

Studies on Korean Species of *Armillaria*

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한국산 병나무버섯균의 종에 관한 연구

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ABSTRACT: One hundred and ninety two isolates of *Armillaria* were obtained from mycelial fans on infected hosts, rhizomorphs, and single basidiospores or trauma tissue of fruiting bodies. Mating tests showed that two of these isolates were *A. mellea*, eight were *A. tabescens*, 20 were *A. ostoyae*, and 162 were *A. gallica*. *Armillaria ostoyae* was mainly isolated from *Pinus koraiensis* and *Quercus spp.*, *A. tabescens* from fruiting bodies on *Pinus densiflora* and *Quercus spp.*, and *A. gallica* from many tree species but not *Pinus koraiensis*. *Armillaria mellea*, *A. gallica*, *A. ostoyae* and *A. tabescens* showed distinct protein banding patterns. Mycelial growth and rhizomorph formation was good on basal medium with ethanol added. *A. gallica* and *A. mellea* formed many rhizomorphs, but *A. ostoyae* did not. *A. gallica* showed the best rhizomorph formation on media with tannic acid and ethanol, but *A. mellea* formed the most rhizomorphs on gallic acid. Rhizomorphs showed monopodial branching for *A. gallica* and dichotomous branching for *A. ostoyae*. Fruiting bodies formed in the laboratory on sawdust media most abundantly by *A. tabescens*. In nature, fruit body formation by *A. tabescens* was from early to mid August. *A. ostoyae* and *A. gallica* fruit bodies were formed from early August to late October. While there are common names in Korea for *A. mellea* and *A. tabescens*, such as mulberry mushroom and mulberry mushroom relative, no common names are available for *A. gallica* and *A. ostoyae*. Therefore, we refer to *A. gallica* as the *Gastrodia* mushroom because it has been used to produce *Gastrodia* and *A. ostoyae* as the Korean pine mushroom because it is frequently found as mushrooms on Korean pine.

Key words: *Armillaria mellea*, *A. ostoyae*, *A. gallica*, *A. tabescens*, *Pinus koraiensis*, Protein banding pattern.

Although members of the genus *Armillaria* cause root rot of coniferous trees, especially *Pinus koraiensis*, these fungal species are very useful because of their symbiotic relationship with *Gastrodia* and as edible mushrooms (fruiting bodies). Vahl (34) described *Agaricus melleus*, and the species was recognized in the tribe *Armillaria* by Fries (5). *Armillaria* was recognized at the genus level by Kummer (10), and *A. mellea* (Vahl:Fr.) Kummer is now recognized as the type species. Later, Karsten (8) erected the genus *Armillariella*, also with *A. mellea* as the type

species. *Armillaria* is the preferred genus name.

Species of *Armillaria* have been characterized by their fruiting bodies (17, 27), cultural characteristics (20, 21, 22), pathogenicity (16, 21, 28, 35), rhizomorphs, mating tests with testers (2, 3, 7) and protein banding patterns (11, 18). About 36 species, including 7 species in Europe (7, 26), nine species in North America (3, 15) and three species in Australia (8) were recorded.

The importance of this genus has increased in such oriental countries as Korea, China and Japan with the use of *Armillaria* to artificially produce *Gastrodia* through a symbiotic relationship between the

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plant and the fungus. Also, damage to *Pinus koraiensis* by *Armillaria* has become a serious problem. Therefore, research was needed to identify the *Armillaria* species present in Korea. This study was conducted to identify the species by mating studies, describe their morphology of fruiting bodies and rhizomorphs, and to contrast the protein banding patterns of the Korean species.

