5-2019

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John S. Desmarais

University of Louisville

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GENETIC CROSSTALK AND ITS EFFECTS ON VIRULENCE AND MATING IN

USTILAGO MAYDIS.

By

John Desmarais

Submitted in Partial Fulfillment of the Requirements for Graduation summa cum laude and for
Graduation with Honors from the Department of Biology

University of Louisville
Louisville, Kentucky

March 25, 2018
Abstract

Ustilago maydis is a model organism for study of fungal mating and host infection. Two compatible haploid mating types must mate to form a dikaryon in order for the fungus to infect its host, corn (Zea mays). There are a variety of genetic mechanisms that regulate mating and infection in the fungus, many of which coregulate both processes. The aim of this study was to investigate how alteration of certain regulatory proteins in U. maydis affects these basic processes as well as how alteration of genes involved in signaling pathways can affect the expression of one another. Primarily, the focus was on exploring the role of the ptn1 gene. This gene was disrupted and therefore found to be involved in tumorigenesis and teliospore development during infection. Additional disruption of the ustl and unh1 genes, which play opposite roles in tumorigenesis, was attempted on these ptn1 deletion strains of U. maydis using a high fidelity CRISPR-Cas9 system to examine the influence of these genes on the ptn1 pathway. Further experiments were intended to generate ptn1 deletion mutant strains of U. maydis in which the pdc1 gene, encoding a major protein involved in cellular regulation, was simultaneously overexpressed. Phenotypes of pdc1 overexpression mutants of U. maydis were examined for both effects on U. maydis cells in culture and during plant infection. Ptn1 deletion strains were found to be more susceptible to cell wall stress, displayed lesser aerial hyphae formation during mating, and showed reduced infection symptoms including reduced tumor size and reduced number of tumors. Overexpression of pdc1 led to no changes in mating or virulence in wild type strains but may exert an added effect on ptn1 deletion strains and their corresponding phenotypes.
**Introduction**

*U. maydis* is an obligate fungal parasite of *Zea mays* (corn) and is one of the most useful genetic model organisms for the study of fungal host-pathogen interaction mechanisms. Like many other fungal species, *U. maydis* must undergo a dimorphic change from two compatible haploid mating types to an infectious dikaryotic form to infect its host. Infection by the fungus elicits the production of galls or tumors in all above-ground parts of the plant and inside these galls the reproductive spores of *U. maydis* develop. In mammals, the **PTEN** signal transduction pathway is involved in the regulation of biological processes such as programmed cell death, metabolism, cell growth, cell proliferation, and suppression of tumorigenesis. The **PTEN** ortholog in *U. maydis*, *ptn1*, is believed to be involved in a signal transduction cascade that regulates mating, tumorigenesis and spore development during infection. An ongoing study in the lab is aimed at investigating the role of the *ptn1* signal transduction pathway in plant infection by *U. maydis* through functional characterization of the *ptn1* gene. Mutant strains of *U. maydis* in which *ptn1* has been deleted display a decrease in virulence, including a decrease in tumorigenesis and tumor size. Moreover, there is a severe reduction in spore production in the tumors that do result from infection by the *ptn1* mutants (Cid et al. 2008; Vijayakrishnapillai et al. 2018).

Additional literature revealed two other genes involved in infection and tumor production by *U. maydis*, *unh1* and *ust1*. The *unh1* gene is similar to *ptn1* in that it is required for tumor maturation and spore development (Doyle et al. 2016); in contrast, *ust1* prevents spore formation in haploid fungal strains and places a limit on tumorigenesis and tumor size during infection (Chacko et al. 2009; García-Pedrajas et al. 2010). Other experiments have found that *ust1* gene
deletion disruption mutants display abnormal morphology and pigmentation, such that haploid fungal strains without a copy of the gene appear grittier and darker and diploid double deletion strains produce darker, more melanized tumors during infection (Islamovic et al. 2015). An aim of this study was to examine how deletion of these genes would affect virulence and mating of mutant *U. maydis* strains in which the *ptn1* gene was already deleted.

