FY20 USWBSI Project Abstract

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Project Title: Mechanisms of Ammonium Sensing and Ammonium Suppression of DON

Biosynthesis

PROJECT 1 ABSTRACT

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Fusarium graminearum is a causal agent of Fusarium head blight (FHB) and a producer of deoxynivalenol (DON). Ras2 GTPase and its potential downstream cAMP-PKA and Gpmk1 pathways all are important for regulating DON biosynthesis and plant infection. Whereas non-preferred nitrogen sources including arginine and putrescine induce DON biosynthesis, ammonium suppresses TRI gene expression. The global nitrogen transcriptional regulator Are1 also plays a critical role in regulating DON biosynthesis and three ammonium permease (MEP) genes in F. graminearum. Our preliminary data showed that MEP2 functions as an ammonium sensor and its cytoplasmic tail (CT or Mep2-CT) is essential for functions. The mep2 mutant was defective in ammonium repression of DON production. However, to date, it is not clear how the nitrogen availability signal recognized by a membrane protein Mep2 is relayed to intracellular targets and what is its functional relationship with Are1.

The goal of this study is to understand how ammonium sensing leads to the suppression of DON production. Based on our preliminary data and phenotypes of *are1*, *ras2* and *mep2* mutants, we hypothesized that Mep2-CT interacts with Ras2 and nitrogen availability signals are relayed to cAMP-PKA or Gpmk1 for regulating Are1 activation and DON biosynthesis. Objective 1 aims to identify and characterize the amino acid sequences of Mep2-CT responsible for ammonium suppression of DON production. The roles of Mep2 in regulating Are1 and genes responsible for the uptake and utilization of arginine or putrescine also will be determined. For objective 2, besides characterizing the interaction and functional relationship between Mep2 and Ras2, we will examine the roles of cAMP-PKA in ammonium repression. Objective 3 will determine the roles of Are1 in *TRI* gene expression and Mep2 functions. Are1 may directly regulate *TRI* expression and phosphorylation of Are1 by PKA/Gpmk1 may connect Ras2-Mep2 with DON biosynthesis.

Overall, results from proposed experiments will be helpful to better understand the transcriptional regulation of *TRI* gene expression and DON biosynthesis in *F. graminearum*, and genetic mechanisms for ammonium suppression of secondary metabolism, which is a common phenomenon in fungal pathogens. Inhibiting DON biosynthesis can be used to control FHB or avoid mycotoxin contamination. Proposed study fits the research area of PBG on characterizing plant-fungal interactions to identify genes that may be useful to reduce DON contamination in barley and wheat. It is a project based on recent progresses.