

Isolation and screening of *Trichoderma* strains antagonistic to *Sclerotinia sclerotiorum* and *Sclerotinia minor*

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Aislamiento y selección de cepas de *Trichoderma* antagonistas a *Sclerotinia sclerotiorum* y *Sclerotinia minor*

Resumen. *Sclerotinia sclerotiorum* (Ss) y *Sclerotinia minor* (Sm) afectan severamente más de 400 especies de plantas, y pueden causar reducciones en rendimiento por arriba del 50%. Con el propósito de obtener microorganismos potenciales para el control de ambos patógenos, se realizó el aislamiento de cepas de *Trichoderma* a partir de muestras de suelo con diferente uso agrícola y forestal. Setenta y un aislados fúngicos de *Trichoderma* (IBA) fueron obtenidos a través de técnica de trampas con esclerocios. Subsecuentemente, se evaluó su capacidad micoparasítica sobre micelio y esclerocios de ambos fitopatógenos mediante cultivos duales in vitro. Los más altos porcentajes de colonización (>90%) de las cepas de *Trichoderma* sobre Ss y Sm fueron observados para los aislamientos IBA-3, IBA-4, IBA-38, e IBA-54. En general, la habilidad de los aislamientos de *Trichoderma* para parasitar y degradar esclerocios fue agresiva en los aislamientos IBA-3, IBA-4, IBA-23, IBA-38, IBA-46, IBA-54, e IBA-56. Con base en criterios morfológicos, cuatro de los más prominentes aislamientos fueron identificados como *T. viride* (IBA-4), *T. pseudokoningii* (IBA-23), *T. harzianum* (IBA-38), y *T. virens* (IBA-46). Estas cepas de *Trichoderma* tienen importante potencial como antagonistas de Ss and Sm.

Palabras clave: micoparasitismo, antibiosis, degradación de esclerocios

Abstract. *Sclerotinia sclerotiorum* (Ss) and *Sclerotinia minor* (Sm) drastically affect more than 400 plant species, and may cause crop yield reductions up to 50%. With the purpose to obtain potential microorganisms to control both pathogens, the isolation of *Trichoderma* strains from soil samples with different agricultural and forestry use was performed. Seventy-one fungal *Trichoderma* isolates (IBA) were obtained through sclerotia-trap techniques. Subsequently their mycoparasitic activities on mycelium as well as on sclerotia of both phytopathogens were assessed by in vitro dual cultures. The highest percentages of colonization (>90%) of the *Trichoderma* strains on Ss and Sm were found for the isolates IBA-3, IBA-4, IBA-38, IBA-54. The ability of *Trichoderma* strains to parasitize and to degrade sclerotia in general was aggressive for the strains IBA-3, IBA-4, IBA-23, IBA-38, IBA-46, IBA-54, and IBA-56. Based on morphological criteria, four of the most prominent strains were identified as *T. viride* (IBA-4), *T. pseudokoningii* (IBA-23), *T. harzianum* (IBA-38), and *T. virens* (IBA-46). These *Trichoderma* strains have important potential as antagonists to Ss and Sm.

Key words: mycoparasitism, antibiosis, sclerotia degradation.

Recibido 5 de agosto 2009; aceptado 10 de marzo 2010.

Received 5 August 2009; accepted 10 March 2010.

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Introduction

The soil-borne fungi *Sclerotinia sclerotiorum* (Lib.) de Bary and *Sclerotinia minor* (Sm) Jagger have worldwide distribution in temperate and subtropical climates and cause significant losses to horticultural and ornamental crops (Willette and Wong, 1980; Mónaco *et al.*, 1998; Rollan *et al.*, 1999; Zago *et al.*, 2001; Agrios, 2005). There are 408 plant species (75 families and 278 genera) that host Ss (Boland and Hall, 1994). In contrast, Sm may infect at least 94 plant species belonging to 21 families and 66 genera (Melzer *et al.*, 1997).