Mutant strains of *U. maydis* were made where *ptn1* was deleted using a homologous recombination construct with a hygromycin resistance marker. CRISPR-Cas9 deletion constructs were used to attempt *ptn1* expression disruption as well (Schuster et al. 2016, 2018; Shi et al. 2017). Further experiments intended to generate double mutants where expression was reduced for both *ptn1* and either *ust1* or *unh1*. Changes in mating and virulence of these mutant strains were predicted and have been assessed by assays on charcoal media and controlled plant infection in order to observe any possible crosstalk between the *ptn1* pathway and either the *ust1* or *unh1* genes. Initial attempts at these double deletion experiments found that the plasmid used for generating the Cas9 construct was not functioning correctly. Therefore, these experiments were put on hold and focus was shifted to another set of experiments.

14-3-3 proteins are involved in signal transduction and cell cycle regulation, among other cellular maintenance functions. In other fungal systems, such as yeast, determining the exact role of each 14-3-3 protein in cellular processes has proved difficult, as many isotypes exist for each protein and each protein can have numerous binding partners (Kakiuchi et al. 2007). *U. maydis*, however, only possesses one 14-3-3 protein homolog, known as Pdc1. This presents an opportunity to study a single 14-3-3 protein’s function within a fungal system. Pdc1 protein specifically is believed to regulate a signal transduction cascade that interacts with *ptn1* by regulating the function of a signaling protein that is required for normal cellular growth and
viability, Rho1. Similarly, Pdc1 is required for viability in haploid cells. Although the physical interaction between the Rho1 signaling protein, Pdc1, and Ptn1 has been documented, the exact effects of Pdc1 on ptn1 transcriptional activity are still unclear (Pham and Perlin 2010). To examine the effects of pdc1 on ptn1, pdc1 overexpressor strains of U. maydis were generated. This upregulation of pdc1 activity is predicted to exert an effect on symptoms associated with ptn1 deletion.

**Materials and Methods**

**DNA Isolation**

The mutant U. maydis colonies were inoculated into YEPS (yeast extract-peptone-sucrose) media and allowed to shake overnight at room temperature. The fungal genomic DNA was isolated by microextraction using PCI (25 phenol:24 chloroform:1 isoamyl alcohol) followed by ethanol precipitation. The samples were vacuum dried and TE (10 mM Tris/1 mM EDTA, pH 8.0) solution was added to allow for proper storage (Hoffman and Winston 1987). Presence of DNA was confirmed by electrophoresis in agarose gels or measurements using a Nanodrop 2000 spectrophotometer.

**PCR Specifications**

Polymerase chain reaction (PCR) was used to amplify plasmid and fungal genomic DNA. PCR was carried out in a T100 ThermalCycler (Bio-Rad Laboratories, Hercules, CA, USA). For PCR amplification reactions, Platinum Taq GreenHot Start DNA Polymerase (Life Technologies Corporation) was used. (Vijayakrishnapillai et al. 2018). The relevant PCR steps and
temperatures for each step are shown in Table 1. Primers used for gel screening of transformants and target primers used in attempted CRISPR-Cas9 gene disruption are shown in Table 2.

Table 1. Polymerase Chain Reaction Conditions.

<table>
<thead>
<tr>
<th>Step</th>
<th>Duration</th>
<th>Temperature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 minutes</td>
<td>94 °C</td>
<td>Initial denature</td>
</tr>
<tr>
<td>2</td>
<td>30 seconds</td>
<td>94 °C</td>
<td>Cycle denature</td>
</tr>
<tr>
<td>3</td>
<td>30 seconds</td>
<td>60 °C</td>
<td>Annealing of primers</td>
</tr>
<tr>
<td>4</td>
<td>3 minutes</td>
<td>72 °C</td>
<td>DNA Elongation (1 minute per 1 kilobase pairs)</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>Repeat steps 1-4 34 times</td>
</tr>
<tr>
<td>6</td>
<td>5 minutes</td>
<td>72 °C</td>
<td>Final elongation</td>
</tr>
<tr>
<td>7</td>
<td>Hold</td>
<td>4 °C</td>
<td>Termination</td>
</tr>
</tbody>
</table>

Table 2. Primers used in study.

