Study of PMT target specificity in *Ustilago maydis*

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ABSTRACT

Ustilago maydis is a pathogenic fungus responsible for the corn smut fungus disease, which causes a significant loss in mayze production every year.

U. maydis requires a pheromone-receptor response for infection, in which the two compatible strains FB1 and FB2 mate and develop an invasive hypha inside the plant (**Fig. 1).**

The PMT is a family of well-conserved O-mannosylating proteins with a total of 3 proteins in *U. maydis* (Pmt1, Pmt2 and Pmt4). These proteins are substrate-specific, since each deletion shows a different phenotype: Δpmt1 shows no differences, Δpmt2 is deleterious and Δpmt4 is unable to infect, lacks adhesion and is sensitive to SDS. We wanted to investigate whether the domains (named PMT, MIR and 4TMC) in Pmt4 are responsible for the substrate specificity. To test it, we built four different strains containing PMT chimerical proteins in a FB1 Δpmt4 strain. [1,2]

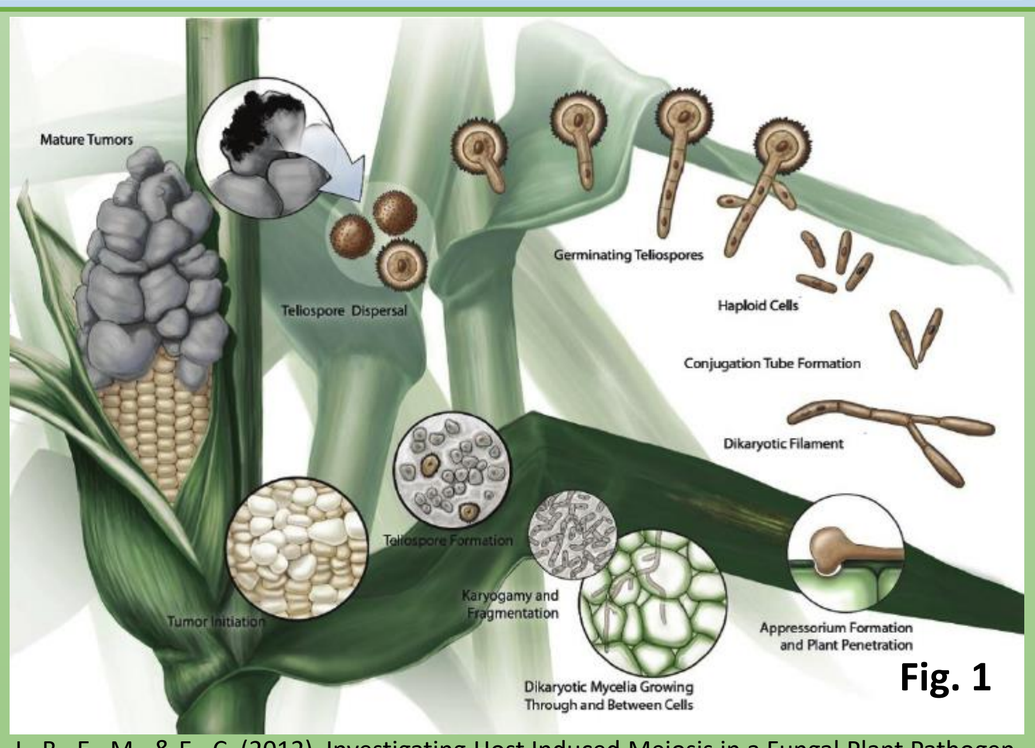
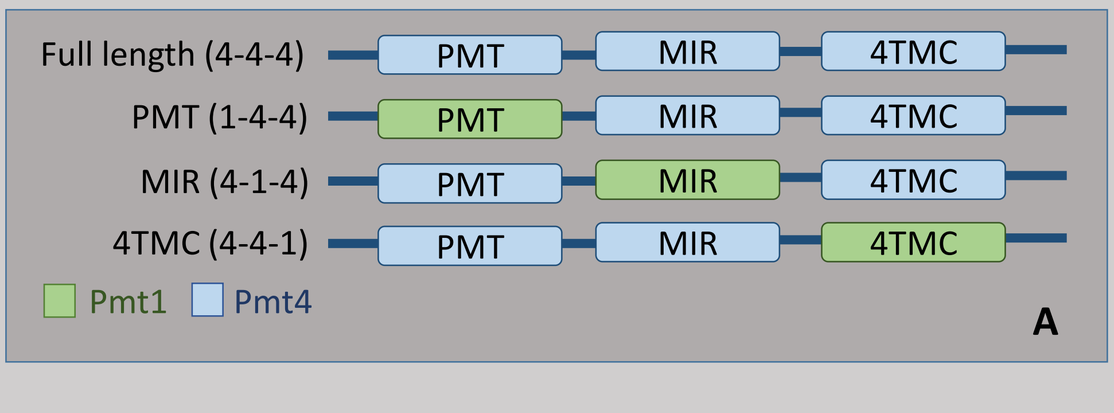