The genus *Sclerotinia* produces sclerotia that remain in the soil under adverse climatic conditions for several years (Willette and Wong, 1980; Mónaco *et al.*, 1998; Rollan *et al.*, 1999). The sclerotia of Ss as well as those of Sm may germinate by forming abundant mycelium; however, only Ss can germinate carpogenically by producing apothecia which carry on ascospores that represent the main source for newly initiating infection in plant tissues (Willette and Wong, 1980; Mónaco *et al.*, 1998). This may provide ecological advantage to this fungus for rapidly colonizing and infecting new hosts.

On the other hand, the fungal members of *Trichoderma* are cosmopolites that grow in different substrates such as vegetal residues and degraded wood (García-Garza *et al.*, 1997; Mónaco *et al.*, 1998; Rollan *et al.*, 1999; Zago *et al.*, 2001; Harman *et al.*, 2004; García *et al.*, 2005). The species of *Trichoderma* are characterized by being: 1) highly reproductive which allow them to survive under unfavorable conditions, 2) effective on nutrient utilization, 3) rhizosphere modifiers by inducing changes on pH and/or on microbial populations, 4) strongly aggressive to phytopathogenic fungi, 5) plant growth promoter agents, and 6) promoters of plant defense mechanisms (Dal *et al.*, 1997; García-Garza *et al.*, 1997; Harman *et al.*, 2004; García *et al.*,

2005). In addition, some *Trichoderma* species have been assessed as biological control agents because of their antagonistic and hyperparasitic ability (Harman *et al.*, 2004; González-Cárdenas *et al.*, 2005; Ávila-Miranda *et al.*, 2006; Benítez *et al.*, 2006).

Trichoderma species show several antagonistic mechanisms towards pathogens (Howell, 2002; Chaube *et al.*, 2003; Brozová, 2004) which include: 1) competition for nutrients and space, 2) release and/or secretion of antibiotic compounds such as trichodermin, trichothecenes, trichorzianins, or gliotoxins; 3) exerting indirect toxic effects due to volatile compounds, and 4) having mycoparasitic capabilities.

During the interaction fungus-fungus several complex mechanisms such as release of antibiotic compounds and lytic enzymes, hyphae attachment, and direct penetration of the host, may occur simultaneously. The mycoparasitism is based on the relationship in which one fungus obtains directly or indirectly its nutrients by invading another fungus (Gao *et al.*, 2005). The ability of a fungus to parasitize another one is partially determined by physical, chemical, or nutritional conditions (Benítez *et al.*, 2006). Barnett and Binder (1973) classified mycoparasitism into two types: necrotrophic and biotrophic. Necrotrophic or destructive mycoparasitism typically results in death of the fungal host, and necrotrophic mycoparasites tend to be more aggressive with wide range of hosts, but without a specialized mode of action (Werner and Zadworny, 2003).

In the biotrophic mycoparasitism, the parasite development is favored by the constant contact with the host living cells. For instance, the primary antagonistic response between *Trichoderma* and the phytopathogen involves growth towards the susceptible hyphae, probably by positive chemotropism. Once *Trichoderma* detects its host, its hyphae develop a profuse branching by which the antagonist gets contact on it. Once the mycoparasite reaches the host its hyphae often roll or grow up along the pathogen mycelium

(Dwivedi and Shukla, 2002).

Studies about the diversity of *Trichoderma* fungi for their application in integrated management of fungal diseases has received little attention in Mexico. For instance, some *Trichoderma* species have been successfully tested on controlling either *Fusarium oxysporum* or *Sclerotium cepivorum* under field conditions (González-Cárdenas *et al.*, 2005; Ávila-Miranda *et al.*, 2006). Thus, the aim of this research consisted on isolating *Trichoderma* fungal strains from different soils of Mexico to assess their antagonistic ability towards *Sclerotinia sclerotiorum* and *S. minor* under *in vitro* conditions.

Materials and methods

Pathogen isolation

The pathogens were isolated and purified in the Laboratory of Soil Microbiology (Colegio de Postgraduados) from soil samples of *Sclerotinia*-infested plots, collected in Salamanca, Guanajuato. *Sclerotinia sclerotiorum* (Ss) and *S. minor* (Sm) were activated in potato-dextrose-agar (PDA, Baker®). The cultures were kept at room temperature (~20 °C) in the dark for 15 days until sclerotia production.

