www.czasopisma.pan.pl PAN POLSKA AKADEMIA NAUK www.journals.pan.pl

JOURNAL OF PLANT PROTECTION RESEARCH Vol. 54, No. 4 (2014)

DOI: 10.2478/jppr-2014-0049

Mycoparasitic nature of *Bionectria* sp. strain 6.21

Itamar Soares de Melo[1], *Angela Maria Montes Peral Valente*[1], *Vanessa Nessner Kavamura*[1]*, *Elke Simoni Dias Vilela*[1], *Jane Louise Faull*[2]

[1] Laboratory of Environmental Microbiology, Embrapa Environment, Jaguariúna, SP 13.820-000, Brazil
[2] Department of Biological Sciences, Birkbeck College, University of London, Malet Street, London, WC1E 7HX, UK

Received: March 13, 2014
Accepted: October 19, 2014

Abstract: In this study, a *Bionectria* sp. strain isolated from citrus rhizosphere was evaluated for its potential in inhibiting the growth of *Rhizoctonia solani* and *Pythium aphanidermatum*. It was demonstrated that *Bionectria* sp. 6.21 inhibited the growth of *P. aphanidermatum* and *R. solani*. In dual cultures, however, the antagonist only parasitised *R. solani*. Regarding the assay involving *P. aphanidermatum*, a lack of mycoparasitic ability was demonstrated. Crude extract of *Bionectria* completely inhibited the mycelial growth of both fungi. It appears that the main mechanism involved in the antagonism of *Pythium* by *Bionectria* is through antibiotic production. The antagonistic fungus released extracellular secondary metabolites. The metabolites were found to be inhibitory to both plant pathogenic fungi. From the crude extract, eleven fractions were obtained and tested for their antifungal properties. Two of them showed very strong activity against *P. aphanidermatum*. The obtained results indicated that this biocontrol agent has both antibiotic and mycoparasitic properties. On the other hand, evidence obtained from Scanning Electron Microscopy (SEM) suggests the involvement of an enzymatic process, with enzymatic digestion playing a major role in the parasitism of *Bionectria* sp. 6.21. In conclusion, these results provide evidence that mainly due to mycoparasitism, this strain has the potential to become a good candidate for biological control.

Key words: antifungal activity, *Bionectria* sp., enzymes, plant diseases

Introduction

All living organisms are subjected to parasitism or competition. The study of these interactions has led to the identification of many potential opportunities for the use of living organisms to protect crops against fungal and bacterial diseases. Several studies have been conducted in the search for mycoparasites acting as biological control agents and used for protecting crops against a number of important soil borne plant pathogens (Kumar *et al*. 2011; Consolo *et al*. 2012; Kumar *et al*. 2012).

Biological control is an environmentally-friendly alternative to the use of synthetic fungicides (Abdalla *et al*. 2014). It is a complex process and involves distinct phases. The modes of action are well known for some fungi (Vinale *et al*. 2006). These mechanisms are reviewed by Junaid *et al*. (2013) and include antibiosis through the production of compounds with antibiotic activity, mycoparasitism or hyperparasitism, competition, enzymes with cell wall-lytic activity, and induction of systemic resistance.

Mycoparasitism involves several steps. First, the host needs to be recognised by the mycoparasite. Several responses will then be triggered (Steindorff *et al*. 2014). These could lead to the attack of the host through the production of enzymes which degrade the cell wall (Junaid *et al*. 2013). There are several enzymes being used in large scale application or being explored for commercial uses (Chavan and Deschpande 2013). Some enzymes such as chitinolytic and endoglucanases play an important role in the mycoparasitism of several plant pathogens (Viterbo *et al*. 2002) because they attack their cell wall polymers (Adams 2004). The production of secondary metabolites, such as antibiotics, also play an important role and they have been extensively investigated. Some of these studies not only isolated microorganisms with the ability to control pathogens, but also identified the molecules responsible for such action, as observed in the studies performed by Vinale *et al*. (2006) and Xiao *et al*. (2014). They obtained new molecules with activity against *Rhizoctonia solani, Pythium ultimum, Gaeumannomyces graminis* var. *tritici* and *Alternaria solani*. There are several natural products used as fungicides, which have been identified so far (Gerwick and Sparks 2013).

Naturally occurring biological control is an effective factor that leads to an ecological balance between pathogens and their antagonists. The most prominent and extensively studied biocontrol fungal agents are *Trichoderma* spp., *Gliocladium* spp., *Coniothyrium minitans*, *Pythium oligandrum*, *Clonostachys rosea*, *Talaromyces flavus*, and *Pythium nunn* (Papavizas 1985; Adams 1990). However, naturally occurring new species of soil fungi should be assessed for their biocontrol ability.

Novel natural products obtained from microorganisms offer opportunities for innovation in agrochemical

*Corresponding address:
van_nessner@yahoo.com.br

discovery (Strobel and Daisy 2003). On the other hand, there has been little commercial success in this field, and this failure may reside in the lack of understanding of the mode of action and the antagonists' ecology as well as inadequate knowledge of production methods and formulation for commercial use (Deacon and Berry 1993; Gnanamanickam 2002; Whipps *et al*. 2007).

