academicJournals

Vol. 10(15), pp. 521-527, 21 April, 2016
DOI: 10.5897/AJMR2016.7958
Article Number: D820D6E58168
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

In vitro activities of *Trichoderma* species against *Phytophthora parasitica* and *Fusarium oxysporum*

E. Osorio-Hernández[1]*, J. Hernández-Morales[2], V. Conde-Martínez[2], A. C. Michel-Aceves[3], J. A. Lopez-Santillan[1] and J. A. Torres-Castillo[4]

[1]Facultad de Ingeniería y Ciencias de la Universidad Autónoma de Tamaulipas, Cd. Vitoria, 87000, Tamaulipas, México.
[2]Colegio de Postgraduados, Montecillo, 56230, Estado de México, México.
[3]Centro de Estudios Profesionales del Colegio Superior Agropecuario del Estado de Guerrero, Colonia Centro. Iguala, 40000, Guerrero, México.
[4]Universidad Autónoma de Tamaulipas-Instituto de Ecología Aplicada, Cd. Vitoria, 87029 Tamaulipas, México.

Received 9 February, 2016; Accepted 8 April, 2016

***Trichoderma* species usually produce chitinases and glucanases with inhibitory effects on several phytopathogens. In this work, the effects of such enzymes activities were evaluated on *Phytophthora parasitica* and *Fusarium oxysporum*. Enriched enzyme extracts were obtained from *Trichoderma asperellum, Trichoderma virens, Trichoderma gamsii* and *Trichoderma logibrachiatum*, which were grown on supplemented media with chitin or lamminarin. Chitinase and glucanase activity were confirmed by spectrophotometric methods. *T. longibrachiatum* showed the highest level of total and specific activity for both hydrolytic enzymes. Filtrated media were submitted to antagonism assays and results showed that chitinases from *T. longibrachiatum* inhibited 15% of *P. parasitica* and 45% of *F. oxysporum* mycelial growth;on the other hand, no inhibition was detected to *P. parasitica* with any other media. Glucanase activity from *T. longibrachiatum* showed 40% of mycelial growth inhibition on *F. oxysporum* and no other species showed inhibitory effects.**

Key words: Antibiotic, biological control, enzymes, phytopathogens.

INTRODUCTION

Phytophthora parasitica and *Fusarium oxysporum* infect a wide range of host plants and cause significant damage on important crops around the world (Leslie and Summerell, 2006; Martin et al., 2014). To date, most of the phytosanitary management has been done through chemical control strategies; nevertheless, some strains of *P. parasitica* and *F. oxysporum* have developed resistance to several fungicides (Lucas et al., 2008). Due to this, searching for alternative biological strategies to control plant diseases has been growing in recent years

*Corresponding author. E-mail: eosorio@uat.edu.mx, osorio@hotmail.com.

(Anitha and Rabeeth, 2010). Amongst these, the use of *Trichoderma*, has received huge attention, because of its adaptability and diverse action mechanisms (mycoparasitism, antibiosis, competence and induction of endogenous plant defense) (Vinale et al., 2014). *Trichoderma* species are widely used in conventional agriculture and for biopesticide industry as biological agents or as enzymes sources. *Trichoderma* spp. produce several enzymes involved in their biological processes, including chitinases, glucanases, cellulases, proteases and amylases (Gajera et al., 2012; González et al., 2012); among these, chintinases and β-glucanases are the more relevant in the mycoparasitism. Chitinase 46-KDa (CHIT 46) affects the growth and cell wall of *Sclerotium rolfsii* and *Rhizoctonia solani* (Lima et al., 1999); β-1,6 glucanase, chitinase and protease produced by *Trichoderma harzianum* and *Trichoderma viride* also showed the ability to degrade cell walls of *S. rolfsii* (Parmar et al., 2015); furthermore, an extracellular alkaline-protease produced by *T. harzianum* of 18.8 kDa and active at pH 7 and 8 hydrolyzed cell-wall proteins from *Crinipellis perniciosa*, a pathogen with high resistance to antagonist microorganisms. *P. parasitica* and *F. oxysporum* also are sensible to lytic enzymes, as demonstrated by Budi et al. (2000), where the confrontation of several hydrolytic enzymes (chitinases, cellulases, proteases and pectinases) from *Paenibacillus* species promoted cell-wall and cell disorganization in some phytopathogens. The ability of *Trichoderma* spp. to parasite fungal phytopathogens is well known, and it is associated with formation of rolling, hooks and apresoria used to penetrate cell walls combined with enzymes, including chitinases and glucanases (Zeilinger and Omann, 2007). These enzymes attack cell walls structures formed almost exclusively of polysaccharides as chitin and glucanes (Küçük and Kivanc, 2004). Based on this, the main goal of this work was the quantification of chitinase and glucanase activities produced by four *Trichoderma* spp. and the evaluation of the inhibitory effect on the mycelial growth of *P. parasitica* and *F. oxysporum*.

