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Hypomyces chrysospermus ACL-01 isolated from *Boletus edulis* and its effect against fungal cereal pathogens

Hypomyces chrysospermus ACL-01 aislado a partir de *Boletus edulis* y su efecto sobre hongos patógenos de cereales

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Abstract

In this study, the *Hypomyces chrysospermus* ACL-01 fungus was isolated from the fruiting body of edible basidiomycete *Boletus edulis*, collected in Acaxochitlán in the State of Hidalgo, Mexico popularly known as tlacoayel. The antagonistic effect of the isolated fungus against different fungal cereal pathogens was evaluated. The dual confrontation test and scanning electron microscopy analysis revealed that this ascomycete grows on the basidiomycetes *Sporisorium reilianum* and *Tilletia* sp., causing loss of cell viability. However, for the ascomycetes *Bipolaris sorokiniana* and *Pyrenophora teres f. teres* no effect was observed. On other hand, *Stenocarpella maydis* and *Fusarium* sp. inhibited the development of *H. chrysospermus* ACL-01. The isolated strain produced extracellular enzymatic activities of the acid and basic proteases, chitinase and laccase. Cellulase and lipase activities were not found.

Palabras clave: *Hypomyces chrysospermus*, *Boletus edulis*, *Tilletia* sp., *Sporisorium reilianum*.

Resumen

En este estudio se aisló el hongo *Hypomyces chrysospermus* ACL-01 a partir del cuerpo fructífero del basidiomiceto comestible *Boletus edulis* mejor conocido como tlacoayel, colectado en Acaxochitlán Hidalgo, México. El efecto antagónico de este ascomiceto se evaluó sobre diferentes fitopatógenos de cereales. Las pruebas de confrontación y el análisis por microscopía electrónica de barrido revelaron que el hongo en estudio crece sobre los basidiomicetos *Sporisorium reilianum* y *Tilletia* sp. causándoles pérdida de la viabilidad celular. Sin embargo, para los ascomicetos *Bipolaris sorokiniana* y *Pyrenophora teres f. teres* no se observó ningún efecto. Por otro lado, *Stenocarpella maydis* y *Fusarium* sp. inhiben el desarrollo de *H. chrysospermus* ACL-01. La cepa aislada produce las enzimas extracelulares; proteasas ácida y básica, quitinasa y lacasa. Las actividades celulolíticas y lipolíticas no fueron encontradas.

Keywords: *Hypomyces chrysospermus*, *Boletus edulis*, *Tilletia* sp., *Sporisorium reilianum*.

1 Introduction

Hypomyces chrysospermus is a cosmopolitan fungal parasite in boletes that grows by invading the entire fruiting body and causing it to rot. At the onset of the infection process, the fungus appears as a whitish layer that later turns to a gold colour before acquiring a brownish-red granular appearance. This ascomycete belongs to the order of the Hypocreales, some of which have been recognized as mycoparasites (Sahr *et al*., 1999). The most widely studied of these taxa are the genus *Trichoderma*, which inhibit the development of other fungi by the competition for nutrients, the production of antibiotics, and mycoparasitism. In the latter, the fungus attacks the host directly by penetrating it and causing its death (Benítez *et al*., 2004, Almeida *et al*., 2007). Some species of *Trichoderma* have been reported to affect crops of edible basidiomycete fungi, such as *Agaricus bisporus* (champignon), *Pleurotus ostreatus* (oyster mushrooms) and *Lentinula edodes* (Shiitake) by causing green mold disease. Finally, some species of this genus have been isolated for use as efficient

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biofungicides that provide biological control of serious diseases known to adversely affect agriculture (Schuster and Schmoll 2010; Wang *et al*., 2016).

Plant diseases caused by fungus contribute directly to the destruction of economically important cereal crops produced primarily for human and animal consumption, such as wheat, corn and barley (Savary *et al*., 2006; Sobhy *et al*., 2014). In the case of wheat, diseases like smuts and spot blotch caused by different species of *Tilletia* and *Bipolaris sorokiniana*, respectively, are particularly harmful (Kumar *et al*., 2009; Garg *et al*. 2014). In corn, *Sporisorium reilianum* is the causal agent of head smut, while *Stenocarpella maydis* and some species of *Fusarium* generate different types of rot and are important mycotoxin producers (Bensch and van Staden 1992; Parry *et al*., 1995; Ghareeb *et al*., 2011; Qiu and Shi 2014; Álvarez-Cervantes *et al*., 2016). *Pyrenophora teres f. teres* is a barley pathogen that triggers the development of net blotch (Ellwood *et al*., 2010). Currently, the application of chemical compounds in crop fields constitutes the most common strategy for controlling phytopathogens. However, fungicides of biological origin are an environmentally-friendly alternative and they have two important advantages: first, microorganisms do not develop resistance to fungicides as they do with chemical substances; and second, the use of fungicides can decrease the contamination of agricultural land (Whipps 2001; Duffy *et al*., 2003; Makovitzki *et al*., 2007; Chávez-Magdaleno *et al*., 2018; Ramos-Guerrero *et al*., 2020).

While *H. chrysospermus* has been recognized as a mycoparasite (Sahr *et al*., 1999), its potential effect on fungi that are pathogenic for plants has not yet been studied. Thus, the purpose of this work is to analyze the effect of one strain of *H. chrysospermus*, isolated from *Bolletus edulis* -an edible fungus commonly known as tlacoayel- against six fungal cereal pathogens: four ascomycetes and two basidiomycetes.

2 Materials and methods

2.1 Fungus strains

The strains used were: *S. reilianum*, *Tilletia* sp., *B. sorokiniana* and *B. teres f. teres*, of the collection of the Chapingo Autonomous University (Mexico), kindly donated by Dr. Gerardo Leyva Myr. The *S. maydis* was provided by Dr. Dan Jeffers of the collection of the International Maize and Wheat Improvement Center (Mexico), while *Fusarium* sp. was obtained from the microorganism collection of the "Integral Use of Biological Resources" research group at the Polytechnic University of Pachuca (Mexico).

2.2 Culture media and growing conditions

The strains were maintained at room temperature in tubes containing YEPD medium (1% yeast extract, 2% peptone, 2% glucose, and 2% agar) and mineral oil (Stebbins and Robbins 1949). The media used to activate the microorganisms in each experiment were YEPD and a *Pleurotus ostreatus* fruiting body infusion (PFB). This last medium was prepared by boiling 250 g of fruiting bodies -obtained at a local market- for 20 min in 800 mL of distilled water, followed by filtration. The solution was supplemented with 3 g of glucose and adjusted to 1 L with distilled water. 2% agar was added to the solid media. The incubation temperature for *S. reilianum*, *Tilletia* sp., *S. maydis* and *Fusarium* sp. was 28 ºC, and for *B. sorokiniana* and *B. teres f. teres*, 20 ºC.