In the course of our studies on the occurrence of antagonists in the rhizosphere, it has been noted that one fungal strain strongly inhibited the growth of some pathogenic fungi. These findings stimulated an investigation into the antagonistic activity of this isolate and its biological characterisation. The first objective of this study was to investigate the mycoparasitic activity of this fungal strain against two soil-borne plant pathogenic fungi, *R. solani* and *Pythium aphanidermatum* that cause seedling damping-off disease, using Scanning Electron Microscopy (SEM) techniques. The second objective was to evaluate the significance of secondary metabolites, and cellulase and chitinase enzyme production.

Material and Methods

Fungal strains

Bionectria sp. strain 6.21, isolated from citrus rhizosphere, and previously selected for its potential in inhibiting the growth of *Rhizoctonia* sp., was used in this study. *R. solani* and *P. aphanidermatum* were obtained from the Collection of Microorganisms of Environmental and Agricultural Importance of the Brazilian Agricultural Research Corporation (Embrapa), located in Jaguariúna, SP, Brazil.

Fungal identification

The identification of the fungal strain was carried out using both molecular and classical taxonomy techniques. For molecular identification, the internal transcribed spacer (ITS) domains of the rRNA gene were used. Amplification was performed with the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') as described by White *et al*. (1990). Sequencing of the DNA was carried out using an ET Dynamic Terminator Kit in a MegaBACE™ 1000/Automated 96 Capillary DNA sequencer (GE Healthcare, USA). The sequences obtained were adjusted using the Seq/Man module of the Lasergene software suite (DNASTAR/Inc.). A consensus sequence was obtained using the Bioedit software (v.7.0.5.3; Ibis Biosciences). To reach species level identification by rRNA gene sequencing, the consensus sequence was aligned with all sequences of related species retrieved from the GeneBank database using the Fasta 2.0 program (Altschul *et al*. 1997).

For classical taxonomy, morphological observations were performed using SEM. The method used was similar to that described by Melo and Faull (2004). The specimens were examined on a Field Emission Scanning Electron Microscope (Leo 982 Zeiss+Leica).

Detection of antagonistic activity

The dual-culture technique (Dennis and Webster 1971) was used to study the interactions of *Bionectria* sp. against the plant pathogenic fungus *R. solani*. Petri dishes containing Potato Dextrose Agar (PDA) were inoculated on one side with a 5-mm mycelia disc from a fresh culture of *Bionectria* sp. The opposite side was inoculated with a 5-mm mycelia disc of the pathogenic fungus. Plates were inoculated for 6 to 12 days in the dark at 28°C. After the pathogenic fungi were overgrown by the antagonist, the organisms were transferred to fresh PDA medium to determine the viability of each pathogen.

The interactions of the organisms were monitored by light microscopy, and regions of the parasitism were marked and selected for SEM. Preparation of the material was performed according to Melo and Faull (2004). The images were observed in a Field Emission Scanning Electron Microscope (Leo 982, Zeiss+Leica).

Extracellular enzyme production

Endoglucanase production was determined by growing the strain of *Bionectria* sp. in semi-solid medium, containing wheat bran, 100%; yeast extract, 6%; distilled water, to a final volume of 100 ml and then measuring reducing sugars released from carboxymethylcellulose incubated with the culture supernatants. Enzyme activity was expressed as units; each unit corresponds to 1 µmol of glucose equivalents released per minute.

For chitinase production, the fungus was grown in the following medium: bactopeptone – 0.1%; urea – 0.03%; $(NH_4)_2SO_4$ – 0.14%; $MgSO_4 \times 7H_2O$ – 0.03%; $CaCl_2 \times 6H_2O$ – 0.03%; glucose – 0.5%, as described by Lima *et al*. (1997). The chitinase activity was measured according to the method described by Fleuri *et al*. (2009), using colloidal chitin. A unit of the enzyme activity was defined as 1 µmol of N-acetylglucosamine formed in the conditions of this study. The different carbon sources used in both assays is due to the fact that the production of lytic enzymes is induced by appropriate substrates (Piegza *et al*. 2014). The design adopted in the enzyme activity was completely randomised with five treatments (culturing time) and three replications. The program used was ASSISTAT 7.5 beta. The means were compared using the Tukeyś test at a significance level of 5%.

Extraction of bioactive metabolites and antifungal assays

Bionectria sp. 6.21 was grown on PDA at 28°C for 7 days, and afterwards five plugs (5-mm mycelia disc) were inoculated into 500 ml Erlenmeyer flasks containing Potato Dextrose Broth (PDB) and incubated at 28°C under agitation (150 rev/min) for eight days. The culture was filtered and the aqueous phase extracted with dichloromethane (3 × × 250 ml). The pooled organic phase was dried over anhydrous sodium sulfate, filtered and the solvent evaporated in a rotatory evaporator.

The bioactive extract was fractionated by column chromatography over silica gel with gradient elution with hexane, hexane-ethyl acetate (10 : 0; 5 : 5; 3 : 7; 2 : 8, and

0 : 10 v/v). The crude extract and 11 fractions obtained from the fractionation of the crude extract were evaluated for antifungal activity against *R. solani* and *P. aphanidermatum* using the paper assay disc method as described by Rodrigues *et al*. (2004).