Isolation of *Trichoderma* strains

The sclerotia-trap technique was utilized to obtain antagonistic filamentous fungi to *S. sclerotiorum* (Ss) and *S. minor* (Sm). Fourteen soil samples from six states of Mexico were collected in order to get a wide range of ecosystems from which fungal strains may be isolated: 1) for Jalisco, soil was taken from the rhizosphere of mesquite vegetation (*Prosopis* sp.); 2) for Guanajuato, soil was collected from zones in which lettuce and garlic crops are cultivated; 3) for Oaxaca, soil samples were obtained from the rhizosphere of litchi trees, 4) for Veracruz, soil samples were collected from deciduous tree forest, 5) for Puebla, soils samples were taken

from the Ixta-Popo reserve whose dominant vegetation consists of pine trees, and 6) for the Estado de Mexico, soil was collected from horticultural zones of Xochimilco and Chapingo.

Small nylon bags (1cm²) were prepared in which 20 sclerotia of each pathogen were separately placed. Sclerotia were previously disinfected superficially with sodium hypochlorite at 2% for 5 min and rinsed three times with sterile distilled water. Bags were placed in duplicate in canning glass jars filled at three quarters of their capacity, at 2 cm depth in the respective soil samples. These experimental units were incubated at room temperature (~20 °C) at soil field capacity conditions. The isolation of antagonists was carried out by extracting two sclerotia at 48 h, 72 h and 96 h. Sclerotia were rinsed with sterile distilled water to eliminate excess of soil, and subsequently placed on PDA-plates and incubated at room temperature (~20 °C) in the dark, then, the sclerotia-emerging antagonistic fungi of the genus *Trichoderma* were selected (Mónaco, 1989; Menéndez and Godeas, 1995).

In vitro confrontation of antagonists versus pathogens, and measurement of the antagonist colonization percentage on the pathogen

The pathogens as well as the isolated *Trichoderma* strains were confronted via dual cultures (Rollan *et al.*, 1999). All fungi were activated in Petri dishes with PDA. Each *Trichoderma* strain was confronted to either Ss or Sm by triplicate, in which, an agar disk of the pathogen was placed at one side of a new petri dish, and the disk with the potential antagonist was placed at the opposite side of the plate. Petri dishes were incubated at room temperature in darkness for 15 days. Plates without fungal confrontation for each fungus were also utilized as controls. Antagonism activity was monitored by performing both daily measurements of fungal colony growth and direct observation of the plates, during 10 days. Afterwards, the colonization percentage of

Trichoderma over the fungal colony of each pathogen was estimated according to the following equation (Rollan *et al.*, 1999): $C = [(DCAP/DSP) \times 100]$, where: C=colonization percentage; DCAP=distance covered by the antagonist on the pathogen colony over the axis which separates both fungi, DSP=distance between sowing points (6.5 cm). For our purposes a colonization of the *Trichoderma* strains higher to 70% was considered as an effective colonization over both pathogens. A completely randomized experimental design was set for each *Trichoderma* strain, and data were analyzed by ANOVA analysis.

Preparation of mycoparasitized sclerotia in dual cultures

After confronting antagonists versus both pathogens, the most effective mycoparasitic *Trichoderma* strains were selected. Thus, 10 mL of formaldehyde 10% were added during 4 h, to each plate (Riddell, 1950). Subsequently, nine sclerotia were extracted depending on the position (first, second and third) by which they were parasitized by the *Trichoderma* strains. The damage of sclerotia due to the *Trichoderma* attack was evaluated under stereoscopic microscope as well as by performing a hardness test consisting in pressing the sclerotia with the tip of a dissection needle according to the following criteria: 1) Hard, in which sclerotia at the moment of exerting pressure with the dissection needle, their structure keep their shape and hardness; 2) Soft, in which sclerotia show little or zero resistance at pressure with dissection needle, and their structure maintain its form; 3) Hollow, where sclerotia visually maintain their structure, but at the moment of pressure, the structure gets perforated or ruined; and, 4) Out of shape (disintegrated), in which sclerotia do not show any structure, without shape and without hardness.

