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ORIGINAL PAPER

In vitro ectomycorrhizal specificity between the Asian red pine *Pinus densiflora* and *Tricholoma matsutake* and allied species from worldwide Pinaceae and Fagaceae forests

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Abstract *Tricholoma matsutake* produces commercially valuable, yet uncultivable, mushrooms (matsutake) in association with pines in the Far East and Scandinavia and with both pines and oaks in the foothills of Tibet. Other matsutake mushrooms, such as *Tricholoma anatolicum* from the Mediterranean regions and *Tricholoma magnivelare* and *Tricholoma* sp. from the North Pacific Coast area of Canada and North America as well as Mexico, respectively, are associated with pines or oaks in their natural habitats. *Tricholoma bakamatsutake* and *Tricholoma fulvocastaneum* from Asia produce moderately valuable matsutake mushrooms and are solely associated with Fagaceae in nature. In this study, we demonstrate for the first time that matsutake mushrooms from Scandinavia, Mediterranean regions, North America, and Tibet form ectomycorrhizae with *Pinus densiflora* similar to the Far East *T. matsutake*. In general, worldwide *T. matsutake* and the symbionts of Pinaceae colonize the rhizospheres of *P. densiflora* as well as *T. matsutake* isolated from the host plant. However, *T. fulvocastaneum* and *T. bakamatsutake* formed a discontinuous Hartig net and no Hartig net, respectively, and colonized to a lesser extent as compared to *T. matsutake*. The data suggest that conifer-associated matsutake mushrooms in their native habitat will associate symbiotically with the Asian red pine.

Keywords *Tricholoma matsutake* · *Pinus densiflora* · Ecophysiology · Ectomycorrhizal symbiosis · Edible mushrooms

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Introduction

The ectomycorrhizal basidiomycete *Tricholoma matsutake* (S. Ito et Imai) Sing (= *Tricholoma nauseosum* Kytt., *Tricholoma zangii* Z. M.) produces the most prized mushroom (matsutake) in association with pines, including *Pinus densiflora* Sieb. et Zucc. in the Far East and *Pinus sylvestris* L. in Scandinavia, and with both pines and oaks in the foothills of Tibet. Other matsutake mushrooms, such as *Tricholoma anatolicum* H. H. Doğan & Intini from the Mediterranean regions and *Tricholoma magnivelare* (Peck) Redhead and *Tricholoma* sp. from the North Pacific Coast

area of Canada and North America as well as Mexico, respectively, produce fruit bodies morphologically similar to matsutake in association with other Pinaceae plants in their natural habitats. *Tricholoma bakamatsutake* Hongo and *Tricholoma fulvocastaneum* Hongo from Asia are solely associated with Fagaceae. None of these matsutake mushrooms has yet been cultivated, and the mechanisms involved in their symbioses remain inadequately investigated. Neither has the systematics of these apparently related mushroom species been definitively established (Masui 1927; Hamada 1974; Ogawa 1978; Singer 1986; Imazeki and Hongo 1987; Hosford et al. 1997; Wang et al. 1997; Lefevre and Müller 1998; Bergius and Danell 2000; Intini et al. 2003; Lim et al. 2003; Maurice et al. 2003; Chapela and Garbelotto 2004; Matsushita et al. 2005; Peter 2006; Smith and Read 2008). The phylogenetic analysis of matsutake mushrooms in relation to fungal ecology, especially their host specificity, is the basis of evolutionary interpretation. However, the mycorrhizal structures as well as the physiological aspects have received little study. To clarify the nature of the mycorrhizal ecophysiology of matsutake mushrooms, experimental analyses of the mycorrhizal associations will likely prove useful.

Symbiosis between *T. matsutake* and *P. densiflora* is demonstrated by the Hartig net structure of mycorrhizae, which is characterized by a thin and undifferentiated fungal sheath on the lateral root tips and by the plant-promoting effect after fungal colonization (Yamada et al. 1999a, b, 2006; Gill et al. 2000; Guerin-Laguette et al. 2000, 2004). We have provided genomic evidence that *T. matsutake* and its apparently related mushroom species have diverged from their ancestor and become established as individual species in a rather limited geographical distribution during the later evolutionary period, probably around the last ice age 12,000 years ago (Murata et al. 2002, 2005, 2008; Murata and Babasaki 2005).