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence</th>
<th>Use (Relevant Fig.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pdc Int primer</td>
<td>5'-AGTCACCTCTATCTTTGTTG-3'</td>
<td>Confirm pdc1 overexpression (Fig. 8)</td>
</tr>
<tr>
<td>EndOtef primer</td>
<td>5'-AGATCCGGTCTTCTTCTCT-3'</td>
<td>Confirm pdc1 overexpression (Fig. 8)</td>
</tr>
<tr>
<td>Hyg Sph1 new RT</td>
<td>5'-TTTGGTCATGCTCAGGCTCATGTGGACA-3'</td>
<td>Confirm presence of hyg cassette (Fig. 4)</td>
</tr>
<tr>
<td>Hyg Mfe1 Lt</td>
<td>5'-TTTCAATTGTGGCGAAGCTGGAACTAC-3'</td>
<td>Confirm presence of hyg cassette (Fig. 4)</td>
</tr>
<tr>
<td>PTEN QRT LT</td>
<td>5'-TTTGGTCATGCTCAGGCTCATGTGGACA-3'</td>
<td>Confirm presence of ptn1 gene (Fig.4)</td>
</tr>
<tr>
<td>PTEN INT RT</td>
<td>5'-TAGAGGATGAG grid 3'</td>
<td>Confirm presence of ptn1 gene (Fig.4)</td>
</tr>
<tr>
<td>ptn1 Target</td>
<td>5'-GATCGTGACACGTGGTGG-3'</td>
<td>CRISPR-Cas9 Gene Disruption</td>
</tr>
<tr>
<td>ust1 Target</td>
<td>5'-GGAATCGGCAAACAGCAGCTG-3'</td>
<td>CRISPR-Cas9 Gene Disruption</td>
</tr>
<tr>
<td>unin Target</td>
<td>5'-GCGAGGTGATGAGTGGCA-3'</td>
<td>CRISPR-Cas9 Gene Disruption</td>
</tr>
</tbody>
</table>

Gene Disruption Construct Development

Constructs for the disruption of ptn1 (see Fig. 1) or for the overexpression of pdc1 (see Fig. 2) were generated in vitro and plasmids bearing these were transformed into *Escherichia coli* strain DH5α using cells that had been made chemically competent for DNA transformation (Chung et
al. 1989) and selection on agar plates containing 200 μg ampicillin per ml (Wang et al. 1988). The hygromycin-resistance-conferring *ptn1* deletion construct was isolated and purified from the transformed bacterial cells using standard alkaline lysis protocols for plasmid purification (Sambrook and Russell 2006). The isolated construct was transformed into *U. maydis* protoplasted cells exposed to the relevant DNA by plating onto YEPS plates with a final concentration of 150 μg hygromycin per ml (Bösch et al. 2016). Hygromycin is a fungicide that acts by inhibiting protein synthesis. The plates were incubated at 26 °C for 3-5 days before colony growth was assessed. Suspected transformant colonies that were conferred hygromycin resistance were re-plated onto YEPS-hygromycin agar plates and stored for subsequent experiments.

![Diagram of the *ptn1* deletion construct](image)

**Fig. 1. Mechanism of *ptn1* deletion construct.** A plasmid containing a hygromycin resistance marker (HygR) flanked by DNA homologous to flanking regions of the *ptn1* gene was transformed into *U. maydis* to delete *ptn1* by homologous recombination of the plasmid into the fungal genome.
CRISPR-Cas9 Construct Development

