Mycoscience (2010) 51:366–372
DOI 10.1007/s10267-010-0053-8

FULL PAPER

Identification of *Armillaria* species associated with *Polyporus umbellatus* using ITS sequences of nuclear ribosomal DNA

Gen Kikuchi · Hiroki Yamaji

Received: 19 June 2007 / Accepted: 2 February 2010 / Published online: 27 April 2010

Abstract The sclerotia of *Polyporus umbellatus* were collected from three locations in Japan and three locations in China. All the collected sclerotia were adhered to by rhizomorphs of the symbionts. When the sclerotium of *P. umbellatus* was cross sectioned, the internal part of the sclerotium was cream colored, and many black regions surrounding the invading rhizomorphs were observed. The surrounding zone contained string-like, gelatinous masses composed of hyphae, and its outside was brown in color. All isolates were similar in colony morphology and grew well on PDA medium with well-developed rhizomorphs. All the isolates showed typical morphology of *Armillaria*. The isolated fungi were identified via the ITS region of the nuclear ribosomal DNA sequence. Phylogenetic analysis based on the neighbor-joining method showed that all the isolates clustered with fungi belonging to *Armillaria* species. Among them, five species (*A. sinapina*, *A. calvescens*, *A. gallica*, *A. cepistipes*, and *A. nabsnona*) and the symbiont formed a highly supported clade. We report on the case where *Armillaria* has a relationship in the sclerotium of *Polyporus umbellatus*.

Keywords *Armillaria* · ITS · *Polyporus umbellatus* · Sclerotia · Symbionts

G. Kikuchi (✉) · H. Yamaji
Botanical Raw Materials Division, Botanical Raw Materials Research Department, Tsumura & Co., 3586 Yoshiwara, Ami-machi, Inashiki, Ibaraki 300-1192, Japan
e-mail: kikuchi_gen@mail.tsumura.co.jp

Introduction

Polyporus umbellatus Fries, belonging to a genus of Polyporaceae, has been considered as a species of wood-rotting fungi. This fungus forms underground sclerotia, and it distributes in the nearer soil surface, such as 10–15 cm below the surface, being rarely found below 30 cm, in broad-leaved forests in Japan, China, Korea, and temperate regions of the Northern Hemisphere (Lee 1988; Imazeki and Hongo 1989; Chen and Chen 2000; Stamets 2000). The dried sclerotia of *P. umbellatus* have been used as a crude drug for Chinese medicine in Asian countries such as Japan and China (Wei et al. 1983; Ohta et al. 1996). The sclerotia of *P. umbellatus* have been produced by cultivation on Chinese farms, using artificial infection with *Armillaria* species (Yao et al. 2006). Moreover, a symbiotic relationship between the sclerotium and *Armillariella mellea* (a synonym of *Armillaria mellea*), the culture of strains of *P. umbellatus*, the extraction of polysaccharides of *P. umbellatus*, and the cultivation of *P. umbellatus* on Chinese farms were summarized for China (Xu et al. 2003). On the other hand, Imazeki and Hongo (1965) described that the sclerotium of *P. umbellatus* was formed adhering to the living roots of deciduous species belonging to *Quercus* and *Alnus*. Therefore, in Japan, there has been no report about the symbiotic relationship between the sclerotium of *P. umbellatus* and *Armillaria*.

Previously, the identification of filamentous fungi and mycorrhizal fungi has been difficult because of the absence of fruiting bodies during most of the year. Recently, DNA analysis has been applied to identify these fungi, and the identification of mycorrhizal fungi has been reported in some achlophyllous orchids (Taylor and Bruns 1997). Ribosomal DNA sequences, in particular the 5.8S rDNA and flanking internal transcribed spacer regions ITS1 and

ITS2, have been used to study the phylogenetic relationships for a number of plant pathogenic fungi (Jasalavich et al. 1995; Morales et al. 1995), including the genus *Armillaria* (Anderson and Stasovski 1992).

In this study, the sclerotia of *P. umbellatus* and sclerotia-adhering-fungi were collected from six sites in Japan and China; sclerotia-adhering fungi were isolated from the sclerotia. The isolated fungi were identified via culture morphology on potato dextrose agar (PDA) medium and the nuclear ribosomal DNA ITS region sequence.