However, whether these matsutake mushrooms can share the same host plant species is unclear, which may inform us of taxonomic and ecological relationships among these ectomycorrhizal symbionts and may lead to the development of a breeding strategy and a seedling-based mushroom cultivation method. In the present study, we tested ectomycorrhization of several matsutake mushrooms with the Asian red pine *P. densiflora* in vitro, examined their mycorrhizal behavior based on anatomical observations, and conducted a plant growth response analysis.

Materials and methods

Organisms

The descriptions of the fungal strains are given in Table 1. We previously described *T. anatolicum* MC1 as *T. matsutake* MC1 (Murata et al. 1999, 2005). However, after a reevaluation of the original basidiomata specimen according to Intini et al. (2003) and our sequence analysis of the internal transcribed spacer (ITS) region within the rDNA (DDBJ accession no. AB510357), we verified the specimen to be *T. anatolicum*. Furthermore, *Tricholoma* sp. MX1 was previously identified as *T. matsutake* MX1 (Murata et al. 2005). However, this strain could be *T. magnivelare*, which was found recently in a Mexican population based on the sequence of the rDNA ITS region (DDBJ accession no. AB 510472; Chapela and Garbelotto 2004). Given the uncertainty in the morphological identification of the basidiomata specimens and basidiospores, we regard this strain as *Tricholoma* sp. MX1. *Tricholoma ustale* (Fr.) P. Kumm. forms typical *Tricholoma* ectomycorrhizae (Agerer 1987–2009) and is common in both coniferous and fagaceous forests in Japan. *Rhizopogon rubescens* (Tul. & C. Tul.) Tul. & C. Tul. is a suilloid, which is one of the well-known conifer-associated ectomycorrhizal fungal groups (Bruns et al. 2002).

Seeds of *P. densiflora* were obtained from Ibaraki Prefectural Forestry Institute and stored at 4°C until use.

In vitro mycorrhization

Mycorrhizae were synthesized as described previously (Yamada et al. 2006). The substrates included 250 ml of a dried vermiculite–sphagnum moss mixture (80:1, *w*/*w*) and 100 ml of modified Norkrans' C (MNC) liquid medium (Yamada et al. 1999b) with a 0.2% glucose, which were packed into a 500-ml glass bottle, autoclaved at 121°C for 45 min, cooled to room temperature, and then used for the experiment. Fungal inocula were prepared as follows. After culturing in the MNC liquid medium, fungal mycelia equivalent to 0.3 g dry weight were washed with sterile distilled water, dissected into several segments with fine forceps, and dispersed throughout the substrate in the glass bottle. Then, an axenically germinated *P. densiflora* seedling on MNC agar was planted in the bottle. The transparent polycarbonate cap of the glass bottle had an aeration hole that was sealed with a fluorocarbon membrane filter (pore size=0.45 μm; Milliseal, Millipore, Yonezawa, Japan). These spawns were incubated at 20°C under continuous 140 μmol $m^{-2}s^{-1}$ light intensity for 5 months. Sterilized distilled water was added to the spawns a few months after the start to compensate for evaporative water loss. Three to five replicates were made for each fungus–plant combination.

Data analysis

After a 5-month incubation period, pine seedlings were removed from the spawns. Plant shoots were separated from

Table 1 Fungal strains tested

Fungal strain		Origin			Remarks
Species	ID	Locality	Host plant	Year	
Tricholoma matsutake	Y1	Ibaraki, Japan	*Pinus densiflora*	1997	=NBRC 33136, ATCC MYA-915
T. matsutake	CH1	China	No record (commercially harvested)	1998	=ATCC MYA-919
T. matsutake	BH1	Bhutan	No record (commercially harvested)	1998	
T. matsutake	AT925	Sweden	Putatively *Pinus sylvestris*	2001	
T. anatolicum	MC1	Morocco	No record (commercially harvested)	1998	=ATCC MYA-929
T. anatolicum	TK S6-1	Fethiye, Turkey	*Cedrus libani*	2006	
T. magnivelare	CA1	Canada	No record (commercially harvested)	1998	
Tricholoma sp.	MX1	Mexico	No record (commercially harvested)	1998	=ATCC MYA-921
T. bakamatsutake	B1	Ibaraki, Japan	*Quercus serrata*	1993	=NBRC 33138, ATCC MYA-927
T. fulvocastaneum	WK-N1	Wakayama, Japan	Putatively *Quercus phillyraeoides*	1998	=ATCC MYA-204325
T. ustale	AT612	Ibaraki, Japan	*Pinus densiflora*	1997	=NBRC 33139, ATCC MYA-922
Rhizopogon rubescens	AT631	Ibaraki, Japan	*Pinus thunbergii*	1998	