Results

Molecular characterization

Data from sequencing analyses were used to characterise the antagonistic fungus isolated from citrus rhizosphere. The strain's ITS1 – 5.8 S – ITS2 partial sequences were compared to ITS sequences of organisms represented in the GenBank database. Results revealed that the fungus showed a similarity to the genus *Bionectria*, with a 92% similarity with *Bionectria* sp. AM 9444351. Further studies must be carried out to elucidate the phylogenetic assignment of this strain and if necessary, a polyphasic taxonomic approach should be included.

Classical taxonomic characterization

Images obtained from field emission scanning electron microscope, revealed ultrastructural features that support the positioning of the strain into the genus *Bionectria* (Fig. 1). Fine structures of conidial surfaces and conidial *en masses* on phialides and conidiophores could be detected. Conidial *en masses* on phialides are formed and viewed in figures 1A and B. The verticillate conidiophores frequently bearing 2–5 phialides at each conidiophore terminus and phialides which are frask-shaped are viewed in figure 1C. The conidia, on verticillate conidiophores, are oblong-ellipsoidal to cylindrical, presenting ends broadly rounded and a narrow or apiculate base (Fig. 1D).

Microscopical observations on the mycoparasitic nature of *Bionectria* sp.

The interaction of *Bionectria* sp. and *R. solani* in dual culture, revealed some typical hyphal interactions. Mycoparasitism was clearly indicated by the extensive *Bionectria* sp. hyphae coiling over *Rhizoctonia,* as observed by SEM (Fig. 2). The smaller hyphal diameter of *Bionectria* sp. compared to *R. solani* allowed the two fungi to be easily distinguished. Hyphae of *Bionectria* sp. often coiled over *R. solani* (Fig. 2A) and also penetrated the host hyphae (Fig. 2B and C), resulting in the ultimate collapse of the host cells (Fig. 2D). The invaded hyphal cells disintegrated, probably due to the action of enzymes, one of the mycoparasitism's mode of action. Shrinkage and collapse of the parasitised hyphae were common in *R. solani*.