Results

Antagonist isolation

From the 14 soil samples, 71 isolates of filamentous fungi (IBA) were obtained and 84% of them corresponded to the genus *Trichoderma*, showing high competition and inhibition of sclerotia germination of Ss and Sm. The soil samples from where most of the fungal isolates were obtained corresponded to Oaxaca and Jalisco with 24 and 18 fungal isolates respectively. In contrast, the smallest numbers of fungal isolations were obtained from Veracruz with eight isolations and from the Estado de Mexico (Xochimilco and Chapingo) with seven isolates; whereas from Guanajuato only seven isolates were obtained. Antagonists from Puebla soil samples could not be isolated.

In vitro confrontation of fungal isolates and pathogens

The 71 isolated antagonists (Table 1), were classified according four categories of visual parasitism: a) Category 4, characterized by presenting 91-100% of short term mycelium invasion, its accelerated growth of cotton-like mycelium on the pathogen, the formation of small heaps by which sclerotia were enveloped, thus causing sclerotia-degradation, the mycelium of the antagonist had intense green color and formed a yellowish brown halo at the meeting zone of both mycelia, b) Category 3, in which 51-90% of invasion on the pathogen mycelium was observed, with small cotton-like growth and scarce formation of mycelial heaps on the sclerotia and inducing softening, the mycelium of the antagonist had intense green to olive green color and showed a brown halo only where mycelia met each other; c) Category 2, presented 1 to 50% of mycelial invasion, slow growth, not very much cotton-like mycelium on the pathogen mycelium nor the sclerotia, moreover, the antagonist strains induced the new formation of sclerotia by the pathogen, light green color of the antagonist; and d) Category 1, did not show

Table 1. Description and grouping of antagonists based on the degree of parasitism which was categorized by visual description on two pathogens *Sclerotinia sclerotiorum* and *Sclerotinia minor*

Degree of Macroscopic Mycoparasitism	Fungal Strain (State of Origin)
4	IBA-1, IBA-4, IBA-23, IBA-38, IBA-56 (Jalisco); IBA-46, IBA-54 (Guanajuato); IBA-22 (Oaxaca); IBA-3, IBA-37 (Veracruz).
3	IBA-55, IBA-63, IBA-67 (Estado de Mexico); IBA-33 (Jalisco); IBA-35, IBA-47, IBA-53, IBA-69 (Guanajuato); IBA-65, IBA-61, IBA-41 (Oaxaca); IBA-5, IBA-17, IBA-30, IBA-32 (Veracruz).
2	IBA-28 (Estado de Mexico); IBA-9; IBA-13, IBA-18, IBA-25, IBA-44 (Jalisco); IBA-7, IBA-10, IBA-14, IBA-15, IBA-21, IBA-26, IBA-36, IBA-42, IBA-43, IBA-70, IBA-71 (Oaxaca); IBA-2, IBA-6, IBA-29, IBA-34 (Veracruz).
1	IBA-27, IBA-39 (Estado de Mexico); IBA-12, IBA-24, IBA-57, IBA-58, IBA-59, IBA-60, IBA-66 (Jalisco); IBA-20, IBA-45 (Guanajuato); IBA-11, IBA-19, IBA-31, IBA-40, IBA-62, IBA-64, IBA-68 (Oaxaca); IBA-8, IBA-16, IBA-48, IBA-49, IBA-50, IBA-51, IBA-52 (Veracruz).

antagonistic effect on the pathogen growth, the mycelium and sclerotia formation was similar to the control.

In general, most of the fungi showed antagonistic effects to both pathogens, but only 10 *Trichoderma* strains were selected on the basis of their classification as Category 4.

During the *Trichoderma*-Sm confrontation a zone of progressive inhibition was observed which may be in part attributed to the fast growth of *Trichoderma*. After the inhibition, a marked hyperparasitic effect of the strains IBA-22, and IBA-37 was detected. In addition a small orange-reddish zone was observed as a result of the invasion on the pathogen's mycelium. The antagonistic ability of *Trichoderma* was maintained for seven days by showing a mycoparasitic effect on both pathogens. Figure 1 shows the effects of three of the best *Trichoderma* isolates (IBA-4, IBA-38, and IBA-56) in their respective confrontation with *S. sclerotiorum* and *S. minor*.