MATERIALS AND METHODS

Biological material

P. parasitica and *F. oxysporum* were isolated from Roselle plants (*Hibiscus sabdariffa L.*) with specific symptoms in the Laboratory of Biochemistry from the Colegio de Postgraduados, Campus Montecillo, Estado de Mexico, Mexico. *P. parasitica* was identified based on taxonomic keys from Erwin and Ribeiro (1996) and *F. oxysporum* was identified through taxonomic keys of Leslie and Summerell (2006).

To evaluate several *Trichoderma* species, *Trichoderma virens* (T6), *Trichoderma asperellum* (T9), *Trichoderma gamsii* (T13) and *Trichoderma longibrachiatum* (T19) were used. *Trichoderma* spp. were obtained from strain collection at the Instituto of Fitosanidad, Colegio de Postgraduados, Campus Montecillo, Estado de Mexico, Mexico. Species were identified with morphology and ITS sequencing by Esparza-Luna (2009) (unpublished data).

Chitinases and glucanases production

Trichoderma spp. were grown in liquid media to produce chitinases and glucanases according to (Almeida et al., 2007). Erlemeyer flasks were filled with 200 ml of culture media (1 L was prepared by mixing 10 g of KNO_3, 5 g of KH_2PO_4, 2.5 g of $MgSO_4/H_2O$; 2 mg of $FeCl_3$, 150 ml of V8 commercial juice, 10 g de PVP and distilled water to 1 L). Salts for media preparation were purchased from J. T. Baker (PA, USA), PVP was purchased from Sigma-Aldrich, MO, USA and the commercial juice was obtained from JUMEX (MO, Mexico). Two culture media were prepared by addition of 1% of chitin from shrimp shells (Sigma-Aldrich, MO, USA) and 0.1% of laminarin from *Laminaria digitata* (Sigma-Aldrich, MO, USA) to induce chitinases and glucanases production, respectively. The final pH of media was 6 and then was autoclaved at 121°C for 20 min. Separately, each species was inoculated in a flask with the addition of 1 ml of 1×10^6 conidia/ml and incubated at 25°C under 12h:12 h (L:D) in continuous stirring (Thermo Scientific MaxQ 4000 Incubated Shake, MA, USA) for 5 days for chitinase and eight days for glucanase production. After incubation, media were filtered through paper Whatman No. 1 (Maidstone, UK) and centrifuged at 5,000 rpm for 15 min, supernatant was adjusted to 20 ml and used as source for protein quantification, chitinases and glucanases assays and for the inhibition assays (sterilized through a 0.22 µm filter (Millex® Millipore, MA, USA)).