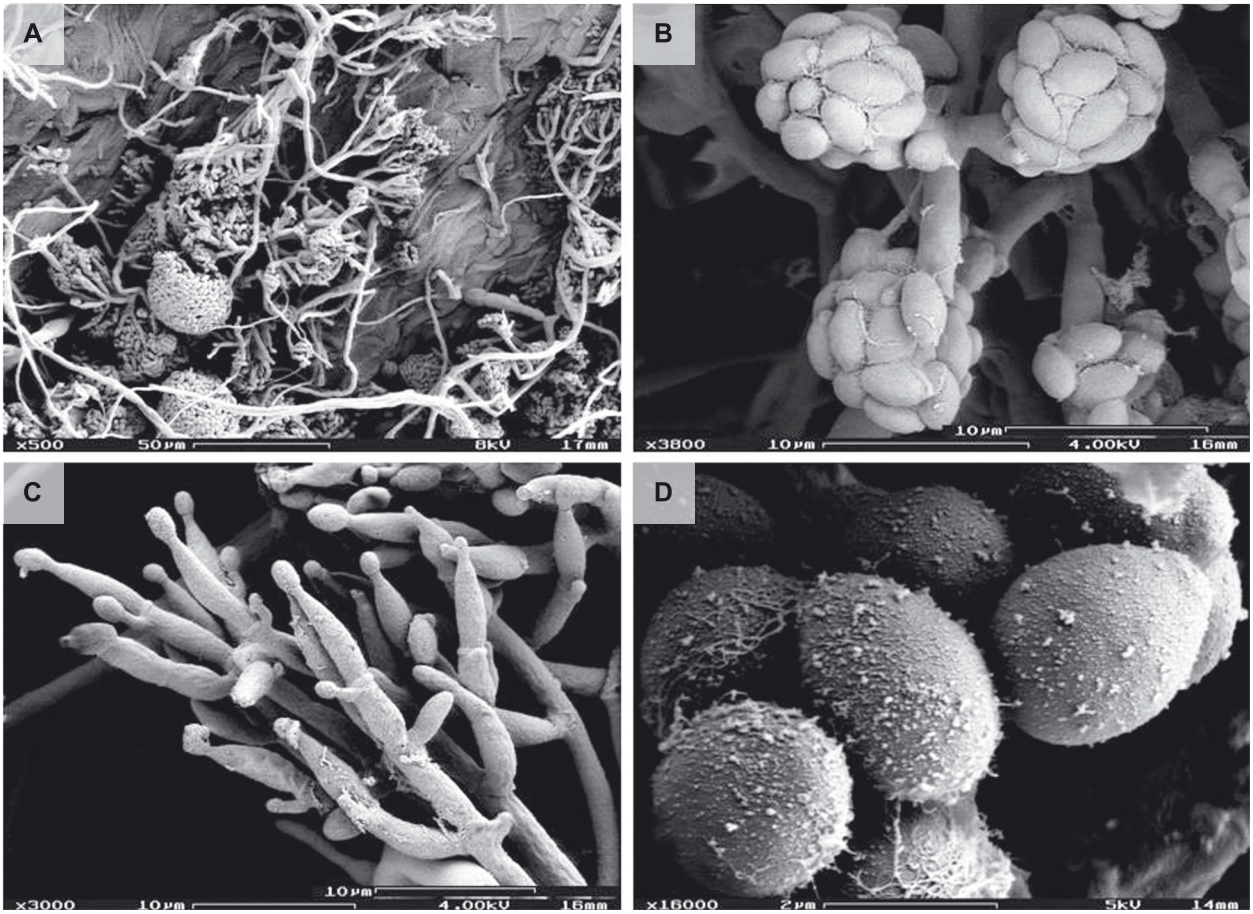

Fig. 1. Ultrastructural observations of *Bionectria* sp. 6.21 through SEM, supporting its positioning into the genus *Bionectria*: A and B – conidial mass showing tight aggregation of conidia; C – phialides, and D – conidia. The bars in each figure represent the magnification, in µm

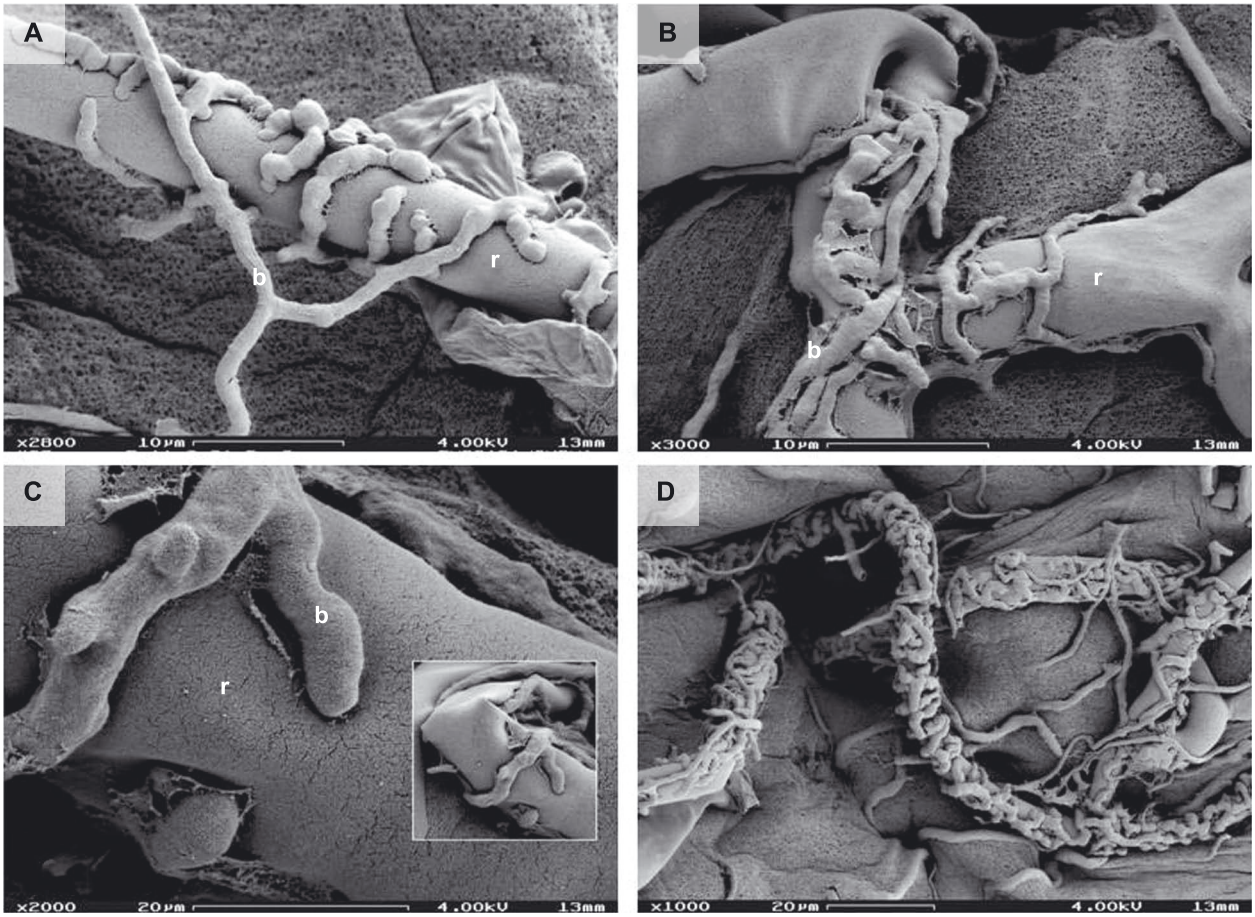

Fig. 2. SEM micrographs showing the mycoparasitism of *Bionectria* sp. 6.21 on *R. solani*. The interaction of both fungi shows clear evidence of fast destruction and lysis of the host: A – condensed coiling around hyphae of *R. solani* (r) by *Bionectria* sp. (b) after attraction and attachment; B and C – penetration of host hyphae by the antagonist; D – lysis of the invaded host cells

Enzyme production

When *Bionectria* sp. 6.21 was grown in semi-solid medium containing wheat bran as a sole carbon source, it produced extracellular endo-1,4-*β*-glucanase (CM-cellulase). A marked increase in specific activity was found after nine days of incubation (Fig. 3). Enzyme production decreased after this period of time.