MATERIALS AND METHODS

Isolation of *Armillaria* species and morphological characteristics of fruiting bodies. Isolates of *Armillaria* species were established from fruiting bodies, mycelial fans or rhizomorphs on infected hosts. Haploid mycelium was isolated from single basidiospores and diploid mycelium was isolated from the flesh of stipes or infected plant tissues. Fruiting bodies were collected mostly from August to October when they were occurring in nature. When collecting fruiting bodies, we kept records of collecting dates, locations, hosts and existence of rhizomorphs or mycelial fans. Fruiting bodies were described using the criteria of Singer (29). Photographs of fresh specimens were taken, and dried specimens were stored.

Classification of Korean biological species using tester strains. Mating tests were carried out using tester strains (B274, B286, B474) in the collection of T. C. Harrington (USA) and tester strain E22 from Kari Korhonen in Finland. Haploid x haploid or diploid x haploid pairings were made between haploid isolates or diploid isolates from Korea and haploid tester strains placed approximately 5~10 mm apart on MEA medium (1.25% malt extract, 1.5% agar, distilled water 1000 ml) and incubated for five weeks at 25°C. The pairings were interpreted as compatible or incompatible on the basis of the macroscopic mycelial appearance of the tester strain. After 20 days incubation at 25°C, subcultures were obtained from the contact region in haploid x haploid matings; or the edge of haploid mycelia in diploid x haploid matings. fluffy aerial mycelium was indicative of different species, whereas flat crustose mycelium was indicative that the mated mycelia were of the same species.

Classification of the genus *Armillaria* using protein banding pattern. Isolates cultured on MEA medium were inoculated into liquid medium and incubated.

Harvested mycelium was washed with distilled water, excess water was removed and the mycelium ground with liquid nitrogen. Mycelial proteins were extracted in 1.0 ml of buffer (0.06 M Tris-HCl at pH 6.8) soaking overnight at 4°C, and then centrifuging the slurry at 15000 x g for 15 mins. The supernatants were collected and used for protein electrophoresis. Seventy microliter of the sample (30 µl protein mixed with 30 µl homogeneity buffer and 10 µl dye solution) were subjected to electrophoresis in a 12% polyacrylamide gel until the tracking dye had travelled to a point 0.5 cm from the bottom of the gel. Current conditions were 16 mA for the stacking gel and 24 mA for the main gel, and the run time was about 5 hours. After electrophoresis, the gel was stained overnight in 0.25% Coomassie blue solution containing water (50%), acetic acid (10%) and methanol (40%). The gel was destained with 7% acetic acid and 5% methanol in water until the solution became clear. Polymorphism in protein bands among the species was expressed as binomial matrix codes, and interspecies similarity was calculated as similarity coefficients as calculated by Dice (1973). Similar species were clustered using UP-GMA (Unweighted Pair Group Method using Arithmetic means; Ludwig and Reynolds, 1988). The computer program Numerical Taxonomy System using Multi-variat Statistical Program was used for analysis.

Rhizomorph formation. Identified isolates were inoculated on oak stem pieces and buried in the ground. Growth of rhizomorphs and formation of fruiting bodies were later observed. Each isolate was also inoculated onto small oak pieces, 5 cm×10 cm, and incubated in the sand box for one year at 25°C. After that, rhizomorph formation and branching patterns were noted.

Fruiting body formation of the isolates. Identified isolates were inoculated onto 4% MEA medium with small oak pieces in 250 ml flasks. They were cultured at 23°C until the each medium was colonized and moved to the incubator at 18°C to induce fruiting body formation.

RESULTS

Isolation of *Armillaria* species and morphological characteristics of fruiting bodies. One hundred and ninety two isolates of *Armillaria* were obtained from

Table 1. Mating tests of Korean isolates of *Armillaria* species from Kangwon province with tester strains

Species	Representative	Tester				No. of determined species/ No. of total tested
		274(M)	286(O)	474(G)	E22(T)	
<i>A. mellea</i>	KNU-A5	+	-	-	-	2/192
<i>A. ostoyae</i>	KNU-A130	-	+	-	-	20/192
<i>A. gallica</i>	KNU-A75	-	-	+	-	162/192
<i>A. tabescens</i>	KNU-A901	-	-	-	+	7/192