2.3 Isolation of the fungus

The mycoparasitic fungus was isolated from fruiting bodies of the edible mushroom *B. edulis* (Tlacoayel) collected from the locality of Tlamimilolpa, municipality of Acaxochitlán, state of Hidalgo, Mexico. The isolated fungus presented yellow cottony growth with rot. The fruiting bodies were cut into 1-cm^3 fragments, placed in a 2% sodium hypochlorite solution for 10 min, and then washed twice with sterile distilled water. The fungal material was set in plates with solid YEPD and incubated at 28 °C for 7 d. The plates were observed every 24 h, and the yellow colonies that grew on the fungal tissue were selected and preserved in YEPD.

2.4 Identification of the isolated fungus

Initial identification of the isolated strain was made by means of microscopic morphology characterization, according to Beug *et al*. (2014). The microculture of the fungus was stained with lactophenol blue cotton. The isolated fungus was identified using molecular methods based on the amplification and sequencing of the Internal Transcribed Spacer (ITS) region. DNA was extracted using a modified CTAB method (Cetyl Trimethylammonium Bromide), as is described by Wagner *et al*. (1987). The following universal primers

were used for ITS amplification: ITS5 forward 5´-GGAAGTAAAAGTCGTAACAAGG-3´ and ITS4 reverse 5´-TCCTCCGCTTATTGATATGC-3´ (White *et al*. 1990). The PCR conditions were an initial denaturation at 95 ºC for 4 min, followed by 30 cycles at 95 ºC for 45 s, 52 ºC for 45 s, and 72 ºC for 45 s, with a final extension at 72 ºC for 5 min. The PCR products were cleaned using the QIAquick PCR Purification Kit (Qiagen, USA) according to the manufacturer?s instructions, and then sequenced in both directions using an automated DNA sequencing system (Applied BioSystems model 3730XL). The two strands of DNA were assembled and edited manually in BioEdit v7.0.5 software (http://www.mbio.ncsu.edu/BioEdit/). Multiple sequence alignments were performed using Clustal X (Thompson 1997). An analysis of maximum likelihood (ML) was made in PAUP*(v.4.0b10) (Swofford 1998), using a heuristic search strategy with tree bisection-reconnection. To verify the results, each node was assessed using a bootstrap analysis based on 5000 pseudoreplicates with 10 random-taxon-addition replicates per pseudoreplicate.

2.5 *Test to measure the degree of antagonisms between the fungus isolated and the filamentous fungal cereal pathogens*

The fungus strain was evaluated for its antagonistic effect against the phytopathogenic filamentous fungi mentioned above. For the filamentous fungi, 0.5-cm^2 square was cut from each of the fungi: *B. sorokiniana*, *B. teres f. teres*, *S. maydis* and *Fusarium* sp., obtained from the cultures in plates with YEPD medium and inoculated at one end of the plate with PFB medium. At the other end, equal-size squares of the isolated fungus strain were inoculated. The plates with *B. sorokiniana* and *B. teres f. teres* were incubated at 20 ºC for 10 d, while those with *S. maydis* and *Fusarium* sp. were incubated at 28 ºC for 4 d. As described by Bell *et al*. (1982), the following criteria for measuring antagonism in *Trichoderma* was used: class 1= the isolated fungus grew over the phytopathogen until it covered the entire surface of the medium; class 2= the isolated fungus grew over the phytopathogen until it covered 2/3 of the surface of the medium; class 3= the isolated fungus and the phytopathogen grew until each one covered half of the surface of the medium and neither organism appeared to dominate the other; class 4= the phytopathogenic fungus grew until it covered ¾ of the dish, impeding encroachment by the isolated fungus; class 5= the phytopathogenic fungus grew until it covered the entire dish, thus impeding development of the isolated fungus.

2.6 *Test to measure the degree of mycoparasitism between the fungus isolated and the yeasts cereal pathogens*

For the yeast *S. reilianum* and *Tilletia* sp., a pre-inoculum in YEPD was prepared and incubated at 28 ºC for 24 h at 150 r.p.m. The inoculum was inoculated with an initial absorbance of 0.2 at 600 nm to 10 mL of the same medium in 50-mL flasks and incubated at 28 ºC for 48 h at 150 r.p.m. A total of 100 μL were used to inoculate the surfaces of the plates with PFB medium. Once the inoculum had diffused into the agar, a 0.5-cm^2 square of a culture of the isolated fungus obtained from a culture in YEPD was taken and inoculated into the centre of the plate. The plates were incubated at 28 ºC and observed every 24 h for 5 d. The degree of mycoparasitism was measured according by Galarza *et al*. (2015), for *Trichoderma* with some modifications as is described below: 0= no invasion over the levaduriform growth; 1= the isolated fungus grew until it covered 25% of the levaduriform growth; 2= the isolated fungus grew until it covered 50% of the levaduriform growth; 3= the isolated fungus grew until it covered 75% of the levaduriform growth; 4= the isolated fungus grew until it covered 100% of the levaduriform growth. To verify that the isolated fungus inhibited of yeasts development, 0.5 cm^2 samples were taken from two sections in the test plates, where only the yeasts grew and where the isolated fungus grew over the yeasts. Each sample was inoculated in 50 mL of YEPD broth in 250-mL flasks and incubated at 28 °C for 5 d at 180 r.p.m. The presence of growth of the yeasts, of the isolated fungus, or both, in the cultures was evaluated by optical microscopy at 40X, measuring as follows: (++++)=the yeasts or filament fungus were observed in the 100% of field. (+++) = the yeasts or filament fungus were observed in the 75% of field. (++)=the yeasts or filament fungus were observed in the 50% of field. (+)=the yeasts or filament fungus were observed in the 25% of field.(-)= no yeasts or mycelium growth was observed. All tests were performed in triplicate and observed every 12 h.

2.7 Scanning electron microscopy (SEM)

To measure the interaction between the fungi in question, 0.5-cm^2 samples were taken from the plates in which the tests were conducted on days one, two, and three after inoculation. The samples were fixed in glutaraldehyde vapours at 50 ºC for 1 h and then placed in 1% osmium tetroxide for 1 h. They were then dried for 24-48 h in a desiccator containing $CaCl_2$, mounted on specimen holders, and coated with gold. Images were captured and digitalized using a JEON microscope (model JSM-5800LV Japan) connected to a computer.

2.8 Agar plate assays for enzymatic activity

Plates with a diameter of 90 mm with different media were used to determine enzymatic activity. They were inoculated in the centre of the plate with 0.5 cm^2 of the isolated fungus obtained from a 7-day culture in PFB medium and then incubated at 28 ºC. All tests were observed every 24 h.

The lipolytic activity was observed in the plates whit 3% olive oil, 1% Tween 80, 2% agar and 10 mg/mL of rhodamine B. A positive test was indicated by the presence of an orange halo around the colony when exposed to UV light (Kouker and Jaeger 1987).

The acid protease activity was determined by preparing plates as follows: 1.2 g of albumin in 60 mL of 0.5 M citrate buffer at pH 4 was pasteurized for 20 min at 75 ºC for 24 h. It was then mixed with 480 mL of a sterile medium containing 0.17% Yeast Nitrogen Base without amino acids and ammonium sulphate (YNB) and 2% agar. Next, 60 mL of sterile 0.5 M citrate buffer (pH 4) was added to the solution. The same medium was used to determine alkaline protease activity, but with 10 g of skim milk prepared in 0.5 M phosphate buffer at pH 6.8, and the YNB medium supplemented by the same buffer (Nelson and Young 1986).