*E. coli* colonies were provided with a CRISPR-Cas9 plasmid via bacterial transformation (Wang et al. 1988) (see Fig. 3). The plasmid DNA was isolated via an alkaline lysis protocol (Sambrook and Russell 2006). Putative sites for targeted mutation of each of the *ptn1*, *ust1*, and *unh1* genes were selected using the E-CRISP online tool (www.e-crisp.org) to select for the highest fidelity for sgRNA cut sites by the ultimate Cas9 construct. Primers containing the genome information for each cut site was obtained from an outside company (Eurofins Scientific, Louisville, Kentucky). Cut sites on the Cas9 vector for restriction enzymes MscI and XbaI were identified to open the vector for introduction of the sgRNA target site genome information. Primers for each target site for each respective gene targeted for disruption were designed with MscI and
Xba1 restriction cut sites. These primers were purified from agarose gel by GENECLERICAN® II Kit (Qbiogene, Inc., Carlsbad, California) and amplified by PCR. Target primers and amplified Cas9 vector DNA were digested together by Msc1 and Xba1 to introduce the target sites into the vector. The final construct was then transformed into *U. maydis* cells by plating an STC-polyethyleneglycol solution containing the protoplasted cells along with the construct DNA onto YEPS agar plates with a final concentration of 150 μg/mL hygromycin (Bösch et al. 2016). The plates were incubated at 30 °C for 3-5 days before colony growth was assessed. Suspected transformant colonies were regularly replated onto YEPS-carboxin plates to gradually eliminate Cas9 construct DNA to reduce the likelihood of off-target effects due to Cas9.

**Fig. 3. Cas9 Construct for Attempted Gene Deletions.** Target DNA sequences for each gene were inserted into the vector in the space between the highlighted enzymes. The construct would then be guided to the complementary sequence in the targeted gene to cut the DNA, resulting in disruption of the target gene.
Stress Tests
Cultures (4 mL) of *U. maydis* in YEPS media, shaken at 260 rpm, were grown overnight at room temperature. Cells were grown to an optical density at 600 nm (OD$_{600}$) of 0.8 and cells were resuspended in sterile dH$_2$O to an OD$_{600}$ of 1.6. The parent inoculum and three sequential 1:10 serial dilutions of the fungal strains were spotted onto media containing 1 mM Congo Red and observations were made after incubation at 26 °C for 48 hours (Ram and Klis 2006). Congo Red is a cell wall stressor that acts by binding to the proteins that form the fungal cell wall. This prevents complete lateral attachment of cell wall proteins, compromising rigidity of the cell wall (Kopecká and Gabriel 1992).

Mating Assay
Cultures (4 mL) of *U. maydis* in YEPS media, shaken at 260 rpm, were grown overnight at room temperature. Cells were grown to an OD$_{600}$ of 0.8, and cells were resuspended in sterile deionized water to an OD$_{600}$ of 1.6 prior to spotting. 10 μL aliquots of haploid strains of opposite mating type background were co-spotted onto PDA charcoal plates and mating reactions were observed after incubation at 26 °C for 24 hrs. and 48 hrs (Gold et al. 1997). A positive mating reaction produced a white “fuzz” phenotype of aerial hyphae production.

Plant Infection and Infection Assessment
Cultures (4 mL) of *U. maydis* in YEPS media grown overnight at room temperature were diluted using 50 mL fresh YEPS liquid media and grown overnight again to an OD$_{600}$ of 1.0, harvested and resuspended in sterile dH$_2$O to an OD$_{600}$ of 3 prior to infection. Plant infection was
performed by inoculating week-old maize seedlings (Golden Bantam seeds, Bunton Seed Co.,
mating types were mixed prior to plant infection. One-week old seedlings of Zea mays were
infected with the indicated strains of U. maydis by injecting the seedlings with inoculum
containing a mixture of compatible strains. Mock injections were performed by injecting water
into the seedlings as a control. The virulence of each treatment group was scored by a disease
index (DI) on a scale of 0 to 5, where 0 = No Symptoms, 1 = Chlorosis, 2 = Small leaf tumors, 3
= Large leaf tumors or small stem tumors, 4 = Large stem tumors with bending, and 5 = Death.
Thirty plants were used in each experiment with plants scored for disease symptoms on 14 and
21 days post-infection (dpi). A haploid a1b1 background mutant strain crossed with a
haploid a2b2 mutant strain was compared in each case to the corresponding wild type cross. The
data was analyzed using Kruskal-Wallis test followed by post-hoc comparison (Gold et al. 1997;
Vijayakrishnapillai et al. 2018).

