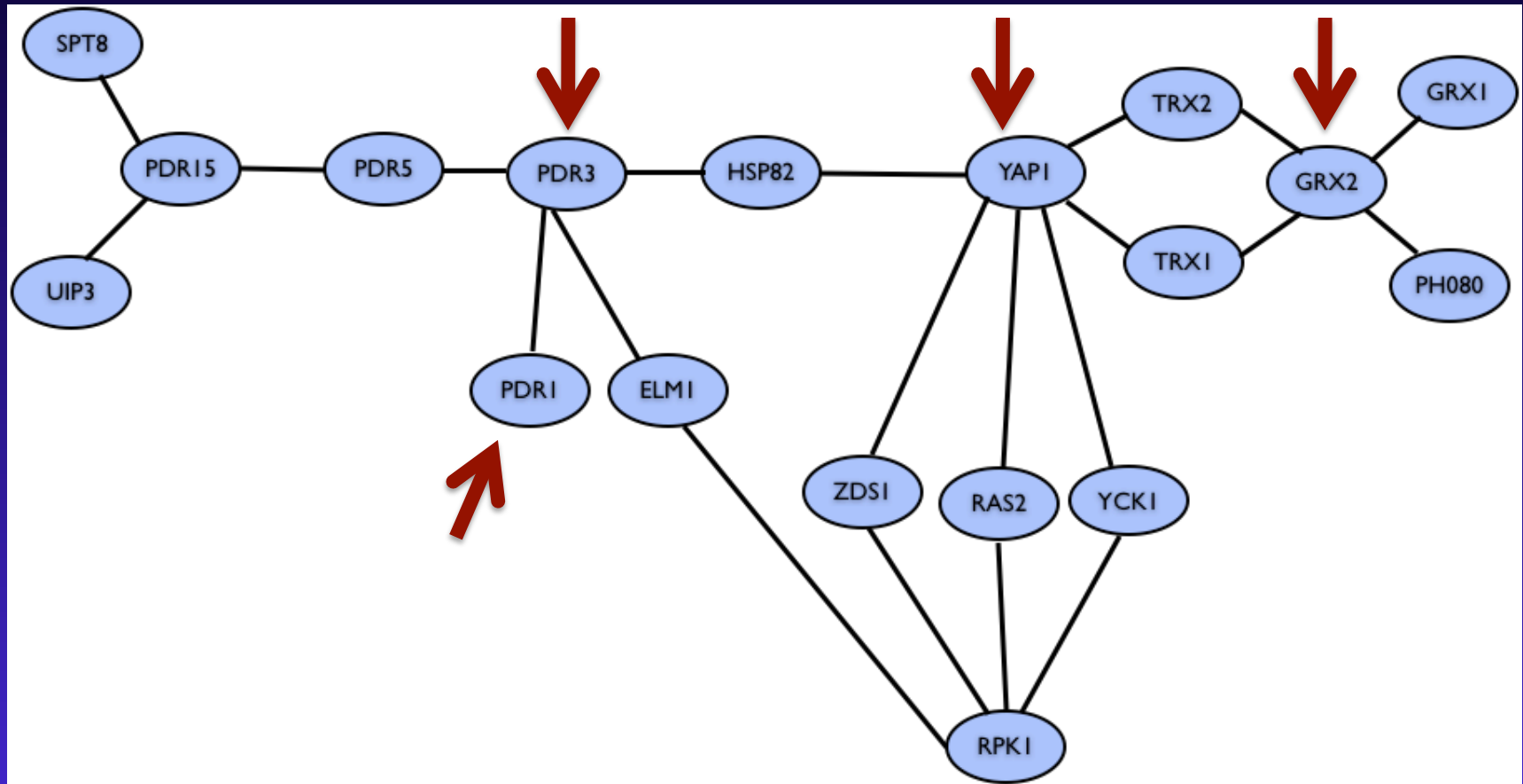
RT-PCR



RT-PCR



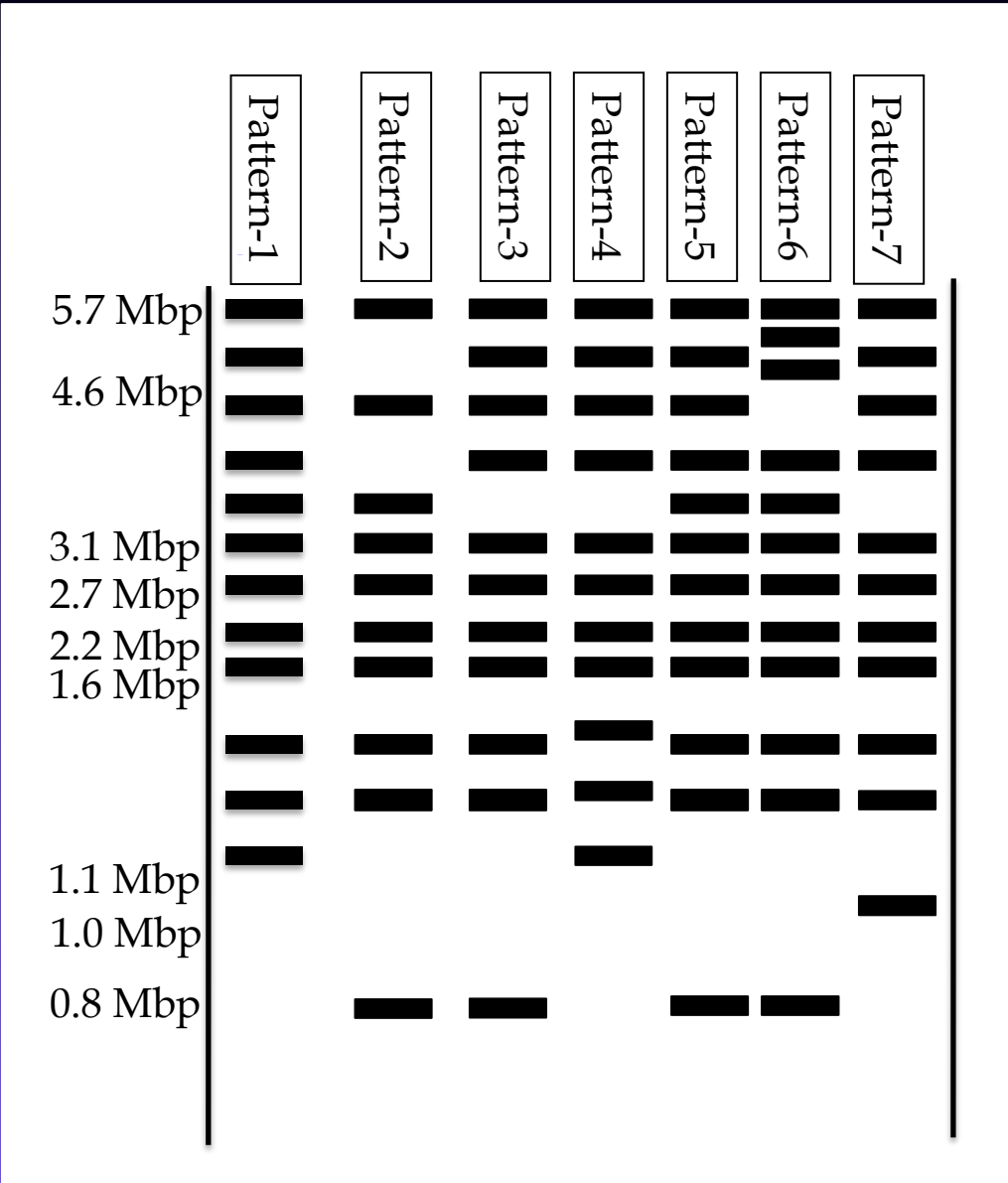