Tester: M=*A. mellea*, O=*A. ostoyae*, G=*A. gallica*, T=*A. tabescens*.

fruiting bodies, mycelial fans on infected hosts, rhizomorphs and single basidiospores. As a result of mating tests (Table 1), 2 isolates were identified as *A. mellea*. These two isolates were from land under cultivation of *Gastrodia*. No fruiting bodies were identified as *A. mellea*. Eight isolates of *A. tabescens* were isolated from fruiting bodies formed on *Acer ginnale* at Mt. Chiak, the stump of *Pinus densiflora* in Soakcho, and *Quercus spp.* in Kangwon Univ. Forest. Twenty isolates of *A. ostoyae* were mostly isolated from fruiting bodies formed on the ground near *Pinus koraiensis* trees that had been dead for 10 years. One hundred and sixty two isolates of *A. gallica* were isolated from fruiting bodies formed on the stumps of 14 host species including *Quercus spp.*

Fruiting bodies of *A. ostoyae* Herink (Fig. 1-1) formed on *Quercus spp.* in Chunchongun of Kangwon province were morphologically similar to the fruiting bodies formed on *Pinus koraiensis* (Fig. 1-2). The pileus, 27~52 mm broad, had a dry surface, was at first hemispherical, then convexed or expanded, scaly and colored greyish-yellow to brownish-yellow, and the margin was inrolled. The pileus tissue was 4~7 mm thick, white, and the taste and smell were mild. Lamellae were 12~16 mm×2 mm size and short decurrent, subclosed and white or creamy. The stipe was 70~120 mm long 5~12 mm thick, subequal or somewhat tapering toward the base, with a fibrillose surface, white or creamy to brown, dark-brown at the base, and covered with yellow scales. The partial veil was greyish-yellow and became a prominent annulus, colored dark-brown around the edge.

As Fig. 2 shows, spores of *A. ostoyae* were ellipsoid or short ellipsoid with a thin cell wall and 7.5~8 μm ×5.5~6.1 μm . The basidium was 37 μm ×9.5 μm , produced 4 spores, was club shaped and had a

clamp connection at the base. No pleurocystidia existed, and cheilocystidia of 15~19 μm ×5~9 μm were fusoid or fusoid-ventricose, sharp-pointed, bent or rounded at the end, with a thin cell wall and scattered. Cells comprising the scales of the pileus surface were 1~24 μm thick, cylindrical or somewhat expanded, with thick cell walls but no clamp connections.

Fruiting bodies of *A. gallica* were formed on *Alnus japonica* in Kangwon University (Fig. 1-3). The pileus was 32~70 mm broad, having a dry or hygroscopic surface, at first hemispherical, then convex or expanded, scaly and colored brown to brownish-yellow, and the margin was at first inrolled. The pileus tissue was white, and the taste and smell were mild. Lamellae were 16 mm×5 mm, short decurrent, subclosed and greyish-yellow. The stipe was 70~80 mm×4~8 mm subequal or clavate sometimes bent and brownish-yellow to greyish-yellow. The partial veil was a cobweb and became barely discernable.

As Fig. 3 shows spores of *A. gallica*, which were ellipsoid to egg shaped, 8~9.5 μm ×5.6~6 μm with a thin cell wall. The basidium produced 4 spores and formed a clamp connection at the base. No pleurocystidia existed, and cheilocystidia of 17~31 μm ×5.4~9.5 μm were ninepin-shaped to fusoid, mucronate, elongated at the end, somewhat bent, with a thin cell wall, and a clamp connection was formed at the base. Scales of the surface were 10.5~28 μm and cylindrical to saccate.

Fruiting bodies of another form of *A. gallica* were collected from *Quercus spp.* (Fig. 1-4) at Dongsanmyun in Chunchongun. The pileus was 28~32 mm broad, having a dry or hygroscopic surface, at first hemispherical, then convex or expanded, greyish-yellow, and the margin was inrolled. Lamellae were 16 mm×4 mm, short decurrent, subclosed, yellowish-white, and sometimes brown spots appeared.

