African Journal of Biotechnology Vol. 11(42), pp. 9955-9960, 24 May, 2012
Available online at http://www.academicjournals.org/AJB
DOI:10.5897/AJB11.1446
ISSN 1684-5315 © 2012 Academic Journals

Full Length Research Paper

Molecular detection of *Cylindrocarpon destructans* in infected Chinese ginseng roots and soil

Junfan Fu*, Jiaman Sun, Rujun Zhou and Xuerui Yan

Plant Protection College, Shenyang Agricultural University, No. 120, Dongling Road, Shenhe District, Shenyang, 110866, People's Republic of China.

Accepted 7 May, 2012

Ginseng (*Panax ginseng* C.A. Meyer) is one of the most important medicinal plants in China, but its yields are often reduced by a variety of root pathogens. The root rot of ginseng is a destructive soil-borne disease caused by *Cylindrocarpon destructans* (teleomorph: *Neonectria radicicola*). A species-specific polymerase chain reaction (PCR) assay was developed for rapid detection of *C. destructans* in diseased ginseng roots and artificially inoculated soil. One pair of specific primers was designed from comparisons of nucleotide sequences of the nuclear ribosomal internal transcribed spacer (ITS) regions of 22 fungal isolates from northeast of China. Under stringent PCR conditions, primers CD-F and CD-R amplified only a 450 bp fragment from *C. destructans* DNA but not from other pathogens or negative control. The sensitivity of detection was 1 pg genomic DNA per 25 µl PCR reaction volume, and *C. destructans* could be specifically detected with CD-F/CD-R from infected ginseng roots and soil. The approach outlined here could be further utilized as a rapid and reliable tool for the diagnosis and monitoring of the root rot of ginseng.

Key words: *Panax ginseng*, *Cylindrocarpon destructans*, internal transcribed spacer (ITS), polymerase chain reaction (PCR), molecular detection.

INTRODUCTON

Ginseng (*Panax ginseng* C.A. Meyer) widely cultivated as a medicinal herb in northeast China, has become an economically important cash crop for growers (Wang, 2001). The dried root is highly valued for its medicinal properties, and is widely used in Chinese traditional medicine (Punja and Rahman, 2005a). With the expansion of ginseng cultivation, seedling, foliar, and root diseases have become more apparent, of which root diseases, caused by several root-infecting fungi have significantly reduced root quality and yield (Buonassissi, 1990; Li, 1993; Punja, 1997; Rahman and Punja, 2005b).

Cylindrocarpon root rot of ginseng caused by *Cylindrocarpon destructans* (teleomorph: *Neonectria radicicola*) is one of the major threats to stable ginseng production (Ahn and Lee, 2001). The disease can result in yield losses of up to 25 to 30% (Chung, 1975; Wang, 2001; Seifert et al., 2003) and limits the re-use of fields for successive ginseng crops (Reeleder and Brammall, 1994; Reeleder, 2002). *C. destructans*, which is a common soil-inhabiting fungus (Fu, 2002), was confirmed to be the causal agent of root rot in ginseng sites (Punja, 1997; Reeleder and Brammall, 1994; Reeleder et al., 1999). Assessment of disease risk from soil-borne fungal pathogens has often been based on the number of colony-forming units (c.f.u) by dilution plating on selective media (Davet, 2000). However, the classical methods of diagnosis are time-consuming and laborious, requiring isolation of the fungus from diseased tissue (Chao, 1999). The fungus grows slowly, colonies arising from diseased tissue are often overgrown by more rapidly growing fungi and rare germination of chlamydospores makes the spread plate method of soil samples unusable (Reeleder, 2002; Seifert et al., 2003). Furthermore, the typical symptoms could not be observed before ginseng roots are infected seriously. Therefore, it is very difficult to diagnose the root disease by the classical methods. An effective and sensitive diagnostic system for

*Corresponding author. E-mail: fujunfan@hotmail.com. Tel: +86 024 8849 2892. Fax: +86 024 88487148.