The chitinase production was obtained in the first 48 h of incubation (Fig. 4). When this fungus was grown in culture medium containing chitin as a sole carbon source, a maximum chitinase yield was found after 182 h of fermentation.

Antifungal activity

Bionectria sp. 6.21 was able to inhibit the growth of *R. solani* and *P. aphanidermatum*. The inhibition of *P. aphanidermatum* was often at a reasonable distance, leaving a clear zone of inhibition between the two organisms. The host fungus or the inoculum plugs were eventually killed, as proved by the test of viability on carrot segments (data not shown).

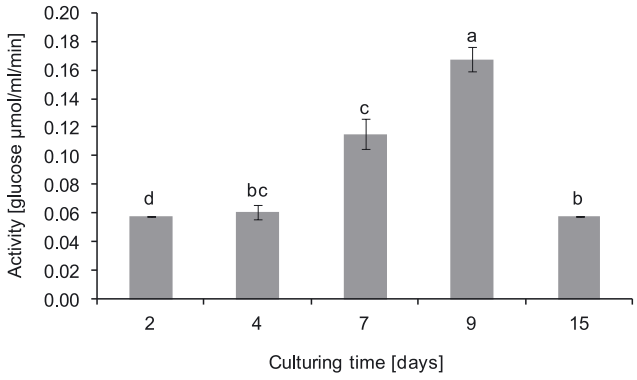

Fig. 3. Endoglucanase activity of *Bionectria* sp. 6.21 grown in semi-solid medium containing wheat bran. Error bars represent standard deviations. Different letters indicate significant differences at $p < 0.05$ according to Tukey's test ($n = 3$)

The crude extract produced by *Bionectria* 6.21 inhibited the fungus *P. aphanidermatum* while exhibiting a weak inhibition over *R. solani* (Fig. 5). Only two fractions F-4(9:1) and F-5(8:2), obtained from the purified crude extract, showed strong antifungal activity against *P. aphanidermatum* (Fig. 6).

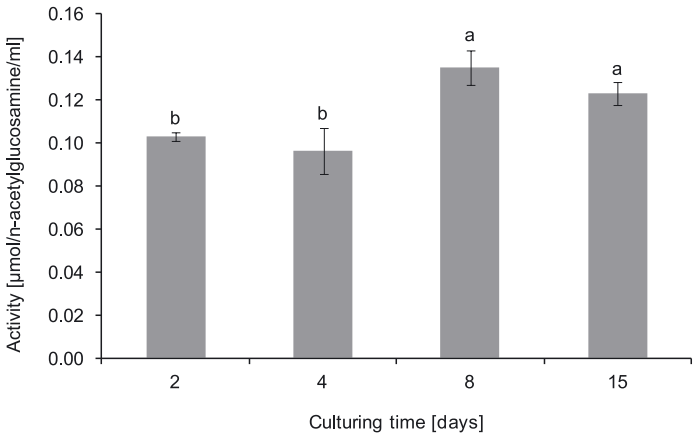

Fig. 4. Chitinolytic activity of *Bionectria* sp. 6.21 grown in culture medium containing chitin. Error bars represent standard deviations. Different letters indicate significant differences at $p < 0.05$ according to Tukey's test ($n = 3$)