The chitinase production was detected in plates containing a medium with 10% colloidal chitin from shrimp shells and 2% agar. To obtain colloidal chitin, 40 g of chitin was mixed with 400 mL of 37% HCl. This solution was agitated in a shaker at 180 r.p.m. for 50 min, and 2 L of cold distilled water was added to the mixture. Agitation continued for 12 h at room temperature. Colloidal chitin was obtained after filtration and washing with distilled water. The paste was sterilized in an autoclave at 120 ºC for 15 min and subsequently stored at 4ºC until further use (Roberts and Selitrennikoff 1988). In the case of the above-mentioned enzymes, a clear halo around the colony was considered a positive test.

Another medium was used to detect cellulase. It contained 0.2% ammonium nitrate, 0.1% dipotassium phosphate, 0.05% magnesium sulphate, 0.05% potassium chloride, 0.2% carboxymethyl cellulose, 0.2% casein peptone and 0.2% agar. To reveal cellulase activity, 30 mL of a 0.2% red Congo solution was added to the dish, after which it was incubated for 30 min at room temperature and the washed in a solution of 1 M NaCl until a clear halo appeared around the colony, indicating a positive test (Andro *et al*., 1984).

In the case of laccase production, Kirk medium containing 1% glucose, 0.5% ammonium tartrate, 0.02% magnesium sulphate, 0.2% monopotassium phosphate, 0.001% calcium chloride, 2% agar and 2 mM ABTS [2,2?-Azino-bis (3-ethylbenzotiazoline-6-sulphoonico acid)]. The presence of a green halo around the colony indicated a positive test (Chairattanamanokorn *et al*., 2006).

The experiments were performed in triplicate, and enzymatic activity was expressed as Potential Index (PI) using the following formula:

PI= Diameter of halo of activity (cm)/Diameter of fungal growth (cm).

2.9 Statistical analysis

The analysis of variance (ANOVA) was made to determine the statistically significant differences in the enzymes production from *H. chrysospermus* ACL-01, as well as, in the degree of mycoparasitism between this fungus and the yeasts *S. reilianum* and *Tilletia* sp.

3 Results

The fungus strain that was isolated from the fruiting bodies of *B. edulis* it called *H. chrysospermus* ACL-01. In the plate culture with YEPD agar medium, this fungus presented radial mycelium growth of a bright yellow color with white edges of a dusty appearance and produced a diffusible yellow pigment that later turned orange.

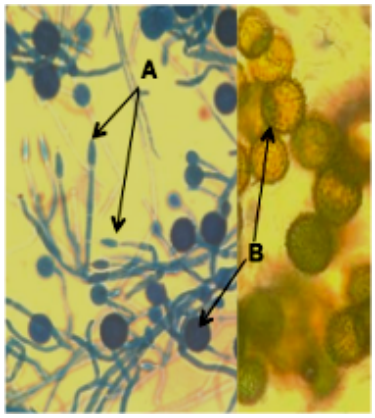

Fig. 1. Microscopic morphology of the *H. chrysosperm* ACL-01 fungus isolated from fruiting bodies of the comestible fungus *Boletus edulis* (Tlacoayel). Septate mycelia were observed with elliptical conidia (A) and aleuriospores (B). Optical microscopy, at 40X.

Table 1. Test to corroborate the mycoparasitism in different sections in the test plates.

Day	Growth presence in YEPD broth*							
	S. reilianum		*H. chrysospermus* ACL-01		*Tilletia* sp		*H. chrysospermus* ACL-01	
	S1s	S2s	S1s	S2s	S1t	S2t	S1t	S2t
1	++++	++	-	++	++++	++	-	++
2	++++	-	-	++++	++++	+	-	+++
3	++++	-	-	++++	++++	-	-	++++
4	++++	-	-	++++	++++	-	-	++++
5	ND	-	-	++++	++++	-	-	++++

S1= Section where only the *S. reilianum* or *Tilletia* sp grew. S2= Section where *H. chrysospermus* ACL-01 grew over the yeasts. ND= *S. reilianum* was not present in the plate. s= *S. reilianum* and *H. chrysospermus* ACL-01 interaction. t= *Tilletia* sp and *H. chrysospermus* ACL-01 interaction.
* The presence of growth of the *H. chrysospermus* ACL-01 or of the yeasts in the cultures was evaluated by optical microscopy at 40X measuring as is described in Materials and Methods. The tests were made by triplicate.

Table 2. Extracellular enzymatic activities form *H. chrysospermus* ACL-01 in plate.

Enzyme	PI*		
	Days		
	3	5	7
Acid Protease	1.3 ± 0.2	1.6 ± 0.1	1.7 ± 0.1
Alkaline protease	1.6 ± 0.1	1.9 ± 0.1	2.1 ± 0.1
Chitinase	1.3 ± 0.2	1.9 ± 0.1	1.9 ± 0.1
Laccase	1.5 ± 0.5	1.5 ± 0.6	1.9 ± 0.1

* The activity is expressed as Potential Index (PI), which was calculated as described in Materials and Methods. The tests in plate were made by triplicate.