C. destructans is desirable.

PCR technique offers advantages over traditional methods of detection and diagnosis because the fungi do not need to be cultured, prior to detection by PCR (Zhang et al., 2006). Recently, some diagnostic methods based on PCR have been developed for plant pathogen detection (Hamelin et al., 1996; Seifert et al., 2003; Ippolito et al., 2004; Zhang et al., 2005; Langrell, 2005; Zhang et al., 2006; Wang et al., 2008; Martin et al., 2009; Huang and Kang, 2010). However, to the best of our knowledge, little work has been done on detection of *C. destructans* in ginseng roots and soil. In view of this, the aim of this research was to develop a species-specific PCR detection assay for *C. destructans*, primarily for detection from ginseng roots and soil. In this study, PCR primers derived from internal transcribed spacer (ITS) sequences for the specific detection was described, and the utility of the assay was assessed.

MATERIALS AND METHODS

Source of isolates

The strains used in this research were obtained from diseased ginseng roots and infested soil, located at the major ginseng cultivation areas in China (Table 1). The fungal isolates were cultured on lima bean agar (LBA) plates (*Phytophthora* spp. and *Pythium* spp.) or on potato dextrose agar (PDA) (other fungi) plates and grown at (20 ± 2°C) in the dark, in an incubator for 1 week. Identification of the isolates was made based on the morphological, cultural, and sporulation characteristics (Domsh, 1980; Samuels and Brayford, 1990). All single-spore isolates were stored either on LBA at 10°C or on PDA at 4°C (Zhang et al., 2005).

Mycelium and conidia preparation

Five pieces of agar culture (ca. 2 × 2 × 4 mm), obtained from the advancing margin of 7-day old colonies, growing on plates of LBA (*Phytophthora* and *Pythium*) or PDA (other fungi) were placed in 100 ml of potato dextrose liquid medium in a 250 ml flask. After incubation in the dark at 20°C on a shaker for 2 weeks, the mycelia were then filtered through filter paper and rinsed with sterile distilled water, lyophilized, and stored at -20°C until DNA extraction (Hamelin et al., 1996). Spore suspensions of the fungus *C. destructans* were prepared by adapting the method of Zhang et al. (2005). Briefly, conidia were prepared by growing plate cultures on PDA at 20°C, for 2 weeks in darkness, to induce sporulation. Conidia were harvested from the plates by rubbing the surface mycelium gently with a rubber swab, and collecting the spores in distilled water. Hyphal debris was removed from the spores by centrifuging the crude spore preparation, through a 40% sucrose pad, with the spores settling to the bottom of the tube and the rest of the cellular debris remaining on the surface of the sucrose pad. Spores were adjusted to the desired concentrations (1×10^4 spores/ml), by counting them in a hemocytometer for soil inoculation.

Soil preparation and inoculation

Soil artificially inoculated was collected from Shenyang Agricultural University research farm, at Shenyang, Liaoning province (41° 47′ N, 123° 27′ E) in August 2010. Soil was steam-pasteurized at 74°C for 30 min, air-dried, sieved through a 4 mm mesh, mixed and stored at room temperature until use (Kernaghan et al., 2007). Soil dilution procedures were carried out, using a semiselective modified Rose Bengal agar medium (MRBA) to confirm that pasteurization had eliminated viable propagules (Reeleder et al., 2003). 5 ml *C. destructans* spores suspensions were inoculated into 5 g of twice-autoclaved soil substrate in 15 ml conical tubes. The tubes were vortexed at maximum speed for 1 min, freeze-dried for 3 weeks, ground in liquid nitrogen to produce a fine powder, and stored at -70°C, prior to DNA extraction (Zhang et al., 2006).

DNA extraction procedures

Genomic DNA from all microorganisms (Table 1) was extracted, using the fungal DNA Kit (OMEGA), according to the manufacturer's instructions.

