

Development of Immunocapture Real-Time PCR to detect *Fusarium* species in Grains and Foods

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Concern for *Fusarium* species

- Trichothecenes, zearalenone, fumonisins
- FDA advisory levels
- Regulated in other countries
- Mycotoxins survive processing
- *Fusarium* mycotoxins in foods

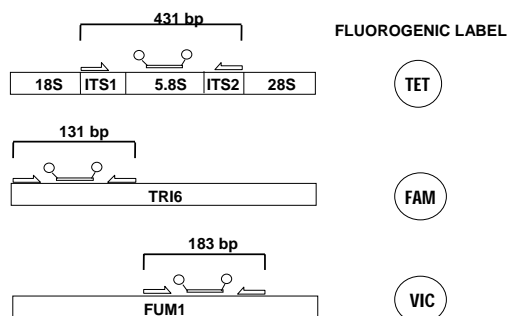
Objective of Research

- To develop immunocapture of *Fusarium* species using antibodies
- To make PCR primers specific for *F. graminearum* and *F. verticillioides*
- To use immunocapture real-time PCR to detect *Fusarium* species in foods and grains

PCR Primers for *Fusarium* species

- General - DNA flanking the 5.8S rDNA
- *Trichothecene* - *Tri6* regulatory gene for biosynthesis
- Fumonisin - *Fum1* polyketide synthase gene for biosynthesis

Primers and Probes Developed



Immunocapture Methods

- To use immunocapture qPCR to detect *Fusarium* species in foods
- Antibodies produced to *F. graminearum* and *F. verticillioides*
- Developed immunocapture qPCR
- Problem – release of DNA from conidia

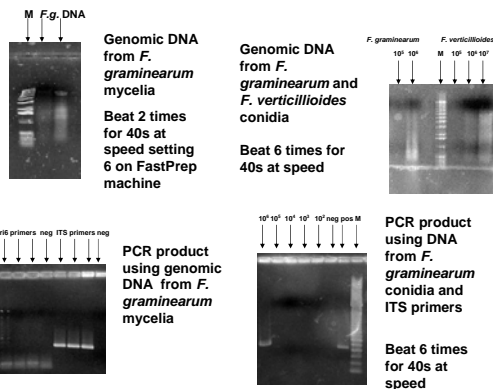
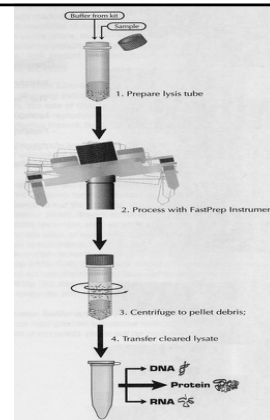
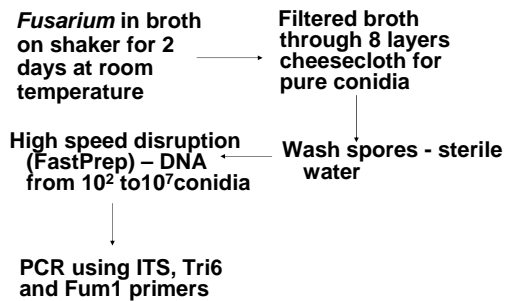
Proposed Future Research from 2004

1. Procedures to break conidia for qPCR
2. Simplify immunocapture before qPCR
3. Test in industry to determine cost effectiveness

1. Disruption of Conidia

- Enzymes to lyse conidia
- Microwave
- Vortex with beads, sand, etc.
- FastDNA® SPIN Kit with FastPrep® Instrument

Disruption of Conidia



2. Immunocapture qPCR

- Simplify immunocapture before qPCR
- Depends on disruption of conidia

3. Proposed Processing Research

- **Test in industry to determine cost effectiveness**
- **Proposal submitted to USDA NRI**
- **Ranked High Priority but not funded**

Summary of Research

- **Methods – enzymes, beads, microwave – no results**
- **FastPrep® Kit and Instrument**
- **Recommended procedure – no DNA**
- **Microscopic slides – intact conidia**
- **Too much beating destroys DNA**
- **Need to develop proper protocol**

Future Research

- **Continue to refine FastPrep® Method**
- **Combine with qPCR**
- **Use with food and grain samples**
- **Future funding questionable**

Genetic Detection Research

- **Monitor genetics for fungal epidemics**
- **Library of PCR primers**
- **Conventional, real-time, multiplex PCR**
- **Fungi that produce major mycotoxins**
 - aflatoxins, fumonisins, ochratoxin, patulin, trichothecenes, zearalenone