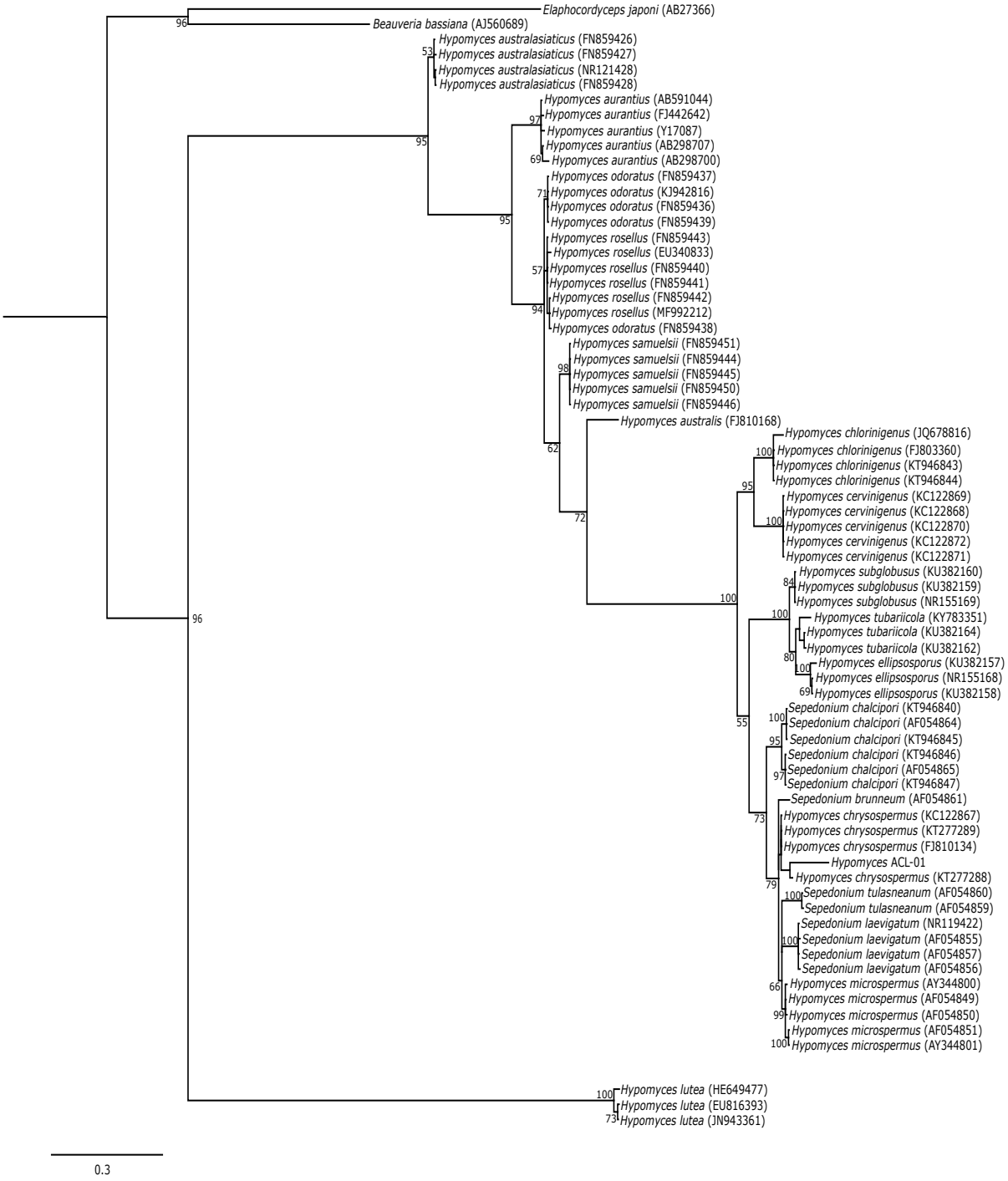

Fig. 2. Maximun likelihood phylogenetic analysis of the nucleic acid sequence of ITS of *H. chrysosperm* ACL-01 and their taxonomic relatives.

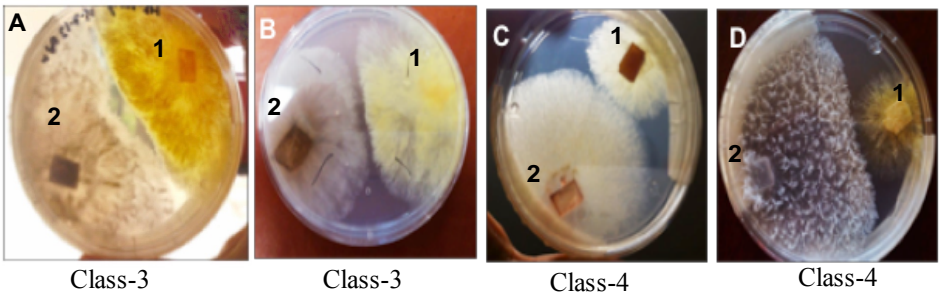

Fig. 3. Confrontation tests of *H. chrysospermus* ACL-01 (1) against phytopathogenic filamentous fungi (2). A) *B. sorokiniana*, B) *B. teres f. teres*, C) *Fusarium* sp., D) and *S. maydis*. In A) and in B) *B. sorokiniana* and *B. teres f. teres* grown invading a half in the plate the same to *H. chrysospermus* ACL-01, whit interaction degree class 3. In C) and d) *Fusarium* sp. and *S. maydis* inhibited the development of *H. chrysospermus* ACL-01, whit interaction degree class 4.

The mycelia were septate and hyaline with elliptical conidia, acute apexes. After 72 h of incubation the presence of spherical yellow aleuriospores of 19.2 ± 1.1 μm with spiny surfaces (Fig. 1). Phylogenetic analysis of the ITS showed that the fungus was related to *H. chrysospermus* (Fig. 2). The sequence was deposited in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) under access code KX459424.1.

The interaction tests of the filamentous fungi *B. sorokiniana* and *B. teres f. teres* with *H. chrysospermus* ACL-01 indicated a class-3 type interaction (Fig. 3A-B). In contrast, *Fusarium* sp. and *S. maydis* presented an antagonistic effect on the growth of *H. chrysospermus* ACL-01, with a class-4 degree of interaction (Fig. 3C-D).

No statistically significant differences were observed in the mycoparasitism degree of *H. chrysospermus* ACL-01 against *S. reilianum* and *Tilletia* sp. ($F=2.98$, $p>0.05$), in where the values were of 4 and 3 after 5 days respectively to each yeast (Fig. 4).

H. chrysospermus ACL-01 sporulated on *S. reilianum* during their interaction. The same effect was not observed in *H. chrysospermus-Tilletia* sp. relationship. To verify these observations, samples were taken from different sections of the plates in which tests were conducted between *H. chrysospermus* ACL-01 and the yeasts under study. At day five, a sample was taken of the section of the plate in which *H. chrysospermus* ACL-01 grew over the levaduriform growth. Only the fungus grew was observed, suggesting that it invades the growth of the yeasts, inhibiting their development and causing their death (Table 1).

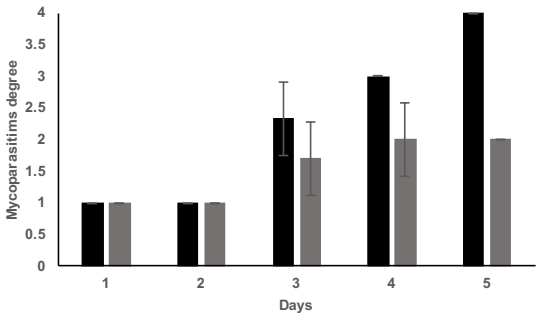

Fig. 4. Mycoparasitism degree between *H. chrysospermus* ACL-01 and the yeasts cereal pathogens. ■ *S. reilianum*, ■ *Tilletia* sp. The mycoparasitism degree of *H. chrysospermus* ACL-01 against *S. reilianum* is higher than *Tilletia* sp. after 4 d of interaction.

The interaction of *H. chrysospermus* ACL-01 with *S. reilianum* was observed where there was growth over yeast colonies under SEM (Fig. 5). Here, the growth of the filamentous fungus was above the yeast growth (Fig. 5A). The Fig. 5B and C, show the yeasts of *S. reilianum* and the mycelia with elliptical conidia of *H. chrysospermus* ACL-01, respectively. The presence of aleuriospores with spiny surfaces was observed on top of the yeasts (Fig. 5D-E). The mycelia of the filamentous fungus were introduced into the yeast growth (Fig. 5F). In the section where the yeast growth was covered completely by *H. chrysospermus* ACL-01 at day three (Fig. 5G), the presence of yeasts between the mycelium of *H. chrysospermus* ACL-01 was observed (Fig. 5H). However, these cells were not viable (Table 1).

