Mycorrhizal association of isolates from sporocarps and ectomycorrhizas with *Pinus densiflora* seedlings*

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Thirty-two isolates from sporocarps of 27 species of macromycetes, 43 isolates from ectomycorrhizas of *Pinus densiflora* (Japanese red pine) and 1 isolate from an ectomycorrhiza of *Quercus myrsinaefolia* were tested for the ability to form mycorrhizas with *P. densiflora* seedlings in glass tubes. Ten isolates from sporocarps of *Hebeloma* sp., *Laccaria bicolor*, *Lactarius chrysorrheus*, *Suillus granulatus*, *Scleroderma areolatum*, *Russula mariae* and *R. nigricans* had formed ectomycorrhizas by 8 months after transplantation. Twenty isolates taken from mycorrhizas including of *Cenococcum geophilum*, *R. mariae* and *R. nigricans* formed ectomycorrhizas. The synthesized mycorrhizas were classified based on morphological characteristics such as hyphal arrangement of their fungal sheath, and appearance of cystidia and emanating hyphae. Twenty-one mycorrhizal types were recognized.

Key Words——association; ectomycorrhiza; in vitro; mycorrhizal synthesis; *Pinus densiflora*.

Many epigeous and hypogeous mycorrhizal macromycetes exist in forests (Trappe, 1962; Arnolds, 1992). The association between several macromycetes and ectomycorrhizas has recently been shown from field observations using a technique of tracing hyphal connections between sporocarps and mycorrhizas (Ingreby et al., 1990; Agerer, 1993). However, these results may need to be confirmed by reproducible laboratory experiments using pure cultures of mycorrhizal fungi and axenic plants. Although this approach has been used for more than 70 years (Harley and Smith, 1983), only a small number of fungal species have been proved experimentally to form ectomycorrhizas with host plants (Malajuczuk et al., 1982; Molina and Trappe, 1982; Godbout and Fortin, 1985; Taylor and Alexander, 1989; Warcup, 1990).

Zak and Bryan (1963) isolated 40 fungal colony types (tentatively 40 species) of fungi from ectomycorrhizas in several pine forests and used them to synthesize mycorrhizas on pine seedlings. However, only 3 of them were identified, i.e. *Laccaria laccata* (Scop.: Fr.) Berk. & Br., *Rhizopogon roseolus* (Corda) Hollós and *Cenococcum geophilum* Fr. despite comparisons with colony types of isolates from sporocarps occurring in pine forests. Isolation from mycorrhizas is ideal for identifying the causal fungi or for comparison with isolates from sporocarps (Zak and Bryan,1963; Lamb and Richards, 1970; Hutchison, 1991).

Although several mycorrhizal synthesis methods are available (Peterson and Chakravarty, 1991), they produce somewhat abnormal mycorrhizal associations, for example, abnormally thickened fungal sheaths under high concentrations of external glucose (Duddridge and Read, 1984), and changes in mycorrhiza-forming ability of a particular fungal isolate with a host species (Malajuczuk et al., 1982; Miller et al., 1991). Therefore, it should be shown that experimentally synthesized mycorrhizas have morphologically identical characteristics with field-sampled mycorrhizas, as reported by Zak (1976), Alexander (1981), and Godbout and Fortin (1985).

There are many ectomycorrhizal macromycetes in Japanese red pine forests (Imazeki and Hongo, 1987, 1989), but there are few reports of morphological study of ectomycorrhizas both from the field and produced synthetically.

The objective of this study was to determine the mycorrhizal fungal associates of Japanese red pine by mycorrhizal synthetic experiments using isolates obtained from epigeous sporocarps of macromycetes and from ectomycorrhizas, and to determine the morphology of the synthesized mycorrhizas.

Materials and Methods

Sampling of sporocarps of macromycete and ectomycorrhizas Both sporocarps and ectomycorrhizas were sampled from two reforested stands of about 40-year-old *Pinus densiflora* Sieb. et Zucc. on the campus of the University of Tsukuba. The area sampled covered about 2500 m² and had sparsely growing *Chamaecyparis obtusa* Endl., *Carpinus tschonoskii* Maxim., *Castanea crenata* Sieb. & Zucc., *Cryptomeria japonica* D. Don, *Quercus myrsinaefolia* Blume, and shrubs of *Aucuba japonica* Thunb., *Eurya japonica* Thunb., *Rhododendron kaempferi* Planch., and *Rhus verniciflua* Stokes. The samples were

collected weekly from May 1992 to November 1993. Sporocarps were collected gently and put into paper bags. Mycorrhizas (mostly pine roots) were sampled by cutting out soil blocks (ca. $15 \times 15 \times 10$ cm) under the litter layer (ca. a few centimeters) with scissors, and they were kept in polyethylene bags. Both types of samples were immediately taken to the laboratory.