Materials and methods

Collection of *Polyporus umbellatus*

The sclerotial samples of *P. umbellatus* were collected from six sites (Table 1). The identification of these sclerotia was based on morphological characteristics of basidiomata and sclerotia (Fig. 1a,b) (Imazeki and Hongo 1989). The surface of sclerotia collected in all locations that were attached to and penetrated by rhizomorphs were considered as symbiotic fungi (Fig. 2). These sclerotia were distributed in the soil at depths of 10–15 cm, where many rhizomorphs of symbiotic fungi were distributed. In many cases, these rhizomorphs were connected with a broad-leaved tree. The host tree of symbiotic fungi was determined by tracing the rhizomorphs. Rhizomorphs of symbiotic fungi was observed within sclerotia tissues (Fig. 3).

Isolation

Unidentified symbionts of each *P. umbellatus* collection were isolated on PDA medium, either from hyphae taken from the tissues of the sclerotia or from rhizomorphs attached to the surface of the sclerotia. Stock cultures of these vegetative diploid isolates were maintained on PDA medium in Petri dishes at 25°C in the dark.

DNA isolation and amplification

Total DNA was extracted from isolated and cultivated symbionts using a DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions.

Polymerase chain reaction (PCR) amplifications were achieved using universal primers for ribosomal DNA regions (White et al. 1990). For ITS1 and ITS2 regions, primer sets ITS5 and ITS2, and ITS3 and ITS4, were used, respectively. We used the following thermocycle protocol: (94°C, 2 min) × 1 cycle, (94°C, 1 min; 48°C, 2 min; 72°C, 3 min) × 30 cycles, and (72°C, 15 min) × 1 cycle. The PCR reaction mixture consisted of 10× gene-Taq Buffer (Nippon Gene), 5 µl; dNTP mix (Nippon Gene), 4 µl; forward primer (10 pmol/µl), 1 µl; reverse primer (10 pmol/µl), 1 µl; gene-Taq (Nippon Gene), 0.25 µl; dimethyl sulfoxide (DMSO), 5 µl; double-distilled water (DDW), 32.5 µl; and template DNA, 1.25 µl.

PCR products were separated from other by-products using 2% TAE [Tris-acetate-ethylenediaminetetraacetic acid (EDTA)] agarose gel electrophoresis. The desired bands were cut out and purified using a GFX PCR DNA and Gel Band Purification Kit (Amersham Biotech).

Sequencing the PCR products

We sequenced the purified PCR products using the BigDye Terminator ver. 3.1 Cycle Sequencing Kit and Model 3100 automated sequencer (Applied Biosystems), following the manufacturer's instructions. For sequencing, we used the same primers as those used for amplification. The sequences were aligned manually. When ambiguous (additive) sites were found, they were coded according to the IUPAC (IUB) codes.

Homology analysis

All the obtained sequences were subjected to the BLAST search service of GenBank, and analogous data were obtained. ITS sequence data of *A. calvescens*, *A. cepistipes*, *A. sinapina*, *A. gallica*, and *A. nabsnona* were obtained from DDBJ/EMBL/GenBank (see Table 1). For all the sequenced data and obtained data, multiple sequence alignment was carried out using Clustal X ver. 1.6 (Thompson et al. 1997) and manually adjusted by inserting gaps.

Phylogenetic analysis

Phylogenetic relationships were analyzed using the neighbor-joining (NJ) method (Saitou and Nei 1987) using PAUP* 4.0b10 (Swofford 2004), and the topology was tested with 1,000 bootstrap trials (Felsenstein 1985). All characters were weighted equally. Indels and ambiguous states were equally treated with four nucleotides as independent characters, respectively. For outgroups, we applied four *A. mellea* sequences (AM1-4), because Kim et al. (2006) estimated that this species was in a basal position of *Armillaria* based on large subunit sequences of nuclear ribosomal DNA.