Results

ptn1 Deletion Strains

The hygromycin homologous recombination construct produced ptn1 deletion (Δptn1)
strains in three haploid mating type backgrounds of U. maydis. The 1/2 (a1b1), 2/9 (a2b2), and
FB1 (a1b1) Δptn1 mutant strains were confirmed as transformants by PCR amplification using
primers specific to the ptn1 gene and primers specific to the substituted hyg (hygromycin-
resistance) gene. Confirmed transformants displayed no band for ptn1 and a band for hyg when
compared to control (WT) strains of the same background, which show a band for ptn1 and no
band for hyg (see Fig. 4).
Stress tests of the mutant strains were performed on Congo red media to assess growth differences from the wild type. These tests showed less growth and more susceptibility to cell wall stress for each mutant strain when compared to WT strains (see Fig. 5).
Additionally, mating assays on charcoal media showed that mutant strains displayed lower mating affinity and growth when crossed with WT strains bearing an intact \textit{ptn1} gene, and extremely lower mating and growth when compatible \textit{Δptn1} mutants were crossed. This supports previous findings that \textit{ptn1} plays an important role in mating, and even having one copy of the gene missing has consequences for mating (see Fig. 6).

![Mating assay trials for \textit{Δptn1} mutant strains.](image)

\textbf{Fig. 6. Mating assay trials for \textit{Δptn1} mutant strains.}

Mating assay for \textit{Δptn1} strains. Crosses involving \textit{Δptn1} strains did not display as much aerial hyphae development during mating when compared to wild type. Differences in mating between strains are show by the circles.

The disruption of \textit{ptn1} gene expression has adverse effects on the virulence of \textit{U. maydis} as well. Plant infection trials consisting of groups of plants infected with each compatible mutant mating pair (1/2 x 2/9 and FB1 x2/9) yielded significantly less advanced phenotypical markers of infection (number and size of galls) for mating pairs that consisted of one or more mutant strains compared to WT infection (see Fig. 7).
Attempted gene disruptions using CRISPR/Cas9

Target cut sequences for each of the *ust1* and *unh1* genes were selected and cloned into a high fidelity CRISPR-Cas9 deletion construct in order to disrupt gene expression at those loci. The construct was introduced into *E. coli* and later isolated via plasmid minipreps. The *ust1* target construct was transformed into the 1/2 and 2/9 mating type backgrounds via PEG-mediated transformation and suspected transformants were screened by PCR amplification of the disrupted region. PCR screening could not confirm any transformants and sequencing attempts did not provide adequate reads for interpretation. Concurrent experiments in the lab using the plasmid used here for the Cas9 construct have also been unsuccessful. These CRISPR-Cas9 deletion attempts have been halted until new, reliable plasmid vector is obtained.

*Pdc1* Overexpressor Strains

In order to investigate the influence of *pdc1* on *ptn1*, overexpressor strains in which the constitutive *otef* promoter drove *pdc1* gene transcription were generated in the 1/2 and 2/9 mating type backgrounds. These mutants were confirmed by electrophoresis on agarose gel using...
primers specific to *otef* and *pdc1* (see Fig. 8). Wild type strains of *U. maydis* did not display a band as they do not have an *otef* promoter.

![Image](image1.png)

**Fig. 8. Pdc1 OX Gel Confirmation.** *Pdc1* overexpressor transformants were confirmed using end primers specific to the *otef* promoter and internal *pdc1* gene primers. Wild type strains that lack *otef* do not display a band. A) 1/2 *pdc1* OX. B) 2/9 *pdc1* OX. C) *otef-pdc1* containing plasmid. D) 1/2 wild type. E) 2/9 wild type.

Stress tests of these strains were performed to examine if any changes in growth were brought about by overexpression of *pdc1*. Stress tests of both overexpressor strains show no phenotypic change when compared to the wild type strains, suggesting that cell wall viability was not compromised by *pdc1* overexpression (see Fig. 9).

![Image](image2.png)

**Fig. 9. Pdc1 OX Mutant Stress Tests.** *Pdc1* overexpressor mutants displayed no differences in growth from the wild type.
Additionally, mating assays of both strains displayed no change in mating filamentation when crossed with each other or when crossed with compatible wild type strains that have a normal copy of the \textit{pdc1} gene (see Fig. 10). These tests imply that \textit{pdc1} overexpression does not directly affect the typical mating response of \textit{U. maydis}.

