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**Project Title: Structural and Functional Studies of Trichothecene Biosynthetic Enzymes.**

## PROJECT 1 ABSTRACT

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There are two components to this proposal. First, it is planned to obtain fundamental knowledge about the enzymes responsible for trichothecene biosynthesis in *Fusarium sporotrichoides* and *F. graminearum* by determining the three dimensional structures and molecular mechanisms of all of the enzymes in the biosynthetic pathway. Second, it is planned to use this structural information to create modified or new agents capable of inactivating or degrading the mycotoxins responsible for the damage caused by Fusarium Head Blight. This latter component will be accomplished by directed evolution utilizing the biosynthetic enzymes as templates for new degradative enzymes. The strategies for accomplishing this type or protein engineering are now well established, but all require a knowledge of three dimensional structure. At the present time little is known about the molecular structure of the enzymes responsible for synthesizing trichothecene mycotoxins. Thus, the specific aims of the project are to:

1. clone, express, and purify the biosynthetic enzymes in the trichothecene biosynthetic pathway.
2. determine the structure of the trichothecene 3-*O*-acetylase from *F. sporotrichoides* (Tri101) and *F. graminearum* and identify the molecular basis for its specificity. Tri101 is the first structural priority, since this enzyme has been shown to provide partial protection against the spread of *F. graminearum* in transgenic wheat.
3. perform a structural analysis for each of the enzymes in the pathway that proves to be soluble and amenable to crystallographic study. Where possible structures will be determined in the presence of substrates or products.
4. apply directed evolution to the most suitable structural templates and screen for protection against T-2 toxin and deoxynivalenol in *Saccharomyces cerevisae*.

The work proposed here will certainly establish a greater understanding of how trichothecene mycotoxins are synthesized, however this alone will not contribute directly to better methods for controlling FHB. The second component to this study is directed to providing better agents for inactivating the toxins. Although this latter research is speculative, it has the potential to make a significant contribution to the control of FHB at the molecular level.