Results

Morphological observations of *P. umbellatus*

At each collection site, rhizomorphs of symbionts were adhered to and penetrated by the rugged sclerotia of

Table 1 Isolate from *Polyporus umbellatus* and *Armillaria* species used in phylogenetic analysis

Species	Strain	Origin	Source tissue	Host	GenBank accession number
A. ostoyae	AO 1	NH, USA	Multisporous	Un	AY213552
A. ostoyae	AO 2	WA, USA	Basidioma	Un	AY213553
A. ostoyae	AO 3	ID, USA	Basidioma	Un	AY213554
A gemina	AG 1	NY, USA	Basidioma	Un	AY213555
A. gemina	AG 2	NY, USA	Basidioma	Un	AY213556
A. gemina	AG 3	NY, USA	Basidioma	Un	AY213557
A. gemina	AG 4	WV, USA	Unknown	Un	AY213558
A. calvescens	ACA 1	QC, Canada	Basidioma	Un	AY213559
A. calvescens	ACA 2	MI, USA	Basidioma	Un	AY213560
A. calvescens	ACA 3	MI, USA	Basidioma	Un	AY213561
A. calvescens	ACA 4	MI, USA	Basidioma	Un	AY213562
A. sinapina	ASI 1	BC, Can	Basidioma	Un	AY213563
A. sinapina	ASI 2	BC, Can	Basidioma	Un	AY213564
A. sinapina	ASI 3	WA, USA	Basidioma	Un	AY213565
A. sinapina	ASI 4	MI, USA	Multisporous	Un	AY213566
A. sinapina	ASI 5	MI, USA	Multisporous	Un	AY213567
A. mellea	AM 1	VA, USA	Multisporous	Un	AY213584
A. mellea	AM 2	VA, USA	Multisporous	Un	AY213585
A. mellea	AM 3	WI, USA	Basidioma	Un	AY213586
A. mellea	AM 4	NH, USA	Multisporous	Un	AY213587
A. gallica	AGA 1	BC, Canada	Basidioma	Un	AY213568
A. gallica	AGA 2	MI, USA	Basidioma	Un	AY213569
A. gallica	AGA 3	MI, USA	Basidioma	Un	AY213570
A. gallica	AGA 4	WI, USA	Basidioma	Un	AY213571
A. nabsnona	ANA 1	ID, USA	Basidioma	Un	AY213572
A. nabsnona	ANA 2	BC, Canada	Basidioma	Un	AY213573
A. nabsnona	ANA 3	AK, USA	Multisporous	Un	AY213574
A. cepistipes	ACE 1	BC, Canada	Basidioma	Un	AY213581
A. cepistipes	ACE 2	BC, Canada	Basidioma	Un	AY213582
A. cepistipes	ACE 3	WA, USA	Basidioma	Un	AY213583
A. tabescens	AT 1	SC, USA	Stalk	Un	AY213588
A. tabescens	AT 2	GA, USA	Basidioma	Un	AY213590
A. tabescens	AT 3	SC, USA	Unknown	Un	AY213589
Symbiont 1	ArHA 1	Takausutyou Asahikawa, Hokkaido, Japan	*P. umbellatus*	Un	AB300716
Symbiont 2	ArHH 1	Sanbusigaiti Hurano, Aza Hokkaido, Japan	*P. umbellatus*	*Betula ermanii*	AB300717
Symbiont 3	ArSF 1	Fujinomiya city, Shizuoka Pref., Japan	*P. umbellatus*	*Zelkova serrata*	AB300718
Symbiont 4	ArST 1	Shaanxi Prov., China	*P. umbellatus*	*Quercus* sp.	AB300719
Symbiont 5	ArSB 1	Shaanxi Prov., China	*P. umbellatus*	*Quercus* sp.	AB300720
Symbiont 6	ArHe 1	Henan Prov., China	*P. umbellatus*	Un	AB300721

Host tree was determined by tracing *Armillaria* rhizomorphal connections between *Polyporus umbellatus* and roots of tree

Un unidentified broad-leaved trees

P. umbellatus (Figs. 2, 3). When the sclerotium of *P. umbellatus* was cross sectioned, the internal part of sclerotium was cream colored. Black regions surrounding the invading rhizomorphs of symbionts into the surface of sclerotium were observed. The surrounding zone contained string-like, gelatinous masses composed of hyphae, and the outside of the region was brown in color (Fig. 3).