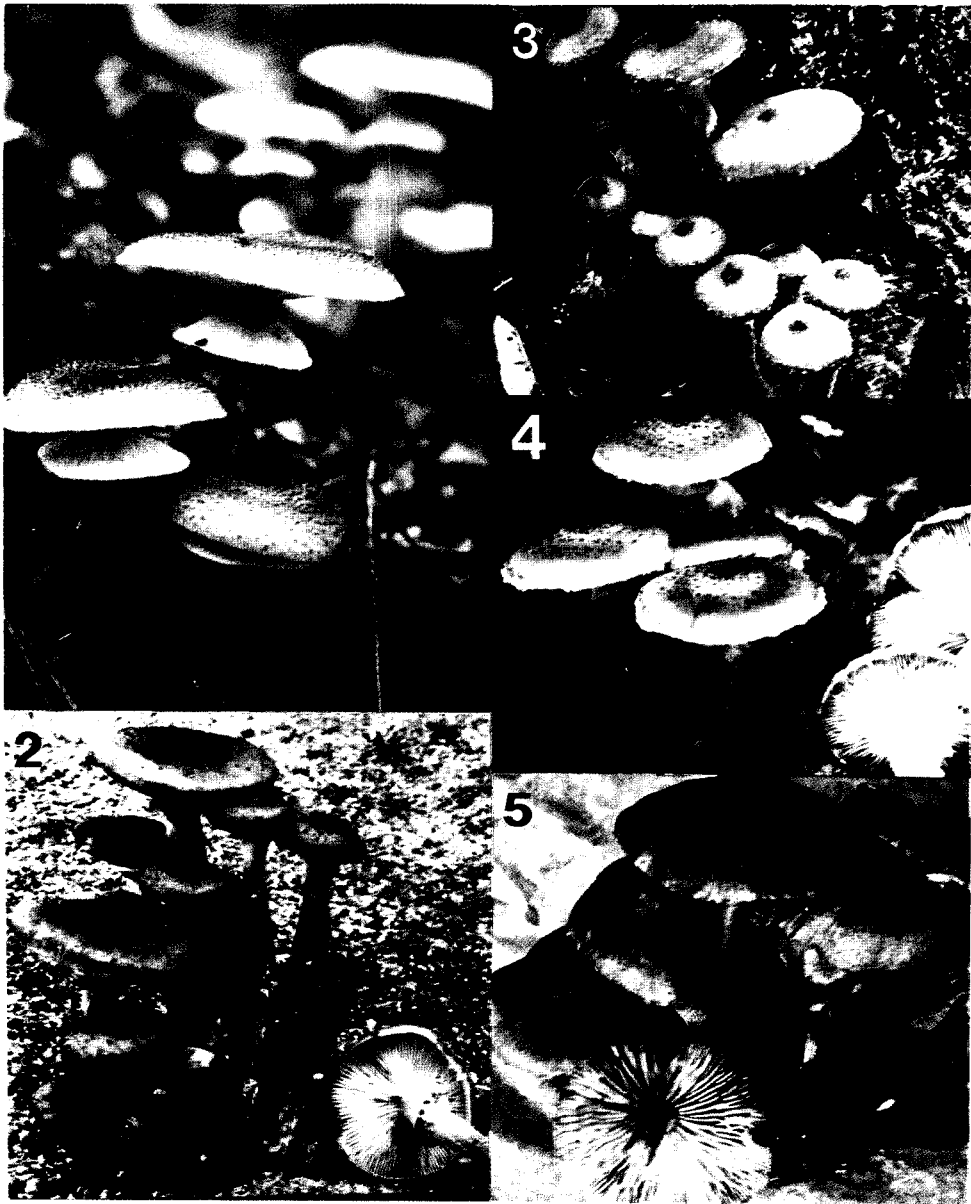


Fig. 1. 1. Fruit bodies of *Armillaria ostoyae* on *Quercus variabilis*. 2. Fruit bodies of *Armillaria ostoyae* on *Pinus koraiensis*. 3. Fruit bodies of *Armillaria gallica* on *Alnus japonica*. 4. Fruit bodies of *Armillaria gallica* on *Quercus variabilis*. 5. Fruit bodies of *Armillaria tabescens* on *Pinus densiflora*.

