Armillaria Root Rot Caused by Armillaria Tabescens On Prunus Salicina in a Korean Garden

Cha, Joo Young
Field Science Center for Northern Biosphere, Hokkaido University

Lee, Si Young
The Professional Graduate School of Disaster Prevention Technology, Kangwon National University

Chun, Kun Woo
Division of Forest Resource, College of Forest and Environmental Sciences, Kangwon National University

Lee, Sang Yong
Division of Forest Resource, College of Forest and Environmental Sciences, Kangwon National University

他

https://doi.org/10.5109/16103

出版情報：九州大学大学院農学研究院紀要. 54 (2), pp.273-277, 2009-10-29. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **54** (2), 273–277 (2009)

Armillaria Root Rot Caused by *Armillaria Tabescens* On *Prunus Salicina* in a Korean Garden

Joo Young CHA[1], Si Young LEE[2], Kun Woo CHUN[3], Sang Yong LEE[3,*] and Shoji OHGA

Laboratory of Forest Resources Management, Division of Forest Ecosphere Management, Department of Forest and Forest Products Sciences, Kyushu University, Sasaguri, Fukuoka 811–2415, Japan

(*Received May 18, 2009 and accepted July 13, 2009*)

The *Armillaria* species causing *Armillaria* root rot on *P. salicina* in Korea was identified as *A. tabescens* based on the morphology of the basidiomata and analysis of DNA sequences. This work reports for the first time in Korea a pathogenic fungus of *A. tabescens* on *P. salicina*. From phylogenic analysis, *A. tabescens* isolate categorized it as a North American species except for the Czech species. However, the European species from Italy and France were distinctly distinguished from the isolate.

INTRODUCTION

Armillaria species exist as pathogens, saprobes, or necrotrophs on a wide range of hosts (Gregory *et al*., 1991; Hood *et al*., 1991; Kile *et al*., 1991; Fox, 2000). *Armillaria* root rot is a serious disease mainly of woody plants, caused by *Armillaria* (Fr.: Fr.) Staude. *Prunus* spp. hosts are very susceptible to *Armillaria*, especially almond (Guillaumin *et al*., 1989; Gregory *et al*., 1991; Tsopelas and Tjamos, 1997) and cherry (Aoshima and Hayashi, 1981; Sahashi, 2004). On the other hand, most reports on infection of almond or cherry come from older literature and refer to *A. mellea* sensu lato (Rhoads, 1965; Aoshima and Hayashi, 1981). Thus, more information is needed to determine which species of *Armillaria* attack these plants.

Armillaria tabescens (Scopoli) Emel (synonym: *Clitocybe tabescens*) has always been distinguished from other *Armillaria* species because its basidiomata do not bear an annulus on the stipe (Tsopelas and Tjamos, 1997). However, basidiomata of the fungus are not very common in nature, and they appear for only short periods of time.

In Korea, *A. ostoyae* has previously been reported as a pathogen on Korean white pine. On the other hand, other basidiomata of *Armillaria* species, *A. tabescens*, have been collected from maple, oak, and red pine (Sung *et al*., 1994). But their biological and ecological aspects of pathogen or saprophyte including real symptoms were not discussed in detail. Moreover, the symptoms of the disease and the morphology of cultures from vegetative isolates of *A. tabescens* are indistinguishable from those of other *Armillaria* species. In fungal molecular methods, polymerase chain reaction (PCR) analysis of the internal transcribe spacer (ITS) region of nuclear rDNA has proven to be reliable methods for identification at species level (Burns *et al*., 1991; Karen *et al*., 1997).

Here we report on the symptoms of *Armillaria* root rot and basidiomata formation of *A. tabescens* on *P. salicina* planted in a Korean garden. The fungus was identified from the characteristics of the basidiomata, and from the analysis of the rDNA internal transcribed spacer (ITS) region. A phylogenic analysis including other continental isolates of *A. tabescens* was conducted.