NBRC NITE Biological Resource Center, Japan, *ATCC* American Type Culture Collection

the root system, dried at 60°C for 24 h, and weighed. The root system was collected under a dissection microscope using fine forceps after washing off the substrate with tap water. Small portions of the fungal-colonized root tips were hand-sectioned and inspected for the ectomycorrhizal structure under a differential interference contrast Nomarski microscope (Yamada et al. 2001). The rest of the root system was separated into small segments to measure root length. The extent of fungal colonization was assessed by a gridline intersection method (Brundertt et al. 1996). After these analyses, root tips were dried at 60°C for 24 h and weighed. All numerical data were statistically analyzed with one-way ANOVA (KaleidaGraph ver. 3.6 J, Hulinks, Tokyo, Japan) and used to determine the extent of the symbiotic effect on plants.

Results

Mycorrhiza formation at root tips

All species of matsutake mushrooms other than *T. fulvocastaneum* and *T. bakamatsutake* from various geographic regions, including the Far East, Tibet, and Scandinavian strains of *T. matsutake*, Mediterranean strains of *T. anatolicum*, the Canadian strain of *T. magnivelare*, and the Mexican strain of *Tricholoma* sp., developed typical ectomycorrhizal anatomy on *P. densiflora* (Table 2, Fig. 1a–f). The external morphology of these mycorrhizal tips was the same as that of the Japanese *T. matsutake* Y1, and no distinct differences in mycelial organization were observed surrounding the root tips. Of the Fagaceae-associating matsutakes, *T. fulvocastaneum* showed discontinuous Hartig net development at the *P. densiflora* root cortex, and *T. bakamatsutake* showed neither intercellular nor intracellular association (Table 2, Fig. 1g, h). However, *T. bakamatsutake* colonized the lateral root surface moderately, and these mycelia often developed many chlamydospore-like structures (Fig. 1i, j), which are commonly observed in the mycorrhizal mycelia in forests (Ogawa and Ohara 1978; our unpublished data). *T. ustale* and *R. rubescens*, which were regarded as control conifer symbionts, showed a typical ectomycorrhizal structure, i.e., dichotomously branched mycorrhizal tips, well-organized and differentiated fungal sheath mycelia, and developed rhizomorphs (data not shown).

Table 2 Ecotomycorrhization tested with *P. densiflora* host

Fungal strain	Hartig net at the root cortex
T. matsutake Y1	+
T. matsutake CH1	+
T. matsutake BH1	+
T. matsutake AT925	+
T. anatolicum MC1	+
T. anatolicum TK S6-1	+
T. magnivelare CA1	+
Tricholoma sp. MX1	+
T. bakamatsutake B1	–
T. fulvocastaneum WK-N1	+/–
T. ustale AT612	+
R. rubescens AT631	+

(+) continous development was observed, (+/–) discontinous development was observed, (–) not observed

