

Poster

Development of an antifungal drug discovery system against the plant pathogen *Ustilago maydis*



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ABSTRACT

Motivation: Glycosylation is a post-translational process where some sugar residues are added on a protein for its correct function. In this process PMT family protein, which is conserved in several organisms such as *S. cerevisiae*, *U. maydis* or *C. albicans*, acts in the addition of the first sugar residue to the proteins target in ER.

This family protein is formed by some proteins such as PMT1, PMT2 and PMT4, and they show a high homology sequence each other, acting in the same phase of glycosylation but not to the same proteins. So, deletions in these genes generate for example lethal phenotype in the case of PMT2 or a phenotype that loses its capacity to infect and may have a lower adherence in solid or liquid media in the case of PMT4, given that it exists a correlation between adherence and virulence. Therefore, the majority of wall and secreted proteins of pathogen fungus are glycosylated and as those proteins are the interaction between these fungus and its hosts, it thought that glycosylation was essential for the virulence.

To verify the study of virulence in fungus, it deleted PMT4 and PMT1 in *Ustilago maydis* and it could observe that PMT1 was no phenotype but PMT4 was essential for the virulence and adherence in the process of infection in this fungus. So, we focus on PMT4 using *Ustilago maydis*, an pathogen fungus that infects maize, which is a great model organism with an amount advantages in laboratory.

The goals of this project are the search of the domain of PMT4 involved in the virulence through the use of chimeras of this protein and the search of antifungal compounds that can affect virulence and adherence of the fungus *Ustilago maydis* mediated by PMT4.

Methods: To determine the domain of PMT4 involved in the virulence we use chimeras of PMT4 with conserved domains of PMT1 in order to complement Δ PMT4 as a pool gene, using one construction of PMT4 with its own promoter and terminator as a positive control. With these chimeras we carry out essays of adherence in plate in order to observe its phenotypes. To observe the adherence we grow Δ PMT4 and wild type FB1, we plate them in a petri dish with starch's agar, and wash it then.

Results: Due to the lack of nutrients in multi-well plate, we use big dishes due to the optimal growth of the fungus. On the other hand, we have obtained some chimera constructions in *E. coli* DH5- α strain, which will be transformed in *Ustilago maydis* in order to see the phenotypes generated.

REFERENCES

- Fernández A., Elías A. et Ibeas J. (2009). "The O-Mannosyltransferase PMT4 Is Essential for Normal Appressorium Formation and Penetration in *Ustilago maydis*". *The Plant Cell*, Vol 21.
- Fernández A., Elías A. et Ibeas J. (2010). "Protein glycosylation in the phytopathogen *Ustilago maydis*: From core oligosaccharide synthesis to the ER glycoprotein quality control system, a genomic analysis". *Fungal Genetics and Biology*, Vol 47.