At the fungal confrontation of *Trichoderma*-Ss, the pathogen limited the invasion of the antagonist by elevating its mycelium and then, acting as a barrier. At the contact with the antagonistic fungal strain, *Sclerotinia* stopped its growth

and started the formation of sclerotia on the periphery of its fungal colony (Figure 1). The sclerotia development was irregular since few of them had large size or deformations (data non-presented). Twenty-five *Trichoderma* strains completely invaded either the colony or the sclerotia of the pathogen. The average period of colonization of the antagonistic over the pathogen was between 8-10 days.

Measurement of the antagonist colonization percentage

There were observed significant differences ($P < 0.001$) on the colonization percentage of the *Trichoderma* strains on the fungal pathogens, and only ten strains were pre-selected according to the best colonization percentages (IBA-1, IBA-3, IBA-4, IBA-22, IBA-23, IBA-37, IBA-38, IBA-46, IBA-54, and IBA-56). Figure 2 shows the colonization of 10 *Trichoderma* strains on the mycelium of Ss and Sm. The colonization percentage of IBA-54 on the mycelium of both pathogens was higher than 90%, which was obtained in less time (6-7 days) in comparison to the strains IBA-3, IBA-4, and IBA-38 (Figure 2). These four *Trichoderma* strains had excellent abilities for invading the mycelium of both



Figure 1. Mycoparasitism of three *Trichoderma* strains on *Sclerotinia minor* (Sm) and *Sclerotinia sclerotiorum* (Ss). Series a1-5 corresponds to strain IBA-4, series b1-5 corresponds to strain IBA-38, and series c1-5 corresponds to strain IBA-56. T=*Trichoderma* strain. Series 1a, 1b, and 1c=Controls of antagonist strains (Tc); Series 2a, 2b, and 2c=Interaction between the antagonist with Sm; Series 3a, 3b, and 3c=Controls of Sm; Series 4a, 4b, and 4c= Interaction between the antagonist with Ss; and Series 5a, 5b; and 5c=Controls of Ss.

Table 2. Degree of parasitism of 10 *Trichoderma* strains on sclerotia of *Sclerotinia sclerotiorum* (Ss) and *S. minor* (Sm), with respect to position at moment of their confrontation with the mycelium of *Trichoderma*

Key	Sclerotia First position		Sclerotia Second position		Sclerotia Third position	
	Sm	Ss	Sm	Ss	Sm	Ss
IBA-1	3* b	2 c	3 b	1 d	3 abc	1 d
IBA-3	4 a	2 c	4 a	1 d	4 a	1 d
IBA-4	4 a	3 b	4 a	2 c	4 a	1.5 cd
IBA-22	4 a	2 c	3 b	1 d	3 abc	1 d
IBA-23	3 b	3 b	4 a	4 a	4 a	4 a
IBA-37	3 b	2 c	2 c	1 d	2 bcd	1 d
IBA-38	2 c	2 c	2 c	2 c	2 bcd	2 bcd
IBA-46	3 b	3 b	3 b	2 c	3.5 ab	1 d
IBA-54	3 b	3 b	3 b	3 b	3 abc	3 abc
IBA-56	4 a	2 c	3 b	1 d	2 bcd	1 d

*Corresponds to the level of sclerotia degradation as they were parasitized, Where: 1=hard (without damage), 2=soft, 3=hollow, and 4=disintegrated. Means with different letters within the columns are statistically different (Tukey, $\alpha=0.05$), n=3.