The pileus tissue was white, and the taste and smell were mild. The stipe of 50~60 mm×4~5 mm was subequal to clavate, fibrillose surface, and light-yellow to light-brown. The partial veil was cobwebby and became an annulus that quickly disappeared.

As Fig. 4 shows, spores of this form of *A. gallica* were ellipsoid to egg shaped with a thin cell wall,

8.7~9.6 μm×5.7~7.3 μm. The basidium, 38~43 μm×5.7~6 μm in size, produced 4 spores, and had a clamp connection at the base. No pleurocystidia existed, and cheilocystidia were 12.3~34 μm×8.1~12 μm, club shaped or spindle shaped, with a thin cell wall and a clamp connection. Scales of the pileus surface were 52~73 μm×10.5~21 μm, cylindri-

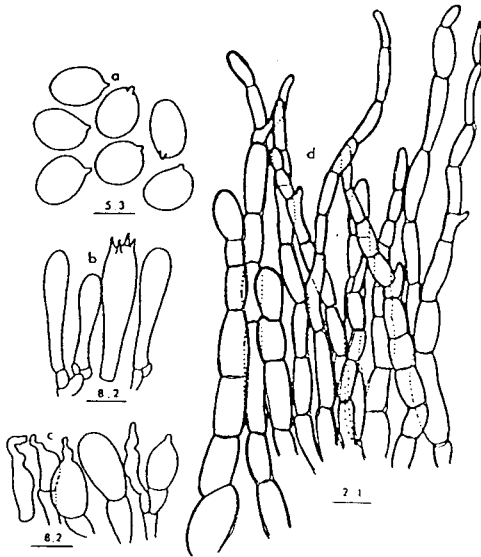


Fig. 2. *Armillaria ostoyae*.

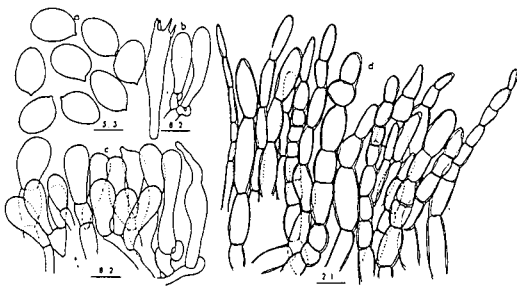


Fig. 3. *Armillaria gallica*.

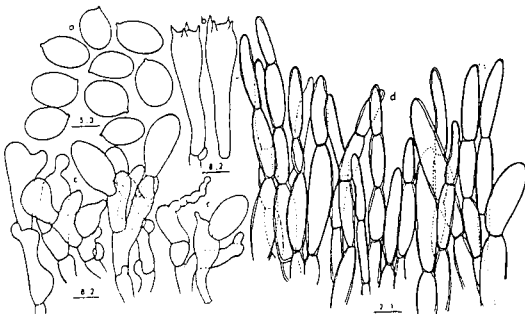


Fig. 4. *Armillaria gallica*.

cal, egg shaped or spindle shaped, with a thick cell wall.