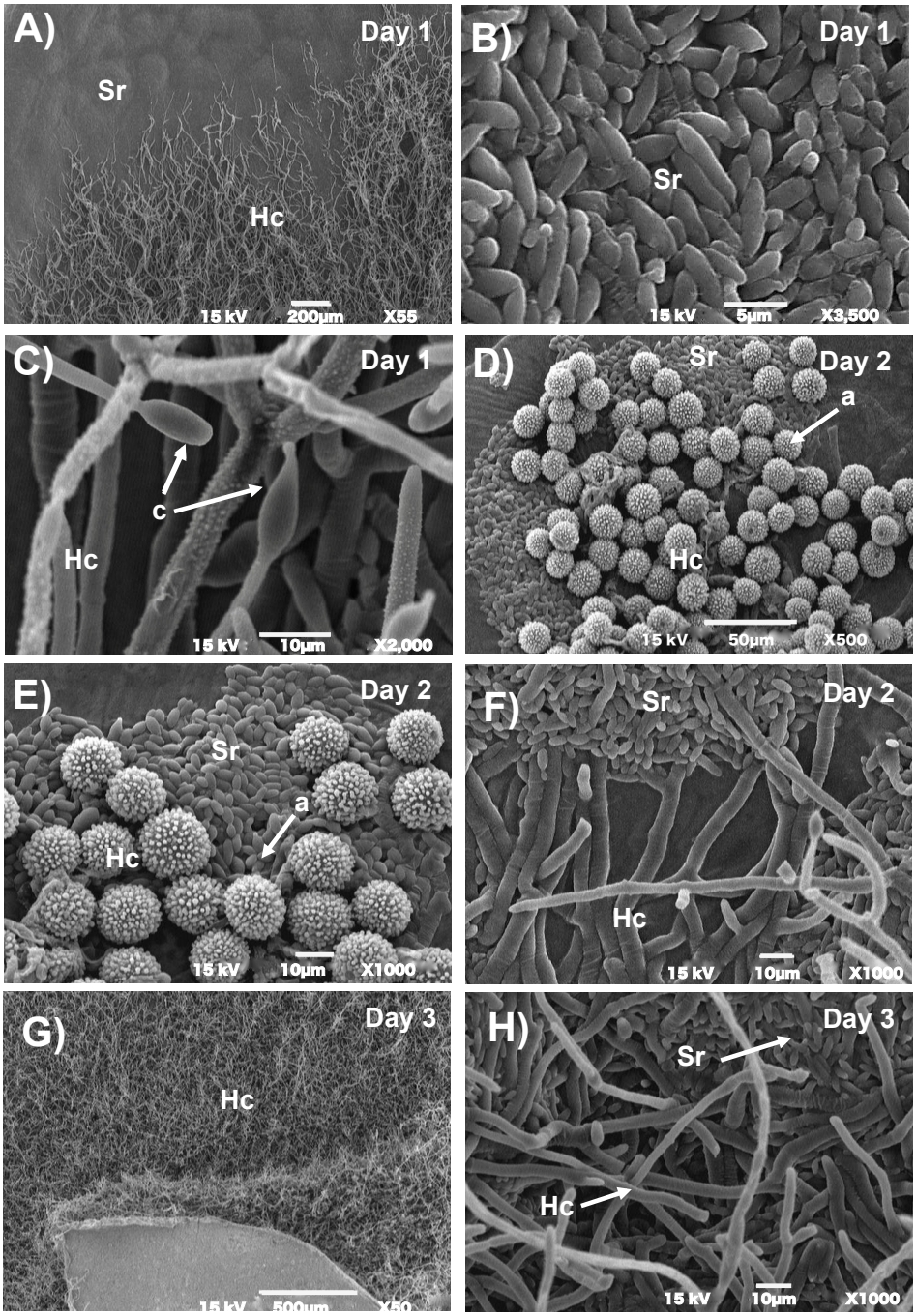

Fig. 5. SEM of the interaction between *H. chrysospermus* ACL-01 (Hc) and *S. reilianum* (Sr). A) Mycelia of *H. chrysospermus* ACL-01 growing above the yeasts of *S. reilianum*. B) Yeasts of *S. reilianum*. C) Mycelia of *H. chrysospermus* ACL-01 with conidia (c). D) and E) Aleuriospores (a) of *H. chrysospermus* ACL-01 above the yeasts of *S. reilianum*. f) Mycelia of *H. chrysospermus* ACL-01 interacting with the yeast growth. G) *H. chrysospermus* ACL-01 growth covering completely the yeasts of *S. reilianum*. H) Yeasts of *S. reilianum* between mycelia of *H. chrysospermus* ACL-01.

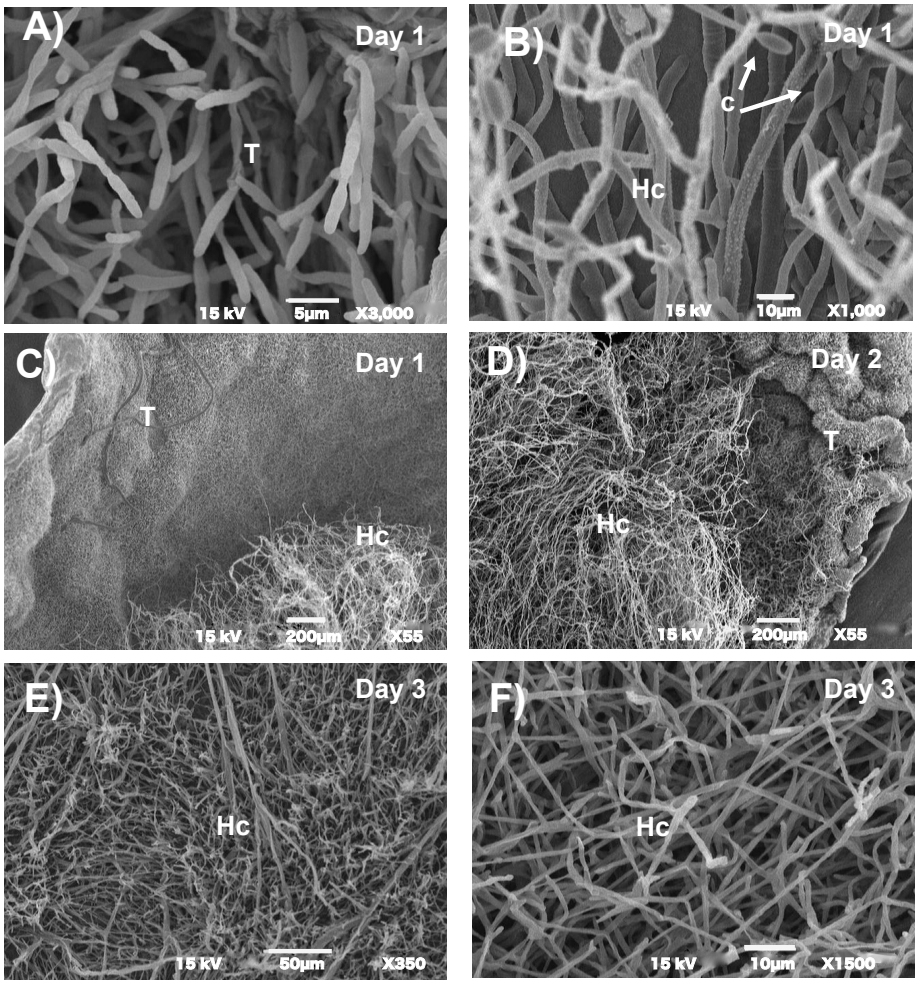

Fig. 6. SEM of the interaction between *H. chrysospermus* ACL-01 (Hc) and *Tilletia* sp. (T). A) Yeasts of *Tilletia* sp. B) Mycelia of *H. chrysospermus* ACL-01 with conidia (c). C) and D) Mycelia of *H. chrysospermus* ACL-01 growing above the yeasts of *Tilletia* sp. E) and F) *H. chrysospermus* ACL-01 growth covering completely the yeasts of *S. reilianum*.