Chitinase activity detection

Five tubes were marked for the experiment, in the first, 1 ml of water was added as a reaction blank, in the remaining tubes 1 ml of filtered media was added and then 1 ml of substrate solution (3.8 mg/ml of chitin in Citrate McIlvaine buffer at pH 5 (prepared with 49 ml of 0.1 M solution of citric acid plus 51 ml of 0.2 M solution of sodium phosphate)) was incorporated. One tube was heated at 95°C in 10 min, after this, heated tube and remaining tubes were incubated at 37°C for 24 h. After incubation, 500 µl of each tube were placed in new tubes and heated at 95°C for 10 min and followed by addition of 100 µl of 0.8 M potassium tetraborate and then tubes were heated again during 5 min. Tubes were incubated at 37°C during 20 min and 3 ml of *p*-dimethylamino benzaldehyde solution (Sigma-Aldrich, MO, USA) (1 g was dissolved in 100 ml of 1.25% HCl - 8.75% aqueous solution) were added. Absorbance was recorded at 544 nm (spectrophotometer JENWAY 6305, OSA, UK). All reactions were done with four replicates and absorbance was compared with a standard curve, prepared with 0, 25, 50, 75, 100, 125, 150, 175 and 200 µg/ml of N-acetylglucosamine in McIlvaine buffer. Chitinase activity was related to release of N-acetylglucosamine by comparison of the recorded absorbance from the initial heated tube minus average absorbance of the remaining tubes. Total chitinase activity (TA) and specific chitinase activity (SA) were calculated according to González et al. (2012) and expressed as µmol of N-acetylglucosamine/h and SA was expressed as µmol of N-acetylglucosamine/µg of protein/h. Protein quantification was according to Bradford (BIORAD, CA, USA) (Bradford et al., 1976).

Glucanase activity detection

Glucanase production was determined according to four filtered *Trichoderma* spp. media. Five tubes were marked as “X” and five as

"Y". One milliliter of 10 mg/ml lamminarin dissolved in 0.1 M citrate buffer pH 5 (0.1 M $C_6H_5Na_3O_7$ and 0.1 M citric acid, in 21:29 ratio) was added in each tube. Then, 250 µl of filtered media were added only in tubes marked as "X" before incubation (4 h at 40°C in a warm water bath). After incubation, 250 µl of filtered media were added in tubes marked as "Y" and then 2 ml of copper reactive (CR) was added to all tubes and immediately heated by immersion in boiling water for 10 min followed by addition of 2 ml of arsenomolybdate. Final volume was adjusted at of 25 ml with water. Solution was stirred in 1 min and finally absorbance was recorded at 595 nm (spectrophotometer JENWAY 6305). Calibration blank was prepared with water (Bruce et al., 1995).

CR solution was prepared previously and consisted of Cu I solution (16.1 g $KNaC_4H_4O_6.4H_2O$, 24 g of Na_2CO_3, 16 g of $NaHCO_3$, 80.44 g of Na_2SO_4 dissolved in distilled water to a final volume of 800 ml) and Cu II solution (4 g of $CuSO_4.5H_2O$, 36 g of Na_2SO_4 dissolved in a final volume of 200 ml), both solutions were mixed before using. The arsenomolybdate solution was prepared by mixing 25 g of $NH_4Mo_7O_{24}.4H_2O$ in 450 ml of water and 21 ml of concentrated H_2SO_4 and 3 g of $Na_2HAs_5O_4.7H_2O$ in 25 ml of water incubated at 37°C for two days. Salts for solutions preparation were purchased from J. T. Baker (PA, USA). Standard curve was made with 25, 50, 75, 100, 125 and 150 µg glucose/ml. Absorbance differences between X and Y were used to detect glucanase activity, detection was done with four replicates. Glucanase activity was calculated according to González et al. (2012), where total glucanase activity was expressed as µmol glucose/h, and glucanase specific activity was expressed as µmol glucose/h/µg of protein.

Chitinase and glucanase activity against fungal phytopathogens

Aqueous fraction of *Trichoderma* spp. cultures with chitinase or glucanase activity were sterilized through 0.22 µm filter (Millex®, Millipore) and mixed separately with warm PDA (Bioxon, Estado de Mexico, Mexico) (ratio 1:1) before filling Petri dishes (8 cm). In each plate, a 5 mm-piece of active mycelium of *P. parasitica* (5 days) or *F. oxysporum* (7 days) was placed and incubated at 25°C. Controls were established using PDA with distilled water (ratio1:1). Radial growth in cm was recorded every two days until the media were completely covered by mycelium (five days for *F. oxysporum* and seven days for *P. parasitica*). To estimate conidial production of *F. oxysporum*, 10 pieces 5 mm-diameter were stirred in 10 ml of distilled water to obtain conidial suspensions which were quantified in a Neubauer chamber (Wertheim, GE). Results from three dishes were recorded and expressed as number of conidia in 1 cm of PDA. Number of colony-forming units (CFU) was evaluated by inoculation of conidia in PDA and counted 48 h later.