Isolation of symbionts

Figure 4 shows surface views of the colony morphology of the six symbionts isolated from sclerotia of *P. umbellatus* and rhizomorphs. All isolates were similar in colony morphology and grew well on PDA medium with well-developed rhizomorphs. All the isolates were characterized

Fig. 1 Basidiomata of *Polyporus umbellatus* arising from sclerotium collected from Fujinomiya, Shizuoka Pref., Japan. **a** The basidioma consists of many small, pale smoky brown, roundish caps. Individual caps 1–4 cm, circular. **b** The undersurfaces of basidiomata are white, and the individual branches are fused together into one solid structure. *Bar* 10 mm

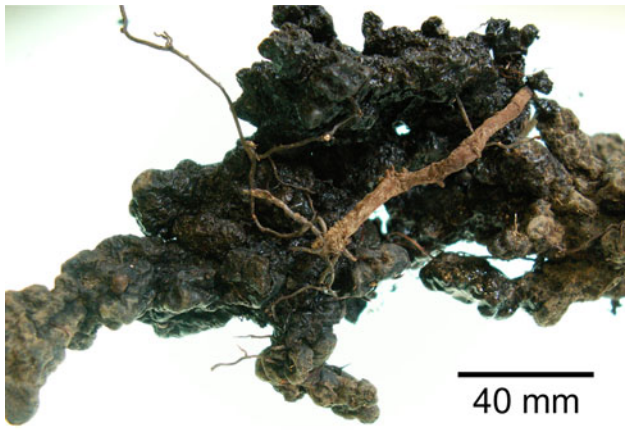

Fig. 2 The sclerotium of *Polyporus umbellatus* collected from Fujinomiya, Shizuoka, Pref., Japan. The surface of the sclerotium was rugged and attached to and penetrated by rhizomorphs

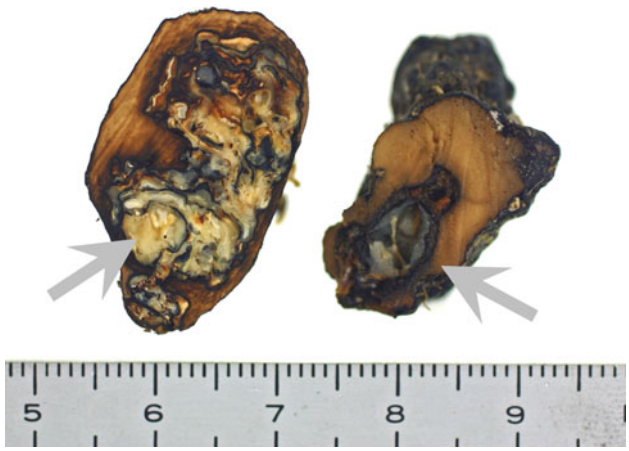

Fig. 3 The internal part of the sclerotium has many black regions surrounding the invading rhizomorphs of symbionts (*arrows*). The surrounding zone contained string-like gelatinous masses composed of hyphae, and the outside region was brown in color