Fruiting bodies of *A. tabescens* were collected from the stump of *Pinus densiflora* in Soakcho (Fig. 1-5). The pileus was 5~10 cm broad, at first flat, then

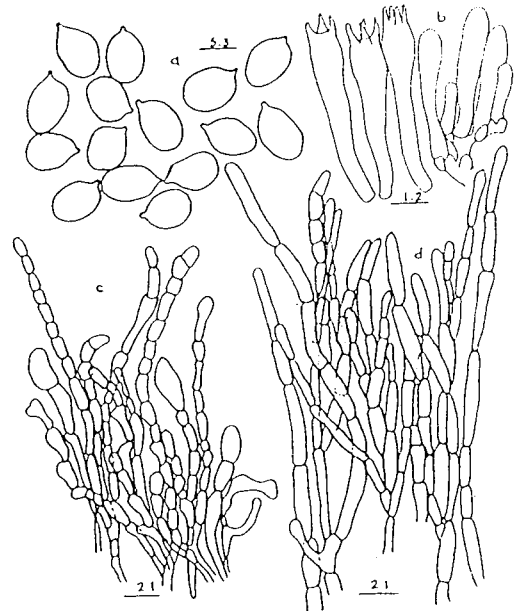


Fig. 5. *Armillaria tabescens*: a. basidiospores, b. basidia, c. cheilocystidia, d. hairs of pileal surface.

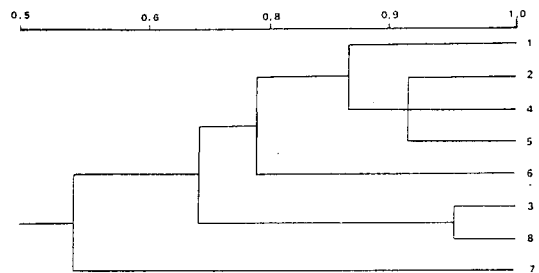


Fig. 6. Results of UPGMA analysis of *Armillaria* species using protein banding pattern. 1. KNU-A110 (*A. gallica*), 2. KNU-A75 (*A. gallica*), 3. KNU-A2 (*A. mellea*), 4. KNU-A993 (*A. gallica*), 5. KNU-A995 (*A. gallica*), 6. KNU-A130 (*A. ostoyae*), 7. KNU-A901 (*A. tabescens*), 8. KNU-A919 (*A. mellea*).

flat to depressed, fibrillose scales in the middle, and colored brownish-yellow to light-brown with radiating lines. Decurrent lamellae were at first white, then brown. The stipe was 50~70 mm×4~16 mm, subequal, slightly twisted, fibrillose surface without lamellae, and the upper part of the stipe turned to brown gradually when mature. Rhizomorphs were formed. Spores of *A. tabescens* were 7.0~9.0 μm×4.0~6.0 μm, white to light-yellow, and ellipsoid (Fig. 5).

Table 2. Mycelial dry weight and rhizomorph formation in liquid media (2% malt) of *Armillaria* species after 4 weeks

Species	Mycelial dry weight	Formation of rhizomorph
<i>A. ostoyae</i> (KNU-A906)	1.82 g	—
<i>A. ostoyae</i> (KNU-A130)	0.92 g	+
<i>A. gallica</i> (KNU-A75)	1.24 g	+++
<i>A. mellea</i> (KNU-A2)	1.40 g	+++
<i>A. mellea</i> (KNU-A919)	1.23 g	+++
<i>A. tabescens</i> (KNU-A901)	0.70 g	—

Classification of the genus *Armillaria* using protein banding patterns. Results of UPGMA analysis using protein banding patterns allowed differentiation of the four groups (Fig. 6). KNU-A110, KNU-A75, KNU-A993 and KNU-A995 had a similarity coefficient of 0.875, whereas KNU-A75, KNU-A993 and KNU-A995 had a similarity coefficient of 0.925. Four isolates mentioned above had a similarity coefficient with KNU-A130 of 0.775, KNU-A2 and KNU-A919 of 0.675 and KNU-A901 of 0.525. Therefore the protein banding patterns clustered the Korean species into four groups: *A. gallica*, *A. ostoyae*, *A. mellea* and *A. tabescens*.

The effect of environment on rhizomorph growth.