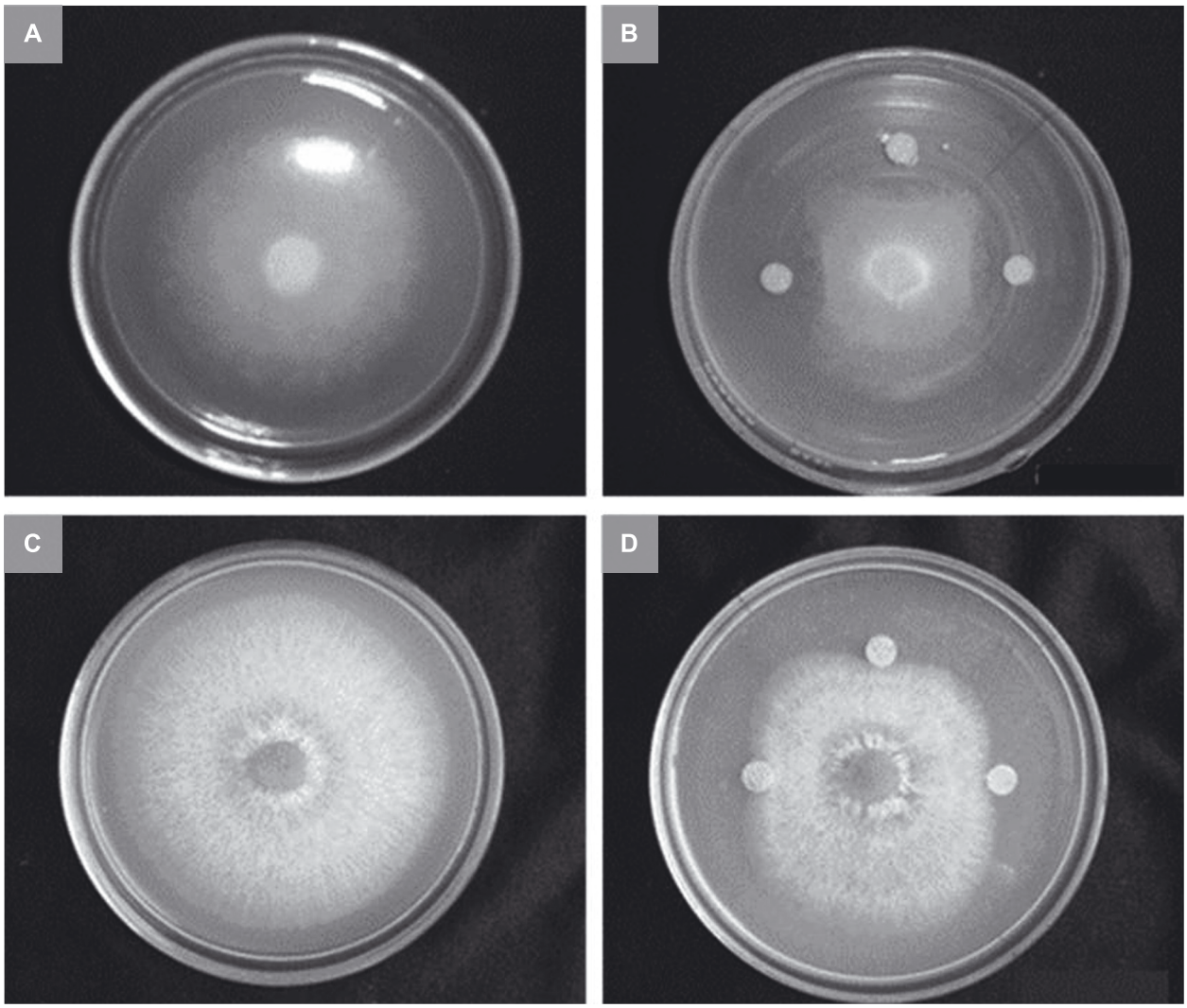

Fig. 5. Antifungal activity of crude extract of *Bionectria* sp. 6.21 against plant pathogenic fungi: A – normal growth of *P. aphanidermatum*; B – inhibition of mycelial growth of *P. aphanidermatum* by the crude extract; C – normal growth of *R. solani* and D – inhibition of mycelial growth of *R. solani*

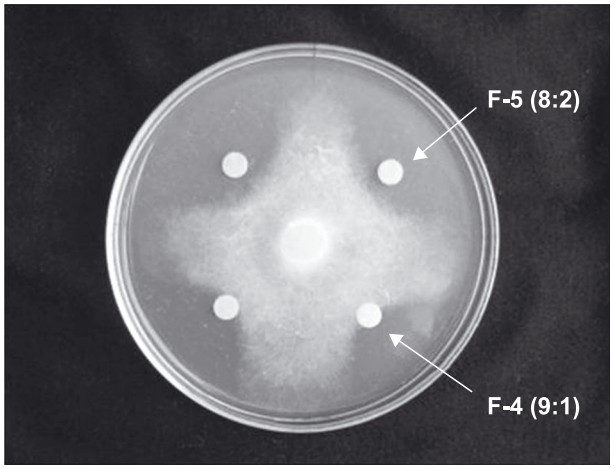

Fig. 6. Antifungal activity of the fractions F-4(9:1) and F-5(8:2) of *Bionectria* sp. 6.21 against *P. aphanidermatum*

Discussion

The genus *Bionectria* comprises a series of destructive fungal species with anamorphs in *Clonostachys*, some of which are used as biocontrol agents of fungal plant pathogens. The fungus is also important as a decomposer of debris and insects, and as a producer of enzymes and mycotoxins (Rossman 1996). A new polyketide glycoside, bionectriol A, was produced by a culture of *Bionectria* sp., which was isolated from a fungus-growing ant *Apterostigna dentogerum* (Freinkman *et al*. 2009). Other diastereomeric polyketide glycosides, reselipins 3A – 3E have been isolated from the anamorphic *Clonostachys candelatrum* on the basis of their positive anthelmintic activity (Ayers *et al*. 2010). *Clonostachys rosea* (Link: Fries) Schroers, Samuels, Seifert, and Gram; teleomorph *Bionectria ochroleuca* (Schweinitz) has been frequently associated with cysts of *Heterodera* spp., *Globodera* spp. and with sclerotia of *Sclerotinia sclerotiorum*, *Phymatochichum ommvorum*, *R. solani*, *Botrytis* spp. and plant materials (Sutton *et al*. 1997). That is due to the fact that this fungus is a versatile and effective biological control agent against soil, seed-borne and foliar diseases.

According to Junaid *et al*. (2013), parasitism involves growth of the biocontrol agent towards the host as well as attachment, coiling, and the final attack and dissolution of cell wall by the activity of enzymes as observed in this study. The observations are reminiscent of necrotrophic parasitism where the antagonist obtains nutrients from the host hyphae. Thus, *Bionectria* sp. exhibited hyperparasitic activity.