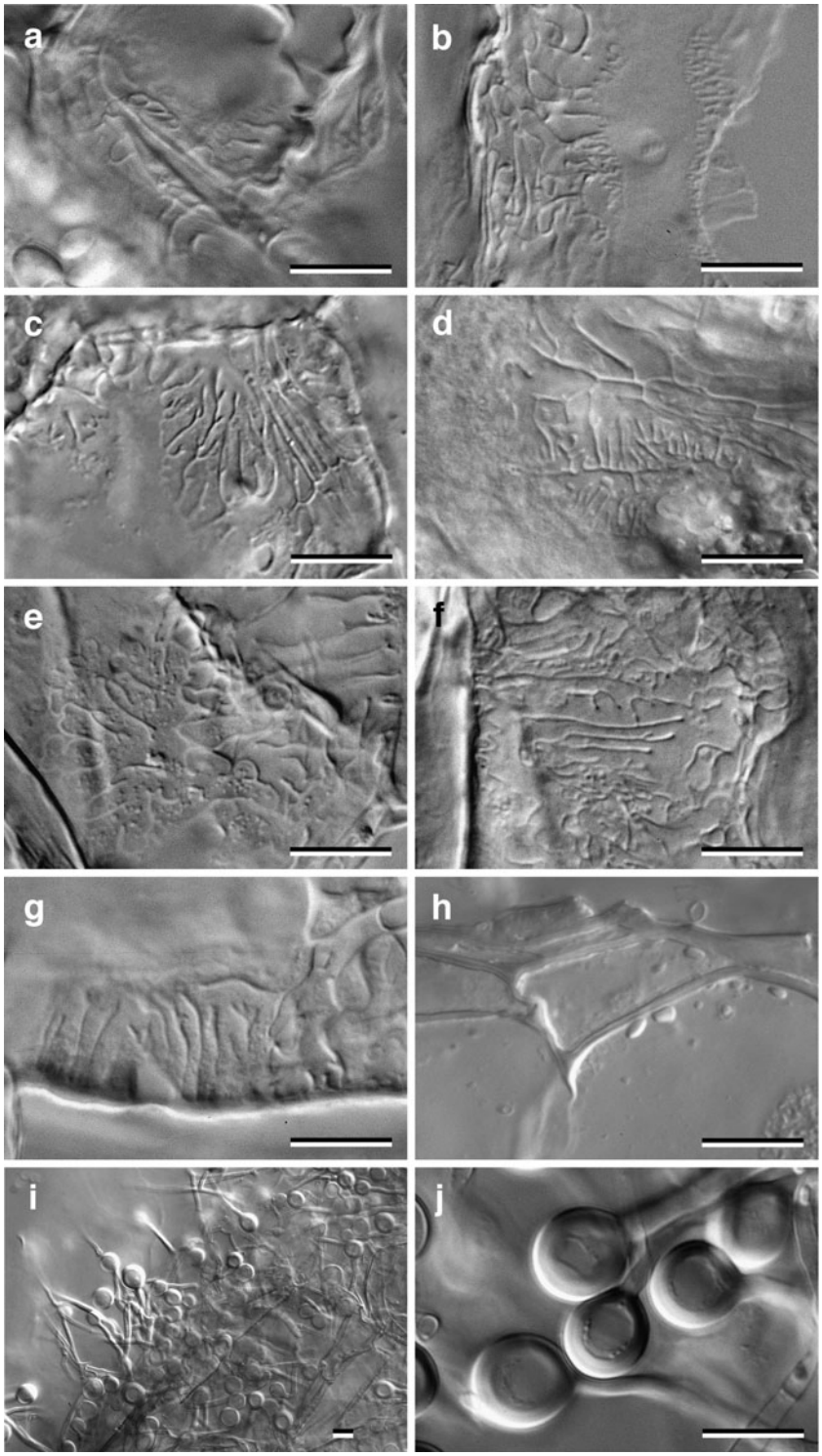

Fig. 1 Morphological and anatomical characteristics of mycorrhizae between matsutake mushrooms and *Pinus densiflora*. Hartig net structures on *P. densiflora* associated with *Tricholoma matsutake* CH1 (**a**), *T. matsutake* BH1 (**b**), *Tricholoma anatolicum* TK S-06 (**c**), *T. anatolicum* MC1 (**d**), *Tricholoma magnivelare* CA1 (**e**), *Tricholoma* sp. MX1 (**f**), and *Tricholoma fulvocastaneum* WK-N1 (**g**). Fungal colonization of *Tricholoma bakamatsutake* on *P. densiflora* (**h**, **i**) and its chlamydospore-like structure (**i**, **j**) on the root surface. *Bars* represent 10 μm

Colonization at the root systems

The vegetative growth of matsutake mushrooms, including *T. matsutake* Y1 on the red pine root system, was markedly slower and less than those of *T. ustale* and *R. rubescens* that developed ectomycorrhizae throughout their root systems. The extent of colonization was apparently correlated with the levels of mycelial growth on the MNC agar plate. For example, among the matsutakes, *T. magnivelare* grew rather vigorously, and *T. bakamatsutake* grew much more slowly (data not shown). Therefore, fungal colonization was not solely determined by host plants but rather by nutrients in the growth conditions, though all *Tricholoma* examined were able to associate with *P. densiflora* to the same extent (see above).

Effect of the symbiont on *P. densiflora* growth

All matsutake isolates were associated with the same degree of plant shoot growth, root growth, and biomass development, and no significant differences were noted among the fungal species (Table 3). *T. ustale*, used as a control, showed the shortest root length and lowest plant biomass among the fungi tested. A similar trend was noted in *R. rubescens*, which exhibited a lower trend for host growth, especially in root weight. The vegetative colonization was the best in *R. rubescens*, and the second highest was in *T. ustale*, although the latter was not significantly different from that in *T. magnivelare*. Among matsutake mushrooms, *T. magnivelare* showed significantly larger colonized root length than that of *T. bakamatsutake* and *T. fulvocastaneum*, both of which have a specific association with fagaceous hosts in nature. The root/shoot ratio was the smallest in *R. rubescens*, and no significant difference was observed among the matsutake mushrooms. No symptoms of plant growth inhibition were observed among the fungi tested.