MATERIALS AND METHODS

Sample collection, isolation, and identification

Prunus salicina Lindl planted in Korean garden where is located in 1162, Hojeo–myeon, Wonju–si, Kangwon–do in KOREA was used in this study. Symptoms of *Armillaria* root rot were observed on 2 August 2007, and basidiomata that formed on 23 September 2007 were collected from the stem and butt of a dead tree. Macro–morphological characteristics of the basidiomata were described for species identification of *Armillaria*, and dried specimens were stored in the herbarium of Kangwon National University, Korea. Before the specimens were dried, vegetative isolates from tissue of the basidiomata were cultured on potato dextrose agar (PDA) medium at 25 °C in an incubator. The culture was used for observation of colony morphology and DNA extraction. A Munsell soil color chart (1994) was used to analyze diagnostic basidiomata colors.

DNA extraction and sequencing

Fungal DNA was extracted from 50 to 200 mg of fleshy mycelium using the DNeasy Plant Mini kit (Qiagen, USA) according to the manufacturer's instructions. The internal transcribed spacer (ITS) region, including 5.8 S rDNA, was amplified using primers specific for the higher fungi ITS1–f (Gardes and Bruns, 1993) and ITS4–b (White *et al*., 1990) regions. Each

[1] Field Science Center for Northern Biosphere, Hokkaido University, Nayoro 096–0071, Japan
[2] The Professional Graduate School of Disaster Prevention Technology, Kangwon National University, Samcheok 245–711, Korea
[3] Division of Forest Resource, College of Forest and Environmental Sciences, Kangwon National University, Chuncheon 200–701, Korea
* Corresponding author (E–mail: sangyong@kangwon.ac.kr)

50 μl mixture contained 5 μl G–Taq buffer (10X), 4 μl dNTP (2.5 M), 5 μl Tuning buffer (Cosmo Genetech Co.) (5X), and 0.5 μl G–Tag DNA polymerase (Cosmo Genetech Co.). Amplifications were performed using a 2720 Thermal Cycler (Applied Biosystems). Initial denaturation at 94 °C for 3 min was followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 48 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were purified using the LaboPass PCR purification kit (COSMO Co. Ltd). Sequencing of the purified products was carried out using a BigDye® Terminator v3.1 Cycle Sequencing Kit and the GeneAmp® PCR System 9700. The BigDye® XTerminator™ Purification Kit was used for purification of the reaction products, and an Applied Biosystems 3730xl DNA Analyzer was used for analysis. All processes were carried out according to the manufacturer's instructions.

Analysis of DNA sequence data

ITS sequences were compared to the GenBank database at the DNA Data Bank of Japan (DDBJ) using the basic local alignment search tool (BLAST) program, and the homology for *A. tabescens* and other *Armillaria* species was calculated. All sequences used in the analysis, except the isolate used in this study, have been deposited into GenBank (Table 1).

Phylogenic analysis was performed for each data set using neighbor–joining (Saitou and Nei, 1987). The ITS sequences were initially aligned using CLUSTAL W in DDBJ. Neighbor–joining analysis was performed using the Tamura–Nei model for estimation of evolutionary distance, and relative support for nodes in resulting trees was generated using 1000 bootstrap replicates (Felsenstein, 1985).

RESULTS

Sample collection, isolation, and identification

On 2 August 2007, an approximately 15–year old *P. salicina* tree, 15 cm diameter at breast height (DBH) and 5 m in height, was observed with the entire canopy collapsed with leaves attached, after looking healthy the previous year (Fig. 1 A). The dying tree was excavated to a depth of about 10 cm, and the butt and root collar were examined for the presence of mycelial fans under the bark (Fig. 1 B). Rhizomorphs were observed on the surface of the roots. Basidiomata of *Armillaria* that had developed on the stems were collected on 23 September 2007 (Fig. 1 C).

In culture, the *Armillaria* isolate produced a sparse flat whitish to brownish mycelium with abundant cylindrical rhizomorphs (Fig. 1 D). Macro–morphological features of the basidiomata are described in detail as follows: pileus 8.4–86.8 mm diameter, when young, convex, sometimes conical then plano–convex and finally planar and uplifted to depressed, sometimes irregularly undulating with age. Surface dry, when young, reddish yellow (7.5YR–7/6), strong brown (7.5YR–5/6), and reddish brown (7.5YR–8/6) at center and reddish yellow (5YR–6/8) to yellowish red (5YR–5/6) toward the margin, usually covered with distinct brownish scales more densely concentrated toward the center. Lamellae white when young, to light reddish brown (5YR–6/4) later,