Enzymes capable of hydrolysing cell walls of fungi have been shown to include an array of polysaccharidases such as cellulase and chitinase. Chitinolytic enzymes and endoglucanases are thought to play an important role in the mycoparasitism of several plant pathogens (Elad *et al*. 1982; Benhamou and Chet 1996; Viterbo *et al*. 2002; Ayers *et al*. 2010). They are key enzymes in the lysis of fungal cell walls (Mitchell and Alexander 1963; Henis and Chet 1975). Some phytopathogenic fungi, such as *R. solani*, harbor chitin-cell walls, and others like *P. aphanidermatum,* contain cellulose as the main component of the cell wall. Chitin and cellulose share similarities in the structure and mechanisms of enzymatic degradation (Ferrari *et al*. 2014). Since some fungal cell walls are rich in chitin (Peberdy 1990; Mahadevan and Crawford 1997), the application of chitinase-producing microorganisms in biocontrol of fungal phytopathogens is promising. In this study, the cellulolytic and chitinolytic nature of *Bionectria* 6.21 is shown. The production of these enzymes by *Bionectria* indicates a potential for their involvement in the penetration of the host fungus as observed by SEM analysis.

In this study, there is strong evidence that the mode of action used by *Bionectria* sp. strain 6.21 in the control of *R. solani* is mainly mycoparasitic, having cell wall degrading enzymes involved in the fungal attack. The strong inhibitory activities of *Bionectria* sp. besides the lytic enzyme producing ability, suggests that this fungus may be a potential candidate for biological control. This strain should be subjected to further *in vivo* tests in order to evaluate its potential for practical use. Furthermore, the antifungal metabolites produced by this isolate may serve as model substance for the synthesis of novel natural pesticides. The understanding of the mode of action of *Bionectria* sp. is important for the design of future biocontrol studies.

References

Abdalla S.A., Algam S.A.A., Ibrahim E.A., Naim A.M.E. 2014. *In vitro* screening of *Bacillus* isolates for biological control of early blight disease of tomato in shambat soil. World J. Agric. Res. 2 (2): 47–50.

Adams D.J. 2004. Fungal cell wall chitinases and glucanases. Microbiology 150 (7): 2029–2035.

Adams P.B. 1990. The potential of mycoparasites for biological control of plant diseases. Annu. Rev. Phytopathol. 28: 59–72.

Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl. Acids Res. 25 (17): 3389–3402.

Ayers S., Zink D.L., Mohn K., Powell J.S., Brown C.M., Bills G., Grund A., Thompson D., Singh S.B. 2010. Anthelmintic constituents of *Clonostachys candelabrum*. J. Antibiot. 63 (3): 119–122.

Benhamou N., Chet I. 1996. Parasitism of sclerotia of *Sclerotium rolfsii* by *Trichoderma harzianum*: Ultrastructural and cytochemical aspects of the interaction. Phytopathology 86 (4): 405–416.

Chavan S.B., Deshpande M.V. 2013. Chitinolytic enzymes: an appraisal as a prodcut of commercial potential. Biotechnol. Prog. 29 (4): 833–846.

Consolo V.F., Mónaco C.I., Cordo C.A., Salerno G.L. 2012. Characterization of novel *Trichoderma* spp. isolates as a search for effective biocontrollers of fungal diseases of economically important crops in Argentina. World J. Microbiol. Biotechnol. 28 (4): 1389–1398.

Deacon J.W., Berry L.A. 1993. Biocontrol of soil-borne plant pathogens: concepts and their application. Pest Manag. Sci. 37 (4): 417–426.

Dennis C., Webster J. 1971. Antagonistic properties of species-groups of *Trichoderma*: III. Hyphal interaction. Trans. Br. Mycol. Soc. 57 (3): 363–369.

Elad Y., Chet I., Henis Y. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. Can. J. Microbiol. 28 (7): 719–725.

Ferrari A.R., Gaber Y., Fraaije M.W. 2014. A fast, sensitive and easy colorimetric assay for chitinase and cellulase activity detection. Biotechnol. Biofuels 7 (1): 37–44.

Fleuri L.F., Kawaguti H.Y., Sato H.H. 2009. Production, purification and application of extracellular chitinase from *Cellulosimicrobium cellulans* 191. Braz. J. Microbiol. 40 (3): 623–630.

Freinkman E., Oh D.C., Scott J.J., Currie C.R., Clardy J. 2009. Bionectriol A, a polyketide glycoside from the fungus *Bionectria* sp. associated with the fungus-growing ant, *Apterostigma dentigerum*. Tetrahedron Lett. 50 (49): 6834–6837.

Gerwick B.C., Sparks T.C. 2013. Natural products for pest control: an analysis of their role, value and future. Pest Manag. Sci. 70 (8): 1169–1185. DOI 10.1002/ps.3744.

Gnanamanickam S.S. 2002. Biological Control of Crop Diseases. Marcel Dekker Inc., New York, 480 pp.

Henis Y., Chet I. 1975. Microbiological control of plant pathogens. Adv. Appl. Microbiol. 19: 85–111.