Discussion

Based on the accepted Latin names, matsutake mushrooms can be provisionally divided into three groups with respect to their natural host plant taxa; i.e., *T. anatolicum* solely associates with *Cedrus* in Pinaceae (Intini et al. 2003), *T. fulvocastaneum* and *T. bakamatsutake* solely associate with Fagaceae (Imazeki and Hongo 1987; Sanmee et al. 2007), and *Tricholoma caligatum*, *T. magnivelare*, and *T. matsutake* associate with both plant families (Hosford et al. 1997; Kytovuori 1988; Chapela and Garbelotto 2004; Matsushita et al. 2005; Xu et al. 2008). In the present study, we provided evidence that a group of matsutakes distributed in the Northern Hemisphere will associate with the Asian red pine, although they only develop a symbiotic relationship with specific host plants in their native geographic habitats.

Recently, we demonstrated that Asian *T. matsutake* consists of two genetic groups: one from the Far East and the other from Tibet (Murata et al. 2008). While *T. matsutake* in the Far East and Scandinavia are solely associated with conifers, the population in Tibetan regions (= Southwestern China through Bhutan) has been found in both Fagaceae and Pinaceae forests (Matsushita et al. 2005; Bao et al. 2007; Xu et al. 2008). In this study, *T. matsutake* from the Tibet area, which should have the potential to develop mycorrhizae on both coniferous and fagaceous hosts in nature, formed a typical Hartig net structure on *P. densiflora*. Canadian *T. magnivelare* CN1, which also has the potential to develop mycorrhizae on both pine and oak hosts (Chapela and Garbelotto 2004), developed a typical Hartig net structure on *P. densiflora* as well. However,

Table 3 Host plant growth response to mycorrhizal association with various matsutake strains

Fungal strains[a]	Shoot height (cm)			Root length (cm)			Fungus colonized			Dry weight (mg)						Root/shoot ratio (*w/w*)		
				Total						Shoot			Root					
T. matsutake Y1 (6)	7.03 (0.27)	ab	A	1374 (42)	a	A	118.3 (10.8)	c	AB	516 (15)	ab	A	272 (5)	a	A	0.530 (0.017)	a	A
T. matsutake CH1 (4)	7.40 (0.41)	ab	A	1516 (122)	a	A	94.8 (20.6)	c	AB	502 (21)	ab	A	280 (23)	a	A	0.557 (0.032)	a	A
T. matsutake BH1 (4)	7.75 (0.33)	ab	A	1079 (109)	ab	A	86.5 (15.4)	c	AB	559 (34)	a	A	253 (9)	ab	A	0.469 (0.036)	ab	A
T. matsutake AT925 (5)	7.60 (0.93)	ab	A	1211 (103)	ab	A	96.0 (14.7)	c	AB	501 (34)	ab	A	244 (19)	ab	A	0.488 (0.026)	ab	A
T. anatolicum MC1 (4)	7.38 (0.15)	ab	A	1171 (67)	ab	A	112.0 (21.3)	c	AB	482 (15)	ab	A	227 (10)	ab	A	0.471 (0.019)	ab	A
T. magnivelare CA1 (4)	7.70 (0.45)	ab	A	1397 (205)	a	A	179.3 (31.7)	bc	A	481 (28)	ab	A	230 (19)	ab	A	0.481 (0.043)	ab	A
Tricholoma sp. MX1 (4)	7.95 (0.34)	a	A	1250 (121)	ab	A	82.5 (39.4)	c	AB	521 (17)	ab	A	233 (18)	ab	A	0.449 (0.036)	ab	A
T. bakamatsutake B1 (5)	7.16 (0.26)	ab	A	1203 (73)	ab	A	38.4 (7.7)	c	B	471 (26)	ab	A	244 (16)	ab	A	0.524 (0.037)	a	A
T. fulvocastaneum WK-N1 (4)	7.25 (0.50)	ab	A	1318 (185)	ab	A	66.0 (19.2)	c	B	547 (34)	a	A	304 (31)	a	A	0.555 (0.044)	a	A
T. ustale AT612 (5)	5.52 (0.27)	b		725 (128)	b		287.6 (47.3)	b		395 (53)	b		115 (17)	c		0.488 (0.026)	ab	
R. rubescens AT631 (5)	7.40 (0.55)	ab		1037 (152)	ab		484.2 (72.3)	a		516 (27)	ab		187 (20)	bc		0.358 (0.021)	b	

Numbers in parenses are the replicate of plant seedlings.