DNA was extracted from infected ginseng roots according to Hamelin et al. (1996). A 100 mg sample of diseased ginseng roots was excised and ground in 400 µl of Qiagen extraction buffer (100 mM Tris-HCl, pH 9.5, 2% CTAB, 1.4 M NaCl, 1% polyethylene glycol 8000, 20 mM EDTA and 1% β-mercaptoethanol) mixed with approximately 10 mg of diatomaceous earth. Extracts were vortexed, incubated at 65°C for approximately 1 h, extracted once with 400 ml of phenol: chloroform: isoamyl alcohol (25:24:1), and centrifuged at 10,000 × g for 5 min. The supernatant was transferred to a new microtube, precipitated with one volume of isopropanol, and centrifuged at 10,000 × g for 5 min. Pellets were washed with 70% ethanol, air dried overnight, and resuspended in 20 µl of TE buffer, pH 8.0. Healthy ginseng roots were also processed as above for DNA extraction.

Total DNA from the artificially infested soil was extracted, following the research methods presented by Kernaghan et al. (2007). Soil (5 g) infested with *C. destructans* was placed in 50 ml disposable centrifuge tubes, with 3.5 ml of autoclaved 1 mm diameter zirconium oxide beads, 5 ml extraction buffer (60 mM sodium phosphate buffer (pH 8.0), 10 mM $CaCl_2$, 0.05% SDS and 100 µg proteinase K). Tubes were then shaken on a commercial paint shaker at maximum speed for 20 min, followed by centrifugation at 5,000 × g for 10 min at 10°C. Supernatants were then transferred to new, autoclaved tubes. EDTA and potassium acetate (pH 5.5) were added to provide final concentrations of 100 and 300 mM, respectively. Tubes were vortexed, placed on ice for 20 min and then centrifuged at 10,000 × g for 10 min. The resulting supernatants (2.5 ml) were transferred to new tubes containing an equal volume of a 1:0.2 v/v mixture of 3 M sodium acetate and 100% isopropanol (mixture pH 5.3), incubated at -20°C for 1 h then centrifuged again. The resulting pellets were washed with 80% ethanol, air-dried and frozen at -80°C, prior to use.

ITS amplification, sequencing and primer design

The entire region contains portions of the small subunit (18S), both internal transcribed spacers (ITS), and the 5.8S of the rDNA repeat unit. DNA was first amplified with oligonucleotides ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White, 1990), to ensure the presence of amplifiable DNA. Amplifications were performed in volumes of 25 µl, containing 3 µl 10× PCR Buffer, 1 µl of the dNTP mixture (2.5 mM), 1 µl of each of the 10 µM primer solutions, 0.2 µl (5U μl^{-1}) of Taq DNA polymerase (TaKaRa Biotechnology (Dalian) Company Lmited, China), 1 µl of template DNA, and 17.8 µl of sterile nanopure water (SDW). PCR reactions were incubated in an ABI Veriti™ 96 thermal cycler (USA), starting with an initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 45 s, annealing at 55°C for 30 s, and extension at 72°C for 60 s, with a

Table 1. Species used in this study for testing the specificity of *Cylindrocarpon destructans* oligonucleotide primers.

Number	Isolate	Geographic origin	Year isolated	Primer set	
				ITS1/ITS4	CD-F/CD-R
1	*Cylindrocarpon destructans*	Ji'an, Jilin	2010	+	+
2	*Cylindrocarpon destructans*	Ji'an, Jilin	2010	+	+
3	*Cylindrocarpon destructans*	Kuandian, Liaoning	2009	+	+
4	*Cylindrocarpon destructans*	Kuandian, Liaoning	2008	+	+
5	*Cylindrocarpon destructans*	Fusong, Jilin	2008	+	+
6	*Cylindrocarpon destructans*	Fusong, Jilin	2008	+	+
7	*Cylindrocarpon destructans*	Fusong, Jilin	2008	+	+
8	*Cylindrocarpon destructans*	Fusong, Jilin	2008	+	+
9	*Cylindrocarpon destructans*	Fusong, Jilin	2008	+	+
10	*Fusarium xysporum*	Ji'an, Jilin	2010	+	-
11	*Fusarium xysporum*	Fusong, Jilin	2009	+	-
12	*Fusarium solani*	Fusong, Jilin	2009	+	-
13	*Fusarium equiseti*	Fusong, Jilin	2009	+	-
14	*Rhizoctonia solani*	Fusong, Jilin	2009	+	-
15	*Alternaria panax*	Fusong, Jilin	2008	+	-
16	*Alternaria panax*	Ji'an, Jilin	2010	+	-
17	*Pythium debaryanum*	Ji'an, Jilin	2010	+	-
18	*Phytophthora cactorum*	Ji'an, Jilin	2010	+	-
19	*Sclerotinia schinseng*	Fusong, Jilin	2008	+	-
20	*Trichoderma* sp.	Ji'an, Jilin	2010	+	-
21	*Penicillium* sp.	Ji'an, Jilin	2010	+	-
22	*Aspergillus* sp.	Ji'an, Jilin	2010	+	-

