Understanding endophyte-mediated dollar spot resistance in red fescue as a new approach to improving management of dollar spot in creeping bentgrass

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Dollar spot is one of the most problematic diseases for many golf courses, particularly on creeping bentgrass, often requiring repeated applications of fungicides. In addition to creeping bentgrass, dollar spot can also be a problem on strong creeping red fescue. However, when strong creeping red fescue is infected with the symbiotic fungal endophyte *Epichloë festucae*, the plants exhibit resistance to dollar spot (Clarke et al., 2006). How infection by the endophytic fungus confers disease resistance to the host red fescue is not known. Resistance to fungal pathogens is not an established effect of endophyte infection of other grass species, and may therefore be unique to the fine fescues.

We are characterizing an *E. festucae* antifungal protein that we first identified through a large scale transcriptome study comparing endophyte-free and endophyte-infected red fescue plants, with the goal of identifying plant or fungal genes that may be involved in the observed disease resistance (Ambrose and Belanger, 2012). The *E. festucae* antifungal protein is a secreted protein and is highly expressed in the infected plant tissues. Most *Epichloë* species do not have a gene for a similar antifungal protein. These features make it a good candidate for involvement in the unique resistance to fungal pathogens observed in endophyte-infected red fescue. Understanding the mechanism behind the endophyte-mediated disease resistance in the fine fescues may lead to new approaches for dollar spot management in other grass species, such as creeping bentgrass. The objective of this project is therefore to characterize the endophyte antifungal protein and determine if it does play a role in the disease resistance.

We partially purified the endophyte antifungal protein from infected plant tissue and confirmed that it did have activity against the dollar spot fungus in a plate assay. However, it is difficult to obtain enough of the protein directly from the infected plants to test its activity on a larger scale. We therefore have expressed the protein in the yeast *Pichia pastoris* to generate larger amounts of the protein. *P. pastoris* has become a highly successful system for the expression of secreted proteins (Ahmad et al., 2014). The *E. festucae* antifungal protein was expressed in *P. pastoris* and proteins from the culture filtrate were analyzed on an SDS polyacrylamide gel (Fig. 1). A band at the expected size was the major protein. Sequence analysis of the protein band indicated it was indeed the antifungal protein. The antifungal protein was partially purified from the yeast culture filtrate and found to have activity against the dollar spot fungus in several different assays. The results from one assay are shown in Fig. 2.

In summary, the results we have obtained from the *E. festucae* antifungal protein support the hypothesis that it may be a component of the disease resistance seen in endophyte-infected strong creeping red fescue.

References

Ahmad, M., Hirz, M., Pichler, H., Schwab, H. (2014) Protein expression in *Pichia pastoris*: recent achievements and perspectives for heterologous protein production. App. Microbiol. Biotechnol. 98:5301-5317

Ambrose, K.V., Belanger, F.C. (2012) SOLiD-SAGE of endophyte-infected red fescue reveals numerous effects on host transcriptome and an abundance of highly expressed fungal secreted proteins. PLoS ONE 7(12):e53214

Clarke, B.B., White, J.F. Jr., Hurley, R.H., Torres, M.S., Sun, S., Huff, D.R. (2006) Endophyte-mediated suppression of dollar spot disease in fine fescues. Plant Disease 90:994-998

Bullet Points

1. The fungal endophyte *Epichloë festucae* produces an abundant antifungal protein that is not found in most *Epichloë* species.

2. We have expressed the *E. festucae* antifungal protein in yeast and partially purified it from the culture filtrate.

3. The partially purified *E. festucae* protein had antifungal activity against the dollar spot fungus. It may therefore be a component of the well-established unique endophyte-mediated disease resistance seen in strong creeping red fescue.

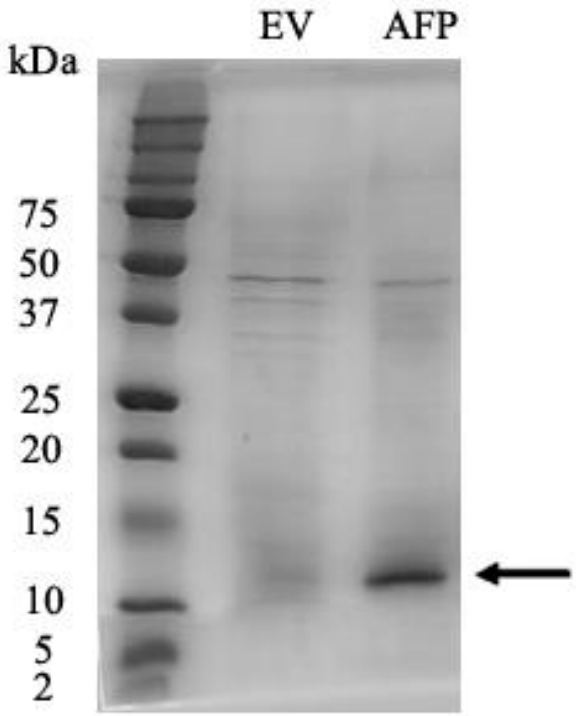

Fig. 1. SDS-polyacrylamide gel of proteins from the culture filtrate of the yeast *P. pastoris* transformed with the empty vector (EV) or with the vector containing the antifungal protein coding sequence (AFP). The arrow indicates the band confirmed by sequence analysis as being the antifungal protein.

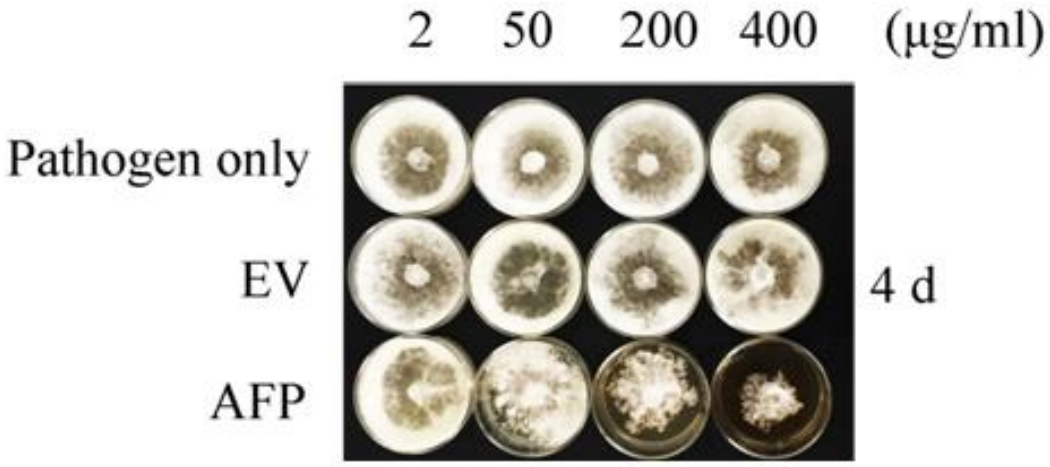

Fig. 2. Inhibition of growth of the dollar spot fungus by the partially purified antifungal protein from the P. pastoris culture filtrate. Different concentrations of the antifungal protein or proteins from the culture filtrate of the empty vector control were incorporated into the agar and a small piece of the dollar spot fungus was placed in the center of the plate. The results shown are after 4 days.