Each numerical data indicate mean with standard error in parenthesis. Different small letters that follow numerical data indicate significant difference between the data all fungal species at $P<0.05$, and large letters indicate significant difference between matsutake mushrooms at $P<0.05$.

Fagaceae-specific *T. bakamatsutake* and *T. fulvocastaneum* did not confer typical ectomycorrhizae on *P. densiflora*. These observations along with available phylogenetic data (Murata et al. 2002, 2008; Chapela and Garbelotto 2004) suggest that solely Fagaceae-associated matsutakes may lack potential ectomycorrhizal compatibility with pines. Our data predict that California and Mesoamerican *T. caligatum* populations, both of which are solely associated with oaks and phylogenetically allied to *T. bakamatsutake* (Chapela and Garbelotto 2004), will not form ectomycorrhizae with *P. densiflora* in vitro. However, another mycorrhization experiment with a certain Fagaceae host is necessary to conclude this issue in matsutake mushrooms at the intra- and interspecific levels. In addition, higher magnification electron microscopy observations are necessary to specify the precise boundary structures between the Hartig net mycelium and cortical cells, as has been reported by Gill et al. (2000) for the *T. matsutake–P. densiflora* combination.

Recently, the geographic distribution of *T. fulvocastaneum*, the most probable candidate as an ancestor of the *T. matsutake–T. magnivelare* phylogroup (Chapela and Garbelotto 2004), has been revised and expanded from endemic in Japan to the southwestern limit of northern Thailand (Sanmee et al. 2007). Yunnan Province, one of the most productive sites for *T. matsutake* in southwestern China, is geomorphologically connected with northern Thailand and shares the same forest vegetation. In addition, *T. bakamatsutake* is widely distributed in eastern and southeastern Asia, with the southern limit at approximately 9° S in a natural *Castanopsis* forest at the highland area of eastern Papua New Guinea (Otani 1976). Therefore, *T. matsutake* shares common hard wood taxa as hosts with *T. fulvocastaneum* and *T. bakamatsutake* in southwestern China. Estimating the potential competitive effect of those Fagaceae-specific matsutake species with the *T. matsutake* population in southwestern China will be important for preserving and controlling its genetic resources in forests for sustainable matsutake harvests.

Matsutake mushrooms showed different mycelial colonization and host plant growth from the controls. Although the colonization level was generally low in the matsutakes as compared to the controls, their plant growth was almost the same as the control *R. rubescens* and better than that of *T. ustale* (Table 3). This suggests that matsutake mushrooms effectively promote host root length and biomass even at low levels of colonization. The growth of *T. bakamatsutake* on the pine root tips without inter- and intracellular colonization (Fig. 1h) clearly demonstrates the unidirectional transfer of nutrients (mainly carbon) from plant to fungus as a saprobic relationship. As *T. bakamatsutake* cultures grow very slowly on nutrient agar and lack availability of polymeric compounds such as cellulose, lignin, and starch (Ogawa 1978), the tested *T. bakamatsutake* strain utilized soluble exudates from pine roots. At present, no experimental evidence demonstrates that mycorrhization of *T. matsutake* has negative effects on host plant growth (Table 3; Guerin-Laguette et al. 2004, Yamada et al. 2006). The symbiotic nature of matsutake mycorrhizae under forest conditions will be assessed by the transplantation and acclimatization of synthesized mycorrhizal seedlings in the field.

From a practical point of view, the production of mycorrhizal pine seedlings with the *T. magnivelare–P. densiflora* combination was better than *T. matsutake–P. densiflora* because *T. magnivelare* was aggressive in the mycorrhization on *P. densiflora* as compared with the other matsutakes that showed ectomycorrhization (Table 3). Recently, we started an outplanting project of mycorrhizal *P. densiflora* seedlings that were colonized by *T. matsutake* mycelium in vitro and have succeeded in maintaining the mycorrhizal mycelium with active host growth for at least 1 year after outplanting (Kobayashi et al. 2008). However, the survival ratio of the mycorrhizal mycelium has not been high, and growth was poor. Therefore, further suitable fungal–plant combinations that are tolerant to field conditions are desired, as suggested in the case of truffles and several other edible mycorrhizal fungi (Cairney and Chambers 1999). For the *T. anatolicum* cultures tested, the Turkish strain TK S6-1 was obtained from a pure *Cedrus libani* A. Rich. forest. *T. anatolicum* is known to exclusively associate with *Cedrus* species even if other conifers such as *Pinus* spp. are present in the same local areas or in the same forest sites in Turkey (Intini et al. 2003; Doç et al. 2007) and in North Africa (Ogawa 1978), although *T. caligatum sensu lato* is suggested to be distributed allopatrically in various coniferous forests in these areas. Given the mycorrhization level of the *T. anatolicum* strains on *P. densiflora* and the effects on host growth were the same as those of *T. matsutake* strains from Asia and Sweden, *T. anatolicum* can be included as a candidate for an artificial mycorrhization trial with pine hosts as well as *Cedrus*.