Table 1. *Armillaria tabescens* collected in this study and best BLAST match

Species*	Origin**	Gen Bank accession no.	Similarity (%)
Armillaria tabescens	USA/GA	AY213590	98
Armillaria tabescens	USA/GA	AY213589	98
Armillaria tabescens	USA/SC	AY695409	98
Armillaria tabescens	USA/SC	AY213588	97
Armillaria socialis	Czech	DQ784799	97
Armillaria socialis	Czech	DQ784800	97
Armillaria tabescens	Czech	AY175806	96
Armillaria tabescens	USA/SC	DQ109806	96
Armillaria ostoyae	USA/NH	AY213552	93
Armillaria gemina	USA/NY	AY213556	92
Armillaria sinapina	USA/WA	AY213565	92
Armillaria gallica	USA/MI	AY213570	92
Armillaria clavescens	USA/MI	AY213561	92
Armillaria cepistipes	USA/WA	AY213583	92
Armillaria nabsnona	USA/ID	AY213572	92

*: *Armillaria socialis* (DC: Fr.) Herink is synonym to *A. tabescens* (David, 2000)
**: GA, Georgia; SC, South Carolina; NH New Hampshire; NY, New York State; WA, Washington State; MI, Nichigan; ID, Idaho

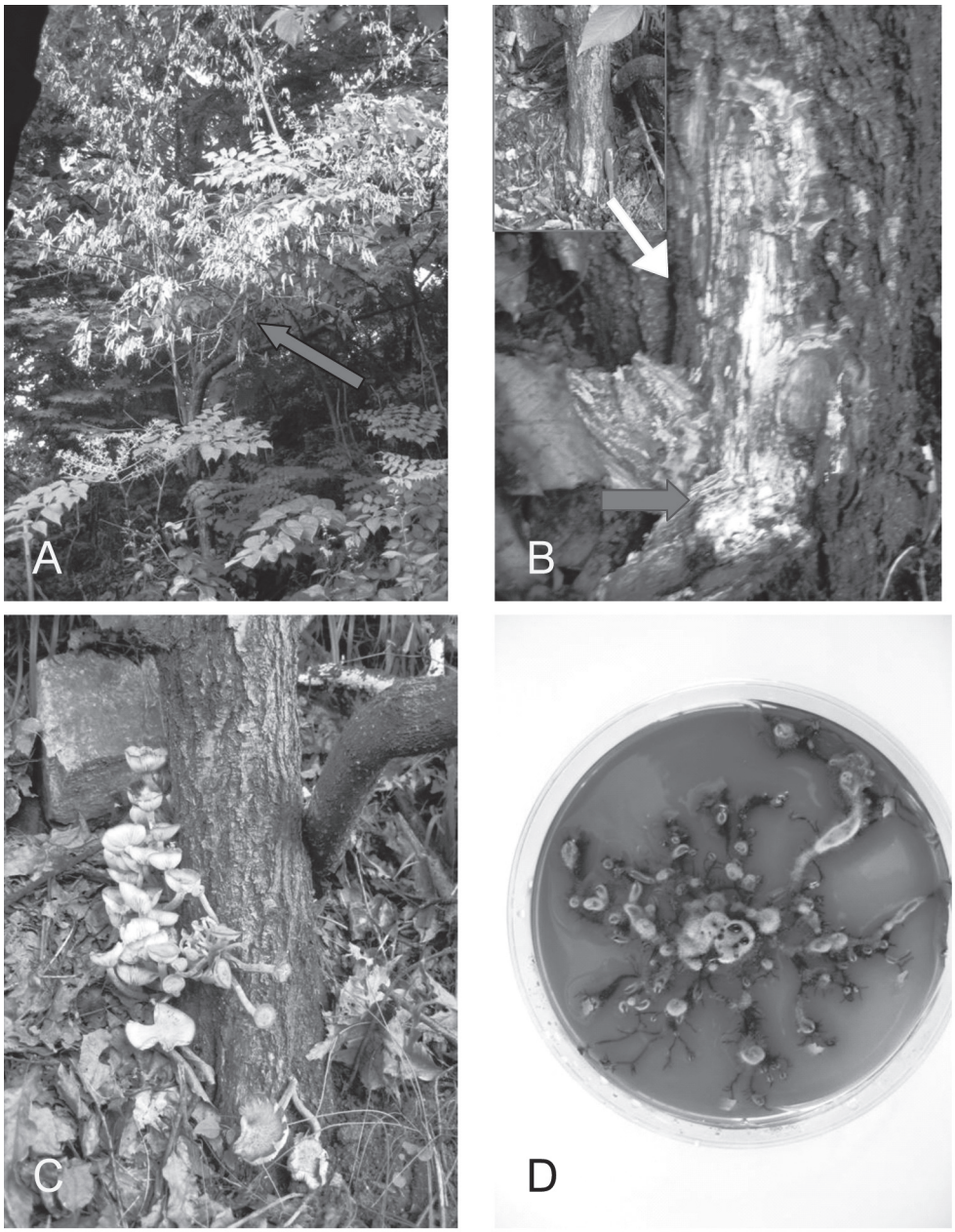

Fig. 1. Dying *P. salicina* (A), mycelial fan under the bark (red arrow, B), basidioma formation (C), and culture on PDA medium (D).

crowded, sinuate, subdecurrent. Stipe central, 33.6–140 mm in length, 4.2–11.2 mm thick at the apex, tapering below, whitish, bruising brown with fibrillose surface without annulus. Habitat stem and root collar, densely caespitose.

DNA sequencing