Experimental design and data analyses

A completely randomized design was used with 9 treatments (4 glucanase activities and 4 chitinase activities and a control) with 5 replicates for two pathogens (*P. parasitica* and *F. oxysporum*). Response variables were mycelial radial growth, the inhibition percentage (calculated with the formula: [I (%) = (1 – T /C) × 100]; where, I= Inhibition percentage of pathogen growth by antagonists, C=radial growth in control, T=radial growth in the treatments), number of conidia and number of CFU. Analyses of variance (ANOVA) was used to detect differences between treatments and comparison means were done by Tukey test (P≤0.05) with SAS program version 9 (2002).

RESULTS AND DISCUSSION

Chitinases and glucanases quantitation

The four *Trichoderma* spp. produced chitinases and glucanases at different levels; in general, chitinase TA (Total chitinase activity) values were between rates of 0.107 and 0.228 µmol of N-acetylglucosamine/h^{-1} and SA (Specific Chitinase activity) rates were from 0.102 to 0.255 µmol h^{-1} μg^{-1} de N-acetylglucosamine/µg of protein. Statistical analyses detect differences (P≤0.0001) between species in TA and AE when chitinase activity was analyzed (Table 1). Total and specific activities were found higher in *T. longibrachiatum*, but this kind of activity was wide spread in all species, which seems typical in *Trichoderma* as reported for strains of *T. harzianum* (Michel-Aceves et al., 2005); however, differences in activities levels between species was evident and coincide with results from González et al. (2012) and Lee et al. (2012), who related such differences to endogenous characteristics, media composition and to culture age; such differences are considered useful by several authors to select *Trichoderma* spp. for biological control. Several studies on *Trichoderma* spp. have demonstrated the secretion of hydrolytic enzymes, including chitinases and glucanases, which have the ability to breakdown cell wall and penetrate the mycelium of other species (González et al., 2011).

It is well known that fungal β-glucanases have roles in glucan mobilization in the cell wall and also during nutrient depletion (Parmar et al., 2015), callose degradation, pathogenesis mechanism of antagonist fungi and nutrition of mycoparasites, for example, β-1,3-glucanases was associated with the lysis and wall degradation of *Sclerotium* spp. Glucanase activities obtained from four *Trichoderma* spp. fluctuated from 0.22 to 0.47 µmol of released glucose, while specific activity varied from 1.24 to 2.78 (Table 2). *T. longibrachiatum* showed the highest activity (0.47 µmol); however, *T. gamsii* and *T. virens* showed the higher specific activity, supporting that production depends on the species, which is similar to that reported by Parmar et al. (2015). Glucanase activity has been reported in several species of *Trichoderma* by Michel-Aceves et al. (2005), where enzyme production was *T. koningii* (1747.2 µmol), *T. longibrachiatum* (1400 µmol), *T. virens* (1045 µmol) and *T. harziamun* (1232 µmol), being higher than levels detected in this work, probably due to the culture conditions or genetic background of each species, similar to results reported by González et al. (2011), who indicated that the β-1,3 glucanase specific activity of *Trichoderma* spp., showed variations associated with variation in the substrate carbon source. On the other hand, Bruce et al. (1995) reported variations of total activity from 50 to 241 µmol of released glucose; nevertheless, specific activity fluctuated between 0.9 and

Table 1. Chitinase activity in *Trichoderma* species cultured in medium supplemented with 1% chitin.

Species	Total chitinase activity (µmol of released N-acetylglucosamine h^{-1})	Specific chitinase activity (µmol of released-acetylglucosamine h^{-1} $µg^{-1}$ of protein)
T. gamsii	0.151[ab]	0.102[b]
T. virens	0.107[b]	0.179[ab]
T. longibrachiatum	0.228[a]	0.255[a]
T. asperellum	0.164[ab]	0.165[b]

[z]Treatments with the same letter are statistically similar between them (Tukey, P ≤0.05).