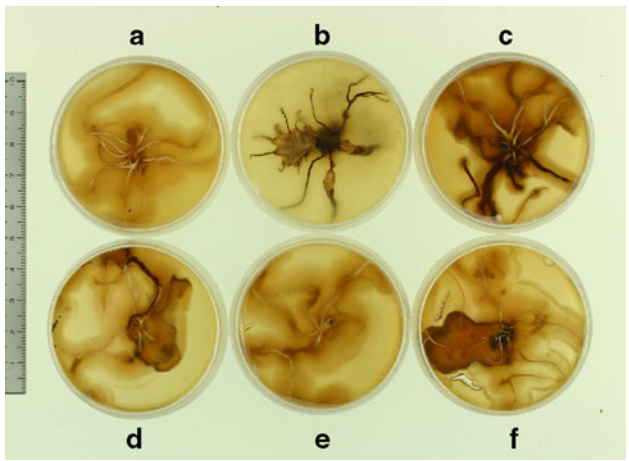

Fig. 4 Symbionts of *Polyporus umbellatus* isolated from the internal part of sclerotia and rhizomorphs. Six symbionts were incubated on potato dextrose agar (PDA) medium at 25°C. All isolates developed rhizomorphs and dark brownish crustose hyphae. **a** ArHA 1 (Hokkaido, Japan). **b** ArHH 1 (Hokkaido, Japan). **c** ArSF 1 (Shizuoka Pref., Japan). **d** ArST 1 (Shaanxi, China). **e** ArSB 1 (Shaanxi, China). **f** ArHe 1 (Henan, China)

by whitish aerial hyphae and long cylindrical rhizomorphs; these showed typical morphology of *Armillaria*.

Fungal isolation and molecular identification

All the fungal isolates were examined for DNA sequence. The sequence data of the ITS region were obtained from the six isolates. The DNA sequences were deposited in DDBJ/EMBL/GenBank nucleotide databases (see Table 1).

BLAST searches showed that the six sequences obtained in this study were analogous to *Armillaria* in Tricholomataceae. ITS sequence data for various *Armillaria* species were obtained from GenBank. Among these ITS sequences of five *Armillaria* species named *A. calvescens*, *A. sinapina*, *A. gallica*, and *A. cepistipes* in North America (Kim et al. 2006) and the six sequences obtained in this study, nucleotide substitutions were observed in the ITS1 and ITS2 regions, respectively. ITS1 showed less variation than ITS2. In contrast, there were no variations in the regions coding for the 5.8S rDNAs.

Most of the genes of this study contained unique sequence types. Sequencing of the rDNA ITS region showed that all the fungal isolates of *P. umbellatus* formed a monophyletic clade.