Isolation of fungi Samples of inside tissues of sporocarps were cultured on modified Norkrans's "C" (Melin, 1959) agar medium (MNC agar medium, 1.0 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 ml of 0.2% $ZnSO_4$, 0.5 g NH_4-tartrate, 0.5 ml of 1.0% Fe-citrate, 50 μg thiamine, 0.23 g casein hydrolysate, 0.5 g yeast extract, 10 g glucose, 15 g agar, and 1000 ml deionized water). Pine ectomycorrhizas were picked up from soil blocks with forceps, washed in tap water, and placed in Petri dishes filled with tap water. Pine roots were easy to distinguish from roots of other plants. Healthy pine ectomycorrhizal tips (ca. 2 to 5 mm) were selected for fungal isolation under a stereoscope. A few tips were transferred to a test tube and washed three times or more if necessary with a sterile detergent solution (10 ml of 0.005% polyoxyethylene (20) sorbitan monooleate) using a flash mixer. After washing, they were observed again under a stereoscope. When debris or soil particles no longer adhered to them, they were placed in sterile deionized water (ca. 20 ml) in a glass tube. The mycorrhizal tips were surface-sterilized with calcium hypochlorite solution (10 g/l) for 1 to 2 min on a clean bench, washed three times with sterile deionized water (ca. 20 ml), and cut into small pieces (0.5 to 2.0 mm long). The pieces were placed on MNC agar medium (3 to 10 pieces per plate), which contained streptomycin (100 mg/l) and tetracycline (50 mg/l) to inhibit bacterial growth. The plates were kept at room temperature (15 to 25°C), and the mycorrhizal pieces were observed daily under a stereoscope. When hyphae appeared, after about a week or more, the fungi were subcultured onto MNC agar medium.

Mycorrhizal synthesis A sterilized cork borer (5 mm in diam) was used to remove 5–10 pieces of each fungal isolate (1 to several months old). Each piece of inoculum was placed in a glass tube (3.6 cm in diam, 9 cm in height, and capacity of ca. 90 ml) containing 12 ml of MNC medium. Then the glass tubes were filled with sterilized vermiculite-peat moss mixture (40 : 1, v/v) containing about 60% (v/v) modified Melin-Norkrans (AMN) liquid medium (0.05 g $CaCl_2$, 0.025 g NaCl, 1.0 g KH_2PO_4, 0.5 g NH_4-tartrate, 1.0 mg $MgSO_4 \cdot 7H_2O$, 5.0 mg Fe-citrate, 0.05 μg thiamine, 0.23 g casein hydrolysate, 0.5 g yeast extract, 1000 ml deionized water). Finally, an aseptic Japanese red pine seedling, 2 to 5 days old, was transplanted into each glass tube, and the top of the tube was wrapped with Parafilm to prevent drying and contamination. For each isolate tested, two replicates and four control seedlings (not inoculated) were made. All glass tubes were placed in an air-conditioned growth chamber (20°C, 16 h light), and intact roots of the seedlings were examined through the glass tubes under a stereoscope for ectomycorrhizal formation.

Microscopical observation of synthesized mycorrhizas When mycorrhizal formation was observed on roots under a stereoscope, seedlings were removed from the glass tubes, and mycorrhizal tips were cut off. The morphological descriptions of mycorrhizas were based on the characteristics used by Chilvers (1968) and Ingleby et al. (1990), including external color of fungal sheath under a stereoscope, hyphal arrangement of the surface layer of the fungal sheath, and form of cystidia and emanating hyphae under a light microscope. Autofluorescence of the fungal sheath to UV irradiation was also observed under a fluorescent microscope.

Results and Discussion

Fungal isolation and mycorrhizal synthesis Thirty-two isolates belonging to 27 species (Table 1) were isolated from sporocarps of 61 species which were collected on the study sites (data not shown), and 44 unidentified isolates (Table 2) were obtained from 108 samples of ectomycorrhizas tested. All isolates were kept on slants of MNC and modified Melin-Norkrans (Marx, 1969) agar medium (MMN) at 20°C.

As shown in Table 1, 10 isolates from sporocarps of seven species formed ectomycorrhizas. The species were *Laccaria bicolor* P. D. Orton, *Lactarius chrysorrheus* Fr., *Hebeloma* sp., *Scleroderma areolatum* Ehrenb., *Suillus granulatus* O. Kuntze, *Russula mariae* Peck and *R. nigricans* (Bull.) Fr. Some species in the same genera have been previously reported to form ectomycorrhizas with their host plants under laboratory or field conditions (Trappe, 1962; Agerer, 1986, 1993). However, this is the first report of mycorrhizal associations by these fungi with *P. densiflora*. Furthermore, this is the first report of establishment of pure cultures from sporocarps of *S. areolatum*, *R. mariae* and *R. nigricans*, and of synthetic mycorrhizas with any host plants.

