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Review article

Cultivation studies of edible ectomycorrhizal mushrooms: successful establishment of ectomycorrhizal associations *in vitro* and efficient production of fruiting bodies

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ABSTRACT

Most edible ectomycorrhizal mushrooms are harvested in forests or controlled tree plantations; examples include truffles, chanterelles, porcinis, saffron milk caps, and matsutake. This study explored recent advances in *in vitro* ectomycorrhizal cultivation of chanterelles and matsutakes for successful ectomycorrhizal seedling establishment and the subsequent manipulation of these seedlings for efficient fruiting body production. Chanterelle cultivation studies have been limited due to the difficulty of establishing pure cultures. However, once pure cultures were established in the Japanese yellow chanterelle (*Cantharellus anzutake*), its ectomycorrhizal manipulation produced fruiting bodies under controlled laboratory conditions. As *C. anzutake* strains have fruited repeatedly under ectomycorrhizal symbiosis with pine and oak seedlings, mating tests for the cross breeding are ongoing issues. As one of the established strains C-23 has full-genome sequence, its application for various type of ectomycorrhizal studies is also expected. By contrast, *Tricholoma matsutake* fruiting bodies have not yet been produced under controlled conditions, despite successful establishment of ectomycorrhizal seedlings. At present, the shiro structure of ≈1L in volume can be provided in two y incubation with pine hosts under controlled environmental conditions. Therefore, further studies that provides larger shiro on the host root system are desired for the outplantation trial and fruiting.

Keywords: Fungal isolation, oak, pine, spore germination, symbiosis

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1. Introduction

Edible ectomycorrhizal mushrooms such as truffles (*Tuber*), chanterelles (*Cantharellus cibarius* Fr. and related species), porcinis (*Boletus edulis* Bull. and related species), saffron milk caps (*Lactarius deliciosus* [L.] Gray and related species), and matsutakes (*Tricholoma matsutake* [S. Ito & S. Imai] Singer and related species) have high economic value worldwide (Zambonelli & Bonito, 2012; Pérez-Moreno, Guerin-Laguette, Arzú, & Yu, 2020), with annual global sales estimated at several billion dollars. These mushrooms are harvested in forests or plantations for both domestic and export markets (Arora, 2008; Tsing, 2015, Pérez-Moreno et al., 2021). In Japan, imported matsutake mushrooms from countries such as China, USA, Canada, Morocco, Turkey, Mexico, and South Korea are valued at 80 to 100 million dollars annually (Aoki et al., 2022), which is equivalent to domestic *T. matsutake* production within Japan, mainly in Nagano and Iwate Prefectures (Yamanaka, Yamada, & Furukawa, 2020). During the past 20 y, truffle imports to Japan have increased such that their economic value in Japan now equivalent to around 10% that of the matsutake mushroom import value (MAFF; https://www.maff.go.jp/e/index.html).

To meet this increasing market demand, truffles have been harvested in tree plantations (Zambonelli, Iotti, & Murat, 2016). However, other edible mushrooms are largely harvested in forests because non-host and tree plantation cultivation techniques have not yet been established. Therefore, these mushrooms must be conserved in natural forests through controlled harvests or forest management to sustain moderate harvest levels for longer (decade-scale) periods (Guerin-Laguette, 2021; Hosford, Pilz, Molina, & Amaranthus, 1997; Pilz et al., 1999, Pilz & Molina, 2002; Furukawa, Masuno, & Takeuchi, 2016, Yamada, Furukawa, & Yamanaka, 2017, Yamanaka et al., 2020). In the Périgord truffle *Tuber melanosporum*, most harvests are derived from tree plantations. In their native European range, as well as in Australia, New Zealand, and North America, several truffle species such as *Tuber aestivum* (Wulfen) Spreng., *Tuber borchii* Vittad., *Tuber brumale* Vittad., and *Tuber lyonii* Butters are harvested in tree plantations established using mycorrhizal seedlings that were grown under greenhouse or laboratory conditions. These mycorrhizal seedlings are usually prepared through spore inoculation to non-mycorrhizal host seedlings. Several years after truffle-associated seedling outplantation, truffle mycelia that survive and adapt to host tree rhizospheres are able to produce fruiting bodies (Zambonelli et al., 2016; Guer-