Fig. 6 show, the ability of *H. chrysospermus* ACL-01 to invade the growth of the yeast *Tilletia* sp. Fig. 6A-B, show the yeasts of *Tilletia* sp. and the mycelium of *H. chrysospermus* ACL-01, respectively. In the section where *H. chrysospermus* ACL-01 covered the growth of *Tilletia* sp, the presence of yeast was not observed (Fig. 6E-F). In this interaction, *H. chrysospermus* ACL-01 did not produce aleuriospores.

H. chrysospermus ACL-01 produced chitinases, laccases and acid and alkaline proteases (Table 2). No statistically significant differences were observed in the production of these enzymes ($F=1.16$, $p>0.05$). No cellulolytic or lipolytic activity was detected in the medium and environmental conditions tested.

4 Discussion

Mycoparasitism is an interesting and important form of microbial interaction based on a sequential process in which a parasitic fungus attacks, penetrates, and causes the death of its host (Howell, 2003). This mechanism has been described for the genus *Trichoderma*, which first shows morphological changes such as coiling around the pathogenic hyphae, the development of an appressorium-like structure, as well as lecithin production, which promotes adhesion (Benítez *et al.*, 2004). The next step involves the production of enzymes that degrade the cell wall

and peptaibol production, that is, peptides with antifungal activity that reflect the production of pores in the membrane and facilitate entry into the host, which subsequently dies and serves as food for the mycoparasite (Atanasova *et al*., 2013).

In the present study, we performed the isolation and identification of the mycoparasitic fungus *H. chrysospermus* ACL-01 from fruiting bodies of *B. edulis*. Several species of *Hypomyces* genera are mycoparasites, specifically *H. chrysospermus* is a cosmopolitan parasite in bolete with a wide of host (Sahr *et al*., 1999; Douhan and Rizzo 2003). For example, the fruiting body infection of *Xerocomus chrysenteron* is often parasitized by *Sepedonium chrysospermum* (the anamorph of *H. chrysospermus*) (Both 2006). However, the association of different yeasts in the fruiting body of *Paxillus involutus* favours infection with *H. chrysospermus* (Yurkov *et al*., 2012). Although, there are reports that *H. chrysospermus* is a mycoparasitic fungus, the mechanisms through which this microorganism functions remain unknown.

The results of the present study demonstrate the ability of *H. chrysospermus* ACL-01 to grow on *S. reilianum* and *Tilletia* sp., leading to a loss of viability in said phytopathogens. On the other hand, although the production of lipases and cellulases in the isolated fungus were not found, extracellular enzymes as chitinases, laccases and acid and alkaline proteases that can degrade cellular components was observed. Lytic enzymes including chitinases, glucanases, proteases, cellulases and lipases produced from mycoparasites, which degrade the fungal cell wall, have been extensively examined in the genus *Trichoderma*, where the host cells show lysis and degradation through a process called exolysis (Benítez *et al*., 2004; Atanasova *et al*., 2013).

SEM revealed no morphological changes in *H. chrysospermus* ACL-01, such as coiling around the pathogen or the development of an appressorium-like structure, as observed in the mycoparasitism of *Trichoderma* (Benítez *et al*., 2004; Almeida *et al*., 2007). However, hyphae invasion of the yeast colony was observed, suggesting that a process of adherence is required.

The *H. chrysospermus* ACL-01 strain produces extracellular laccase activity. In *Trichoderma virens*, a laccase plays an important role in the sclerotic colonization of fungal phytopathogens through the melanin degradation present in these fungal structures. The deletion of the lcc1 gene, which encodes for this enzyme, decreases the fungus ability to colonize the sclerotia of *Botrytis cinerea*, but has no colonizing effect on the sclerotia of *Sclerotinia sclerotiorum*. This finding suggests a distinct mechanism of parasitic action (Catalano *et al*., 2011). In other hand the laccases of *Trichoderma* versicolor can be used for the herbicide glyphosate degradation (Pizzul *et al*., 2009; Me?ndez-Herna?ndez and Loera, 2019). In *H. chrysospermus* ACL-0 these enzymes could be evaluated for the degradation of recalcitrant agrochemicals.

The effect of *H. chrysospermus* ACL-01 on other plant pathogens was evaluated by means of confrontation tests. Notably, the growth of *B. sorokiniana* and *B. teres f. teres* was the same as that of *H. chrysospermus* ACL-01, in where a half of plate was invaded by each fungus, but differed from *Fusarium* sp. and *S. maydis*, which show faster growth and even inhibit the development of *H. chrysospermus* ACL-01. These results show that, under the conditions of this study, the isolated fungus had no effect on the growth of the ascomycetes, and its behaviour was not like a mycoparasite against these fungi. However, the production of antimicrobial compounds by *H. chrysospermus* that inhibit the growth of gram-positive and gram-negative bacteria, yeasts and filamentous fungi has been reported (Dornberger *et al*.,1995; Mitova *et al*., 2006; Nagao *et al*., 2006).

In the natural environment, microorganisms are typically subject to various interactions, including competition, which involve the unequal behaviour of two or more organisms towards the same requirement, whereby the growth of some will be limited (Vey *et al*., 2001; Benítez *et al*., 2004). Different strains of *Trichoderma* have been reported to grow faster in the soil, besides, these microorganisms can naturally resist toxic compounds, including fungicides and pesticides. However, inanition is the most common form of death in microorganisms, reflecting the fact that competition over limited nutrients inhibits the growth of some of these microbes. Thus, *Trichoderma* produces siderophores that act as iron-chelating agents, limiting the growth of other fungal strains (Chet *et al*., 1997; Benítez *et al*., 2004). A similar process may have occurred in the present study of the interactions of *H. chrysospermus* ACL-01 with *B. sorokiniana* and *B. teres f. teres*, where the limited growth of these two microorganisms reflected competition for space and nutrients. In contrast, *Fusarium* sp. and *H. chrysospermus* ACL-01 showed unequal development, with the former showing a faster rate of growth. Moreover, *S. maydis* presented antibiosis.

