Methods for Investigating the Role of Siderophores in Clinical and Agricultural Isolates of *Fusarium*

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The fungal genus *Fusarium* is comprised of many ubiquitous species that present challenges as both clinical and environmental pathogens (Coleman, 2015). Despite the availability of many modern techniques for genetic modification, it is challenging and sometimes impossible to alter this genus. Specifically, deletion of multiple genes requires an extensive, tedious process with often unsatisfactory results.

The goal of this project was the design and generation of a recyclable dominant selectable marker cassette that, upon insertion into a gene causing a mutation, would result in the disruption of transcription and termination of any product being created.

Like many other fungal genera, *Fusarium* produces secondary metabolites known as siderophores to chelate iron from its surroundings (Greenshields et al., 2007). Upon creation of the recyclable dominant selectable marker construct, the plasmid would be tested in one of the three primary siderophore-producing genes in a member of the *Fusarium solani* species complex (FSSC), which can infect both agricultural and clinical hosts. Following insertion into a single gene generating the desired mutation, this construct could then be modified to lose the dominant selectable marker but maintain a small insertion of DNA keeping the siderophore encoding gene nonfunctional.

A similar strategy would be used to disrupt and mutagenize the other siderophore-producing genes. Once produced, the triple mutant of all the siderophore producing genes would then be cultured to examine its ability to survive compared to the wild type. We hypothesize that upon the production of this mutant, it will show that iron plays a more significant role in the FSSC than previously thought. These mutants will fail to survive and will not cause as severe symptoms as the wild type in their hosts.

So far, the construct is still being designed due to challenges experienced in its assembly. Multiple molecular techniques and strategies have been taken to approach the difficulties, but further work is required to ensure the production of the proper vector. Once the plasmid vector can be utilized, though explicitly designed for the inhibition of the production of siderophores, it is not limited in its application solely for this purpose. It will be able to disrupt any gene or group of genes, allowing for a more efficient investigation of the many unknown biochemical pathways in *Fusarium*.

The importance of this research project lies in the commonality of *Fusarium*, the lack of a method to modify the fungus efficiently, and the few options available to treat its infections. This project will provide information about the siderophores of *Fusarium*, which can be utilized to treat and prevent diseases in clinical and agricultural environments. Additionally, the created vector will be utilized to investigate other metabolic pathways and the development of other novel treatments.

Statement of Research Advisor

Iron serves as an important cofactor for many enzymes and is a limiting nutrient during fungal virulence within a host. Many fungi have evolved several mechanisms to overcome this limitation, including synthesizing many iron-binding compounds such as siderophores. As generation of multiple mutations within a single fungal strain is difficult, Harrison's project aimed to develop a method to overcome this challenge. His research has demonstrated that construction of this novel vector is not straight forward and will require a time consuming and tedious process of multiple cloning steps.
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References

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Authors Biography

Harrison P. Estes is a junior-year student and Undergraduate Research Fellow pursuing a B.S. degree in Applied Biotechnology at Auburn University. He has played key research roles in determining methodology for the assembly of the vector. From Pike Road, Alabama, Harrison has spent the previous two years in Coleman Laboratories contributing to three projects in molecular mycology, fungal ionomics, and virulence studies of clinical and agricultural isolates of *Fusarium*. Once in graduate school, he plans to pursue a Ph.D. and continue his research career focusing on agricultural biotechnology, medical mycology, and fungal genomics.

Jeffrey J. Coleman is an Associate Professor in the Department of Entomology and Plant Pathology at Auburn University. After obtaining his Ph.D. in Plant Pathology at the University of Arizona, Dr. Coleman conducted postdoctoral research in medical mycology at Harvard Medical School. Since joining the faculty at Auburn University in 2014, he has developed a laboratory focused on investigating fungal pathogenesis using advanced molecular techniques.