Table 2. Glucanase activity in *Trichoderma* species cultured in medium supplemented with 0.1% laminarin.

Species	Total activity (µmol h^{-1} de glucose)	Specific activity (glucose µmol h^{-1} $µg^{-1}$ de protein)
T. gamsii	0.35[abz]	2.78[a]
T. virens	0.28[ab]	2.44[a]
T. longibrachiatum	0.47[a]	1.24[b]
T. asperellum	0.22[b]	1.26[b]

[z]Treatments with the same letter are statistically similar between them(Tukey, P ≤0.05).

Table 3. Effect of chitinase activity from *Trichoderma* species on mycelial growth and reproductive potential of *P. parasitica* (Pp) and *F. oxysporum* (Fo).

Species	Growth of *Pp* (cm)	Growth of *Fo (cm)*	Number of Conidia *Fo*	Number of CFU of *Fo*
T. virens	7.9[az]	7.8[a]	11.3[a]	153[a]
T. gamsii	7.7[a]	8.0[a]	5.6[a]	56[c]
T. asperellum	7.5[a]	7.8[a]	6.6[a]	138[ab]
T. longibrachiatum	6.7[b]	4.4[c]	6.3[a]	89[bc]
Control (Water)	3.4[c]	5.9[b]	2.0[a]	41[c]

[z]Treatments with the same letter are statistically similar between them (Tukey, P ≤0.05). CFU (Colony-forming units).

4.44 µmol h^{-1} $µg^{-1}$ of glucose/mg protein and was in the range of results reported here, which is indicative that enzyme production is also influenced by culture media composition and the *Trichoderma* spp. evaluated.

Evaluation of chitinase and glucanase activity against *P. parasitica* and *F. oxysporum*

Production of enzymes by *Trichoderma* as part of their mycoparasitic process, where chitinases and glucanases are the most relevant enzymes has been reported (Küçük and Kivanç, 2008; Parmar et al., 2015). Gajera et al. (2012) and Parmar et al. (2015) indicated that chitinases and glucanases produce the lysis of fungal cell walls in some phytopathogens. Based on this, chitinase effects on *P. parasitica* mycelium growth was different between the *T. gamsii, T. asperellum* and *T. longibrachiatum* (Tables 3 and 4). On the other hand, enzyme activity obtained from *T. asperellum, T. virens* and *T. gamsii* induced abundant mycelial growth of *P. parasitica*, which could be attributed to enzyme inhibitors possibly produced.

Antagonistic activity could be also influenced by specific recognition at the molecular level, which determines the success or failure for the control; for example, the glucanase AGN13.1 recognized the cell wall of *Aspergillus niger, Botrytis cinerea, Colletotrichun acutatum, F. oxysporum, Penicillium aurantiogriseum* and *R. solani* but did not affect other evaluated fungi (Ait-Lahsen et al., 2001). This suggests that no evident inhibitory effects observed here by the four *Trichoderma*

Table 4. Effect of glucanase activity from *Trichoderma* spp., on mycelial growth and reproductive potential of *P. parasitica* (*Pp*) and *F. oxysporum* (*Fo*).

Species	Growth of *Pp* (cm)	Growth of *Fo* (cm)	Number of conidia in *Fo*	CFUof *Fo*
T. virens	7.7[az]	8.0[a]	8.3[a]	87[bc]
T. gamsii	7.3[b]	8.0[a]	8.3[a]	124[ab]
T. asperellum	7.2[b]	8.0[a]	9.6[a]	149[a]
T. longibrachiatum	7.2[b]	4.8[c]	3.3[a]	97[abc]
Control	3.4[c]	5.8[b]	2.0[a]	41[c]

[z]Treatments with the same letter are statistically similar between them(Tukey, P ≤0.05).