![Mating assay of pdc1 OX strains](image)

\textit{Fig. 10. Mating assay for pdc1 OX strains.} Compatible haploid wild type and \textit{pdc1} OX mutant strains of \textit{U. maydis} were crossed. No differences in mating in the mutant strains from wild type were observed.

Plant infection testing of the \textit{pdc1} overexpressor strains was carried out in order to assess if there were differences in infection severity compared to wild type. The mutant strains were not found to have a significant difference in virulence when compared to wild type. These preliminary results suggest that constitutive expression of \textit{pdc1} does not significantly attenuate virulence in \textit{U. maydis}. However, these results are based on a single set of plant infection. More replicates must be carried out in order to confirm these results (see Fig. 11).
This study attempted to show how alteration of \textit{ust1}, \textit{unh1}, and \textit{pdc1} gene expression affected \( \Delta \text{ptn1} \) strains of \textit{Ustilago maydis}. Deletion of the \textit{ptn1} gene in the 1/2, 2/9, and FB1 backgrounds of \textit{U. maydis} were successful and confirmed by PCR amplification. These mutant strains were found not only to be more susceptible to the cell well stressor, Congo red, but also to produce a reduced “fuzz” white phenotype normally associated with compatible mating types during mating assay trials. Due to the apparent role of \textit{ptn1} in mating, it was thought that deletion of even one copy of the \textit{ptn1} gene was enough to attenuate the mating response; this prediction was supported by the mating assays in this paper. Moreover, it appears that mating pairs for which both copies of the \textit{ptn1} gene were absent displayed a more attenuated mating response than the mating pairs where one partner had a copy of the gene. Thus, the normal mating response appears to require both copies of the gene.

If \textit{ptn1} contributes to virulence of the fungus, \( \Delta \text{ptn1} \) strains of \textit{U. maydis} should display a lessened ability to infect corn as well. Plant infection trials for compatible \( \Delta \text{ptn1} \) mutants showed a significant decrease in pathogenicity of infection, displaying smaller and less numerous tumors. Additionally, relatively less mature teliospores were shown to develop within the tumors created.

![Graph showing disease index for WT and \( pdc1^{\text{ef}} \) strains. The graph indicates a significant reduction in disease index for \( pdc1^{\text{ef}} \) compared to WT.]

**Fig. 11. Plant infection for \( pdc1 \) \( \text{OX} \) strains.** Mutant strains displayed no significant reduction in virulence compared to wild type. More replicates must be performed to confirm results.
by the mutant strains (Dalton 2016; Vijayakrishnapillai et al. 2018). This supports previous studies showing that ptn1 is necessary not only for tumorigenesis, but also for further teliospore development in the tumors caused during infection. This attenuation of virulence caused by deletion of the ptn1 gene was of interest in suggesting subsequent experiments to evaluate other contributing genes that might further reduce virulence or to rescue this reduced virulence. Thus, I tried to disrupt two other genes, unh1 and ust1, by a CRISPR-Cas9 system.

As previously stated, ust1 acts as a suppressor of tumorigenesis in haploid strains of U. maydis. Its regulatory action is relaxed after the dimorphic switch during mating to allow for tumor formation during infection. Previous studies have found that haploid ust1 deletion mutants display different phenotypes than the wild type fungus, including a darker pigmentation and grittier appearance. Additionally, mutant strains produce teliospore-like structures in liquid culture (García-Pedrajas et al. 2010). Diploid double deletion mutants have also been shown to produce darker and more numerous tumors during infection (Islamovic et al. 2015). Since Δust1 mutants have been shown to display greatly increased virulence, double deletion mutants in which ptn1 and ust1 are both deleted were intended to be created by CRISPR with the prediction that deletion of ust1 action could complement deficiencies in infection brought about by ptn1 deletion.

