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Growth inhibition of *Colletotrichum gloeosporioides* and *Phytophthora capsici* by native Mexican *Trichoderma* strains

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Abstract

An integrated evaluation to determine the potential of *Trichoderma* against two important phytopathogens is described in the present research. *Phytophthora capsici* and *Colletotrichum gloeosporioides* were the phytopathogens used in the assay to evaluate the inhibition potential by *Trichoderma*. *Trichoderma harzianum, Trichoderma longibranchiatum, Trichoderma yunnanense, Trichoderma asperellum* (T2-10 and T2-31) and *Trichoderma* sp. were evaluated. The *Trichoderma* strains showed a percentage of inhibition on *P. capsici* and *C. gloeosporioides* up to 22.5%. *P. capsici* was mycoparasitized by all *Trichoderma* strains evaluated and also *C. gloeosporioides,* except when it was evaluated against *T. longibranchiatum*. When there was a mycoparasitism, it was 100% over phytopathogen biomass. The production of cellulases and chitinases using sugarcane bagasse and chitin from shrimp shell as unique carbon source were evaluated. The best production of endoglucanase was showed by *T. harzianum* (40.8 U/g), exoglucanase by *Trichoderma* sp. and *T. harzianum* (2.9 and 2.5 U/g, respectively), by last the best value of chitinases was 22.1, 20.5 and 20.1 U/g showed by *T. asperellum* (T2-31), *T. longibranchiatum* and *Trichoderma* sp., respectively. All *Trichoderma* strains evaluated showed to be an excellent potential agent to be used as control pest against *C. gloeosporioides* and *P. capsici*.
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Keywords: Mycoparasitism; Antibiosis; Cellulases; Chitinases; Biocontrol; Biopesticides

1. Introduction

Mexico is a great producer of Tomato (*Solanum lycopersicum*) and Chili (*Capsicum annuum*). The national production reaches up to 2.2 and 2.8 million of tons per year [29]. However, some pests are affecting the production around the world. *Phytophthora capsici* in Tomato and *Colletotrichum gloeosporioides* in Chili are the most harmful fungi causing important crop losses [2,19]. Chemical fungicides (metalaxyl or copper based), laborious process (phytosanitation) and the use of resistant seeds and water management are strategies traditionally used to manage these pathogens, but none of these

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have individually provided adequate control [1,3,5,9,24,32,33]. So, it is necessary to find alternatives such as biological control and restrict the traditional and limited methods to control phytopathogens. The strategy of disease management using biocontrol agents (BCA's) is an eco-friendly way to protect the environmental and human health [12]. Around the world, there are some microorganisms commercialized to inhibit phytopathogens. *Trichoderma* is one of the most studied biocontrol agents against fungal phytopathogens. Mainly, the species of *Trichoderma harzianum, Trichoderma asperellum,* and *Trichoderma virens* has been reported as BCA [9,10,15,32,33]. Several *Trichoderma* strains have been tested against important phytopathogens, such as *Botrytis cinerea, Pseudoperonospora cubensis, Sclerotinia sclerotium, Sphaerotheca fusca, P. capsici, P. megakarya, P. citrophthora, P. palmivora, Phytophthora erythroseptica, Pythium ultimum*, among others [9,10,13,32,33]. A microorganism considerate good BCA must have more than one mechanism of action against phytopathogens, namely synergism. The mechanisms of control include (1) Antibiosis that is related to the production and release of several compounds to retard or inhibit the microorganism's growth [27]. (2) Competition and superficial growth, this mechanism is very effective when the conidia of the pathogens needed exogenous nutrients to germinate [9]. (3) Mycoparasitism, which is accompanied with cell-wall degrading enzymes such as chitinases (endochitinases, exochitinases, and β-*N*-acetilhexosaminidases), cellulases (exoglucanases, endoglucanases, and β-1-3-glucanases) and proteases. These enzymes are used by microorganisms to dissolve the host cell wall and then penetrate the cell to obtain nutrients [23,35]. The aim of this research was to report the effect of *Trichoderma* strains against two important phytopathogens (*P. capsici* and *C. gloeosporioides*) and to determine the potential to control or inhibit them. Cell wall degrading enzymes (chitinases and cellulases) and mycoparasitism were also determined.

2. Materials and methods

2.1. Microorganisms and culture conditions

The strains of *T. harzianum, Trichoderma longibranchiatum, Trichoderma yunnanense, T. asperellum* (T2-10 and T2-31), *Trichoderma* sp. were isolated in a previous work done by Ref. [26]. *P. capsici* were isolated and proportioned by the Department of Agricultural Parasitology of UAAAN (Universidad Autónoma Agraria Antonio Narro, Coahuila, Mexico). The Food Research Department of UAdeC (Universidad Autónoma de Coahuila) proportioned *C. gloeosporioides*. The fungal strains were cultured and conserved in a cryogenic solution of glycerol-milk 8.5%. The strains were activated in sterilized PDA and inoculated during 5 days at 30 °C to conserve at 4 °C.

2.2. In vitro antagonistic activity in dual culture

The six strains of *Trichoderma* mentioned above were evaluated against *P. capsici* and *C. gloeosporioides* in a dual culture. A 5 mm diameter mycelial disc from the growing edge of seven days old *Trichoderma* and phytopathogen cultures were placed on the opposite of the PDA Petri plate (Size 90 × 15 mm) and equal distance apart-distance. The plates were incubated at 28 °C ± 2. Inhibition of phytopathogen growth rate and mycoparasitism were the parameters evaluated. A factorial design was used and each treatment was done in triplicate.

2.3. Microbial enzyme assay

Screening of enzymes activity was assessed by using the cellophane disk method on solid medium culture (SMC) [7]. Czapek-dox culture medium was supplemented with sugarcane bagasse (SCB) and chitin from shrimps shells (CSS) at 30 g/L. Sterilized cellophane disk was placed on the surface of culture medium on Petri plates, and then the plates were inoculated with a mycelial disc from a *Trichoderma* culture (seven days old). The solid medium was incubated at 30 °C for 96 h. The cellophane disk was removed and the solid medium was used for the cellulases and chitinases assay. All treatments were done in triplicate and a factorial design was used. Distilled water (70 mL) was mixed with each sample until complete homogenization. Cellulase activity [28], chitinase activity [30] and reducing sugars [25] were determined in all samples. The Carboxymethylcellulose activity was carried out at 50 °C for 30 min. The reaction consisted of a mix of the sample (1 mL) and substrate (1 mL of Carboxymethylcellulose 1%). The substrate control was done with citrate buffer (1 mL at 50 mM, pH 4.8) and substrate (1 mL). The enzyme control was the mix of the sample (1 mL) and citrate buffer (1 mL). The filter paper activity was carried out at 50 °C for 1 h. Sample (1 mL) and substrate (filter paper Whatman No. 1 (1 cm × 5 cm) and 1 mL of citrate buffer at 50 mM, pH 4.8) were the reaction mix. The control substrate was the mix of citrate buffer

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