Aug 2nd, 11:30 AM - 1:30 PM

**Editing Fusarium graminearum genome with CRISPR/Cas9**

Grace Sack  
*University of Northern Iowa*

Tilahun Abebe  
*University of Northern Iowa*

Let us know how access to this document benefits you

Copyright ©2019 Grace Sack and Tilahun Abebe  
Follow this and additional works at: https://scholarworks.uni.edu/surp  
Part of the Genetics Commons

**Recommended Citation**  
https://scholarworks.uni.edu/surp/2019/all/1

This Open Access Poster Presentation is brought to you for free and open access by the Student Work at UNI ScholarWorks. It has been accepted for inclusion in Summer Undergraduate Research Program (SURP) by an authorized administrator of UNI ScholarWorks. For more information, please contact scholarworks@uni.edu.
Editing *F. graminearum* genome with CRISPR/Cas9

Grace Sack and Dr. Tilahun Abebe

Department of Biology, University of Northern Iowa, Cedar Falls IA, 50614

Abstract

CRISPR/Cas9 technology can be used to mutate genomes and make a pathogenic organism less infectious. We used CRISPR/Cas9 plasmids constructed by a former undergraduate student to mutate three genes in the cereal fungal pathogen *Fusarium graminearum*. One of the genes (*AUR1*) is a visual marker and the other two genes (*MGV1* and *Tri5*) are essential for infection. The CRISPR/Cas9 plasmids also contain a hygromycin B resistance gene for selection. We transformed *F. graminearum* protoplasts and selected colonies on media containing hygromycin B. We then screened the target genes for mutations by PCR and gel electrophoresis. Unfortunately, we were unable to confirm mutations in the transformants. However, many fungi, including *F. graminearum*, are multinucleated and this made it difficult to isolate colonies with genetically identical nuclei (homokaryotes). Further work is needed to isolate homokaryotic cells.

Introduction

*Fusarium graminearum* is a pathogenic fungus that causes scab or *Fusarium* head blight disease in barley and wheat. The disease not only reduces yield, but it also contaminates the kernel with harmful toxins. Therefore, the disease causes economic loss and poses a health risk to humans and animals. Previous studies have shown that CRISPR/Cas9 can be used to introduce mutations in filamentous fungi (Nødvig et al., 2015). In this study, we used CRISPR/Cas9 to mutagenize three genes in *F. graminearum*: *AUR1* (a visual marker), *MGV1* (essential for sexual reproduction and infection), and *Tri5* (important for toxin production and infection). We hypothesize that silencing *MGV1* and *Tri5* could inhibit the ability of the fungus to infect barley.

Methods

1. Grow *F. graminearum*
2. Isolate protoplasts
3. Transform protoplasts with CRISPR/Cas9 plasmids
4. Grow protoplasts into fungal colonies
5. Screen colonies using PCR

Results & Discussion

Recovery of colonies on selective media suggests that they carry the CRISPR/Cas9 plasmids. However, we were unable to detect the plasmid DNA in these colonies by PCR. This might be due to the fact that *Fusarium graminearum* is multinucleated and not all are transformed. Further research is needed to tweak the screening process in order to isolate spores with a single nucleus, and to improve the transformation procedure.

Acknowledgement

We thank CHAS, the UNI Department of Biology, and the Floyd Undergraduate Research Fellowship in Biology for funding the project.

References


Figure 1. Infected (left) vs. healthy barley (right).

Source: USDA

Figure 2. *F. graminearum* growing on artificial medium


Figure 3. Basic plan of the CRISPR/Cas9 plasmids. The single guide RNA (sgRNA) is shown in light green.

Figure 4. *F. graminearum* macrospores used to grow mycelia in liquid culture.

Figure 5. Protoplasts isolated from mycelia for transformation.

Figure 6. Screening putative *F. graminearum* transformants by PCR. Only one band was detected using primers to amplify the target gene in the fungus and primers to amplify a segment of the plasmid DNA. Two bands would have indicated successful transformation.

Conclusions

Recovery of colonies on selective media suggests that they carry the CRISPR/Cas9 plasmids. However, we were unable to detect the plasmid DNA in these colonies by PCR. This might be due to the fact that *Fusarium graminearum* is multinucleated and not all are transformed. Further research is needed to tweak the screening process in order to isolate spores with a single nucleus, and to improve the transformation procedure.