+: 450 bp product amplified by primers CD-F/CD-R; -: no amplified products.

final post extension of 72°C for 10 min. PCR products were examined electrophoretically in a 1% TBE agarose gel (5 V/cm, 60 min), and stained with ethidium bromide for visualization under a UV transilluminator. Images were captured and analyzed, using a Bioimaging Chemi System (UVP, Incorporated, Upland, CA). The expected DNA fragment was gel purified using the PCR purification kit (TaKaRa Biotechnology (Dalian) Company Limited, China). The PCR products were sequenced by Invitrogen Company Limited, (Shanghai, China).

Specific primers for *C. destructans* (CD-F, 5'-TTGTTGCCTCGGCGGTGCCTG-3' and CD-R, 5'-GGGTTTAACGGCGTGGCCACGCT-3') were designed by comparison of the internal transcribed spacer (ITS) regions of different species in Table 1, using the software of primer premier 5.0.

Assessment of primer specificity and sensitivity

To determine the specificity of the primer, DNA extracted from a range of *C. destructans* isolates and other soil-borne pathogens mentioned previously was used as a template for PCR amplification. The reaction system was the same as described for the ITS sequence, the cycle sequencing reaction profile included an initial denaturation for 3 min, at 95°C, followed by 30 cycles of 30 s of denaturation at 94°C, 45 s of annealing at 59°C, 1 min of extension at 72°C, with a final extension of 10 min at 72°C. The sensitivity of the primer pairs was evaluated, using serial dilutions of purified genomic DNA from 1 ng/µl to 1 fg/µl (Wang et al., 2008). 1 µl of each dilution was added to the PCR reaction and amplification was carried out, using optimal conditions and cycling parameters as described above with primer CD-F/CD-R. Negative controls lacking template DNA were carried out in each experiment to test for contaminated reagents. All the assays were repeated twice with three replications.

RESULTS AND DISCUSSION

Primers, specificity and sensitivity

Soil-borne pathogens such as *C. destructans* could be extremely persistent, and the potential for losses as a result of replant decline may exist for decades after the cultivation of ginseng (Reeleder, 2002), as well as for some other perennial crops (Braun, 1995; Mercado-Blanco, 2003). Rapid and reliable detection of *C. destructans* is important for formulating strategies for disease management. The species-specific primer set CD-F/CD-R was designed for *C. destructans,* based on the ITS regions. All DNA preparations from fungi (Table 1) were amplified with the ITS universal primers ITS1/ITS4 (White, 1990), resulting in the fragments ranging from 400 bp to 650 bp, demonstrating that they were of sufficient quality as PCR templates. PCR with