Nineteen isolates obtained from pine mycorrhizas and one from an oak (*Q. myrsinaefolia*) mycorrhiza formed ectomycorrhizas. They could not be identified in culture, but they had regular septa and some had clamp connections (Table 2). Isolates having clamp connections are Basidiomycetes, but the others could not be ascribed to either Ascomycetes or Basidiomycetes.

No mycorrhizas was formed on control plants. When all 30 synthesized mycorrhizas were used to reisolate mycorrhizal fungi, colonies were established from 25 mycorrhizas and they produced the original colony types and hyphal characteristics on MNC agar medium.

Synthesis of mycorrhizas is significant for two reasons in this study. First, isolates originating from sporocarps were shown to have the ability to form mycorrhizas even though the conditions were rather different from the field. Secondly, isolates originating from mycorrhizas were shown to be mycobionts after confirmation of morphological identity between synthesized and original mycorrhizas. On the other hand, isolates that did not form ectomycorrhizas cannot be simply eliminated as mycorrhizal species (Zak and Bryan, 1963), because experimental conditions may influence the results

Table 1. Mycorrhizal formation by sporocarp isolates.

Isolate No. (AT)	Species	Mycorrhizal formation[1]	Clamp connections[2]	Months[3]
105	*Hebeloma* sp.	+	+	3
101	*Laccaria bicolor* P.D. Orton	+	+	1
109	*L. bicolor*	+	+	1
114	*Lactarius chrysorrheus* Fr.	+	−	7
123	*L. chrysorrheus*	+	−	6
107	*Russula mariae* Peck	+	−	6
112	*R. mariae*	+	−	6
111	*R. mariae*	−	−	
110	*R. nigricans* (Bull.) Fr.	+	−	6
113	*Scleroderma areolatum* Ehrenb.	+	−	6
108	*Suillus granulatus* (L.: Fr.) O. Kuntze	+	+	2
119	*Agrocybe erebia* (Fr.) Kühn	−	+	
127	*Amanita esculenta* Hongo & Matsuda	−	−	
116	*Chalciporus piperatus* (Bull.: Fr.) Bataille	−	−	
133	*Clitocybe gibba* (Pers.: Fr.) Kummer	−	+	
103	*Collybia confluens* (Pers.: Fr.) Kummer	−	+	
134	*Collybia* sp.	−	+	
121	*Lactarius hatsudake* Tanaka	−	−	
124	*L. hatsudake*	−	−	
120	*Lepiota* sp.	−	−	
128	*Leucoagaricus rubrotinctus* (Peck) Sing.	−	−	
118	*Naematoloma gracile* Hongo	−	+	
102	*Morchella esculenta* (L.: Fr.) Pers.	−	−	
104	*Marasmius maximus* Hongo	−	+	
106	*Melanoleuca melaleuca* (Pers.: Fr.) Murr.	−	−	
126	*Oudemansiella radicata* (Relhan: Fr.) Sing.	−	+	
132	*Psilocybe* sp.	−	+	
131	*Russula foetens* Pers.: Fr.	−	−	
115	*Russula* sp.	−	−	
129	*Strobilulus stephanocystis* (Hora) Sing.	−	+	
125	*Tylopilus neofelleus* Hongo	−	−	
117	*Xeromphalina cauticinaris* (Fr.) Kühn & Maire	−	+	

[1] +: formed; −: not formed.   [2] +: present; −: absent.   [3] First appearance of mycorrhizas after transplantation.

of mycorrhizal synthesis (Malajuczuk et al., 1982; Miller et al., 1991; Peterson and Chakravarty, 1991).

The first mycorrhizal synthesis was observed with *L. bicolor* (isolate AT109) one month after transplantation (Table 1), whereas *L. chrysorrheus* (AT114) formed ectomycorrhiza seven months after transplantation. Therefore mycorrhiza-forming ability differs among species or isolates. Mycorrhiza isolates also showed differences in the first appearance of mycorrhizas (Table 2).

Among non-mycorrhiza-forming species in the present study, about half of them had a positive effect on seedling height and root length (data not available). In the case of *Marasmius maximus* Hongo (AT126) and *Oudemansiella radicata* (Relhan: Fr.) Sing. (AT104), however, all pine seedlings died by three months after transplantation.

Morphological comparison among synthesized mycorrhizas All synthesized mycorrhizas were considered to be ectomycorrhizas because they had both a fungal sheath

and Hartig net, but not regular intracellular penetrations (Harley and Smith, 1983). According to microscopical observations of mycorrhizal characteristics such as fungal sheath, 30 synthesized mycorrhizas were classified into 21 mycorrhizal types (Table 3). Each fungal species (1 or 2 isolates used) showed a mycorrhizal type. It may be said that the 21 synthesized ectomycorrhizal types were composed of 21 fungal species.