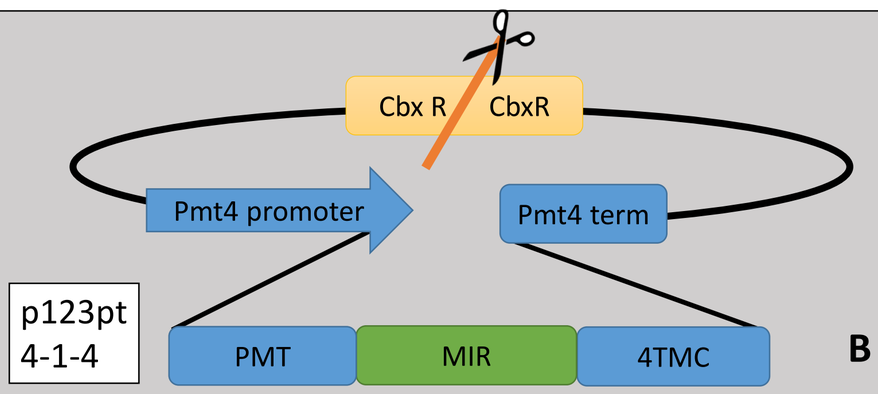
Fig. 1

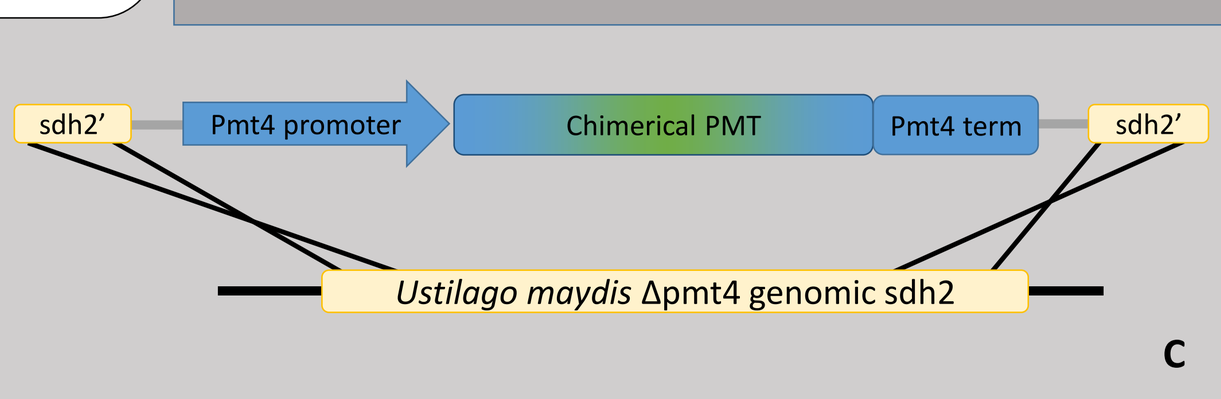
J., B., E., M., & E., C. (2012). Investigating Host Induced Meiosis in a Fungal Plant Pathogen. *Meiosis - Molecular Mechanisms and Cytogenetic Diversity*, (1999).