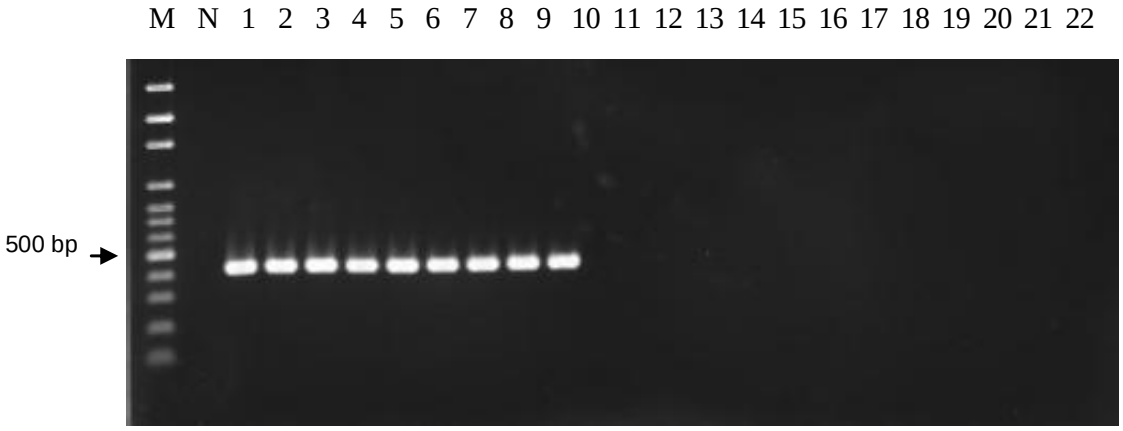

Figure 1. Agarose gel electrophoresis results of PCR amplification with specific primers CD-F/CD-R. Lane M: 100 bp DNA ladder; lane N: negative control; lanes 1 to 9: *Cylindrocarpon destructans* isolates; lanes 10 to 13: *Fusarium* sp.; lane 14: *Rhizoctonia solani*; lanes 15 to 16: *Alternaria panax* isolates; lane 17: *Pythium debaryanum*; lane 18: *Phytophthora cactorum*; lane 19: *Sclerotinia schinseng*; lanes 20 to 22: *Trichoderma* sp., *Penicillium* sp., and *Aspergillus* sp., respectively. Similar results were obtained in three replicates.

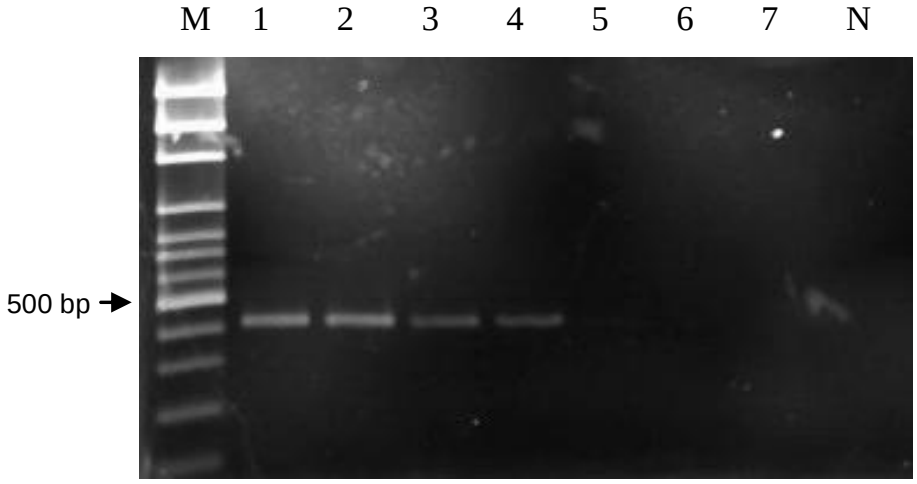

Figure 2. Detection of primer's sensitivity (of the CD-F/CD-R) on the genomic DNA of *Cylindrocarpon destructans*. Lane M: 100 bp DNA ladder; lane N: negative control; lanes 1 to 7: amplified products using DNA at concentrations of 1 ng, 100 pg, 10 pg, 1 pg, 100 fg, 10 fg and 1 fg in a 25 µl PCR reaction volume. Similar results were obtained in three replicates.

primers CD-F/CD-R, amplified DNA from all *C. destructans* isolates, and produced a specific amplicon of approximately 450 bp (Figure 1). However, no amplicon was obtained from any other fungal species tested.