Synthesized mycorrhizas formed by isolate AT107 (*R. mariae*) and AT325 (isolated from a mycorrhiza directly under a sporocarp, which was the origin of AT107) showed the same morphological type (type 3) (Table 3; Fig. 1). Similarly, synthesized mycorrhizas formed by AT110 (*R. nigricans*) and AT309 (isolated from a mycorrhiza directly under a sporocarp, which was the origin of AT110) showed the same morphological type (type 2) (Table 3; Fig. 2). On the other hand, the synthesized mycorrhiza formed by AT109 (*L. bicolor*) was different from that of AT335 (isolated from a mycorrhiza directly

Table 2. Mycorrhizal formation by mycorrhizal isolates.

Isolate No. (AT)	Mycorrhizal formation[1]	Clamp connections[2]	Months[3]	Sampling point of mycorrhiza[4]
				Mycorrhiza was sampled from directly under a sporocarp of:
316	+	−	6	*Agrocybe erebia*
341	+	−	2	*Amanita esculenta*
315	+	−	6	*A. spissacea* Imai
327	+	+	6	*A. vaginata* (Bull.: Fr.) Vitt.
330	+	+	2	*A. vaginata*
310	+	+	4	*Inocybe* sp.
335	+	+	4	*Laccaria bicolor*
329	+	−	5	*Lycoperdon pusillum* Batsch: Pers.
301	+	−	6	*Morchella esculenta*
325	+	−	4	*Russula mariae*
309	+	−	6	*R. nigricans*
331	+	+	2	*Xerocomus subtomentosus* (L.: Fr.) Quél.
337	+	−	5	*X. subtomentosus*
358	+	+	4	ns, s
304	+	−	4	ns
306	+	−	6	ns
307	+	−	3	ns
319	+	−	6	ns
323	+	−	6	ns
353	+	−	4	ns
350	−	−		*Amanita farinosa* Schw.
348	−	−		*A. vaginata*
352	−	−		*Inocybe* sp.
318	−	−		*Inocybe* sp.
324	−	−		*Inocybe* sp.
336	−	−		*Inocybe* sp.
349	−	−		*Inocybe* sp.
338	−	−		*Lactarius chrysorrheus*
342	−	−		*L. chrysorrheus*
326	−	−		*Russula nigricans*
311	−	−		*Russula* sp.
339	−	−		*Neolecta vitellina* Korf & Rogers
351	−	−		*N. vitellina*
302	−	−		ns
308	−	−		ns
320	−	−		ns
321	−	−		ns
322	−	−		ns
332	−	−		ns
333	−	−		ns
343	−	−		ns
344	−	−		ns
345	−	−		ns
346	−	+		ns

1) +: formed; −: not formed.   2) +: present; −: absent.   3) First appearance of mycorrhizas after transplantation.   4) ns: no sporocarp, s: mycorrhiza on a seedling.

under a sporocarp, which was the origin of AT109) (Table 3, Figs. 4, 5).   Type 20, shown by AT341 and AT353, was black mycorrhiza, characteristic of *Cenococcum geophilum* Fr. (Chilvers, 1968; Pigott, 1982; Ingleby et al., 1990; Agerer, 1993).   Therefore we concluded that both the isolates were *C. geophilum*.

When different isolates formed the same morphological type of mycorrhiza, those isolates could not be distin-

Table 3. Classification of synthesized mycorrhizas.

Type	Color[1]	Cystidia[2]	S.s[3]	Emanating hyphae[4]		Isolate No. (AT)
				Cl.	Diam (μm)	
1	WP	acicular (65 μm)	ns	+	2.0–2.5	330
2	WP	lageniform (2 appendages at the apex)	np	−	1.5–2.0	110, 309, 316, 323
3	WP	acicular (20–30 μm, occasionally dichotomous)	np	−	1.5–2.0	107, 112, 325
4	WP	lageniform to subulate	np	−	1.5–2.5	301
5	WP	corniform (appendage)	np	−	1.5–2.5	307
6	WP	horn-like or ampulliform	np	−	2.0–2.5	337
7	WP	hyphae-like to spathulate	np	+	2.0–3.0	327
8	WP	capitate	np	−	2.0–3.0	114
9	WP	no cystidium	rs	−	2.5	315
10	WP	no cystidium	ns	−	2.0–2.5	123
11	WP	no cystidium (Emanating hyphae are covered with small granules and UV-positive.)	np	+	1.5–2.0	105
12	WP	no cystidium	np	+	2.0–3.0	310
13	WP	no cystidium (Hyphae are adhering brown secretions like oil drops.)	np	−	2.0–2.5	108
14	WP	no cystidium	np	−	2.0–2.5	319
15	WP	no cystidium	fp	−	2.0–4.5	113
16	L-DB	club-like to horn-like (dichotomous)	is	+	3.0–5.0	329
17	L-DB	club-like to globules	is	+	2.5–3.5	335
18	L-DB	no cystidium	rs	+	4.0–4.5	331, 358
19	Bl	no cystidium (Hyphae are covered with black dandruff-like ornamentations.)	is	−	3.5–5.0	304, 306
20	Bl	no cystidium	rs	−	2.5–4.5	341, 353
21	Pu	no cystidium	fp	−	2.0–2.5	101, 109

