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Antagonistic capacity of native paraguayan isolates of *Trichoderma* spp. against capsicum pathogens (*Capsicum annuum* var Natalie): *Rhizoctonia solani* and *Sclerotinia sclerotiorum*

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Introduction

In Paraguay, the pepper production is considered a good source of income for small and medium producers, but these crops are affected by their sensitivity to different factors that generate serious economic losses. Among the factors that affect, in addition to the climatic conditions, are mainly phytopathogenic fungi (Chew 2008). Agrochemical products are the most used for their control, but the abuse of them generates serious environmental problems and for human health. *Trichoderma* spp. covers various species of fungi used as biocontrol agents especially against plant pathogens, whose interaction requires their study at chemical biology level (Gams 1998, Ibarra 2006).

The mechanisms of action by which the isolates of the genus *Trichoderma* confront the pathogen are basically of three types: direct competition for space or nutrients, antibiotics, fungistasis by production of secondary metabolites and direct parasitism of the species on phytopathogenic fungi (Guédez 2012)

This work aims to evaluate the antagonistic capacity of native Paraguayan isolates of *Trichoderma* spp. against pepper pathogens (*Capsicum annuum* var. Natalie): *Rhizoctonia solani and Sclerotinia sclerotiorum*, through evaluation of the antagonistic activity against phytopathogens in direct confrontation assays (Bell 1982) and the antibiosis produced by molecules secreted by *Trichoderma* (Malmierca 2012).

Methodology

This work was carried out in the Phytochemical Department of the Facultad de Ciencias Químicas de la Universidad Nacional de Asunción, where 25 Paraguayan native isolates of *Trichoderma* spp. belonging to the FCQ fungal collection, which were isolated from several agricultural regions from the eastern region of Paraguay. Two native isolates of phytopathogens, *R. solani* (FCQ35) and *S. sclerotiorum* (FCQ40) were also used. These fungi were maintained in PDA medium until its use.

For the direct confrontation between *Trichoderma* spp. and Phytopathogen was used the technique of direct confrontation of microorganisms, confronting the *Trichoderma* spp. against phytopathogenic fungi (*R. solani* and *S. sclerotiorum*) in 7 cm diameter Petri dishes.

Antibiosis test on membranes: from the plates containing the *Trichoderma* spp. in active growth, 5 mm diameter mycelial discs was cut and subsequently cultured in the Petri dish center containing PDA culture medium on which a cellophane or dialysis (cutoff, 10 kDa) membrane was previously placed to allow the diffusion of Trichoderma extracellular compounds into the medium. After the removal of the membranes containing the mycelia, the effect of such hydrolytic enzymes plus metabolites (cellophane) or only metabolites (dialysis) on the growth of two different plant-pathogenic fungi was determined. This was incubated for 30 hours in the dark and at temperatures ranging from 25 to 30°C. After the isolates of *Trichoderma* spp.

reached the total coverage of the membrane, its was remove from the plate, taking with him the mycelium of the fungus and remaining in the agar the molecules secreted by *Trichoderma* spp. Phytopathogenic fungi were cultured on the same plates containing the substances secreted by the antagonist. This assay was performed in triplicate.

The following equation was used to calculate the growth inhibition percentage (PIC):

$$PIC = \left[\frac{C - T}{C}\right] \times 100$$

Where C represents the Growth of the control of the phytopathogen, and T represents the growth of the phytopathogen confronted with *Trichoderma*.

For the statistical analysis, the PrismaGraphic software was used, using descriptive statistics and the analysis of variance was performed through ANOVA and later Tukey test or in the case of comparison of means of two groups the Student's t-test was used. A confidence level of 95% was considered, that is, significant differences were considered for $p<0.05$.

Results and discussion

Direct confrontation assays

Comparing the growth inhibition percentage of each *Trichoderma* spp. isolate versus the two phytopathogens at 96 hours, it was observed significant differences between PIC values in the vast majority (ANOVA, $p<0.05$). When analyzing in a paired way the PIC of each phytopathogen, it was observed that in all cases, except for FCQ17, the isolates of Paraguay of *Trichoderma* have a differential behavior. That is to say, the percentage of inhibition that varies each one varies depending on the phytopathogen ($p<0.05$), being in general *S. sclerotiorum* (FCQ40) more sensitive to the action of the *Trichoderma* isolates evaluated (Figure 1).

