ISOLATION AND MOLECULAR CHARACTERIZATION OF SOLOPATHOGENIC STRAIN OF *Ustilago maydis*

Porfirio Raúl Galicia García[1], Maribel Quezada Cruz[1], Israel Jesús Rodríguez Meléndez[1], Erika Villeda Gutiérrez[1], Serafín Cruz Izquierdo[2], Jesús Alarcón Bonilla[1]

[1]Laboratorio de Biotecnología Ambiental, Universidad Tecnológica de Tecámac. Km. 37.5 Carretera Federal México – Pachuca. Col. Sierra Hermosa. CP 55740. Tecámac, Edo. de México. [2]Colegio de Postgraduados. Recursos Genéticos y Productividad-Genética. Km 36.5 Carretera México-Texcoco. Montecillo Edo. de México C.P.56230

*sercruz@colpos.mx

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Introduction. In Mexico, maize smut (*Ustilago maydis*) is a fungus that infects less than 5% of plants maize (*Zea mays* L.) in the nature. However, due to the economic value, there is interest in the production of artificially infected corn ears. Unfortunately, there are not isolated and characterized strains specific for infecting ear corn that can already be used as inoculum. Common methodology consists in culturing teliospores (which are obtained from tumors) in potato dextrose agar (PDA) medium and then mixed in a suspension (1). By following this methodology, less than 60% of infections can be reached, depending on maize genotype and strain inoculated. The goal of this study was to identify and molecularly characterize strains of *U. maydis* in order to use them as inoculum.

Methods. Six tumors were collected from corn ears. From those tumors, individual teliospores were cultured in PDA medium, and 26 isolations were obtained. From each isolation, DNA was extracted and then the rDNA's internal transcribed spacer was amplified using ITS1/ITS4 primers (2). Once the isolations were identified, they were placed in PDA medium and activated charcoal in order to observe Fuzzy+ type mycelium.

Results. All the colonies showed an initial growth similar to that of yeast-like colony in PDA medium. Isolations 19 and 26 amplified a fragment of pb 615 (Figure 1).

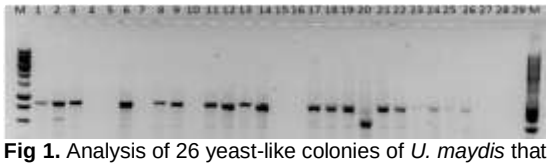

Fig 1. Analysis of 26 yeast-like colonies of *U. maydis* that show a fragment of pb 615 using ITS1/ITS4.

The colonies that amplified showed morphological differences when placed in PDA medium and activated charcoal, varying from a typical yeast-like colony to colonies with foldings that showed signs of mycelium production (1).

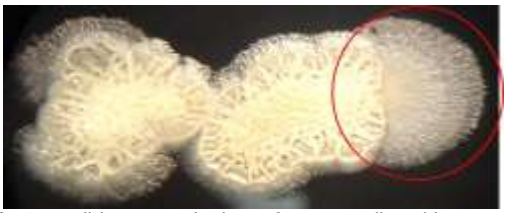

Fig.2 Wild type colonies of U. maydis with Fuzzy+ reaction obtained by culturing teliospores in PDA medium plus 5% activated charcoal.

Colonies were crossed under a dialelic design in PDA medium and activated charcoal. Some colonies showed Fuzzy+ type morphology due to mycelium production (Fig 2) within the same colony. Fuzzy+ type mycelium from two isolations was cultured a second time in PDA medium and activated charcoal. Seventy two hours later, the mycelium was harvested and used to prepare inoculum with 106 yeast-like cells per milliliter. Maize seedlings on the first ligulate leaf and grown under greenhouse conditions, were inoculated with the inoculum. Results were positive, since all the seedlings showed the typical yellowish symptoms.

Conclusions. This method is useful to obtain solopathogenic strains (3), which after being evaluated, by inoculating maize seedlings under greenhouse conditions, resulted in infection percentages greater than 60%.

References.

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