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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 and test their efficacy in barley. In the next two years we plan 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 previous funding cycles. The prospect of success in this first phase is high because the kinetic analysis of these enzymes demonstrate that the Tri101 from *F. graminearum* is 70 fold catalytically more efficient at inactivating DON than the enzyme from *F. sporotrichioides*, where the later enzyme has shown promising but limited effectiveness at controlling FHB in transgenic barley. An important new component of the investigation will be a program to test the improved enzymes against FHB in barley and as such this proposal represents an interdisciplinary collaboration. We also will 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, or inactive or post-translationally modified protein. Thus the specific aims of the project are to:

1. apply protein engineering to the trichothecene 3-*O*-acetylase (Tri101) from *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.
2. insert the improved genes into plasmid pUBK and transform them into barley to create transgenic strains. These will be tested for resistance to FHB once homozygous lines are identified.
3. 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 vivo* studies of Tri101. This will establish a biochemical foundation for assessing the efficacy of Tri101 as a protective agent against FHB.

This proposal is consistent with the research priorities of both PBG and GDER.