1) WP: white to pale color; L-DB: light and dark brown; Bl: black; Pu: purple. 2) Terms are according to Hawksworth et al. (1983).
3) S.s.: hyphal arrangement of the surface of the fungal sheath; rs: regular synenchyma; is: irregular synenchyma; ns: net synenchyma;
   np: net prosenchyma; fp: felt prosenchyma. The terms for hyphal arrangements follow Chilvers (1968) and Ingreby et al. (1990).
4) Cl.: clamp connection (+: present, −: absent).

guished from each other in colony type on both MNC and MMN agar media. Conversely, when synthesized mycorrhizal types were different, colony types between those causal fungi were different. This suggests that the difference between colony types on agar media reflects the difference in morphology of their synthesized mycorrhizas. For example, two isolates of *L. chrysorrheus*, AT114 and AT123, formed apparently different types of mycorrhizas (neither had laticiferous hyphae in the fungal sheath) (Table 3), and their colony types were also different, i.e. the former was white and the latter was yellow (having seta-like aeration hyphae) on MNC agar medium. On the other hand, *L. bicolor* (AT101 and AT109), *R. mariae* (AT107, 112 and 325), *R. nigricans* (AT110, 309, 316 and 323) and *C. geophilum* (AT341 and 353) each had a distinct morphological type. Intraspecific variation of mycorrhizal morphology on a plant species has been shown using isolates (different genotypes) obtained from geographically or vegetationally different areas (Burgess et al., 1994). The present study was carried out using isolates obtained from a very restricted

area. The meaning of the difference between AT114 and AT123 is not clear, but they were consistently different (probably different species). Additional research is necessary to confirm intraspecific stability and variability of mycorrhizal morphology.

Morphological comparison of synthesized and field sampled mycorrhizas In a morphological comparison of 20 synthesized mycorrhizas formed by 20 isolates obtained from field-sampled mycorrhizas, 14 mycorrhizas (formed by isolates AT301, 304, 306, 309, 310, 315, 316, 323, 325, 331, 335, 341, 353 and 358) were morphologically identical with the original ones. This result confirmed data from Alexander (1981) and Godbout and Fortin (1985), indicating that it may be possible to use synthesized mycorrhizas to identify samples from the field. Isolate AT301, which was isolated from an ectomycorrhiza of *Q. myrsinaefolia*, formed the same morphological type as the original one even though the host was Japanese red pine (Fig. 3). Morphological identity of fungal sheaths irrespective of host plant has previously been shown experimentally by Zak (1976) and Godbout and
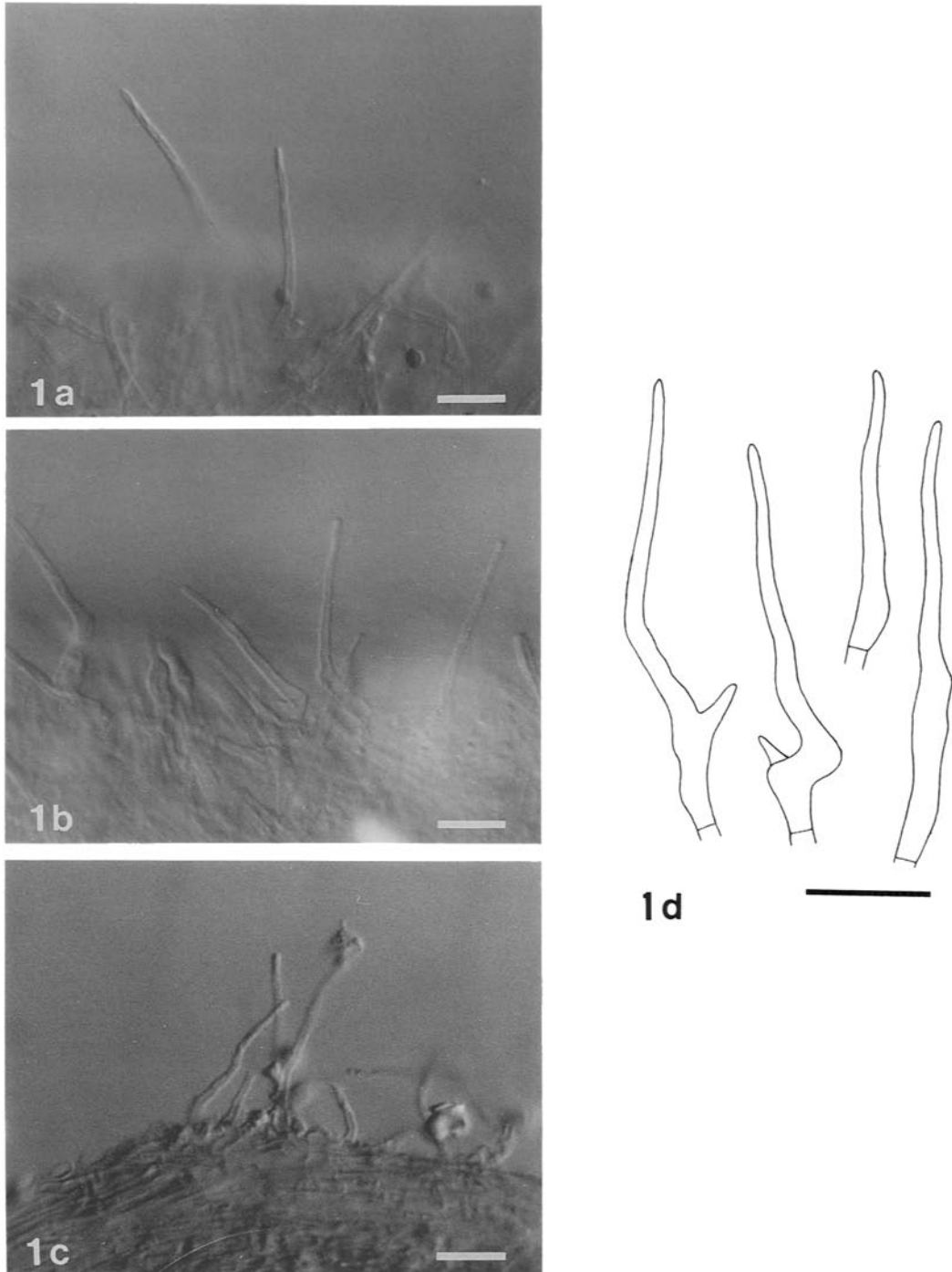
Fig. 1. Morphological characteristics of the ectomycorrhizas formed by *Russula mariae*. a and b; apex of the cystidia on the surface of the mycorrhiza formed by isolate AT107 (a) and AT325 (b). c; apex of the cystidia on the surface of mycorrhiza which is the origin of AT325. d; illustrated cystidia. Each bar shows 10 μm.