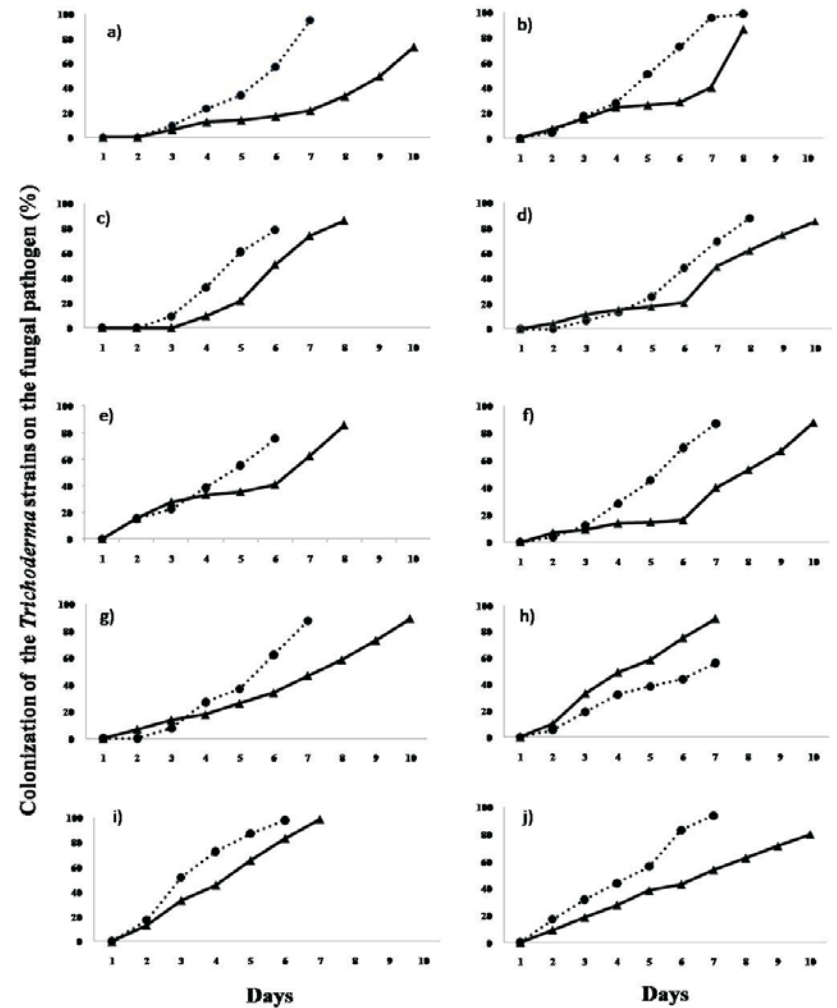


Figure 2. Colonization percentage during the mycoparasitism process of ten *Trichoderma* strains on the fungal colony of two pathogens. a) IBA-1, b) IBA-3, c) IBA-4, d) IBA-22, e) IBA-23, f) IBA37, g) IBA-8, h) IBA-46, i) IBA-54, and j) IBA-56. *Sclerotinia sclerotiorum* (), *Sclerotinia minor* (). (n=3).

pathogens, by which these strains were selected for the next experimental stages.

Mycoparasitism of sclerotia in dual cultures

The zone of attack induced on the pathogen by *Trichoderma* strains, increased with the time, and it was accompanied by the destruction of the pathogen mycelium. In addition, the observed mycoparasitism of sclerotia resulted on severe damage to their structure. Some sclerotia structures were hollow, soft or disintegrated by the activity of the 10 selected strains, likewise, variations in sclerotia size were observed (Figures 3 and 4). Table 2 shows the degree of sclerotia

parasitism after 7 days of the contact between the antagonists with the pathogen. Significant differences in sclerotia degradation were observed (Tukey, $\alpha=0.05$) at the moment of its invasion for the antagonists.

Main damages caused by the IBA-4 strain to Ss-sclerotia consisted on deformations, collapsing, and cracking (Figure 3 b1-b3) when compared to the control (Figure 3 a1-a3); the strain IBA-23 produced collapsing, distortion, and increase in size (Figure 3 c1-c3); while the strain IBA-56 besides affecting and diminishing sclerotia size also produced their collapsing (Figure 3 e1-e3). In contrast, the strain IBA-22 did not produce internal damage of sclerotia, but did