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in-Laguette, 2021). Other groups cultivated in tree plantations include porcinis, saffron milk caps, *Suillus* (e.g., *Suillus bovinus* [L.] Lam. and *Suillus luteus* [L.] Roussel), *Tricholoma* (e.g., *Tricholoma portentosum* [Fr.] Quél.), desert truffles (*Terfezia*), *Lyophyllum shimeji* Hongo, *Rhizopogon roseolus* (Corda) Th. Fr., and *Astraeus* (Wang & Chen, 2014; Yamada et al., 2017; Suwannasai, Dokmai, Yamada, Watling, & Phosri, 2020; Pérez-Moreno et al., 2020; Yamanaka et al., 2020; Guerin-Laguette, 2021). Most other edible mycorrhizal taxa including Caesar's mushrooms, chanterelles, and matsutakes are harvested in managed natural or semi-natural (plantation or secondary) forests because techniques for mycorrhizal seedling have yet to be established for these taxa (Pilz, Norvell, Danell, & Molina, 2003; Yamada, Ogura, & Ohmasa, 2001b, c; Endo, Gisusi, Fukuda, & Yamada, 2013; Endo et al., 2014; Yamanaka et al., 2020; Guerin-Laguette, 2021).

Yellow chanterelles have the highest economic value among edible mycorrhizal mushrooms globally, estimated at over 200,000 metric tons and 1.25 to 1.4 billion dollars wholesale annually (Pilz et al., 2003). Pure culture techniques for the representative European species, *Cantharellus cibarius*, were established by the early 1990s (Danell & Fries, 1990), leading to successful chanterelle fruiting from established mycorrhizal seedlings under greenhouse conditions (Danell & Camacho, 1997; Pilz et al., 2003). However, the outplantation of mycorrhizal seedlings for chanterelle production has been poorly studied. Pure culture of another European chanterelle, *Cantharellus pallens* Pilát, was reported in a single study (Danell & Fries, 1990). In the Japanese yellow chanterelle, *Cantharellus anzutake* W. Ogawa, N. Endo, M. Fukuda & A. Yamada, which is phylogenetically closely related to *C. cibarius*, successful pure culture establishment (Ogawa et al., 2019a), ectomycorrhization, and fruiting through mycorrhizal symbiosis in the laboratory (Ogawa et al., 2019b) have been reported. Other economically important chanterelle species in Europe (e.g., *Cantharellus ferruginascens* P. D. Orton and *C. amethysteus* [Quél.] Sacc.), North America (e.g., *C. roseocanus* [Redhead, Norvell & Danell] Redhead, Norvell & Moncalvo, *C. formosus* Corner, *C. californicus* D. Arora & Dunham, *C. cascadensis* Dunham, O'Dell & R. Molina, and *C. enelensis* Voitk, Thorn, Lebeuf & J.I. Kim), and China (e.g., *C. yunnanensis* W. F. Chiu) remain to be isolated, and very few cultivation trials have been reported. Many matsutake mushroom cultivation trials have been reported in Japan since the 1960s (Matsutake Research Association, 1964; Ogawa, 1978; Yamanaka et al., 2020); however, ectomycorrhizal synthesis of matsutake mushrooms was first validated only in the late 20th century (Yamada, Maeda, & Ohmasa, 1999b; Gill, Lapeyrie, Gomi, & Suzuki, 1999), followed by wide acceptance of the technique (Guerin-Laguette, Shindo, Matsushita, Suzuki, & Lapeyrie, 2004; Kobayashi, Watahiki, Kuramochi, Onose, & Yamada, 2007; Yamada et al. 2010; Vaario, Pennanen, Sarjala, Savonen, & Heinonsalo, 2010; Yamanaka et al., 2014, Jeon & Ka, 2016; Herrera, Wang, Zhang, & Yu, 2022) and matsutake fruiting under controlled environmental conditions with or without a host remains to be validated. Recently, fruiting of the matsutake species *Tricholoma bakamatsutake* Hongo on nutrient medium without a host plant was reported by a private company in Japan, which intended to cultivate *T. bakamatsutake* commercially. The cultivation of other economically important matsutake species such as *Tricholoma murrillianum*, *Tricholoma magnivelare*, *Tricholoma mesoamericanum*, *Tricholoma anatolicum*, and *Tricholoma fulvocastaneum* via mycorrhizal synthesis also remains poorly studied. Therefore, in this review, I examine recent advances in *C. anzutake* and *T. matsutake* cultivation, with a particular focus on host–plant associations in chanterelle and matsutake mushroom production, and discuss future directions in the cultivation of these economically important edible mushrooms and in related mycology fields.