The ITS *Armillaria* isolate from *P. salicina* was 886 bp long. A BLAST search using the ITS 1 and 5.8S gene sequences for this isolate against sequences in DDBJ showed that the DNA sequences of the *Armillaria* isolate were most similar to those identified as A. tabescens (Table 1). On the other hand, the similarity between the isolate and other *Armillaria* species except for *A. tabescens* was less than 93%.

Analysis of DNA sequence data

Twelve *A. tabescens* including the *P. salicina* isolate (KOREA/Kangwon) used in this study formed two main groups in a neighbor–joining tree with bootstrap support of 1000. Group I sequences originated from Italy and France in Europe. The other group was from Korea (KORE/Kangwon), and South Carolina and Georgia in the USA and the Czech Republic in Europe (Fig. 2).

DISCUSSION

The *Armillaria* species causing *Armillaria* root rot on *P. salicina* in Korea was identified as *A. tabescens* based on the morphology of the basidiomata and analysis of DNA sequences. *Armillaria tabescens* is considered a significant pathogen of orchid trees and ornamental

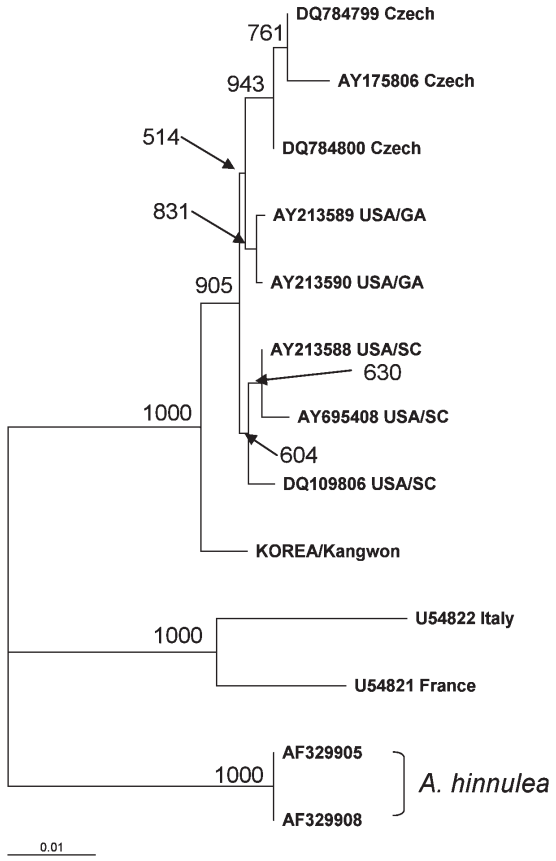

Fig. 2. Phylogram generated from Neighbor–joining analysis of the ITS sequence data used in this study. Bootstrap values are shown on the branch. *Armillaria hinnulea* is used as out group.