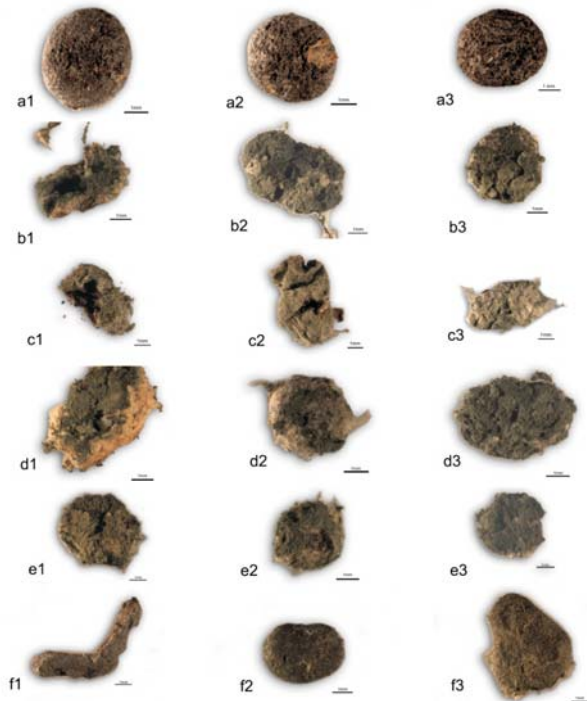


Figure 3. Mycoparasitism of sclerotia of *Sclerotinia sclerotiorum* by five *Trichoderma* strains, where: a) Control, b) IBA-4, c) IBA-23, d) IBA-38, e) IBA-56, and f) IBA-22. Numerals (1-3) refer to the position of sclerotia (1=first, 2=second, and 3=third position) at the moment of meeting with the antagonist in dual cultures, after completely invading the colony of *S. sclerotiorum*. Line = 1 mm.

produce distortion in size, inducing larger sclerotia than the control (Figure 3 f1-f3).

The damages observed for Sm-sclerotia due to three strains (IBA-4, IBA-23, and IBA-38) were more remarkable since they caused disintegration of sclerotia (Figure 4 d1-d3), distortion, and collapsing (Figure 4 b1-b2, c1-c3, d1-d3). Although the strain IBA-22 did not induced damage on sclerotia, it caused structure distortion (Figure 4 e1-e3) in comparison to the control (Figure 4 a1-a3). The most severe damages on the sclerotia structures corresponded to those sclerotia that were firstly reached by the antagonist.

Based on morphological criteria (Bissett, 1984,

1991), four of the most prominent strains were identified as *T. viride* (IBA-4), *T. pseudokoningii* (IBA-23), *T. harzianum* (IBA-38), and *T. virens* (IBA-46), have important potential as antagonists to Ss and Sm.

Discussion

The *in vitro* assay confirmed the effectiveness of *Trichoderma* isolates for controlling the growth of Ss and Sm. The *Trichoderma* isolates showed clear antagonistic effect by significantly inhibiting the growth of both pathogens. Similar

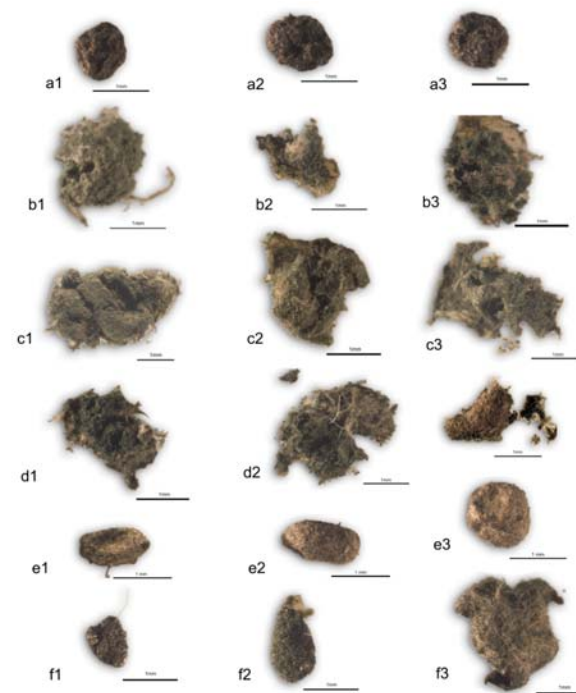


Figure 4. Sclerotia mycoparasitism of *Sclerotinia minor* by five *Trichoderma* strains, where: a) Control, b) IBA-4, c) IBA-23, d) IBA-38, e) IBA-56, and f) IBA-22. Numerals (1-3) refer to the sclerotia position (1=first, 2=second, and 3=third position) at the moment of meeting with the antagonist in dual cultures, after completely invading the colony of *S. minor*. Line = 1 mm.