2. Chanterelle cultivation

2.1. Foundations of chanterelle cultivation

Chanterelles consist of fungi in the family Cantharellaceae (Moncalvo et al., 2006; Hibbett et al., 2014), which is among the most ancestral clades of ectomycorrhizal basidiomycetous fungi (Miyauchi et al., 2020). Yellow chanterelles (*Cantharellus*) and other related groups such as *Craterellus* (e.g., *Craterellus tubaeformis* [Fr.] Quél. and *Craterellus cornucopioides* [L.] Pers.) have common fragrances that are similar to that of apricots, which makes them a delicacy among consumers. Their unique funnel shape and diverse colors (yellow, white, reddish, green, or black) also stimulate the senses.

The first report of pure chanterelle culture establishment described yellow colonies (representative strain 740b) obtained using a spore isolation technique; these colonies had a similar fragrance to the fruiting bodies (Fries, 1979). Strain 740b was identified as true *C. cibarius* using a DNA hybridization technique (Straatsma, Konings, & van Griensven, 1985). Danell and Fries (1990) reported 56 cultured *C. cibarius* and four *C. pallens* strains isolated from fruiting body tissues collected in coniferous forests. Based on established cultures, *in vitro* ectomycorrhization of *C. cibarius* was first achieved by Moore, Jansen, and van Griensven (1989). Ectomycorrhizal synthesis of this fungus with pine and spruce hosts was conducted to obtain mycorrhizal seedlings in a nursery study (Danell, 1994), producing several fruiting events under greenhouse conditions over a period of several months (Danell & Camacho, 1997; Pilz et al., 2003). However, later reports of pure culture, mycorrhization, and artificial fruiting of this fungal species are rare. The Japanese yellow chanterelle *C. anzutake* was previously identified as *C. cibarius* (Ogawa, Endo, Fukuda, & Yamada, 2018); pure cultures of this fungus were isolated from basidioma tissues and ectomycorrhizal root tips (Ogawa et al., 2019a). The established strains showed characteristics similar to those of *C. cibarius*, i.e., yellowish color and an apricot-like smell (Fig. 1A–D). Ectomycorrhizae of several *C. anzutake* strains were synthesized on a *Pinus densiflora* Siebold et Zucc. host *in vitro*, which were subsequently used as mother plants for the preparation of another ectomycorrhizal system on a *Quercus serrata* Murray host (Ogawa et al., 2019b). Under laboratory conditions, pine and oak seedlings grown in 4 L pots hosted 29 fruiting events within 2 y of observation (Fig. 1E–H). In North American yellow chanterelles, no cultivation based on pure culture techniques has been reported; most studies of these mushrooms have focused on their taxonomy and ecology, as well as forest management for their sustainable harvest and conservation (Pilz, Molina, & Mayo, 2006; Dunham, Kretzer, & Pfrender, 2003a; Dunham, O'Dell, & Molina, 2003b; Thorn, Kim, Lebeuf, & Voitk, 2017). The cultivation of other chanterelle taxa such as *Craterellus* has rarely been studied.

2.2. Chanterelle cultivation bottleneck

The limited reports of chanterelle cultivation have highlighted the difficulty of obtaining pure cultures, compared to other edible ectomycorrhizal mushrooms such as those of the suilloid group, *Tricholoma*, *Lactarius*, and *Boletus*. Although *C. cibarius* mycelia grow moderately on nutrient media, success rates for pure culture establishment from basidiomata remain low (Straatsma et al., 1985; Danell & Fries, 1990) because basidioma tissue from *C. cibarius*