Additionally, unh1 was selected for its similarity in function to ptn1, influencing tumorigenesis, teliospore development, and meiosis during infection. Unh1 deletion strains of U. maydis have been found to have reduced teliospore development during infection, with teliospores that did reach maturity displaying abnormal discoloration (Doyle et al. 2016). Strains in which both ptn1 and unh1 were disrupted by CRISPR were to be generated to see if unh1 exerted any influence on the ptn1 pathway by examining whether double mutant strains
displayed even further attenuated infection symptoms than the Δptn1 strains. Trials were attempted using the high fidelity CRISPR-Cas9 deletion construct and proceeded to the point of insertion of target cut sites for each gene into the construct; subsequent isolation of the complete construct was successful. However, transformations of the construct into U. maydis yielded no candidate colonies that were confirmed to be deletion mutants. These experiments were put on hold until a more reliable plasmid could be used to create the construct can be obtained, which was beyond the timeline of this study. This remains an area of interest for future experiments.

The effects of pdc1 on ptn1 was another area of interest for this study. Pdc1 has been shown to be involved in the Rho1-mediated signal transduction pathway and its gene product is believed to interact with Rho1 protein during the signaling process. Further support for the interaction between Pdc1 and Rho1 has been found in experiments showing that overexpression of rho1 mitigated the adverse effects of pdc1 deletion strains of U. maydis (Pham et al. 2009). This study aimed to further investigate the role of Pdc1 in the Ptn1 signal transduction pathway by overexpressing pdc1 and examining changes in mating, cell growth, and infection. Pdc1 overexpression mutants did not display changes in mating or cell growth when compared to wild type. Based on one set of plant infection data, overexpression mutants displayed a slight reduction in virulence that was not found to be statistically significant. Additionally, more replicates of infection using these mutant strains must be carried out to validate these results. These experiments serve to further inform another ongoing study in the laboratory involving U. maydis strains that have both ptn1 deletion and pdc1 overexpression mutations. Preliminary findings of that study suggest that these dual mutation strains of U. maydis may actually display reduced virulence of infection relative to both wild type and Δptn1 strains (unpublished). Further
experimentation is required to observe other differences of the dual mutants from the wild type fungus.

**Conclusions and Limitations**

This study attempted to show the effects of genetic disruption and overexpression on the biological processes of *U. maydis*. It also attempted to investigate how certain gene products involved in signal transduction pathways pertaining to virulence interact with one another. It was found that *ptn1* deletion strains of *U. maydis* show not only reduced pathogenicity, but also reduced mating and growth ability as well. It was planned to further mutate these *ptn1* deletion mutants by CRISPR-Cas9 gene disruption of two other genes of interest, *ust1* and *unh1*. It was predicted that deletion of *ust1* in *ptn1* deletion strains of *U. maydis* might recover some of the pathogenicity of the fungus, whereas it was predicted that deletion of *unh1* in *ptn1* deletion strains would lead to a further reduction in virulence than a *ptn1* deletion alone. However, these experiments could not be completed presently due to the CRISPR-Cas9 construct being ineffective in generating these mutations. This remains as an open area of study for future experiments.

*Pdc1* overexpression mutant strains of *U. maydis* were found not to display significant differences in mating, cellular growth, or virulence from wild type. However, as previously stated, more plant infection trials must be carried out in order to properly understand effects of *pdc1* overexpression on virulence since the present study only includes data from one set of infection trials. The other ongoing study in the Perlin lab that is examining *pdc1* overexpression in *ptn1* deletion mutants will be able to further examine how the signal transduction pathways involving these genes interact. One future direction for this study is the final generation of dual
deletion mutations of *ptn1/ust1* and *ptn1/unh1* using CRISPR-Cas9 in *U. maydis* along with all relevant mating, cellular growth, and infection testing of the dual mutant strains. Another future direction of this study is to generate dual *pdc1* overexpression/*ptn1* deletion mutants of my own on which to perform all relevant tests. Meeting these future goals will facilitate increased understanding of these important regulatory genes and their interactions with one another and will further elucidate important basic genetic mechanisms in *U. maydis*.

**Acknowledgements**

This work was partially supported by a Research Initiation Grant from the Office of the Vice President for Research to the Perlin laboratory and a Summer Research Opportunity Grant to J. Desmarais. I would like to thank Dr. Michael Perlin and Lalu Vijayakrishnapillai for their help in overseeing and guiding this research.
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