results have been reported for *Trichoderma harzianum* isolates against *Phytophthora capsici* in dual cultures (Ezziyyani *et al.*, 2004). The mycoparasitism of the *Trichoderma* strains varied according to its combination with the pathogen species. The inhibition zone produced by some *Trichoderma* strains when facing the pathogen increased with the time and resulted in mycelium and sclerotia destruction. The strain IBA-23 reduced the sclerotia formation of Sm, and this effect agrees with that described by Molina *et al.* (2006). The mycoparasitic effect of the *Trichoderma* strains on sclerotia has ecological implications since not only their structure was destroyed but also this fungal attack may significantly prevent sclerotia germination.

All *Trichoderma* strains were capable of growing and sporulating on the pathogen sclerotia, although not all sclerotia were fully parasitized. The latest effect agrees with results from Rollan *et al.* (1999) in which some *Trichoderma* species are able to parasitize sclerotia, but their effectiveness depended on the target pathogen.

The growth inhibition observed on the fungal colony of both pathogens by the antagonists may be attributed to the release of hydrolytic enzymes such as β , 1-3-glucanase, chitinase, protease, and cellulase (Ezziyyani *et al.*, 2004), which are a key step for initiating the pathogen cell wall degradation during mycoparasitism (Rey *et al.*, 2000). The lysis induced by these enzymatic activities depends on the melanin content of the host, and the melanization is directly related to the fungal development and volatile metabolites (Dal *et al.*, 1997; Brozová, 2004).

Sclerotia formation has an ecological importance due to the fact that it guarantees pathogen persistence in soil. The observed mycoparasitism on sclerotia has special relevance since the sclerotia formation and persistence may be significantly reduced by the presence of an effective fungal hyperparasite. Our results about sclerotia degradation agree with some experimental reports. García-Garza *et al.* (1997) described the degradation of sclerotia by *Trichoderma*

hamatum, which initiated its attack in the melanized layer and then invading, and causing lysis of the inner structure of the sclerotia which had either constrained or hollow appearance. These processes were observed for the strains IBA-23 and IBA-38 on *S. minor*, whose sclerotia show hollows and/or cracks. Bolton *et al.* (2006) described that the inner structure of sclerotia has a fibroid matrix consisting of carbohydrates, mainly β -glucan and proteins; therefore, the internal degradation of sclerotia by antagonists may be partly due to β -glucanase releasing. This may justify in part, why the internal sclerotia degradation is the first process during the antagonist attack, and subsequently the structure of the sclerotia collapses. The elimination of the sclerotia matrix increases the opportunity of being also parasitized by other organisms. García-Garza *et al.* (1997) suggest that sclerotia under field conditions may have an infected surface, allowing the easy penetration by other organisms, and then resulting on the degradation of these persistence structures. The fungal cell wall is a complex structure made up of different polymers that define their degradation or survival. Generally, the cell wall is constituted by an internal layer of crystalline mycofibrils wrapped up in an amorphous matrix, together with one or more external layers. The most abundant components of fungal cell walls are chitin, β -1,3-glucane and glycoproteins, which confer them special characteristics. There is evidence that some nanoproteins are bounded with β -1,3-glucane and chitin fibers, forming a very resistant net (Mohammed *et al.*, 2004); that is why the secretion of lytic enzymes such as glucanase and chitinase by *Trichoderma* make the mycoparasitism be more efficient.

Even though it is true that under field conditions there are other biotic or abiotic factors that may influence the expression of the antagonism, the *in vitro* assays allow the selection of *Trichoderma* species suitable as biocontrol agents, but further research is needed to validate the most prominent strains under systems that resemble natural conditions or under field conditions. It may be concluded that

it is possible to isolate antagonistic fungi from several soils with different use such as forestry and agricultural. In addition, *Trichoderma* strains are appropriate agents for controlling the growth and sclerotia of either Ss or Sm.

Acknowledgements

Authors thank financial support to the grant SEP-CONACYT 58594.

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