Junaid J.M., Dar N.A., Bhat T.A., Bhat A.H., Bhat M.A. 2013. Commercial biocontrol agents and their mechanism of action in the management of plant pathogens. Int. J. Modern Plant Anim. Sci. 1 (2): 39–57.

Kumar K., Amaresan N., Bhagat S., Madhuri K., Udhayaraj P., Srivastava R.C. 2011. Genetic and physiological relatedness of antagonistic *Trichoderma* isolates against soil borne plant pathogenic fungi. Arch. Phytopathol. Plant Prot. 44 (14): 1399–1409.

Kumar K., Amaresan N., Bhagat S., Madhuri K., Srivastava R.C. 2012. Isolation and characterization of *Trichoderma* spp. for antagonistic activity against root rot and foliar pathogens. Indian J. Microbiol. 52 (2): 137–144.

Lima L.H.C., Ulhoa C.J., Fernandes A.P., Felix C.R. 1997. Purification of chitinase from *Trichoderma* sp. and its action on *Sclerotium rolfsii* and *Rhizoctonia solani* cell walls. J. Gen. Appl. Microbiol. 43 (1): 31–37.

Mahadevan B., Crawford D.L. 1997. Properties of the chitinase of the antifungal biocontrol agent *Streptomyces lydicus* WYE-ClOS. Enzyme Microb. Technol. 20: 489–493.

Melo I.S., Faull J.L. 2004. Scanning electron microscopy of conidia of *Trichoderma stromaticum*, a biocontrol agent of witches broom disease of cocoa. Braz. J. Microbiol. 35 (4): 330–332.

Mitchell R., Alexander M. 1963. Lysis of soil fungi by bacteria. Can. J. Microbiol. 9: 169–177.

Papavizas G.C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annu. Rev. Phytopathol. 23: 23–54.

Peberdy J.F. 1990. Fungal cell walls – a review. p. 5–24. In: "Biochemistry of Cell Walls and Membranes in Fungi" (P.J. Kuhn, A.P.J. Trinci, M.J. Jung, M.W. Goosey, L.G. Copping, eds.). Springer, Berlin Heidelberg, Germany, 327 pp.

Piegza M., Szlączka K., Łaba W., Witkowska D. 2014. Effect of carbon source on the production of lytic enzymes by filamentous fungi of the *Trichoderma* genus. Electronic Journal of Polish Agricultural Universities 17 (2) http://www.ejpau.media.pl/volume17/issue2/art-06.html [Accessed: March 9, 2013].

Rodrigues E., Supriya T., Naik C.G. 2004. Antimicrobial activity of marine organisms collected off the coast of South East India. J. Exp. Mar. Biol. Ecol. 309 (1): 121–127.

Rossman A.Y. 1996. Morphological and molecular perspectives on systematics of the Hypocreales. Mycologia 88 (1): 1–19.

Steindorff A.S., Ramada M.H.S., Coelho A.S.G., Miller R.N.G., Júnior G.J.P., Ulhoa C.J., Noronha E.F. 2014. Identification of mycoparasitism-related genes against the phytopathogen *Sclerotinia sclerotiorum* through transcriptome and expression profile analysis in *Trichoderma harzianum*. BMC Genomics 15: 204–218.

Strobel G., Daisy B. 2003. Bioprospecting for microbial endophytes ans their natural products. Microbiol. Mol. Biol. Rev. 67 (4): 491–502.

Sutton J.C., Li D.W., Peng G., Yu H., Zhang P. 1997. *Gliocladium roseum*: a versatile adversary of *Botrytis cinerea* in crops. Plant Dis. 81 (4): 316–328.

Vinale F., Marra R., Scala F., Ghisalberti E.L., Lorito M., Sivasithamparam K. 2006. Major secondary metabolites produced by two commercial *Trichoderma* strains active against different phytopathogens. Lett. Appl. Microbiol. 43 (2): 143–148.

Viterbo A., Ramot O., Chernin L., Chet I. 2002. Significance of lytic enzymes from *Trichoderma* spp. in the biocontrol of fungal plant pathogens. Antonie van Leeuwenhoek 81 (1–4): 549–556.

Whipps J.M., Bennett A., Challen M., Clarkson J., Coventry E., Muthumeenakshi S., Noble R., Rogers C., Sreenivasaprasad S., Jones E.E. 2007. Control of sclerotial pathogens with the mycoparasite coniothyrium minitans. p. 223–243. In: "Novel Biotechnologies for Biocontrol Agent Enhancement and Management" (M. Vurro, J. Gressel, eds.). Springer Netherlands, 374 pp.

White T.J., Bruns T., Lee S., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p. 315–322. In: "PCR Protocol, a Guide to Methods and Applications" (M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White, eds.). Academic Press, San Diego, USA, 482 pp.

Xiao J., Zhang Q., Gao Y.-Q., Tang J.-J, Zhang A.-L., Gao J.-M. 2014. Secondary metabolites from the endophytic Botryosphaeria dothidea of Melia azedarach and their antifungal, antibacterial, antioxidant, and cytotoxic activities. J. Agric. Food Chem. 62 (16): 3584–3590.