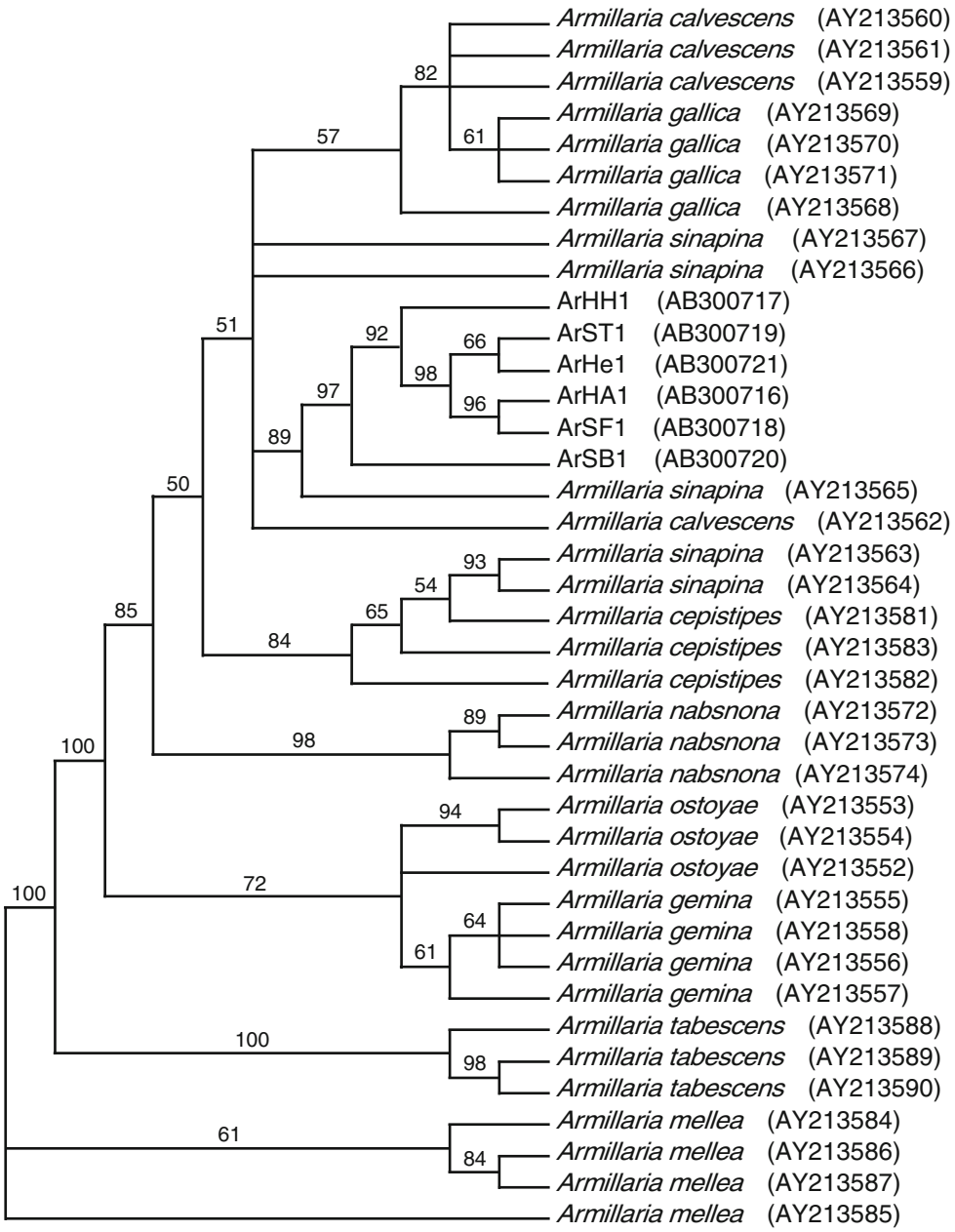

Fig. 5 Neighbor-joining phylogenetic tree showing relationship between symbionts isolated from *P. umbellatus* and North American *Armillaria* species based on DNA sequences of internal transcribed spacer 1, 2, and 5.8S rDNA. *A. mellea* (AY213581, AY213583) is applied as outgroup species. Bootstrap support values more than 50% from 1,000 replicates are shown *above* branches of clades. Accession numbers of GenBank nucleotide database are given for all sequences

All the symbionts of *P. umbellatus* analyzed in this study formed a clade with one of five *A. sinapina* sequences (AS3) with a high bootstrap value (89%). However, *A. sinapina* was paraphyletic and formed a clade with *A. calvescens*, *A. gallica*, and *A. cepistipes* with low bootstrap support (50%). On the other hand, the symbionts were included in a comparatively highly supported clade (85%) with *A. nabsnona* in addition to the former four species. For this clade, *A. ostoyae* and *A. gemina* formed a distinct clade and *A. tabescens* placed in the basal position of *Armillaria* species examined in this study except *A. mellea* (Fig. 5).

Discussion

Symbiotic association of *A. mellea* complex has been reported in some achlophyllous orchids. *Gastrodia elata* is associated with *A. ostoyae*, *A. gallica*, *A. jezoensis*, *A. sinapina*, and *A. singula*. Among them, *A. gallica* was the most common in Hokkaido, Japan (Cha and Igarashi 1995). This species is well known as a saprobe and produces abundant rhizomorphs in soil (Roll-Hansen 1985). Terashita and Chuman (1989) recognized five biological species (*A. borealis*, *A. gallica*, *A. cepistipes*, *A. mellea*, and *A. tabescens*) from *Gaelola septentrionalis*, an achlorophyllos

orchid, whereas Ota et al. (1998) pointed that the *A. borealis* was misidentified with *A. mellea*.

The association of the *A. mellea* complex with *Entoloma abortivum* (Berk. et Curt.) Donk. has been reported by Watling (1974). Watling considered that rhizomorphs of *A. mellea* complex invade the developing basidiomata of *E. abortivum*. However Czederpiltz et al. (2001) suggested that *Armillaria* basidiomata were parasitized by *E. abortivum*. *Armillaria* isolates from carpophoroids of *E. abortivum* and sclerotia of two *Wynnea* species (*W. americana* Thaxter and *Wynnea gigantea* Berk. et Curt.) in Japan were identified using PCR-restriction fragment length polymorphism (RFLP) analysis of the intergenic region (IGR) of ribosomal DNA (Fukuda et al. 2003). In the *Armillaria–E. abortivum* and *Armillaria–Wynnea* association, it is uncertain what kind of relationship exists between them.