Fortin (1985).   On the other hand, host dependency of fungal sheath morphogenesis was reported from Australian herbaceous and shrubby plants (Kope and Warcup, 1986).   From the present study, it is suggested that ectomycorrhizas of *P. densiflora* and *Q. myrsinaefolia* are directly comparable to identify the causal fungi, as in previous reports of the plants of the northern hemisphere (Zak, 1976; Godbout and Fortin, 1985).

Of 20 synthesized mycorrhizas, 3 formed by AT329, AT330 and AT337 had the same cystidia but different hyphal arrangements of the fungal sheath compared with field-sampled ones (hyphal arrangements of the original mycorrhizas of AT329 and AT337 are regular synenchyma, and that of AT330 is irregular synenchyma).   The fungal sheaths of these synthesized mycorrhizas may have been immature.   Changes in hyphal arrangement of
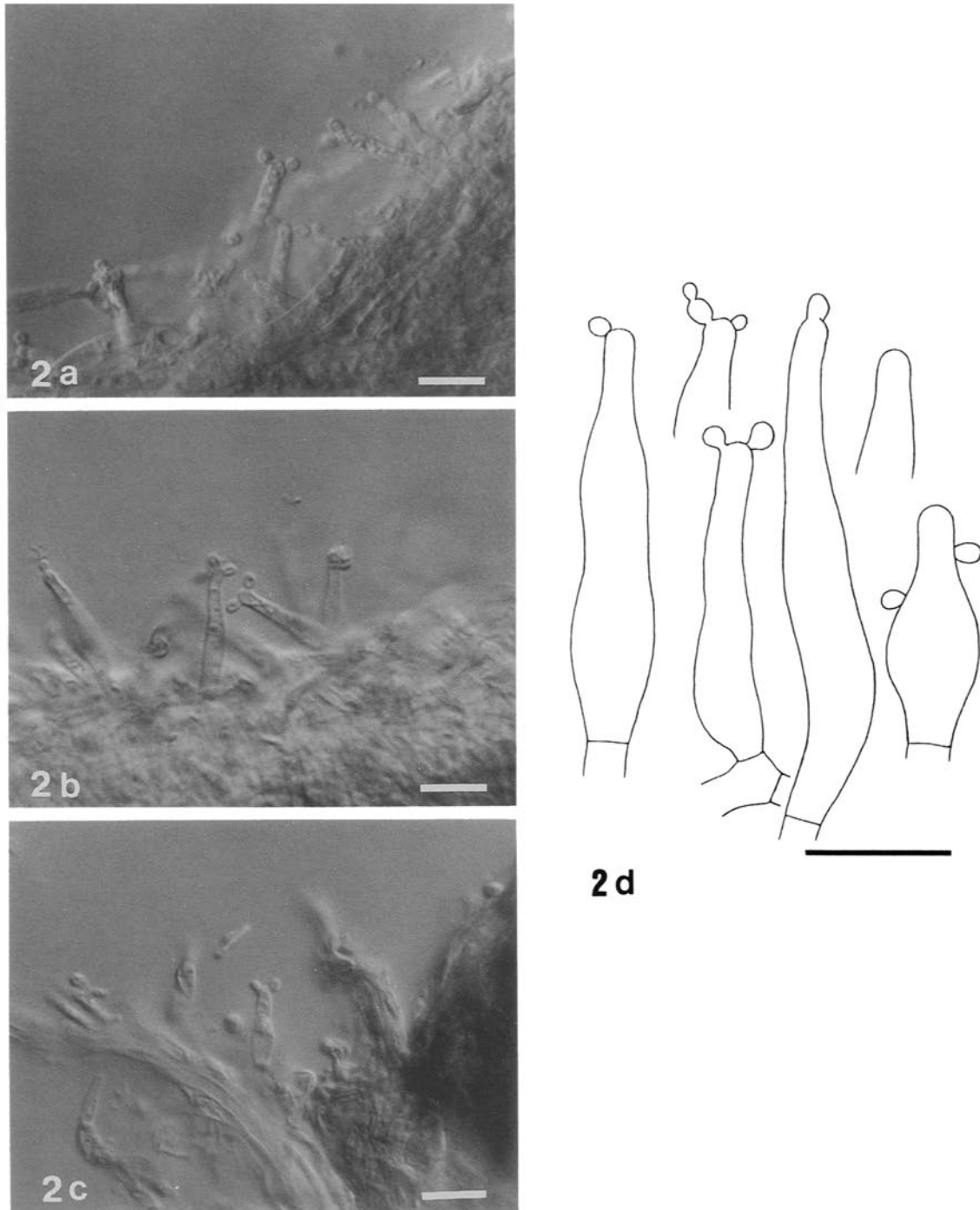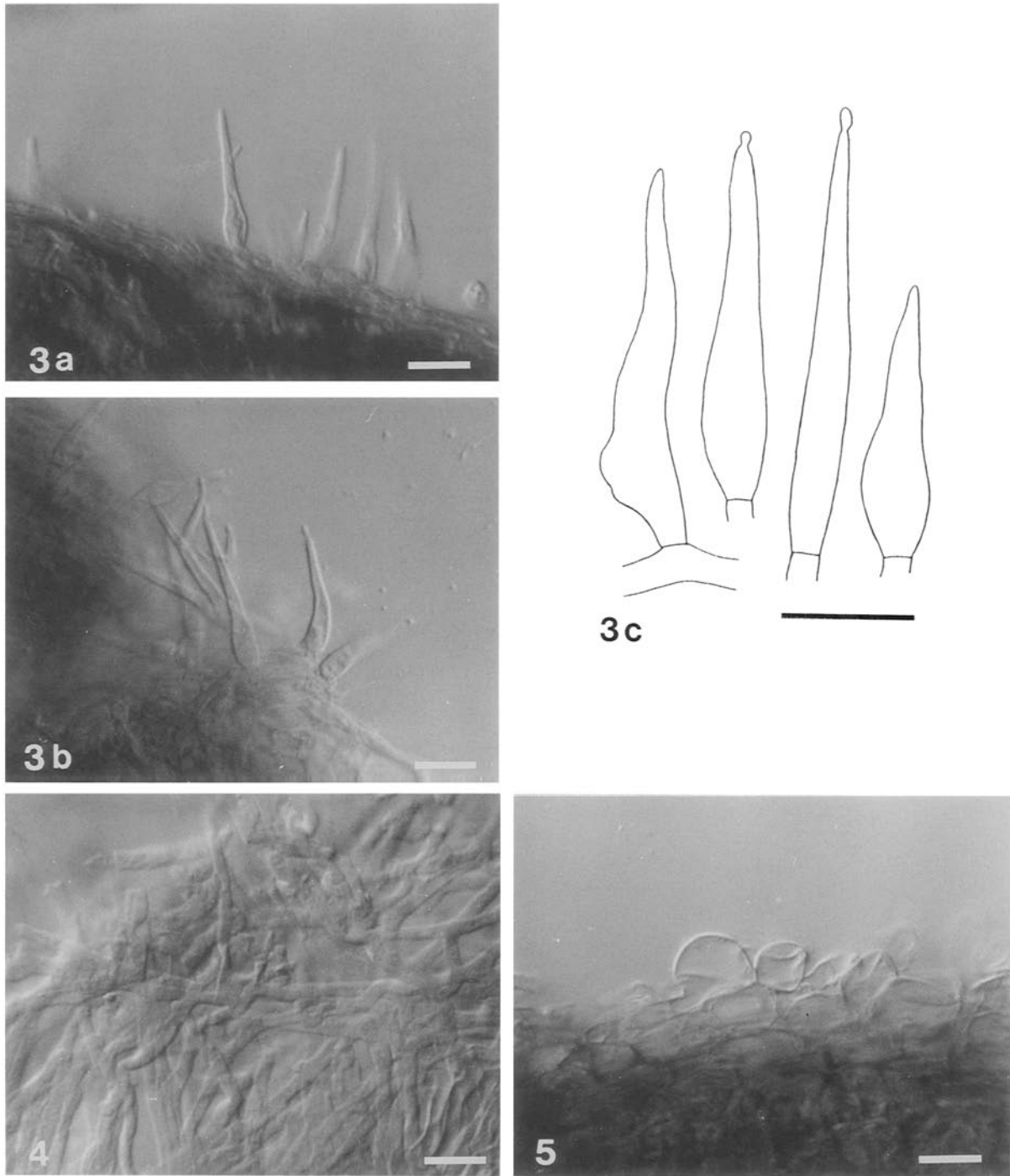
Fig. 2. Morphological characteristics of the ectomycorrhizas formed by *Russula nigricans*. a and b; apex of the cystidia on the surface of mycorrhiza formed by isolate AT110 (a) and AT309 (b). c; apex of the cystidia on the surface of mycorrhiza which is the origin of AT309. d; illustrated cystidia. Each bar shows 10 μm.