Mycelial growth of the identified isolates was observed on 50 ml of 2% ME liquid medium in 250 ml flask for four weeks. As Table 2 shows, *A. gallica* differed in mycelial weight and rhizomorph formation from the other species. Among *Armillaria* species, *A. gallica* showed the best mycelial growth rate, *A. ostoyae* was the second, and *A. tabescens* grew slowly. Rhizomorphs were formed in abundance by most *A. gallica* isolates except the KNU-A993. The growth habit of rhizomorphs on 2% MEA varied among the species: Some of them showed dichotomous branching and some of them formed small side branches while maintaining growth of a main tip (monopodial branching). In the ground, *A. gallica* formed monopodially branched rhizomorphs and *A. ostoyae* formed dichotomously branched rhizomorphs. When *Armillaria* isolates were grown on oak pieces and incubated in sand at 25°C for six months, rhizomorphs of *A. gallica* were the most abundant (Table 3).

Fruiting body formation of the isolates. Among our isolates, *A. tabescens* showed the best fruiting

Table 3. Formation of rhizomorphs on *Quercus* wood inoculated and kept at 25°C for six months

Species	Formation of rhizomorph
<i>A. gallica</i> (KNU-A110)	++++
<i>A. gallica</i> (KNU-A75)	++
<i>A. ostoyae</i> (KNU-A130)	++
<i>A. ostoyae</i> (KNU-A906)	+
<i>A. mellea</i> (KNU-A919)	++
<i>A. tabescens</i> (KNU-A901)	—

Table 4. Season of fruiting body production by *Armillaria* spp.

Species	Season of formation	Host substrate
<i>A. gallica</i>	Sep. 4~Oct. 18	10 species including <i>Quercus</i> spp.
<i>A. ostoyae</i>	Sep. 4~Oct. 13	<i>Quercus</i> spp. <i>Pinus koraiensis</i>
<i>A. mellea</i>	Sep. 2~Nov. 10	<i>Quercus</i> spp. <i>Pinus densiflora</i>
<i>A. tabescens</i>	Aug. 2~Aug. 23	<i>Quercus</i> spp. <i>Pinus densiflora</i>

body formation, and it also formed fruiting bodies on MEA medium. Some isolates made by pairing singles basidiospore strains formed fruiting bodies, but some of them did not. *A. tabescens* formed fruiting bodies well on the sawdust medium. In nature, fruiting body formation of *A. tabescens* was in early August to mid August. *A. ostoyae* was collected in early September to early October. *A. mellea* formed fruiting bodies in early September to middle November in 1993.

DISCUSSION

The genus *Armillaria* is known in Korea for production of edible fruiting bodies and *Gastrodia*. Since the description of *A. mellea* by Vahl (34), many species of *Armillaria* have been shown to differ substantially in pathogenicity (16, 21, 28, 35), fruiting body morphology (12, 28) and cultural characteristics (15, 20, 21, 22). Previously, only *A. mellea* and *A. tabescens* have been recognized in Korea (9, 19, 30~33). In this study, we identified fruiting bodies of three *Armillaria* species in Korea: *A. ostoyae*, *A. gallica* and *A. tabescens* based on morphological or microscopic characteristics. *A. tabescens* fruiting bodies

were characterized by absence of annulus. Two isolates of *A. mellea* were obtained from sites where *Gastrodia* was being produced.

When *Armillaria* isolates were identified using tester strains, two isolates of *A. mellea*, seven isolates of *A. tabescens*, twenty isolates of *A. ostoyae* and one hundred and sixty two isolates of *A. gallica* were identified. *A. mellea* was found only through the mating tests and it is possible that it was introduced to Korea from Japan or some other country with *Gastrodia* production. Through analysis of protein banding pattern, four species of *Armillaria* (*A. mellea*, *A. ostoyae*, *A. tabescens* and *A. gallica*) were identified and these identifications agreed with the mating tests.