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References

Agerer R (ed) (1987–2009) Color atlas of ectomycorrhizae. 1st–14th delivery. Einhorn-Verlag, Schwabisch Gmünd

Bao D, Koike A, Yao F, Yamanaka K, Aimi T, Kitamoto Y (2007) Analyses of the genetic diversity of matsutake isolates collected

from different ecological environments in Asia. J Wood Sci 53:344–350
Bergius N, Danell E (2000) The Swedish matsutake (*Tricholoma nauseosum* syn. *T. matsutake*): distribution, abundance and ecology. Scan J For Res 15:318–325
Brundertt M, Bougher N, Dell B, Grove T, Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture, Chapter 4.3. Measuring root colonisation by mycorrhizal fungi. Australian Centre for International Agricultural Research, Canberra, pp. 184–193
Bruns TD, Bidartondo MI, Taylor DL (2002) Host specificity in ectomycorrhizal communities: what do the exceptions tell us? Integ Comp Biol 42:352–359
Cairney JWG, Chambers SM (1999) Ectomycorrhizal fungi: key genera in profile. Springer, Berlin
Chapela IH, Garbelotto M (2004) Phylogeography and evolution in matsutake and close allies inferred by analyses of ITS sequences and AFLPs. Mycologia 96:730–741
Doç Y, Solak MH, Kalmiş E, Kalyoncu F (2007) Sedir Mantari. Ege University, Izmir
Gill WM, Guerin-Laguette A, Lapeyrie F, Suzuki K (2000) Matsutake—morphological evidences of ectomycorrhiza formation between *Tricholoma matsutake* and host roots in a pure *Pinus densiflora* forest stand. New Phytol 147:381–388
Guerin-Laguette A, Vaario LM, Gill WM, Lapeyrie F, Matsushita N, Suzuki K (2000) Rapid *in vitro* ectomycorrhizal infection on *Pinus densiflora* roots by *Tricholoma matsutake*. Mycoscience 41:389–393
Guerin-Laguette A, Shindo K, Matsushita N, Suzuki K, Lapeyrie F (2004) The mycorrhizal fungus *Tricholoma matsutake* stimulates *Pinus densiflora* seedlings *in vitro*. Mycorrhiza 14:397–400
Hamada M (1974) Matsutake nikki (diaries of matsutake). Kyoto University, Kyoto (in Japanese)
Hosford D, Pilz D, Molina R, Amaranthus M (1997) Ecology and management of the commercially harvested American matsutake mushrooms. USDA Forest Service PNG-GTR-412. USDA Forest Service, Washington
Imazeki R, Hongo T (1987) Colored illustrations of mushrooms of Japan, vol. 1. Hoikusha, Osaka
Intini M, Dogan HH, Riva A (2003) *Tricholoma anatolicum* spec. nov.: a new member of the matsutake group. Micol Veget Medit 18:135–142
Kobayashi H, Terasaki M, Yamada A (2008) Survival of *Tricholoma matsutake* mycelia on the root systems of Japanese red pine seedlings for a year after outplanting. Kanto-Shisniri-Kenkyu 59:325–326 (in Japanese)
Kytovuori I (1988) The *Tricholoma caligatum* group in Europe and North Africa. Karstenia 28:65–78
Lefevre C, Müller WR (1998) CDE18: *Tricholoma magnivelare* (Peck) Redhead+*Pinus contorta* Dougl. var. *latifolia* Engelm. Concise descriptions of North American Ectomycorrhizae. In Goodman DM, Durall DM, Trofymow JA, Berch SM (eds) Concise descriptions of North American Ectomycorrhizae, 4th folio. Mycologue, Sydney
Lim SR, Fischer A, Berbee M, Berch SM (2003) Is the booted tricholoma in British Columbia really Japanese matsutake? BC Journal of Ecosystem and Management 3:1–7
Masui K (1927) A study of the ectotrophic mycorrhizas of woody plants. Mem Coll Sci Kyoto Univ B III 2:152–279