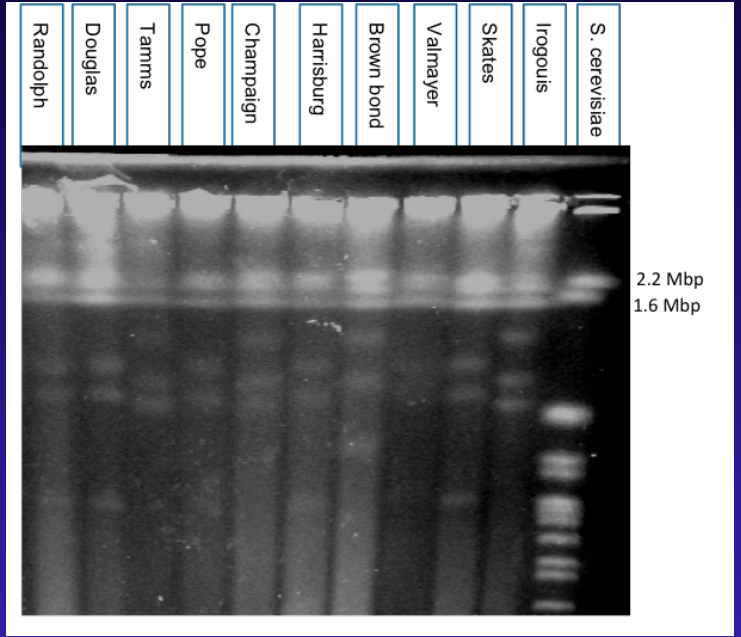
Hypothetical interactome of *F. virguliforme*