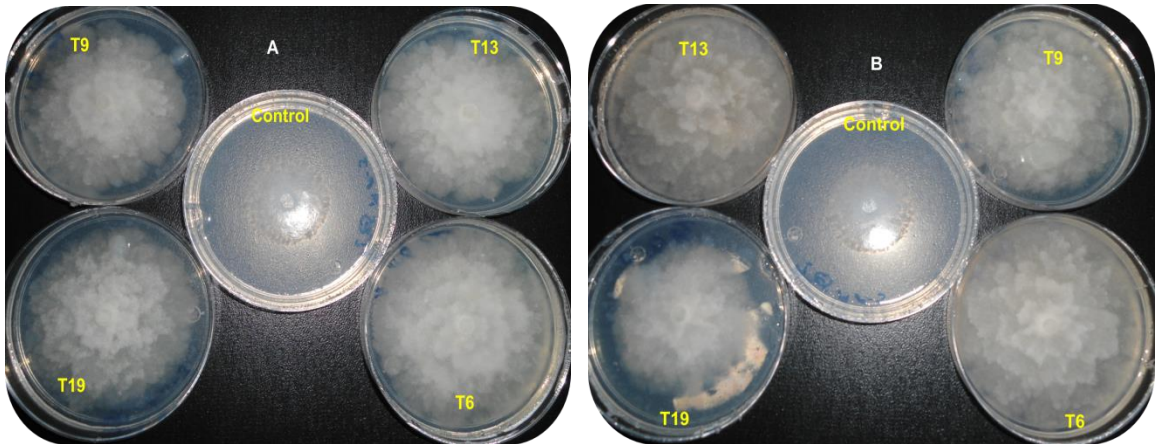

Figure 1. Chitinases (A) and glucanases (B) activity from *T. asperellum* (T9*)*, *T. virens* (T6*)*, *T. gamsii* (T13*)*, *T. longibrachiatum* (T19*)* and Control on *P. parasitica* growth.

spp. could be related to absence of specific recognition on *P. parasitica* and *F. oxysporum*.

Some reports indicated that *P. parasitica* could induce glucanase production to defend itself against antagonists, which in addition to the possible enzyme inhibitor production could be part of factors that influenced the null inhibitory effects; however, this remains for confirmation. Chitinase and glucanase antibiotic effect on mycelial growth of *F. oxysporum* showed significant differences between treatments (P≤0.0001) (Figure 1). *T. longibrachiatum* showed the highest inhibitory effect with a reduction of 40% (4.4 cm) when chitinase activity was evaluated and 45% (4.8 cm) when glucanase were used (Table 3). Michel-Aceves et al. (2005) indicated that glucanases and chitinases produced by *T. koningii* and *T. harzianum* presented variations in their accumulation; nevertheless, also inhibited growth of *F. oxysporum*. Nevertheless, Gajera et al. (2012) and Parmar et al. (2015) reported that a significant positive correlation between percentage growth inhibition (*Macrophomina phaseolina* and *S. rolfsii)* of test fungus and lytic enzymes (chitinase, β-1,3-glucanase and protease) in the culture medium of antagonist treatment established a relationship to inhibit growth of fungal pathogen by increasing the levels of these enzymes.

Similarly to *P. parasitica* results, it was also observed an abundant growth of *F. oxysporum* when challenged with enzymes activities of *T. asperellum, T. virens* and *T. gamsii* (Figure 2); however, *F. oxysporum* inoculated in media containing enzyme activities did not produce violet pigments, typical of this species, which accumulation was observed in control media, indicating that extracts did not affect the growth rate but could have effects at other levels. Such inhibition of pigment accumulation could diminish pathogenic potential, because such pigments usually are associated with pathogenic roles and are important in the interaction phytopathogen-plant (Medentsev et al., 2005). Nelson et al. (1993) reported that *F. oxysporum* produce secondary metabolites like micotoxins, trichothecenes, pigments, antibiotics and phytotoxins that could have influence on the null inhibition of *F. oxysporum* growth. Number of conidia and CFU were different (P≤0.0001) between treatments and it demonstrated a relation with the higher mycelium growth