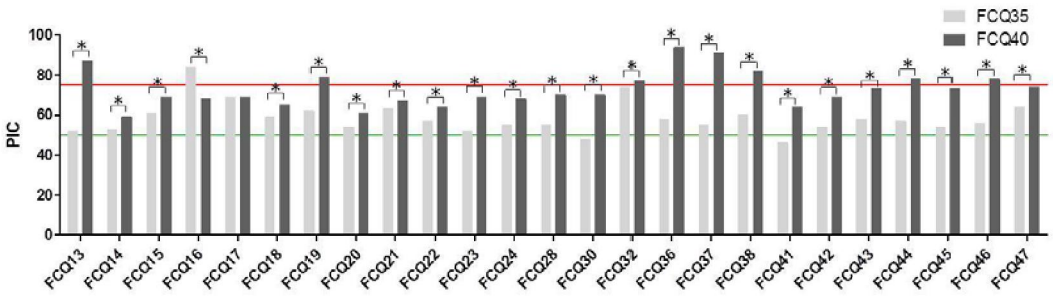

Figure 1. Growth inhibition percentage of FCQ35 and FCQ40 exerted by 25 *Trichoderma* isolates in the Direct Confrontation test. At 96 hours of follow up. The inhibitory activity on both phytopathogens is compared, the green line indicates 50% inhibition, the red line indicates 75% inhibition (* t-Student, $p<0.05$).

The lowest horizontal line in the figure indicates 50% inhibition of phytopathogen growth. According to Figure 1, all *Trichoderma* isolates were able to inhibit phytopathogen growth by over 50%, as well as the red line indicating 75% Inhibition of phytopathogen growth above which the *Trichoderma* isolate is considered to be a good antagonist according to Bell et al. 1982.

Antibiosis assays on membranes

Comparing the data obtained with the cellophane and dialysis membranes, it should be interpreted that the inhibition of phytopathogen growth would be due mainly to metabolites if high inhibition of phytopathogen growth was observed in both plaques with dialysis membranes and plaques with cellophane membranes. In contrast, it can be said that metabolites do not have a clear contribution in antibiosis when inhibition is low or zero in plates treated with dialysis membranes. In addition, inhibition was observed in cellophane plates, in that way it can be inferred that such inhibition is characteristic of the hydrolytic enzymes secreted by *Trichoderma*, or that there could be some synergistic effect between the high and the low molecular weight molecules.

Antibiosis of *Trichoderma* on *Rizoctonia solani* (FCQ35)

The green line in Figure 2 indicates a 50% inhibition of phytopathogen growth, which shows that most *Trichoderma* isolates exert more than 50% inhibition of phytopathogen growth.

According to Figure 2 the isolates FCQ13, FCQ16, FCQ18, FCQ21, FCQ30, FCQ36 and FCQ38 that maintained 100% growth inhibition of *R. solani* in both cellophane and dialysis assay indicate that low molecular weight molecules strongly inhibit growth, and we cannot infer in the activity of the molecules of high molecular weight since in the tests with cellofan are all the molecules secreted by *Trichoderma*. Isolates in which statistically significant differences

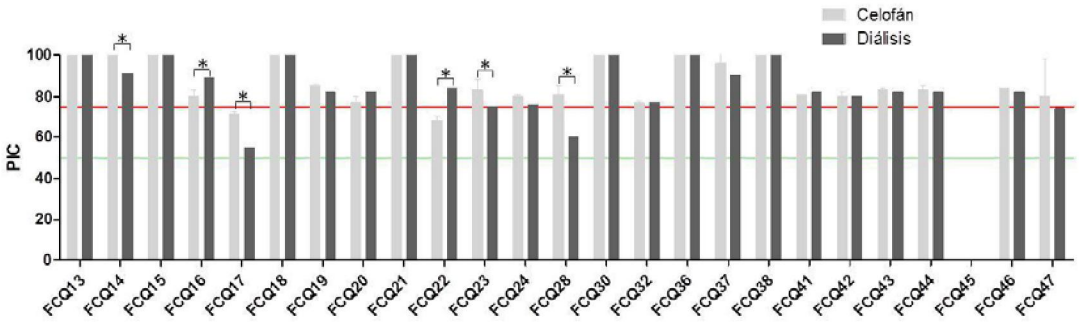

Figure 2. Growth inhibition percentage of *R. solani* (FCQ35) in antibiosis assays. At 48 hours of follow-up. The percent inhibition for cellophane membrane, light gray, and dialysis, dark gray, is plotted. Groups marked with an asterisk indicate significant differences between groups (*t-Student, $p<0.05$)

between cellophane membrane and dialysis are observed, in case the inhibition is greater in the cellophane treated plates, as FCQ14, FCQ17 and FCQ28 gives us indications that the molecules of high molecular weight are producing some effect.

