
# Immunocapture real-time PCR to detect mycotoxigenic mold spores in grains

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## Project Rationale

Currently, there are few commercial rapid methods to detect molds and their spores in agricultural commodities, grains, and foods. In previous research, a protocol was developed to identify *Fusarium* species that produce two major mycotoxins: fumonisins and trichothecenes. This antibody-based method was developed for *Fusarium* species to capture antigens of these mycotoxin-producers, which were then combined with a real-time PCR assay that was based on species-specific and genus-specific primers to identify the *Fusarium* species. The efficiency of spore capture was limited in the previous research because the *Fusarium* spores were difficult to lyse for DNA release. We proposed this new research to help resolve that limitation by: (1) studying physical, enzymatic, and mechanical methods to break mold spores to release DNA for use in real-time PCR, and (2) incorporating the method developed in objective 1 into the immunocapture-qPCR method that uses antibodies produced against *F. graminearum* and *F. verticillioides* and primers that are specific for the *Tri6* gene involved in trichothecene biosynthesis and for the *Fum1* gene involved in fumonisin biosynthesis. In addition, we proposed to develop a library of PCR primers to other mycotoxigenic genera (*Aspergillus* that produce aflatoxins and ochratoxin and *Penicillium* that produce ochratoxin and patulin) for real-time PCR, and to use these primers in multiplex PCR formats to detect all major mycotoxin producers in the same assay. Antibodies to aflatoxin-producing molds and *Penicillium* species were produced in earlier research.

## Project Objectives

- Develop primer sets to detect *Aspergillus* and *Penicillium* species.
- Experiment with different methods to break mold spores of *Fusarium* species.
- Determine the specificity and sensitivity of primer sets and multiplex format.
- Optimize the capture of mold spores and release of DNA used to detect *Fusarium* species in foods and grains.

## Project Highlights

A procedure was developed and optimized using lyticase (Sigma: 5263) to extract DNA from conidia of *Fusarium graminearum* and *Fusarium verticillioides* for use in real-time quantitative PCR (qPCR). This was important because the methods used to extract DNA from other microorganisms do not work for filamentous fungi. Lyticase was mixed with buffer and mercaptoethanol, incubated at 37°C for four or six hours for *F. graminearum* and *F. verticillioides*, respectively, and shaken in a bead-beater to physically disrupt the conidia before analyzing with real-time qPCR. By this method, a minimum of 10 conidia of *F. graminearum* and 1000 conidia of *F. verticillioides* could be detected.

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