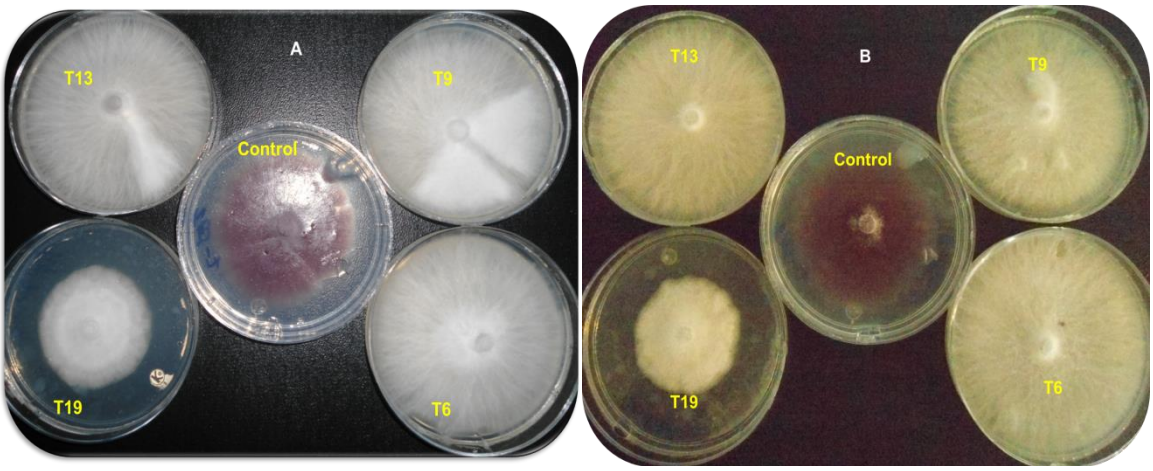

Figure 2. Chitin (A) and glucanase (B) activity of *T. asperellum* (T9), *T. virens* (T6*)*, *T. gamsii* (T13*)*, *T. longibrachiatum* (T19*)* and control on *F.oxysporum* growth.

that presented the higher number of conidia and CFU, demonstrating that an important parameter for reproduction as the conidial viability was not affected, which contrasted with report of Michel-Aceves et al. (2005) who found a 95% reduction in conidial production and around 70% of loss of viability when *T. harzianum* and *T. koningii* were used, rising the arguments to consider that physiological conditions, genetic background and nutritional availability are directly related with antagonistic effects of each *Trichoderma* spp., which should be considered as part of the production management and for control strategies that guarantees successfully plant protection.

Conclusions

All the *Trichoderma* spp. produced hydrolytic activities depending on the carbon source and activity levels were independent of the species. *T. longibrachaitum* was the species with higher total and specific enzyme activities of both activities; it also showed antagonistic effect on both phytopathogens.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors wish to thank Colegio de Postgraduados and Consejo Nacional de Ciencia y Tecnologia (CONACYT) for the providing scholarship to the first author (Scholarship number: 50928) and to the grant SAGARPA-163972.”

REFERENCES

Ait-Lahsen H, Soler A, Rey MJ, de la Cruz J, Monte E, Llobell A (2001). An antifungal Exo-α 1,3-glucanase (AGN13.1) from the biocontrol fungus *Trichoderma harzianum*. Appl. Environ. Microbiol. 67(12):5833-5839.

Almeida FB, Cerqueira FM, Silva RN, Ulhoa CJ, Lima AL (2007). Mycoparasitism studies of *Trichoderma harzianum* strains against *Rhizoctonia solani*: Evaluation of coiling and hydrolytic enzyme production. Biotechnol. Lett. 29:1189-1193.

Anitha A, Rabeeth M (2010). Degradation of fungal cell walls of phytopathogenic fungi by lytic enzyme of *Streptomyces griseus*. Afr. J. Plant Sci. 4:061-066.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Bruce A, Srinivasan U, Staines HJ, Highley TL (1995). Chitinase and laminarinase production in liquid culture by *Trichoderma* spp. and their role in biocontrol of wood decay fungi. Int. Biodeter. Biodegrad. 23(4):337-353.

Budi SW, van Tuinen D, Arnould C, Dumas-Gaudot E, Gianinazzi-Pearson V, Gianinazzi S (2000). Hydrolytic enzyme activity of *Paenibacillus* sp. Strain B2 and effects of the antagonistic bacterium on cell integrity of two soil-borne pathogenic fungi. Appl. Soil. Ecol. 15:191-199.

Phytophthora diseases worldwide. American Phytopathological Society (APS Press).

