Summer Scholars Project Proposal

Title:
Identification of the Location of Ustilago maydis Appressorium Formation in Zea mays Silk Epidermal Cells

Student and Faculty Mentor
XXXX and Dr. XXXX
Department of Biology, Saint Joseph University

Relevant Course Work Already Completed
Biology I: Cells
Biology II: Genetics
Biology III: Organismic
Advanced Cellular Biology
Undergraduate Research on *Ustilago maydis*

Background Information

Ustilago maydis is a fungal pathogen that causes corn smut. There are two phases to the life cycle of *Ustilago maydis*: a haploid yeast-like phase and a dikaryotic filamentous phase. *Ustilago maydis* can infect plants through the leaves or the silks (Christensen, 1963). Once inside the plant, complimentary strains of *Ustilago maydis* will mate and form these dikaryotic filaments and grow within the plant. Since *Ustilago maydis* is a biotrophic pathogen, it is able to grow through the plant without killing it. The fungus is able to grow without any major signs of infection.

Ustilago maydis forms a swollen infection structure known as an appressorium to penetrate the cell wall of the corn cells within the silk or the leaves. Very little is completely understood about the infectious process of *Ustilago maydis* because of their appressoria. Unlike many other types of fungal appressoria, *Ustilago maydis* appressoria lack melanin, which makes them very hard to see since they are not dark or colored like other types of appressoria. The lack of support provided by melanin also means that the appressoria are smaller than the average fungal appressoria.

The small size and lack of coloration in *Ustilago maydis* appressoria has made the fungus somewhat difficult to research. Fluorescent microscopy techniques and stains are used in trying to locate the appressoria within the leaves or silks. The filaments grow inside of the plant for some time before forming appressoria and entering the plant cell. The mechanism of how and why the appressoria form when they do is what was investigated during research in our lab.

Ustilago maydis uses its small appressoria to puncture the cell wall and enter the cell. The interesting part of this process is that the fungal filament punctures the cell wall but manages to keep the plasma membrane of the cell intact. This keeps the cell alive and doesn't alert the rest of the plant that an infection has occurred. The exact mechanisms of breaking through the cell wall and invaginating the plasma membrane are not known. The fungus continues to grow through the plant cells, eventually forming

black tumors filled with diploid spores. These spores can geminate and produce haploid cells that will go through the life cycle again.

While corn smut is not a huge problem among corn crops, the research done with *Ustilago maydis* is very important because *Ustilago maydis* is a good model system for other types of fungal pathogens that are not as easy to handle and study in a lab setting but cause far more damage in nature.

Description of Proposed Project

The purpose of my research has been to try and find how the fungal filaments differentiate between cells in the corn silks and which cells should be penetrated via an appressorium. Last summer, XXXX and I investigated into whether or not fungal filaments differentiated plant cells by size and if appressoria more commonly formed at any specific size of cell. Our data showed that the cell length varied within the different regions of the silk. While the average cell length varied, the appressoria seemed to form on the same average size cell regardless of the region of the silk. While our evidence was far from conclusive, it opened up new ideas and questions.

My research this semester and hopefully throughout the summer is a continuation of that project. One of our ideas was that the appressoria, being weaker than other fungal appressoria, targets cells that are elongating. Cell elongation weakens the cell wall as the cell grows. These cells would be easier to puncture than a cell that is not elongating.

In order to test this idea, a plant hormone, auxin, is used to induce cell elongation within the corn silks. The increased concentration of auxin within the plant would raise the rate of cell elongation. If appressoria were formed on elongated cells due to a weakened cell wall, the amount of appressoria would be greater in these silks than in silks inoculated without auxin.

The project also includes test to determine the effects, if any, of auxin on the fungal cells. This is important because it will help ensure that results seen in the inoculated silks are due to cell elongation, and not due to unknown interactions between the auxin and *Ustilago maydis*. The effectiveness of the auxin would also be tested by observing silk cells to see if they were longer, on average, when treated with auxin.