STRATEGY

The strains were named after the origin of the domains as seen in **A**. To transform *U. maydis* we built a plasmid with the Pmt4 promoter, the chimerical protein and the Pmt4 terminator, creating **p123pt (B),** that can be inserted in *U. maydis* genome via homologous recombination in the Sdh2 gene **(C)**. We selected the PMT chimerical strains in a AR-carboxin medium. [1]







RESULTS

We can see that the adhesion and SDS sensitivity is not complemented in either of the experimental strains, but it is in the positive control **(Fig 2).** The same is true for the infection experiments. This graph is the sum result from three independent infections **(Fig. 3).** [2,3]

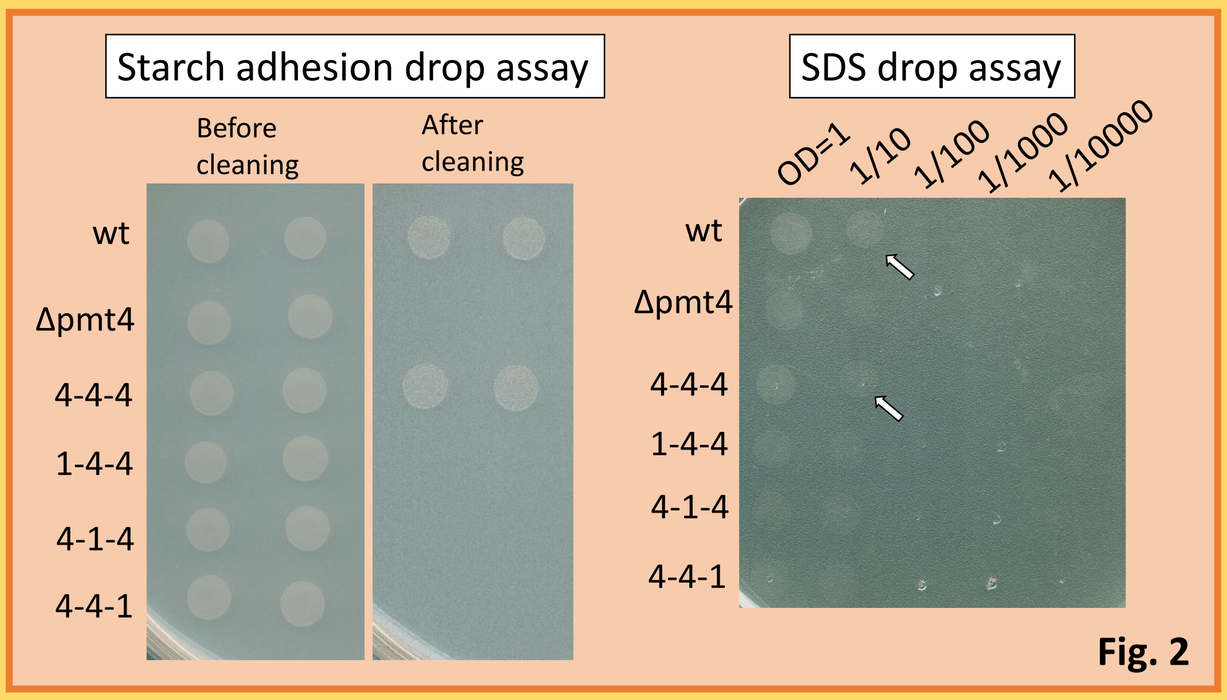


Fig. 2

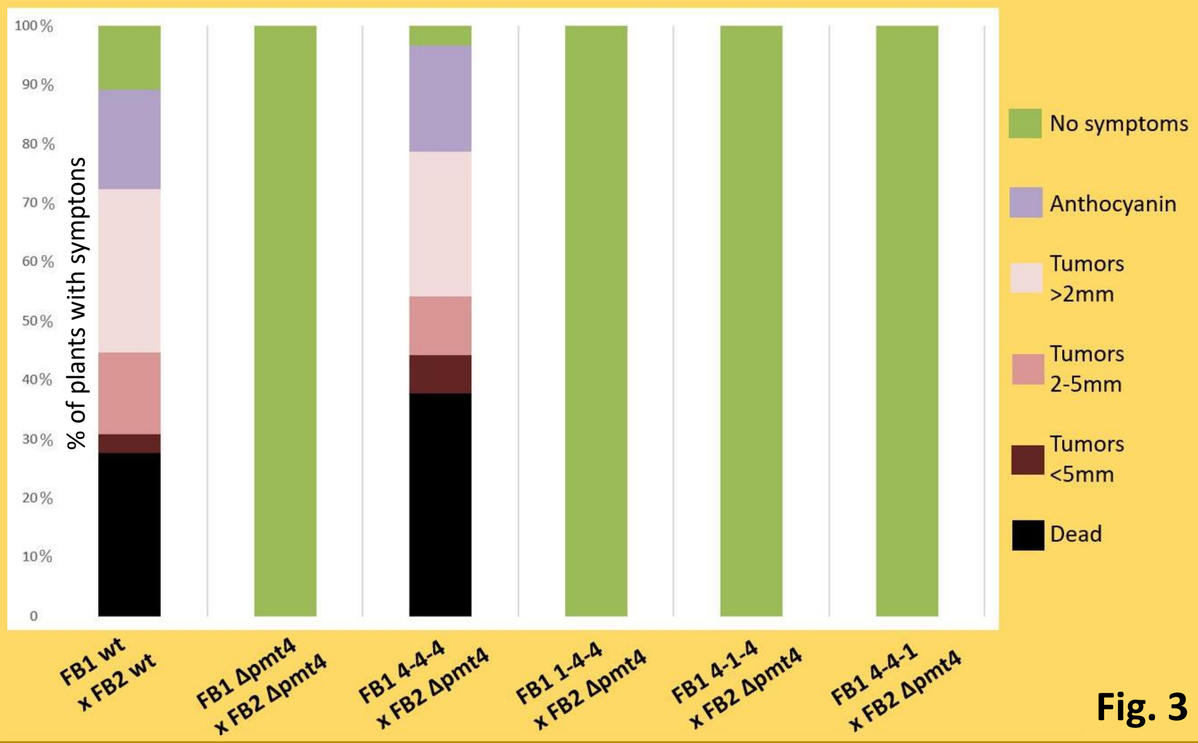


Fig. 3

DISCUSSION AND FUTURE VIEWS

The results indicate that either the protein requires all three Pmt4 domains to work specifically or the chimerical protein is being degraded.

We are also preparing new strains changing Pmt4 domains in the Pmt1 protein to check if Pmt1 is able to gain Pmt4 specificity. The strains got so far and the ones we're in progress of getting are listed in **Table 1**.

Strain	DNA design	*E. coli*	*U. maydis*
4-4-4	Done previously	Done previously	Done previously
1-4-4	Done previously	Done previously	✓
4-1-4	Done previously	Done previously	✓
4-4-1	Done previously	Done previously	Done previously
1-1-1	✓	✓	In progress
4-1-1	✓	✓	In progress
1-4-1	✓	In progress	x
1-1-4	✓	In progress	x

Table 1

References:

1. Blanquero, A., Ramon, R. Development of an antifungal drug discovery system against the plant pathogen Ustilago maydis 21, 2019.
2. Fernández-Álvarez et al., 2012. Identification of O-mannosylated virulence factors in Ustilago maydis. PLoS Pathog. 8.
3. Fernández-Álvarez et al., 2009. The O-mannosyltransferase PMT4 is essential for normal appressorium formation and penetration in Ustilago maydis. Plant Cell 21, 3397–3412.