Esparza-Luna, LL (2009). *In vitro* effectivity natives strains of *Trichoderma* spp. In isolates of *Phytophythora parasitica* D. that was obtained of plants of roselle. Master Thesis, Colegio de Postgraduados, campus Montecillo.

Gajera HP, Bambharolia RP, Patel SV, Khatrani TJ, Goalkiya BA (2012). Antagonism of *Trichoderma* spp. against *Macrophomina phaseolina:* Evaluation of Coiling and Cell Wall Degrading Enzymatic Activities. J. Plant Pathol. Microb. 3:149.

González I, Infante D, Martínez B, Arias Y, González N, Miranda I, Peteira B (2012). Induction of chitinases and glucanases in *Trichoderma* spp. strains intended for biological control. Biotecnología Aplicada 29(1):7-11.

González I, Infante D, Peteira B, Martínez B, Arias Y, González N, Miranda I (2011). Biochemical characterization of promising isolates of *Trichoderma* spp. as biological control agents. II. Expression of glucanase activity. Rev. Protección Veg. 26:23-29.

Küçük C, Kivanc M (2004). *In vitro* antifungal activity of strains of *Trichoderma harzianum*. Turk. J. Biol. 28: 111-115.

Lee CK, Darah I, Ibrahim CO (2012). Production and optimization of cellulase enzyme using *Aspergillus niger* USM AI 1 and comparison with *Trichoderma reesei* via solid state fermentation system. Biotechnol. Res. Int. pp. 1-6.

Leslie JF, Summerell BA (2006). The Laboratory *Fusarium*. Manual. Blackwell Publishing. Ames, Iowa, USA. 2:10.

Lima L, De Marco JL, Ulhoa CJ, Felix CR (1999). Synthesis of *Trichoderma* chitinase which affects the *Sclerotium rolfsii* and *Rhizoctonia solani* cell walls. Folia Microbiol. 44:45-49.

Lucas AJ, Bower LA, Coffey MD (2008). Fungicide resistance in soil-borne *Phytophthora* species. EPPO Bull. 1365-2338.

Martin NF, Blair J, Coffey E, Michael DA (2014). Combined mitochondrial and nuclear multilocus phylogeny of the genus *Phytophthora*. Fung. Genet. Biol. 66:19-32.

Medentsev AG, Arinbasarova AY, Akimenko VK (2005). Biosynthesis of naphthoquinone pigments by fungi of the genus *Fusarium*. Appl. Biochem. Microbiol. 41(5):573-577.

Michel-Aceves AC, Otero-Sánchez MA, Rebolledo-Domínguez O, Lezama-Gutiérrez R, Ochoa-Moreno ME (2005). Production and antagonistic effect of chitinases and glucanases from *Trichoderma* spp. in the inhibition of *Fusarium oxysporum* and *Fusarium subglutinans in vitro*. Revista Chapingo Serie Horticultura 11:273-278.

Nelson PE, Desjardins AE, Plattner RD (1993). Fumonisins, mycotoxins produced by *Fusarium* species: Biology, chemistry, and significance. Ann. Rev. Phytopathol. 31:233-252.

Parmar HJ, Bodar NP, Lakhani HN, Patel SV, Umrania VV, Hassan MM (2015). Production of lytic enzymes by *Trichoderma* strains during *in vitro* antagonism with *Sclerotium rolfsii*, the causal agent of stem rot of groundnut. Afr. J. Microbiol. Res. 9(6):365-372.

SAS OnlineDoc® 9 Copyright © (2002). By SAS Institute Inc. Cary NC USA.

Vinale F, Sivasithamparam K, Ghisalberti EL, Woo SL, Nigro M, Marra R, Lombardi N, Pascale A, Ruocco M, Lanzuise S, Manganiello G, Lorito M (2014). *Trichoderma* Secondary Metabolites Active on Plants and Fungal Pathogens. Open Mycol. J. 8:127-139.

Zeilinger S, Omann M (2007). *Trichoderma* biocontrol: Signal transduction pathways involved in host sensing and mycoparasitism. Gene. Regul. Syst. Biol. 1:227-234.