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Research Category: **PBG** **Duration of Award: 1 Year**

Project Title: Development of improved Enzymes for the Inactivation of Trichothecene Toxins.

PROJECT 1 ABSTRACT

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The primary goal of this proposal is to develop improved enzymes for the inactivation and degradation of fungal mycotoxins associated with Fusarium head blight. This year it is planned to utilize the three-dimensional structure and kinetic properties of trichothecene 3-*O*-acetylase from *F. sporotrichioides* and *F. graminearum* (Tri101) to develop a modified enzyme with improved efficacy towards the inactivation of DON and nivalenol. This will be accomplished by protein engineering starting from the structures and kinetic analyses of Tri101 from *F. sporotrichioides* and *F. graminearum* that were completed during the past year. The prospect of success in this first phase is high because the kinetic analysis of this enzyme suggest that there are already significant differences in specificity between isozymes from different fungi. An important component of the investigation this coming year will be to continue to integrate the *in vitro* studies of Tri101 with the properties of the enzyme expressed in transgenic cereals to investigate whether the limited performance of the transgenic cereals is due to low expression, inactive, or posttranslationally modified protein. Investigation of the oxidative enzymes in the trichothecene biosynthetic pathway will be continued at a reduced level. Much less is known about these enzymes, yet they play vital roles in trichothecene biosynthesis. This will establish a framework for expanding the repertoire of biodegradative agents in future years. Thus the specific aims of the project are to:

1. apply protein engineering to the trichothecene 3-*O*-acetylase (Tri101) from *F. sporotrichioides* and *F. graminearum* to improve the function and stability of the enzyme. This is the first priority, since *tri101* has been shown to provide partial protection against the spread of *F. graminearum* in transgenic wheat. These modified genes will be inserted into plasmid pUBK so that the efficacy of these genes in combating FHB can be tested in barley.
2. correlate the structure and function of the Tri101 protein produced in *E. coli* with that isolated directly from *F. sporotrichioides* and *F. graminearum* and from transgenic barley. This will ascertain the level of activity of the enzyme expressed in transgenic barley and establish a connection between the *in vitro* and *in viv*o studies of Tri101.
3. initiate a structural and biochemical analysis of the oxidative biosynthetic enzyme Tri11 in the trichothecene biosynthetic pathway.

The work proposed here should lead to improved biological agents for inactivating mycotoxins and is expected to aid in the development of better methods for controlling FHB. At a fundamental level these studies will contribute to a greater understanding of how trichothecene mycotoxins are synthesized.