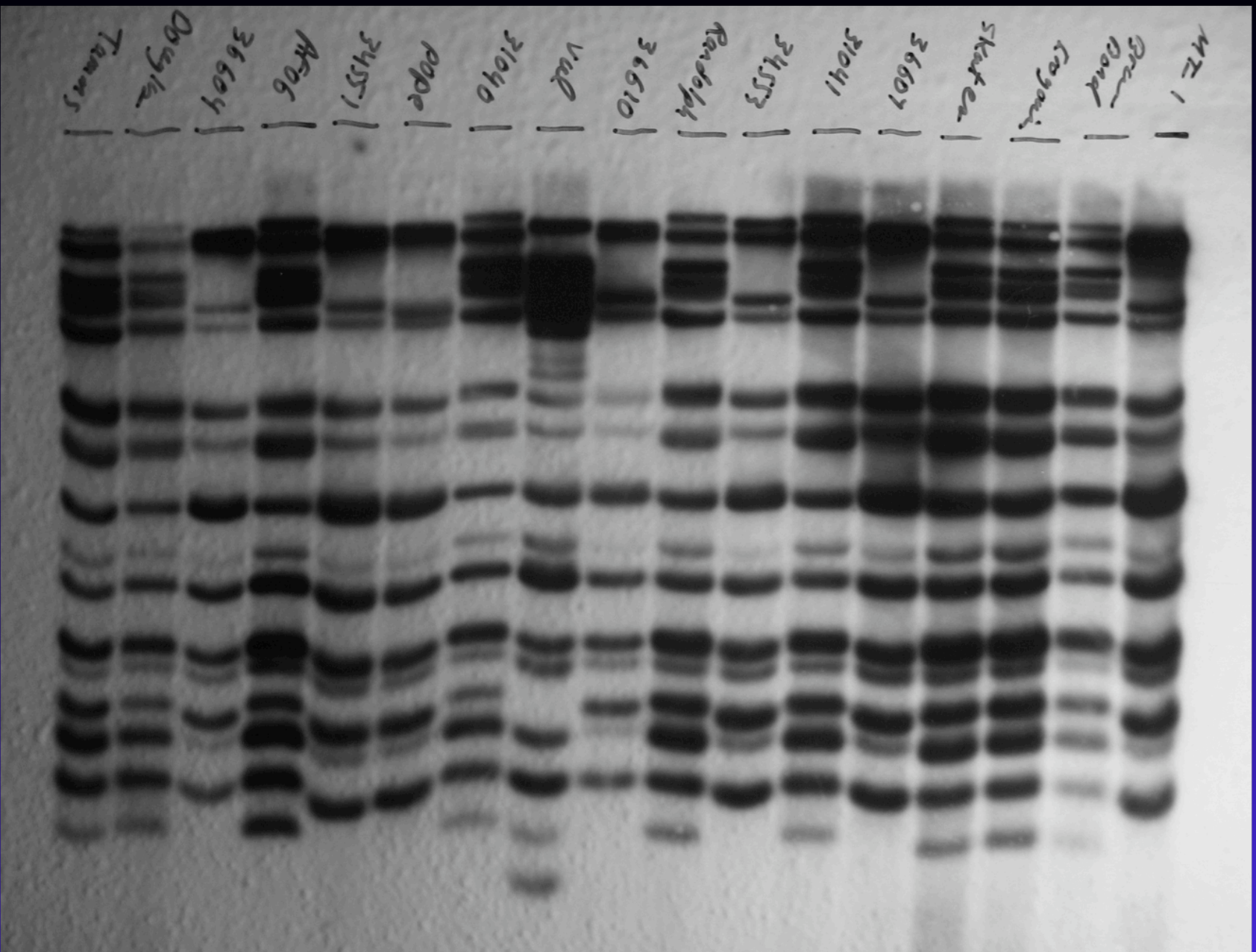


Objectives

- Identification of fungal genes involved in the development of SDS
- Identification and characterization of pathways involved in virulence and pathogenesis
- Detection of the karyotypic variation among *Fusarium virguliforme* isolates

Isolate	Geographical origin
Iowa07	Iowa
Mont-1	provided by NSRC, Illinois
Vick07	Illinois
Af06	Illinois
ARC07	Illinois
Carmi07	Illinois
22292	Illinois
31039	Illinois
31040	Illinois
31041	Illinois
22823	Indiana
22825	Indiana
34551	Argentina
34553	Argentina
36604	Argentina
36607	Argentina
36610	Argentina





Pathogenecity assay



We Appreciate Your Support