Until recently, the effects of *H. chrysospermus* on the ascomycete plant pathogens *B. sorokiniana*, *B.*

teres f. teres, *Fusarium* sp. and *S. maydis* had not been evaluated. Therefore, this report is the first to examine the effects of *H. chrysospermus* ACL-01 on the yeasts *S. reilianum* and *Tilletia* sp., two basidiomycete phytopathogens. Probably this preference is due to the in the natural environmental, this fungus is a parasite of boletes, which are into the basidiomycetes group (Sahr *et al*., 1999). Our results serve to increase the existing knowledge of this fungus mycoparasitic.

Conclusions

H. chrysospermus ACL-0, a strain isolated from *B. edulis* was evaluated in order to determine its effect on six fungal cereal pathogens. The results show that this ascomycete is able to grow over the yeasts *S. reilianum* and *Tilletia* sp. and to cause its death, thereby demonstrating the potential of the fungus to inhibit the development of these basidiomycetes. On the other hand, in the filamentous fungi *Bipolaris sorokiniana*, *Pyrenophora teres f. teres*, *Stenocarpella maydis* and *Fusarium* sp. the same effect was not observed. Even the last two ascomycetes inhibited the development of *H. chrysospermus* ACL-01. Besides in this study the production of extracellular enzymatic activities of acid and basic proteases, chitinase and laccase in this fungus were demonstrated.

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References

Andro, T., Chambost ,J.P., Kotoujansky, A., Catteneo, J., Bertheau, Y., Barras, F., van Gijsegem, F., and Colleno, A. (1984). Mutants of *Erwinia chrysanthemi* defective in secretion of pectinase and cellulase. *Journal of Bacteriology 160*, 1199-1203.

Almeida, F.B.R., Cerqueira, F.M., Silva, R.N., Ulhoa, C.J., and Lima, A.L. (2007). Mycoparasitism studies of *Trichoderma* harzianum strains against Rhizoctonia solani: evaluation of coiling and hydrolytic enzyme production. *Biotechnology Letters 29*, 1189-1193. https://doi.org/10.1007/s10529-007-9372-z

Álvarez-Cervantes, J.,, Hernández-Domínguez, E.M., Tellez-Tellez, M., Mandujano-González, V., Mercado-Flores, Y., and Díaz-Godinez, G. (2016). *Stenocarpella maydis* and *Sporisorium reilianum*: Two pathogenic fungi of maize, In: *Fungal Pathogenicity*, (S. Sultan ed.), Pp. 45-60. INTECH, Croatia. https://doi.org/10.5772/62662

Atanasova, L., Le Crom, S., Gruber, S.., Coulpier, F., Seidl-Seiboth, V., Kubicek, C.P., and Druzhinina, I.S. (2013). Comparative transcriptomics reveals different strategies of *Trichoderma* mycoparasitism. *BioMed Central Genomics 14*, 121. https://doi.org/10.1186/1471-2164-14-121

Bell, D.K., Wells, H.D., and Markham, C.R. (1982). In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathology 72*, 379-382

Benítez, T., Rincón, A.M., Limón, M.C., and Codón, A.C. (2014). Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology 7*, 249-260.

Bensch, M.J., and van Staden, J. (2006). Ultraestructural histopathology of infection and colonization of maize by *Stenocarpella maydis* (=*Diplodia maydis*). *Journal of Phytopathology 136*, 312-318. https://doi.org/10.1111/j.1439-0434.1992.tb01313.x

Beug, M.W., Bessette, A.E., and Bessette, A.R. (2014). *Ascomycete Fungi of North America*. A mushroom reference guide. Texas University. USA. Pp 45-48.

Both, E.E. (2006). Personal encounters with the parasitic bolete. *Field Mycology 7*, 104-110. https://doi.org/10.1016/S1468-1641(10)60485-9

Catalano, V., Vergara, M., Hauzenberger, J.R., Seiboth, B., Sarrocco, S., Vannacci, G., Kubicek, C.P., and Seidl-Seiboth, V. (2011). Use of a non-homologous end-joining-deficient strain (delta-ku70) of the biocontrol fungus *Trichoderma* virens to investigate the function of the laccase gene lcc1 in sclerotia degradation. *Current Genetics 57*, 13-23. https://doi.org/10.1007/s00294-010-0322-2

Chairattanamanokorn, P., Imai, T., Kondo, R., Ukita, M., and Prasertsan, P. (2006). Screening thermotolerant white-rot fungi for decolorization of wastewaters. *Applied Biochemistry and Biotechnology 128*, 195-204.

Chávez-Magdaleno, M.E., Luque-Alcaraz, M.E., Gutiérrez-Martínez, P., Cortez-Rocha, M.O., Burgos-Hernández, A., Lizardi-Mendoza, J., and Plascencia-Jatomea, M. (2018). Effect of chitosan-pepper tree (*Schinus molle*) essential oil biocomposites on the growth kinetics, viability and membrane integrity of *Colletotrichum gloeosporioides*. *Revista Mexicana de Ingeniería Química 17*, 29-45. https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2018v17n1/Chavez

Chet, I., Inbar, J., and Hadar, I. (1997). Fungal antagonists and mycoparasites. In: *The Mycota IV: Environmental and Microbial Relationships*, (D.T. Wicklow, and B. Söderström, eds.), Pp. 165-184. Springer-Verlag, Berlin Germany.

Dornberger, K., Ihn, W., Ritzau, M., Gräfe, U., Schlegel, B., and Fleck, W.F. (1995). Chrysospermins, new peptaibol antibiotics from *Apiocrea chrysosperma* Ap 101. *The Journal of Antibiotics 48*, 977-989.

Douhan, G.W., and Rizzo, D.M. (2003). Host-parasite relationships among bolete infecting *Hypomyces* species. *Mycological Research 107*, 1342-1349. https://doi.org/10.1017/S0953756203008542

Duffy, B., Schouten, A., and Raaijmakers, J.M. (2003). Pathogen self-defence: mechanisms to counteract microbial antagonism. *Annual Review of Phytopathology 41*, 501-538. https://doi.org/10.1146/annurev.phyto.41.052002.095606

Ellwood, S.R., Liu, Z., Syme, R.A., Lai, Z., Hane, J.K., Keiper, F., Moffat, C.S., Oliver, R.P., and Friesen, T.L. (2010). A first genome assembly of the barley fungal pathogen *Pyrenophora teres f. teres*. *Genome Biology 11*, R109. https://doi.org/10.1186/gb-2010-11-11-r109

Galarza, L., Akagi, Y., Takao, K., Kim, C.S., Maekawa, N., Itai, A., Peralta, E., Santos, E., and Kodoma, M. (2015). Characterization of *Trichoderma* species isolated in Ecuador and their antagonistic activities against phytopathogenic fungi from Ecuador and Japan. *Journal of General Plant Pathology 81*, 201-210. https://doi.org/10.1007/s10327-015-0587-x

Garg, S., Pandey, D., Taj, G., Goel, A., and Kumar., A. (2014). TRIPATH: a biological genetic and genomic database of three economically important fungal pathogen of wheat - rust: smut: bunt. *Bioinformation 10*, 466-468. https://doi.org/10.6026/97320630010466

Ghareeb, H., Becker, A., Iven, T., Feussner, I., and Schirawski, J. (2011). *Sporisorium reilianum* infection changes inflorescence and branching architectures of maize. *Plant Physiology 156*, 2037-2052. https://doi.org/10.1104/pp.111.179499

Howell, C.R. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Disease 87*, 4-10. http://dx.doi.org/10.1094/PDIS.2003.87.1.4

Kouker, G., and Jaeger, K.E. (1987). Specific and sensitive plate assay for bacterial lipases. *Applied and Environmental Microbiology 53*, 211-213.