Xu et al. (2003) reported that, in China, only in symbiosis with *Armillariella mellea* can the sclerotium of *P. umbellatus* continue its growth. This study showed that all the sclerotial samples of *P. umbellatus* collected in Japan and China were adhered to and penetrated by rhizomorphs of *Armillaria* species. Therefore, *P. umbellatus* might be parasitic in *Armillaria* species because actively growing sclerotia of *P. umbellatus* are always present with *Armillaria* rhizomorphs in the field and there were no *P. umbellatus* sclerotia putrefied by *Armillaria* in our observation. The fungal growth rate of *P. umbellatus* was low on PDA medium (data not shown). The rhizomorph of *Armillaria* might have some relationship in the growth of *P. umbellatus*.

Armillaria species are widely distributed all over the world and are well known as a cause of root rot (Gibson 1960; Kable 1974; Wargo and Shaw 1985). Previous studies have shown that this genus is composed of several biological species in North America (Anderson and Ullrich 1979), Europe (Korhonen 1978), and Australia (Kile and Watling 1983). In Japan, nine annulate species and one exannulate species have been clearly recognized (Ota et al. 1998). Mating tests are commonly used for the identification of biological species of *Armillaria*, pairing haploid tester strains with unknown haploid or diploid isolates (Guillaumin et al. 1991). However, the results of these tests are often difficult to interpret.

Based on the molecular phylogenetic analysis, this study showed that all the symbionts of *P. umbellatus* were found to belong to *Armillaria*. Among the *Armillaria* species, five species (*A. sinapina*, *A. calvescens*, *A. gallica*, *A. cepistipes*, and *A. nabsnona*) formed a highly supported clade with the symbiont. The five species did not form distinct clades, respectively, except *A. nabsnona*. Anderson and Stasovski (1992) and Kim et al. (2006) also showed their close phylogenetic relationships.

Therefore, we suspended the taxonomic attribution of the symbionts of *P. umbellatus* analyzed in this study at species rank. On the other hand, these symbionts probably belonged to neither *A. mellea*, *A. gemina*, *A. tabescens*, nor *A. ostoyae* because they were discriminated phylogenetically in our analysis.

In conclusion, our study indicated that all the sclerotial samples of *P. umbellatus* collected in the field were adhered to and penetrated by rhizomorphs of *Armillaria* species. We identified the rhizomorphs by plate culture morphology on PDA medium and on the basis of the molecular phylogenetic analysis.

Acknowledgments We are grateful to Dr. Y. Ota (Forestry and Forest Products Research Institute) for valuable information and supplying literature; to Dr. S. Isoda (Showa University) for taking picture of *P. umbellatus*. We also thank Dr. M. Okada (The Kochi Prefectural Makino Botanical Garden), Prof. S. Terabayashi (Yokohama College of Pharmacy), Prof. A. Suzuki (Chiba University), and members of Botanical Raw Materials Division, Tsumura & Co., for useful information and their suggestions.

References

Anderson JB, Stasovski E (1992) Molecular phylogeny of Northern Hemisphere species of *Armillaria*. Mycologia 84:505–516

Anderson JB, Ullrich RC (1979) Biological species of *Armillaria mellea* in North America. Mycologia 71:402–414

Cha JY, Igarashi T (1995) *Armillaria* species associated with *Gastrodia elata* in Japan. Eur J For Pathol 25:319–326

Chen SY, Chen HY (2000) Collection of mushroom prescriptions. Shanghai Science and Technology Literatures Press, Shanghai, pp 315–327

Czederpiltz DLL, Volk TJ, Burdsall HH (2001) Field observations and inoculation experiments to determine the nature of the carpophoroids associated with *Entoloma abortivum* and *Armillaria*. Mycologia 93:841–851

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

Fukuda M, Nakashima E, Hayashi K, Nagasawa E (2003) Identification of the biological species of *Armillaria* associated with *Wynnea* and *Entoloma abortivum* using PCR-RFLP analysis of the intergenic region (IGR) of ribosomal DNA. Mycol Res 107:1435–1441