Additionally, the primer set CD-F/CD-R has proven to be sensitive to detect very low quantities of DNA, by using purified fungal template DNA. Sensitivity was assessed, using serial dilutions of total DNA extracted from *C. destructans*. The PCR produced positive results for all *C. destructans* strains at four concentrations of DNA (Figure 2). In a 25 µl reaction volume assaying *C. destructans*, successful amplification was achieved down to 1 pg of pure genomic DNA, with a clearly visible product on ethidium bromide-stained agarose gels. However, primer-dimer formation at low template concentrations meant that the level of detection was sometimes lower than the level which could be accurately quantified (Kernaghan et al., 2007).

Detection of *C. destructans* from infested ginseng roots

A PCR method was developed to detect *C. destructans*

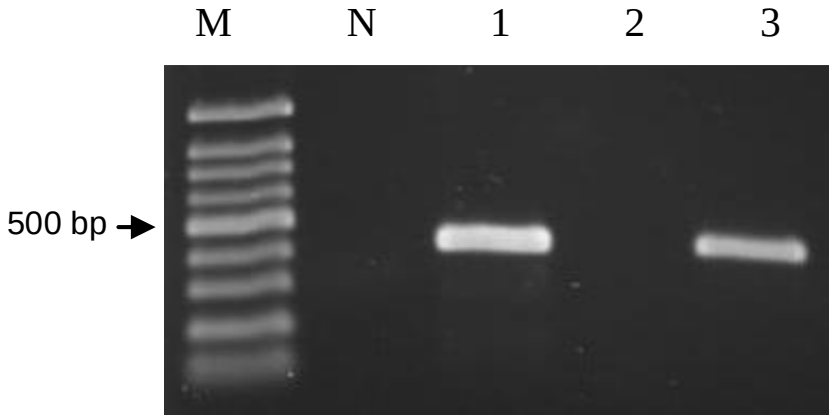

Figure 3. PCR amplification of DNA extracted from diseased ginseng roots. Lane M: 100 bp DNA ladder; lane N: negative control; lane 1: positive control (DNA from pure culture) of *Cylindrocarpon destructans*; lane 2: amplified product using DNA from healthy ginseng roots; lane 3: amplified product using DNA from infected ginseng roots.

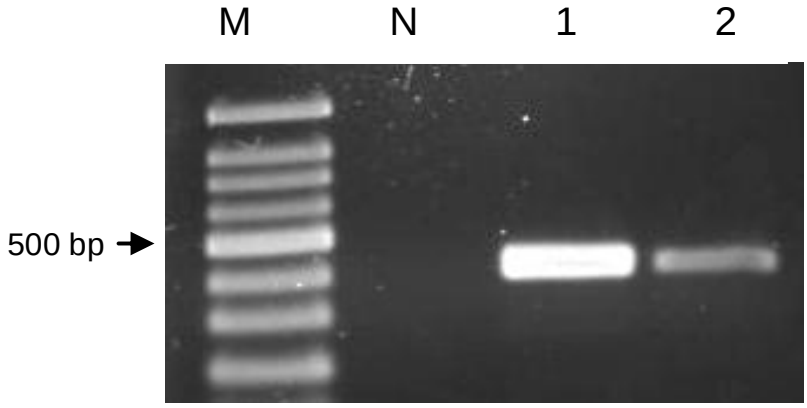

Figure 4. PCR amplification of DNA extracted from artificially infected soil. Lane M: 100 bp DNA ladder; lane N: negative control; lane 1: positive control (DNA from pure culture) of *Cylindrocarpon destructans*; lane 2: amplified product using DNA from diseased soil sample.

from ginseng roots collected from Jilin Province in 2010. Using PCR with primer set CD-F/CD-R, the predicted fragment of 450 bp was obtained from diseased ginseng roots (Figure 3). The fungus *C. destructans* was detected in asymptomatic ginseng in no case. This finding was in agreement with Hamelin (1996), whose study indicated that *C. destructans* could not be detected from nonviable fungal material. This was probably due to the low degree of infection in naturally infected plants, in comparison with those artificially infected, and the presence in field plants of other pathogens which could interfere with the detection (Silvar et al., 2005). Every picked diseased ginseng sample had DNA sequence 100% identical to those of *C. destructans* in the specificity assay. This confirmed that the DNA from infected ginseng that was amplified by primers CD-F/CD-R was derived from *C. destructans*.