Kumar, U., Joshi, A.K., Kumar, S., Chand, R., and Röder, M.S. (2009). Mapping of resistance to spot blotch disease caused by *Bipolaris sorokiniana* in spring wheat. *Theoretical and Applied Genetics 118*, 783-792. https://doi.org/10.1007/s00122-008-0938-5

Makovitzki, A., Viterbo, A., Brotman, Y., Chet, I., and Shai, Y. (2007). Inhibition of fungal and bacterial plant pathogens *in vitro* and *in planta* with ultrashort cationic lipopeptides. *Applied and Environmental Microbiology 73*, 6629-6636. https://doi.org/10.1128/AEM.01334-07

Me?ndez-Herna?ndez, J.E., and Loera, O. (2019). Biotechnological potential of ligninolytic enzymes for pollutant biodegradation in water: from test-tubes to full-scale enzymatic reactors. *Revista Mexicana de Ingenieri?a Qui?mica 18*, 397-417. https://doi.org/10.24275/uam/

izt/dcbi/revmexingquim/2019v18n2/Mendez

Mitova, M.I., Stuart, B.G., Cao, G..H, Blunt, J.W., Cole, A.L., and Munro, M.H. (2006). Chrysosporide, a cyclic pentapeptide from a New Zealand sample of the fungus *Sepedonium chrysospermum*. *Journal of Natural Products 69*, 1481-1484. https://doi.org/10.1021/np060137o

Nagao, K., Yoshida, N., Iwai, K., Sakai, T., Tanaka, M., and Miyahara, T. (2006) Production of sepedonin by *Sepedonium chrysospermum* NT-1 in submerged culture. *Environmental Sciences 13*, 251-256.

Nelson, G., and Young, T.W. (1986). Yeast extracellular proteolyticenzymes for chill-proofing beer. *Journal of the Institute of Brewing 92*, 599-603.

Parry, D.W., Jenkinson, P., and McLeod, L. (1995). *Fusarium* ear blight (scab) in small grain cereals-A review. *Plant Pathology 44*, 207-238. *https://doi.org/10.1111/j.1365-3059.1995.tb02773.x*

Pizzul, L., del Pilar Castillo, M. and Stenstro?m, J. (2009). Degradation of glyphosate and other pesticides by ligninolytic enzymes. *Biodegradation 20*, 751-759. https://doi.org/10.1007/s10532-009-9263-1

Qiu, J., and Shi, J. (2014). Genetic relationships, carbendazim sensitivity and mycotoxin production of the *Fusarium graminearum* populations from maize, wheat and rice in Eastern China. *Toxins 6*, 2291-2309. https://doi.org/10.3390/toxins6082291

Ramos-Guerrero, A., Gonza?lez-Estrada, R.R., Romanazzi, G., Landi, L., Gutie?rrez-Marti?nez, P. (2020). Effects of chitosan in the control of postharvest anthracnose of soursop (*Annona muricata*) fruit. *Revista Mexicana de Ingenieri?a Qui?mica 19*, 99-108. https://doi.org/10.24275/rmiq/Bio527

Roberts, W.K., and Selitrennikoff, C.P. (1988). Plant and bacterial chitinases differ in antifungal activity. *Journal of General Microbiology 134*, 169-176.

Sahr, T., Ammer, H., Besl, H., and Fischer, M. (1999). Infrageneric classification of the boleticolous genus *Sepedonium*: species delimitation and phylogenetic relationships. *Mycologia 91*, 935-943. https://doi.org/10.2307/3761625

Savary, S., Teng, P.S., Willocquet, L., Nutter, and F.W.Jr. (2006). Quantification and modeling of crop losses: a review of purposes. *Annual Review of Phytopathology 44*, 89-112. https://doi.org/10.1146/annurev.phyto.44.070505.143342

Schuster, A., and Schmoll, M. (2010). Biology and biotechnology of *Trichoderma*. *Applied Microbiology and Biotechnology 87*, 787-799. https://doi.org/10.1007/s00253-010-2632-1

Sobhy, I.S., Erb, M., Lou, Y., and Turlings, T.C.J. (2014). The prospect of applying chemical elicitors and plant strengtheners to enhance the biological control of crop pests. *Philosophical Transactions of the Royal Society B: Biological Sciences 369*, 20120283. https://doi.org/10.1098/rstb.2012.0283

Stebbins, M.E., and Robbins, W.J. (1949). Mineral oil and preservation of fungous cultures. *Mycologia 41*, 632-636. https://doi.org/10.1080/00275514.1949.12017806

Swofford, D.L. (1998). PAUP*. Phylogenetic Analysis Using Parsimony, (*and Other Methods), 4.0b4a. Smithsonian Institute. Washington D. C.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. (1997). The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research 24*, 4876-4882.

Vey, A., Hoagland, R.E., and But, T.M. (2001). Toxic metabolites of fungal biocontrol agents. In: Fungi as biocontrol agents: *Progress, Problems and Potential*, (T.M., Butt, C., Jackson, and N. Magan, eds.), Pp. 311-346. CAB International, Bristol U.K.

Wagner, D.B., Furnier, G.R., Saghai-Maroof, M.A., Williams, S.M., Dancik, B.P., and Allard, R.W. (1987). Chloroplast DNA polymorphisms in

Lodgepole and Jack pines and their hybrids. *Proceedings of the National Academy of Sciences of the United States of America 84*, 2097-2100.

Wang, G., Cao, X., Ma, X., Guo, M., Liu, C., Yan, L., and Bian, Y. (2016). Diversity and effect of *Trichoderma* spp. associated with green mold disease on *Lentinula edodes* in China. *Microbiology Open 5*, 709-718. https://doi.org/10.1002/mbo3.364

Whipps, J.M. (2001). Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany 52*, 487-511. https://doi.org/10.1093/jexbot/52.suppl_1.487

White, T.J., Bruns, T., Lee, S., Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications*, (M.A., Innis, D.H., Gelfand, J.J., Sninsky, and T.J., White, eds.), Pp. 315-322. Academic Press, New York, USA.

Yurkov, A., Kruger, D., Begerow, D., Arnold, N., and Tarkka, M.T. (2012). Basidiomycetous yeasts from boletales fruiting bodies and their interactions with the mycoparasite *Sepedonium chrysospermum* and the host fungus *Paxillus*. *Microbial Ecology 63*, 295-303. https://doi.org/10.1007/s00248-011-9923-7