Myxobacteria as Biocontrol Agents against Crop Pathogens

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Introduction
There are approximately 2.1 million farms in the U.S. that annually generate around $400 billion in agricultural sales, including crops and livestock. One ongoing threat to the agricultural industry is pathogens that destroy crops. One category of pathogens is microbes, which cause tens of billions of dollars in crop damage annually. A number of methods are used to control crop disease; chemicals, such as fungicides, are a major strategy. Although generally effective, the use of chemicals is problematic because they are expensive and can be harmful to humans, wildlife, and the environment, and pathogens can develop resistance. An alternative approach is biocontrol, where naturally occurring microbes in the soil are used to control crop diseases. Biocontrol stems from the long-known observation that some soils are naturally suppressive to crop pathogens, while other soils are conducive to diseases. That is, soil contains thousands of different microbial species—and some of these species inhibit the growth of pathogens. Many farmers have known this fact for some time, and for this reason commercial biocontrol products are used. Although these biocontrol agents are useful, their overall effectiveness is limited because little is known about these microbes and how they work.

This project was initiated with the hypothesis that myxobacteria can be used as effective biocontrol agents. This idea was based on the fact that myxobacteria are natural soil predators that kill and consume other microbes while not harming plants. During the course of our earlier studies, we found that myxobacteria kill microbes by two mechanisms. One is dependent on the secretion of products (such as antibiotics) that kill other microbes. We found that a second mechanism depends on cell–cell contact, but the details were not known.

Objectives
Myxobacteria are promising biocontrol agents to help control crop diseases because they, in part, kill other microbes without hurting plants and causing potential harm to humans and the environment. Here we sought to understand the molecular mechanism of cell–cell contact-dependent killing.

Materials and Methods
This study was conducted in the University of Wyoming laboratory of the investigator, who used microbiology, molecular biology, bioinformatics, and microscopy methods.

Results and Discussion
We sought to identify genetic determinants that myxobacteria use to kill competitors. We did this to better understand how myxobacteria might be used as biocontrol agents and how they might be optimized. In prior work, we showed that one mechanism of killing involves the secretion of antibiotics. Since then, we discovered that a second system involves cell–cell contact. By using genetic and bioinformatic methods we discovered that myxobacteria contain a protein delivery system that transfers toxins to adjacent cells (Figure 1). Killing is effective and depends on the delivery genes (tra) and the toxin genes because when either of these components is knocked out by genetic methods, the killing trait is abolished (Figure 1). We further discovered that at least three different toxins are transferred. The toxins are proteins that act as enzymatic poisons that degrade DNA and RNA in the inflicted cells. This work was published in the American Society for Microbiology's Journal of Bacteriology in March 2016 (http://jb.asm.org/content/early/2016/01/12/JB.00964-15.abstract).

In ongoing work we have also characterized a second system involved in cell–cell delivery of toxins; it is called

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the type VI secretion system. This work has broadened our understanding into how myxobacteria kill and has added insight into how they might be genetically manipulated to serve as improved biocontrol agents. Future studies with myxobacteria are needed to test our laboratory findings in field-model systems for protecting crops from disease.

Acknowledgments
This study was supported in part by U.S. Department of Agriculture Hatch funds.

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Keywords: biocontrol, myxobacteria, pathogens

PARP: VIII:2

Figure 1. Cell killing depends on toxin transfer. A) Target cells (white) mixed at a 10:1 ratio with a killer strain (gray) and examined by microscopy after one day. Left, toxin transfer kills target strain or they become filamentous, while a traA mutation (right) blocks toxin transfer and killing. B) In competition experiments killing depends on the toxin gene.