Gibson IAS (1960) *Armillaria* root rot in Kenya pine plantations. Commw For Rev 39:94–99

Guillaumin JJ, Anderson JB, Korhonen K (1991) Life cycle, interfertility, and biological species. In: Show CG, Kile GA (eds) *Armillaria* root disease. Agriculture Handbook, No. 691. USDA. Forest Service, Washington, DC, pp 10–20

Imazeki R, Hongo T (1965) Coloured illustrations of fungi of Japan, vol 2 (in Japanese). Hoikusha, Osaka, p 134

Imazeki R, Hongo T (1989) Colored illustrations of mushrooms of Japan, vol 2 (in Japanese). Hoikusha, Osaka, p 139

Jasalavich CA, Morales VM, Pelcher LE, Seguin-Swartz G (1995) Comparison of nuclear ribosomal DNA sequences from *Alternaria* species pathogenic to crucifers. Mycol Res 99:604–614

Kable PF (1974) Spread of *Armillariella* sp. in a peach orchard. Trans Br Mycol Soc 62:89–98

Kile GA, Watling R (1983) *Armillaria* species from south-eastern Australia. Trans Br Mycol Soc 81:129–140

Kim MS, Klopfenstein NB, Hanna JW, McDonald GI (2006) Characterization of North American *Armillaria* species: genetic relationships determined by ribosomal DNA sequence and AFLP markers. For Pathol 36:145–164

Korhonen K (1978) Interfertility and clonal size in the *Armillaria mellea* complex. Karstenia 18:31–42

Lee JY (1988) Coloured Korean mushrooms. Academy Press, Seoul, p 258

Morales VM, Jasalavich CA, Pelcher LE, Petrie GA, Taylor JL (1995) Phylogenetic relationship among several *Leptosphaeria* species based on their ribosomal DNA sequences. Mycol Res 99:593–603

Ohta K, Yaoita Y, Matsuda N, Kikuchi M (1996) Sterol constituents from the sclerotium of *Polyporus umbellatus* Fries. Nat Med 50:179–181

Ota Y, Matsushita N, Nagasawa E, Terashita T, Fukuda K, Suzuki K (1998) Biological species of *Armillaria* in Japan. Plant Dis 82:537–543

Roll-Hansen F (1985) *Armillaria* species in Europe. Eur J For Pathol 15:22–31

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Stamets P (2000) Growing Gourmet and Medicinal Mushrooms, 3rd edn. Ten Speed Press, Berkeley

Swofford DL (2004) PAUP: phylogenetic analysis using parsimony (and other methods), version 4. Sinauer Associates, Sunderland

Taylor DL, Bruns TD (1997) Independent, specialized invasions of ectomycorrhizal mutualism by two nonphotosynthetic orchids. Proc Natl Acad Sci USA 94:4510–4515

Terashita T, Chuman S (1989) *Armillaria* species isolated from the wild orchid, *Galeola septentrionalis*. In: Proceedings of the 7th international conference on root and butt rots, vernon and Victoria, BC, Canada, 9–16 August 1988, pp 364–370

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876–4882

Wargo PM, Shaw CG (1985) *Armillaria* root rot: the puzzle is being solved. Plant Dis 69:826–832

Watling R (1974) Dimophism in *Entoloma arbortivun*. Bull Mens Soc Linn Lyon 43:449–470

Wei Q, Wu GL, Nei JC (1983) Effect of *Polyporus umbellatus* polysaccharides on glycogen storage, enzyme activities in glyconeogenesis and glycogenolysis of liver in mice bearing hepatoma H22. Zhongguo Yao Li Xue Bao 4:141–143

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Snisky JJ, White TJ (eds) PCR protocols. Academic Press, London, pp 315–322

Xu G, Fu W, Zhao X (2003) Advances in studies on *Polyporus umbellatus* in China. J Fungal Res 1:58–61

Yao L, Cheng H, Yang Z (2006) Guidelines for good agricultural practice of Chinese crude drugs (in Chinese). China Agricultural Press, Beijing, pp 1213–1222