plants in southern USA (Gregory *et al*., 1991) Although the occurrence of the fungus in Europe on cultivated plants are rare, *A. tabescens* on citrus trees in Crosica (Laville and Vogei, 1984) and on pear tree (*Pyrus communis*) in Portugal (Rishbeth, 1985). However, Tsopelas and Tjamos (1997) reported on the attack of almond (*P. amygdalus*) in Greece. Although Sung *et al*. (1994) reported that *A. ostoyae* occur a serious disease of pine (*Pinus koraiensis*) in plantation after clear cut of forest, the studies on the *Armillaria* root rot are rare in Korea. This work reports for the first time in Korea a pathogenic fungus of *A. tabescens* on *P. salicina*.

In morphology, the macro– and micro–scopical characteristics were not distinguish with other continental fungus, *A. tabescens*. It is easy to distinguish *A. tabescens*, the non–annulus *Armillaria*, from other species based on the morphology of basidiomata. Although another *Armillaria* species without an annual ring on the stipe, *A. ectypa* (Fr.: Fr.) D. Lam, has been reported in Europe (Chillai *et al*., 1998) and Japan (Imazeki and Hongo, 1995), it is found in very different habitat from that of *A. tabescens*, which is ligneous, while *A. ectypa* is terrestrial. Moreover, *A. ectypa* has not been reported in Korea yet.

Additionally, a BLAST search in DDBJ of the ITS region identified it as A. tabescens with 96% to 98% sequence homology, strongly supporting the identity of this species. Phylogenic analysis of the *A. tabescens* isolate categorized it as a Northern American species except for the Czech species. However, the European species from Italy and France were distinctly distinguished from the isolate. The bootstrap support for a continental origin of the ITS region was strong with bootstrap support of 1000. Several researchers doubt exists whether *A. tabescens* found in Europe is the same species as the one reported in North America and other places in the world (Tsopelas and Tjamos, 1997). Because the compatibility tests between isolates from Europe and USA showed them to belong to different species (Guillaumin *et al*., 1989, 1993). However, Darmono *et al*. (1993) reported that haploid isolates from Europe were compatible with haploid isolates from USA, and that isolates from different areas of Nroth America all belong to the same species.

As mentioned above, among the continental isolates are slightly complex in mating behavior and some aspect of the pathogenicity to plants. From this study, although the Czech isolates was included in the same group of Korean and USA, the Korean group including USA was clearly separated in genetic. But, it is considered that the diversity from the mating behaviors and DNA analysis is originated from not the continent but environmental condition such as temperature in habitat. Because *A. tabescens* is thermophilic. So, the geographical distribution in the northern temperate hemisphere will be appeared to increase dramatically depending upon continental climatic conditions. Therefore, it can be expected that severe tree diseases such as *Armillaria* root rot caused in *A. tabescens* will increase with global warming and abrupt climatic changes in forest ecosystems. Therefore, the diversity in taxonomy and heredity, and ecology of *Armillaria* spp. including *A. tabescens* will be clarified in regional in the future.

ACKNOWLEDGEMENTS

We thank Drs. Y. Tamai and K. Terashima for critical advice. This study was carried out with the support of 'Forest Science & Technology Projects (No. S210809L010110)' provided by Korea Forest Service in Korea, and of a grant–in–aid from Heiwa Nakajima Foundation in Japan.

REFERENCES

Aoshima, K. and Y. Hayashi 1981 Trap method for detecting *Armillaria mellea* from soil. In: Abstracts of Proceedings of the 17th World Congress of the International Union of Forest Research Organizations (IUFRO), Kyoto, Japan: pp. 621

Burns T. D., T. J. and J. W. Taylor 1991 Fungal molecular systematics. *Ann. Rev. Ecol. Syst*., **22**: 525–564

Chillali, M., H. Idder–Ighili, J. J. Guillaumin, C. Mohammed, B. L. Escarmant and B. Botton 1998 Variation in the ITS and IGS regions of ribosomal DNA among the biological species of European *Armillaria,*. *Mycological Research*, **102**: 533–540

Darmono, T. W., H. H. Burdsall and T. J. Volk 1993 Interfertility among isolates of *Armillaria tabescens in* North America. *Sydowia*, **44**: 105–116

