Progress in the development of tools to investigate the molecular biology of *Fusarium oxysporum* f. sp. *vasinfectum*

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Introduction

Fusarium oxysporum f. sp. *vasinfectum*, (Fov) the causal organism of Fusarium wilt, is a significant threat to the Australian cotton industry. It is therefore essential that various strategies for the development of solutions to this problem are explored. It is hoped that an improved understanding of the genetic factors responsible for the pathogenicity of Fov will uncover novel approaches for disease control. We are implementing a transformation protocol for Fov and developing a project aimed at identifying pathogenicity genes using gene disruption and tagging techniques. We are also generating Fov transformants expressing marker genes to facilitate studies of the interactions of the host and the pathogen.

Transformation of *Fusarium oxysporum* f. sp. *vasinfectum*

The initial goal for transformation of Fov was to generate a line expressing the marker protein GUS. This provided a convenient method for assessment of the effectiveness of the transformation protocol in the development stages, and generated isolates of Fov that are potentially useful for the study of the growth of the pathogen within the plant in experimental systems.

The second goal was to demonstrate the effectiveness of targeted gene inactivation by homologous recombination in Fov using a construct that contains a gene that had been shown by other researchers to be important for pathogenicity in *Fusarium oxysporum* f. sp. *lycopersici* (Fol) in tomato (di Pietro *et al.* 2001) viz. the *fmk1* gene for MAP kinase. We cloned the Fov homologue of this gene and found that the Fol and Fov genes code for identical proteins. Thus members of this MAP kinase gene family appear to be highly conserved in *Fusarium oxysporum* species. The inactivation construct contained the MAP kinase gene sequence disrupted with an insert comprising a hygromycin resistance expression cassette. The aim was inactivation of the native MAP kinase by homologous recombination resulting in insertion of the hygromycin-resistance gene cassette into the native gene sequence.

Methods

Transformation for GUS expression was performed using *Agrobacterium tumefaciens* containing a binary construct containing the GUS gene with the *gpdA* promoter from the plasmid pNOM102 (Punt *et al.*, 1990) and the hygromycin resistance gene with *trpC* promoter from the plasmid pGpdGFP (Sexton & Howlett, 2001) as the selectable marker gene. Transformation for MAP kinase inactivation was performed using *Agrobacterium tumefaciens* containing a binary construct with a homologue of the MAPkinase gene cloned from *Fusarium oxysporum* f. sp. *conglutinans* (nearly identical to Fol and Fov MAPkinase genes) disrupted with a hygromycin resistance expression cassette.
Transformation of Fov was performed using a method based on protocols developed for other *Fusarium oxysporum* species. (Mullins *et al.*, 2001, Diener *et al.*, unpublished results).

Results

For the GUS transformation, 22 putative hygromycin-resistant transformants were obtained. Transformation efficiency was approximately 50 colonies per 10^6 conidia. All putative transformants were GUS-expressing. Five of these lines were found by Southern blot analysis to have stable integration of the GUS gene. Of these lines, two appeared to contain single integration events. Thus, potentially useful marker-gene expressing transformants have been obtained.

For the MAPkinase inactivation, 16 putative hygromycin-resistant transformants were obtained. PCR analysis of these lines demonstrated loss of a band corresponding to the native MAP kinase gene with simultaneous appearance of a larger band corresponding to the inactivated gene. These results are consistent with homologous recombination having occurred giving disruption of the native MAP kinase gene.

Pathogenicity testing with these lines is in progress to demonstrate that the GUS-expressing lines have a similar phenotype to untransformed Fov, and to demonstrate that disruption of the MAP kinase gene has reduced pathogenicity.

Future directions

This work has established a transformation protocol for Fov as the basis for the development of strategies for studying the molecular biology of pathogenicity in the fungus. The following approaches will be considered for the study of Fov virulence.

Random mutagenesis for gene tagging and gene inactivation.

Once the transformation protocol for Fov has been streamlined it will be possible to generate a large number of potential insertion mutants. These mutants would be assessed for loss of pathogenicity by infection of cotton, and non-pathogenic mutants analysed to identify the gene responsible.

Targeted mutagenesis of genes identified as associated with pathogenicity in microarray studies

Comparison of the patterns of gene expression of pathogenic and non-pathogenic isolates of Fov while in contact with a potential host could identify genes that are potentially involved in pathogenic processes. Existing microarray technology could be utilised for such gene expression studies. Inactivation of identified genes using an approach similar to that described for MAP kinase above, could demonstrate the relative importance of each gene in establishing infection.

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