Detection of *C. destructans* in soil

Agricultural field soil is a complex ecosystem with a diverse microbial community (Torsvik, 2002). More than hundreds of different species of *Fusarium*, *Phytophthora*, *Verticillium*, *Pythium*, *Rhizoctonia*, in addition to various bacteria and nematodes, have been found in field soil (Zhang et al., 2005). Specificity is essential for detecting *C. destructans* in soil. The DNA sample extracted from artificially inoculated soil was used for PCR amplification. Agarose gel electrophoresis revealed that the fragment amplified by CD-F/CD-R was of the predicted size (*c.* 450 bp) (Figure 4). This indicates that the PCR product in soil

DNA was from *C. destructans*.

Conclusion

The PCR assay with CD-F/CD-R primers could be used for detecting *C. destructans* from infested ginseng and soil. Although it would be impossible to perform routine diagnostics with PCR on asymptomatic ginseng, the PCR detection method presented here could contribute to improving the management of *Cylindrocarpon* root rot of ginseng. This method, which could be considered a crucial component of plant disease management is very easy to use, and requires minimal training. However, further research is needed to quantify the fungus in soil by real-time PCR.

ACKNOWLEDGEMENTS

This work was supported by Programs for Science and Technology Development of Liaoning province, China (Project No. 2007207002). This paper was edited by Dr. Xuqiao Feng (Food Science College, Shenyang Agricultural University).

REFERENCES

Ahn IP, Lee YH (2001). A viral double-stranded RNA up regulates the fungal virulence of *Nectria radicicola*. Mol. Plant-Microbe Interact., 14: 496-507.

Braun PG (1995). Effects of *Cylindrocarpon* and *Pythium* species on apple seedlings and potential role in apple replant disease. Can. J. Plant Pathol., 17: 336-341.

Buonassissi A, MacDonald LS (1990). Ginseng diseases in British Columbia from 1987-1989. Can. Plant Dis. Surv., 70:77.

Chao CC (1999). Methods for DNA extraction from various soils: A comparison. J. Appl. Microbiol., 86: 937-943.

Chung HS (1975). Studies on *Cylindrocarpon destructans* (Zins.) Scholten causing root rot of ginseng. Reports of the Tottori Mycolog. 12: 127-138.

Davet P, Rouxel F (2000). Detection and Isolation of Soil Fungi. Science Publishers, NH.

Domsh KH, Gams W, Anderson T (1980). Compendium of soil fungi. Academic Press, London, UK.

Fu JF, Yan XR (2002). A summary of researches on *Cylindrocarpon* and Panax root rust rot. J. Shenyang Agri. University, 33(1): 71-75.

Hamelin RC, Berube P, Gignac M, Bourassa M (1996). Identification of root rot fungi in nursery seedlings by nested multiplex PCR. Appl. Environ. Microbiol., 62(11): 4026-4031.

Huang JL, Kang ZH (2010). Detection of *Thielaviopsis basicola* in soil with real-time quantitative PCR assays. Microbiol. Res. 165 (5): 411-417.

Ippolito A, Schena L, Nigro F, Ligorio VS, Yaseen T (2004). Real-time detection of *Phytophthora nicotianae* and *P. citrophthora* in citrus roots and soil. Eur. J. Plant Pathol., 110(8): 833-843.

Kernaghan G, Reeleder RD, Hoke SMT (2007). Quantification of *Cylindrocarpon destructans* f. sp *panacis* in soils by real-time PCR. Plant Pathol., 56(3): 508-516.

Langrell RH (2005). Development of a nested PCR detection procedure for *Nectria fuckeliana* direct from Norway spruce bark extracts. FEMS Microbiol. Lett. 242(1): 185-193.