the fungal sheath, e.g., from prosenchyma to synenchyma, are likely to occur during mycorrhizal maturation (Chilvers, 1968). In fact, an immature fungal sheath of *C. geophilum* showed net synenchyma but a mature one showed regular synenchyma in the present study. Another 3 of the 20 synthesized mycorrhizas, formed by AT307, AT319 and AT327, were apparently different types from the original ones. It is possible that the field-

sampled mycorrhizas used for the fungal isolation and for the microscopical observations were different types.

A problem with mycorrhizal synthesis experiments using seedlings is that mycorrhizal fungal succession is known from field observations (mainly from sporocarp occurrences) (Arnold, 1992; Newton, 1992). If plant physiological changes involving aging affect the association with mycorrhizal fungi, fungal species that have the abili-

Figs. 3-5. Morphological characteristics of the ectomycorrhizas.   3. a; apex of cystidia on the surface of mycorrhiza formed by
isolate AT301 (unknown isolate). b; apex of the cystidia on the surface of mycorrhiza on *Q. myrsinaefolia* which is the origin of
AT301.   c; illustrated cystidia 4 and 5. Surface layer of the fungal sheath formed by isolate AT109 (*Laccaria bicolor*) and AT 335
(unknown isolate obtained from a mycorrhiza directly under a sporocarp, which is the origin of AT109).   Each bar shows 10 μm.

ty to form mycorrhizal associations only with mature
trees may not form them with young seedlings.

In the present study site, 46 types of pine ectomycor-
rhizas were sampled and classified (Yamada, unpublished
data).   Fourteen types out of the 46 were demonstrated
in vitro using 20 fungal isolates obtained from mycorrhi-
zas and 3 isolates obtained from sporocarps of *R. mariae*

(Fig. 1) and *R. nigricans* (Fig. 2).   However, the other spe-
cies (*Hebeloma* sp., *L. laccata*, *L. chrysorrheus*, *S. areola-
tum* and *S. granulatus*) formed new mycorrhizal types
that did not correspond to any of the field-sampled pine
mycorrhizas.   As suggested by Zak and Bryan (1963),
the latter species may have no or a little association with
pine roots in the field.

Many of the causal fungi of the synthesized mycorrhizas remained unidentified in this study. It may be that many ectomycorrhizal species do not form sporocarps on the ground in this study site. At the present time, mycorrhizal synthesis experiments under dual conditions are thought to be ideal for determing the community structure of ectomycorrhizal fungi. In the forest, fungal relationships between ectomycorrhizas formed in the soil and sporocarps occurring on the surface of the forest floor are not clear. Tracing hyphal connections between sporocarps and mycorrhizas is difficult when several types of mycorrhizas are present on a restricted root system. Comparison of isolates from sporocarps and from mycorrhizas provide physiological clues to their relationships, and mycorrhizal synthesis produces physiologically and morphologically integrated information on the causal fungi on the same basis of "in vitro mycorrhizas."

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