In the case of FCQ45 that does not produce inhibition sample, it may require some stimulation for the production of growth inhibitory molecules, or it simply acts using some other mechanism of action other than antibiosis

Antibiosis of *Trichoderma* on *Sclerotinia sclerotiorum* (FCQ40)

The green line in Figure 3 indicates a 50% inhibition of phytopathogen growth, which shows that most *Trichoderma* isolates exert more than 50% inhibition of phytopathogen growth.

According to Figure 2 most of the isolates that maintained 100% growth inhibition of *R. solani* in both cellophane and dialysis trials indicate that metabolites strongly inhibit growth, and we cannot infer in the activity of the hydrolytic

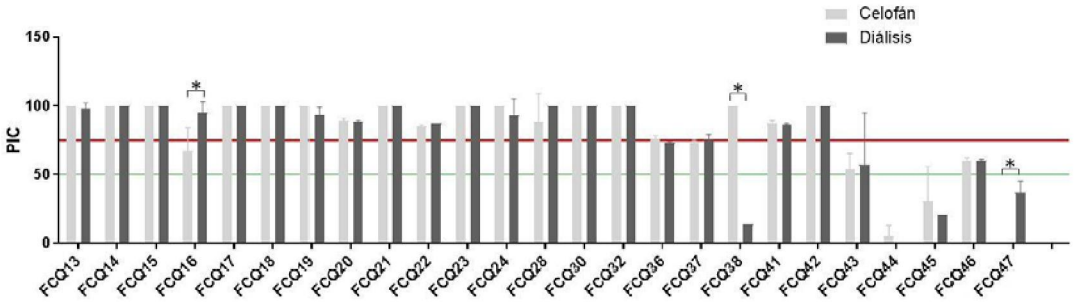

Figure 3. Growth inhibition percentage of *S. sclerotiorum* (FCQ40) in antibiosis assays. At 72 hours of follow-up. The percent inhibition for cellophane membrane, light gray, and dialysis, dark gray, is plotted. Groups marked with an asterisk indicate significant differences between groups (*t-Student, $p<0.05$)

enzymes since in the tests with cellophane are all the molecules secreted by *Trichoderma*. Isolates in which statistically significant differences between cellophane membrane and dialysis are observed, in case the inhibition is greater in the cellophane treated plates, as FCQ38 gives us indications that the molecules of high molecular weight are producing some effect.

Conclusion

It was found that 8 of the 25 *Trichoderma* isolates have significant antagonistic activity on both pathogens in direct confrontation. Furthermore, it was evidenced that the low molecular weight molecules are the ones that most contributed to the inhibitory effect in the membrane antibiosis assay.

Bibliographic references

Bell, DK; Wells, HD; Markham, CR. 1982. In vitro antagonism of *Trichoderma* species six fungal plant pathogens. Phytopathology 72:379-382.

Chew, Y; Vega, A; Rodríguez, M; Jiménez, F. 2008. Principales enfermedades del Chile (*Capsicum annuum* L.). México, INIFAP. p. 1-42.

Ibarra, JE; Castro, MCDR; Galindo, E; Patiño, M; Serrano, L; García, R; Carrillo, JÁ; Pereyra-Alférez, B; Alcázar-Pizaña, A; Olivera, HL; Wong, L; Pardo, L; Muñoz-Garay, C; Gómez, I; Soberón, M; Bravo, A. 2006. Los microorganismos en el control biológico de insectos y fitopatógenos. Revista Latinoamericana de Microbiología 48(2):113-120.

Gams, W, Meyer, W. 1998. What exactly is *Trichoderma harzianum*?. Mycology 90(5):904.

Guédez, C; Cañizalez, L; Castillo, C; Olivar, R. 2012. Evaluación *in vitro* de aislamientos de *Trichoderma harzianum* para el control de *Rhizoctonia solani*, *Sclerotium rolfsii* y *Fusarium oxysporum* en plantas de tomate. Revista de la Sociedad Venezolana de Microbiología 32(1):44-49.

Ibarra-Medina, VA; Ferrera-Cerrato, R; Alarcón, A; Lara-Hernández, ME; Valdez-Carrasco, JM. 2010. Aislamiento y selección de cepas de *Trichoderma* antagonistas a *Sclerotinia sclerotiorum y Sclerotinia minor*. Revista Mexicana de Micología 31:53-63.

Malmierca, MG; Cardoza, RE; Alexander, NJ; McCormick, SP; Hermosa, R; Monte, E; Gutiérrez, S. 2012. Involvement of *Trichoderma trichothecenes* in the biocontrol activity and induction of plant defense-related genes. Applied and Environmental Microbiology 78(14):4856-4868.