Felsenstein, J. 1985 Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**: 783–791

Fox, R. T. V. 2000 Pathogenicity. In: Armillaria Root Rot: Biology and Control of Honey Fungus. Ed. by Fox, R. T. V. Andover, England: pp. 113–136

Gardes, M. and T. D. Burns 1993 ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. *Mol. Ecol.*, **2**: 113–118

Gregory, S. C., J. Rishbeth and S. G. Show 1991 Pathogenicity and virulence. In: Armillaria Root Disease. Agricultural Handbook No. 691. Ed. By Show, C. G. Ⅱ and G. A. Kile, Washington, DC: USDA Forest Service: pp. 76–87

Guillaumin, J. J., J. Pierson and C. Grassely 1989 The susceptibility of different *Prunus* species used as stone fruit rootstocks to *Armillaria mellea* (sensu stricto). In: Proceedings of the 7th International Conference on Root and Butt Rots, Ed. By Morrison, D. J. IUFRO, Vernon and Victoria (CA): pp. 27–43

Guillaumin, J. J., C. Mohammed, N. Anselmi, R. Courtecuisse, S. C. Gregory, O. Holdenrieder, M. Intini, B. Lung, H. Marxmuller, D. Morrison, J. Rishbeth, A. Termorshuizen, A. Tirro, and B. Van Dam 1993 Geographical distribution and ecology of the six *Armillaria* species in Western Europe. *European Journal of Forest Pathology*, **23**: 321–341

Hood, I. A., D. B. Redfern and G. A. Kile, G. A. 1991 *Armillaria* in planted hosts. In: Armillaria Root Disease. Agricultural Handbook No. 691. Ed. By Show, C. G.., Ⅲ; Kile, G. A. Washington, DC: USDA Forest Service: pp. 122–149

Imazeki, R. and T. Hong 1995 Colored illustrations of mushrooms of Japan Vol. I. Hoikusya Publishing Co., LTD. Osaka, Japan: pp. 325 (In Japanese)

Karen, O., N. Hogberg, A. Dahlberg, L. Jonssson and J. Nylund 1997 Inter– and intraspecific variation in the ITS region of rDNA of ectomycorrhizal fungi in Fennoscandia as detected by endonuclease analysis. *New Phytol.*, **136**: 313–325

Kile, G. A., G. I. McDonald and J. W. Byler 1991 Ecology and disease in natural forests. In: Armillaria Root Disease. Agricultural Handbook No. 691. Ed. By Show, C. G. Ⅲ; Kile, G. A. Washington, DC: USDA Forest Service: pp. 102–121

Laville, E. and R. Vogel 1984 Les attaques de pourridie dans les vergers d'agrimes de Corse. *Arboriculture Fruitiere*, **31**: 34–36

Munsell 1994 Munsell soil color charts, revised ed, Macbeth Division of Kollmorgen Instruments Corporation, Baltimore, Maryland

Rhoads, A. S. 1956 The occurrence and destructiveness of Clitocybe root rot of woody plants in Florida. *Lloydia*, **19**: 193–240

Rishbeth, J. 1985 Infection cycle of *Armillaria* and host response. *European Journal of Forest Pathology*, **15**: 332–341

Sahashi, N. 2004 Story of forest fungi in Japan–their diverse functions, ecological roles and conservation–, Tokai University Press, Kanagawa, Japan: pp. 198 (In Japanese)

Saitou, N. and M. Nei, M 1987 The neighbor–joining methods: a new method for reconstructing phylogenic trees. *Mol. Biol. Evol.*, **4**: 406–425

Sung, J. M., K. J. Yang, H. K. Lee and T. C. Harrington 1994 Studies on Korean species of *Armillaria*. *Korean J. Plant Pathol.*, **10**(4): 261–269

Tsopelas, P., and E. C. Tjamos, E. C. 1997 Occurrence and pathogenicity of *Armillaria tabescens* on almond in Greece. *Bulletin OEPP/EPPO Bulletin*, **27**: 455–461

White, T. J., T. D. Bruns, S. Lee and J. Taylor 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications. Ed. By Innis, M. A., D. H. Gelfand, J. J. Sninsky and T. J. White, New York: Academic Press, Inc.: pp. 315–322