Matsushita N, Kikuchi K, Sasaki Y, Guerin-Laguette A, Lapeyrie F, Vaario L-M, Intini M, Suzuki K (2005) Genetic relationship of *Tricholoma matsutake* and *T. nauseosum* from the Northern Hemisphere based on analyses of ribosomal DNA spacer regions. Mycoscience 46:90–96
Maurice J-P, Vairelles D, Jeandroz S, Le Tacon F (2003) Une Station à *Tricholoma caligatum* en forêt communale de greux (vosges). Bull Soc Mycol Fr 119:103–115
Murata H, Babasaki K (2005) Intra- and inter-specific variations in the copy number of two types of retrotransposons from the ectomycorrhizal basidiomycete *Tricholoma matsutake*. Mycorrhiza 15:381–386
Murata H, Babasaki K, Yamada A (1999) Identification of repetitive sequences with motifs of retrotransposons in the ectomycorrhizal basidiomycete *Tricholoma matsutake*. Mycologia 91:766–775
Murata H, Babasaki K, Miyazaki Y, Yamada A (2002) Genetic evidence that two types retroelements evolved through different pathways in ectomycorrhizal homobasidiomycetes *Tricholoma* spp. Biosci Biotechnol Biochem 66:1880–1886
Murata H, Babasaki K, Yamada A (2005) Highly polymorphic DNA markers to specify strains of the ectomycorrhizal basidiomycete *Tricholoma matsutake* based on σ_{marY1}, the long terminal repeat of *gypsy*-type retroelement *marY1*. Mycorrhiza 15:179–186
Murata H, Babasaki K, Saegusa T, Takemoto K, Yamada A, Ohta A (2008) Traceability of Asian matsutake, specialty mushrooms produced by the ectomycorrhizal basidiomycete *Tricholoma matsutake*, on the basis of retroelement-based DNA markers. App Environ Microbiol 74:2023–2031
Ogawa M (1978) The biology of matsutake. Tsukiji-shokan, Tokyo (in Japanese)
Ogawa M, Ohara H (1978) Microbial ecology of 'Shiro' in *Tricholoma matsutake* (S. Ito et Imai) Sing. and its allied species. VIII: *Tricholoma bakamatsutake* Hongo in *Quercus mongolica* var. *grosserrata* forest and *Q. serrata* forests. Trans Mycol Soc Japan 19:391–405 in Japanese
Otani Y (1976) *Tricholoma bakamatsutake* Hongo collected in New Guinea. Trans Mycol Soc Japan 17:363–365 (in Japanese)
Peter M (2006) Ectomycorrhizal fungi—fairy rings and the wood-wide web. New Phytol 171:685–687
Sanmee R, Fell B, Lumyong P, Lumyong S (2007) First record of *Tricholoma fulvocastaneum* from Thailand. Mycoscience 48:131–133
Singer R (1986) The Agaricale in modern taxonomy, 4th edn. Koeltz, Koenigstein
Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Elsevier, Oxford
Wang Y, Hall IR, Evans LA (1997) Ectomycorrhizal fungi with edible fruiting bodies 1. Tricholoma matsutake and related fungi. Eco Bot 51:311–327
Xu J, Sha T, Li Y-C, Zhao Z-W, Yang ZL (2008) Recombination and genetic differentiation among natural populations of the ectomycorrhizal mushroom *Tricholoma matsutake* from southwestern China. Mol Ecol 17:1238–1247
Yamada A, Kanekawa S, Ohmasa M (1999a) Ectomycorrhiza formation of *Tricholoma matsutake* on *Pinus densiflora*. Mycoscience 40:193–198
Yamada A, Maeda K, Ohmasa M (1999b) Ectomycorrhiza formation of *Tricholoma matsutake* isolates on seedlings of *Pinus densiflora in vitro*. Mycoscience 40:455–463
Yamada A, Ogura T, Ohmasa M (2001) Cultivation of mushroom of edible ectomycorrhizal fungi associated with *Pinus densiflora* by *in vitro* mycorrhizal synthesis II. Morphological description of ectomycorrhizas in open-pot soil. Mycorrhiza 11:67–81
Yamada A, Maeda K, Kobayashi H, Murata H (2006) Ectomycorrhizal symbiosis in vitro between *Tricholoma matsutake* and *Pinus densiflora* seedlings that resembles naturally occurring 'shiro'. Mycorrhiza 16:111–116