Li TS, Utkhede RS (1993). Pathological and nonpathological diseases of ginseng and their control. Curr. Top. Bot. Res., 1: 101-113.

Martin FN, Coffey MD, Zeller K, Hamelin RC, Tooley P, Garbelotto M, Hughes KJD, Kubisiak T, Bilodeau GJ, Levy L, Blomquist C, Berger PH (2009). Evaluation of Molecular Markers for *Phytophthora ramorum* Detection and Identification: Testing for Specificity Using a Standardized Library of Isolates. Phytopathology, 99(4): 390-403.

Mercado-Blanco J (2003). Quantitative monitoring of colonization of olive genotypes by *Verticillium dahliae* pathotypes with real-time polymerase chain reaction. Physiol. Mol. Plant Pathol., 63: 91-105.

Punja ZK (1997). Fungal pathogens of American ginseng (*Panax quinquefolium*) in British Columbia. Can. J. Plant Pathol., 19(3): 301-306.

Rahman M, Punja ZK (2005a). Biochemistry of ginseng root tissues affected by rusty root symptoms. Plant Physiol. Biochem., 43: 1103-1114.

Rahman M, Punja ZK (2005b). Factors influencing development of root rot on ginseng caused by *Cylindrocarpon destructans*. Phytopathol., 95(12): 1381-1390.

Reeleder RD, Brammall RA (1994). Pathogenicity of *Pythium* species, *Cylindrocarpon destructans*, and *Rhizoctonia solani* to ginseng seedlings in Ontario. Can. J. Plant Pathol., 16(4): 311-316.

Reeleder RD, Capell BB, Tomlinson LD, Hickey WJ (2003). The extraction of fungal DNA from multiple large soil samples. Can. J. Plant Pathol., 25(2): 182-191.

Reeleder RD, Roy R, Capell B (1999). Seed and root rots of ginseng (*Panax quinquefolius*) caused by *Cylindrocarpon destructans* and *Fusarium* spp. Phytopathology, 89, S65.

Reeleder RD, Roy R, Capell B (2002). Seed and root rots of ginseng (*Panax quinquefolius*) caused by *Cylindrocarpon destructans* and *Fusarium* spp. J. Ginseng Res., 26: 151-158.

Samuels GJ, Brayford D (1990). Variation in *Nectria radicicola* and its anamorph *Cylindrocarpon destructans*. Mycol. Res., 94: 433-442.

Seifert KA, McMullen CR, Yee D, Reeleder RD, Dobinson KF (2003). Molecular differentiation and detection of ginseng-adapted isolates of the root rot fungus *Cylindrocarpon destructans*. Phytopathol. 93(12): 1533-1542.

Silvar C, Duncan JM, Cooke DEL, Williams NA, Diaz J, Merino F (2005). Development of specific PCR primers for identification and detection of *Phytophthora capsici* Leon. Eur. J. Plant Pathol., 112(1): 43-52.

Torsvik V (2002). Microbial diversity and function in soil: from genes to ecosystems. Curr Opin Microbiol., 5: 240-245.

Wang TS (2001). China Ginseng. Liaoning Science and Technology Press, Shenyang, China.

Wang XJ, Zheng WM, Buchenauer H, Zhao J, Han QM, Huang LL, Kang ZS (2008). The development of a PCR-based method for detecting *Puccinia striiformis* latent infections in wheat leaves. Eur. J. Plant Pathol., 120(3): 241-247.

White TJ, Bruns T, Lee S, Taylor J (1990). PCR protocols: A guide to methods and applications. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic Press, New York, USA.

Zhang ZG, Li YQ, Fan H, Wang YC, Zheng XB (2006). Molecular detection of *Phytophthora capsici* in infected plant tissues, soil and water. Plant Pathol., 55(6): 770-775.

Zhang ZG, Zhang JY, Wang YC, Zheng XB (2005). Molecular detection of *Fusarium oxysporum* f. sp *niveum* and *Mycosphaerella melonis* in infected plant tissues and soil. FEMS Microbiol. Lett., 249(1): 39-47.