Rhizomorphs were divided into two types: monopodially branched for *A. gallica* and dichotomously branched for *A. ostoyae*, consistent for reports of these two species elsewhere. Reports of other countries showed similar results (13, 14, 23). *A. gallica* formed net-like rhizomorphs on the surface of the roots, but no attachment to the root bark was found (25). *A. gallica* was less pathogenic than *A. ostoyae* and showed better rhizomorph formation. In general, species of lower pathogenicity tend to produce more rhizomorphs and appear to obtain their nutrition from dead wood (24), and thus most of the collected rhizomorphs in nature were rhizomorphs of *A. gallica*. *A. tabescens* formed fruiting bodies in mid July, but other species formed fruiting bodies in mid September to late October.

A. ostoyae was isolated from mycelial fans of infected *Pinus koraiensis* and *A. gallica* was isolated from fruiting bodies formed on several kinds of dead trees, including *Quercus spp.* In Korea, *Armillaria* species associated with root disease were *A. ostoyae*, *A. mellea* and *A. tabescens*; *A. gallica* showed little pathogenicity to the trees. Therefore, use of *A. gallica* for production of *Gastrodia* in Korea, China and Japan should pose little problem for tree disease.

While there were Korean names for *A. mellea* and *A. tabescens*, such as mulberry mushroom and mulberry mushroom relatives, no common names are available for *A. gallica* and *A. ostoyae*. Therefore, we will refer to *A. gallica* as *Gastrodia* mushroom because it has been used to produce *Gastrodia* and *A. ostoyae* will be referred to as Korea pine mushroom because it forms many fruiting bodies on Korean pine.

要 約

자실체와 이병된 기주의 mycelial fan, 균사속, 자실체의 조직, 단포자 등에서 192개의 *Armillaria*속 균주가 분리되었으며 교배시험에 의하여 *A. mellea*가 2균주, *A. tabescens*는 7균주, *A. ostoyae*는 20균주, *A. gallica*는 162균주로 판명되었다. *A. ostoyae*는 주로 참나무와 잣나무에서 분리되었으며 *A. gallica*는 잣나무를 제외한 여러가지 수종에서 분리되었다. 단백질 band pattern을 이용하여 종분류를 한 결과 교배시험에서와 같이 *A. gallica*, *A. ostoyae*, *A. mellea*, *A. tabescens*로 분류할 수 있었다. 균사생장과 균사속형성은 ethanol을 처리한 기본배지에서 양호하였다. *A. gallica*와 *A. mellea*는 균사속을 잘형성한 반면 *A. ostoyae*는 균사속형성이 부진 하였다. *A. gallica*는 tannic과 ethanol을 처리한 배지에서 균사속 생장이 가장 우수 하였으며 *A. mellea*는 gallic을 처리한 배지에서 가장 우수 하였다. 균사속은 두가지 형태로 monopodial을 나타내는 것은 *A. gallica*이며 dichotomous를 나타낸것은 *A. ostoyae*로 나타났다. 실험실에서는 *A. tabescens*가 자실체를 가장 잘 형성 하였으며 톱밥배지에서의 자실체 형성에서도 *A. tabescens*가 가장 우수 하였다. 자연상태에서 자실체 형성은 *A. tabescens*가 8월 초순부터 8월 중순인 더운 날씨에 많이 형성하나 *A. ostoyae*와 *A. gallica*는 8월 초순에서 10월 하순경에 주로 형성하였으며 KNU-A 919의 자실체는 10월 하순에 주로 형성 하였다. *Armillaria*에 대한 한국명은 *A. mellea*는 뽕나무버섯, *A. tabescens*는 뽕나무버섯부치로 명명되었으나 *A. gallica*와 *A. ostoyae*는 아직 한국명이 없으므로 *A. gallica*는 천마를 재배하는데 사용되는 버섯이므로 천마버섯으로 *A. ostoyae*는 잣나무에 자실체를 다량으로 형성하므로 잣나무버섯으로 명명하고자 한다.

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