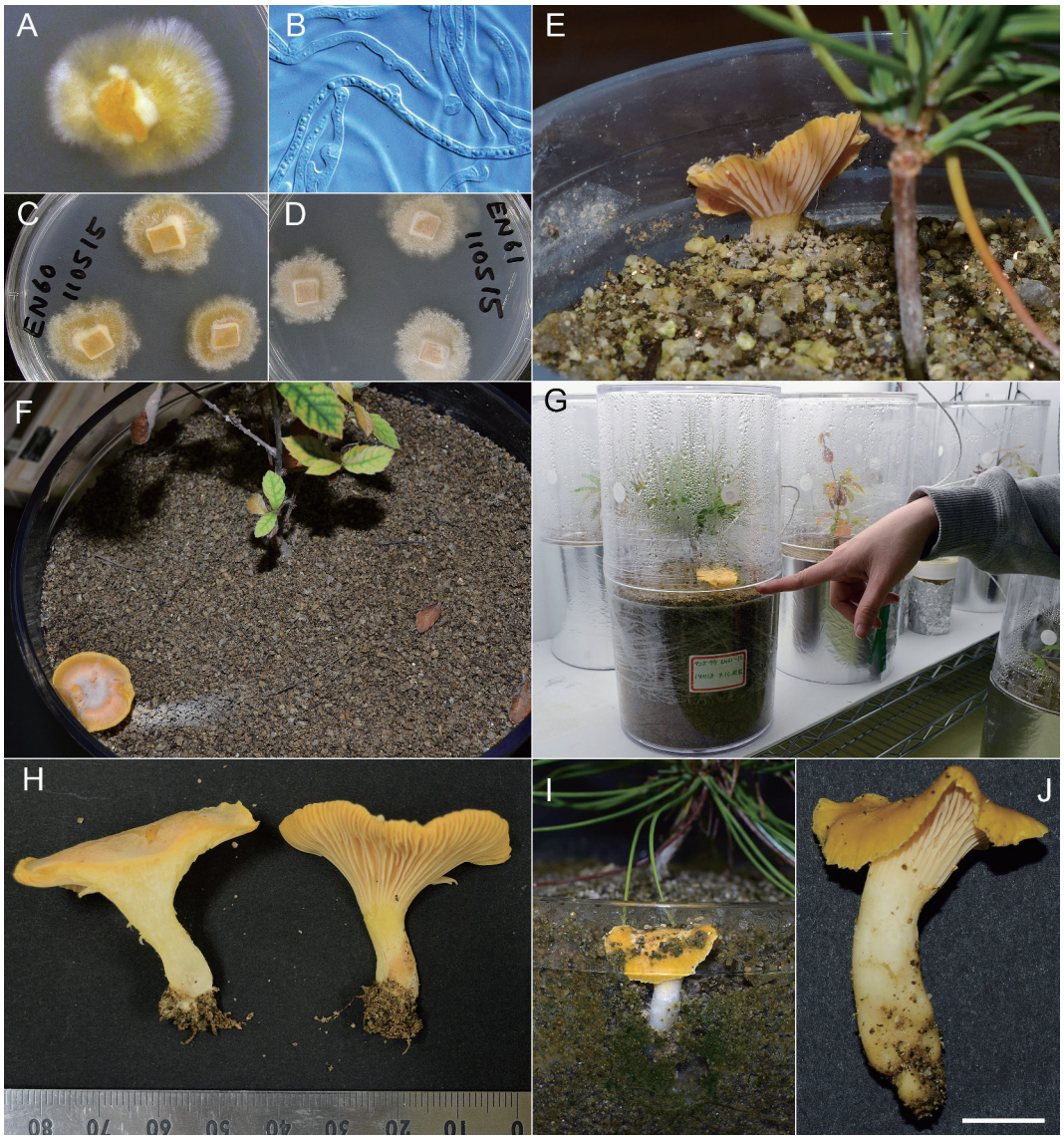

Fig. 1–*Cantharellus anzutake*. A: A colony of strain C-18 on MNC agar plate, which was isolated from a mycorrhizal root tip. B: Hyphae of strain C-18 showing clamp connection and intracellular oily droplet. C, D: Colonies of stains EN-60 and EN61 isolated from mycorrhizal root tips. E: Fruiting of strain EN-61 associated with *Pinus densiflora* host in a 250 mL pot, photographed Oct 2012. F: Fruiting of strain EN-51 associated with *Quercus serrata* host in a 4 L pot, photographed May 2015. Please note the deposited basidiospores on the soil surface, which were released form the basidioma. G, H: Fruiting of strain EN-61 associated with *Pinus densiflora* host in a 4 L pot, photographed May 2015. I, J: Fruiting of strain C-23 associated with *P. densiflora* host in a 4 L pot, photographed Aug 2021. In the details of experimental systems of A–D and E–H, please see Ogawa et al. (2019a) and Ogawa et al. (2019b), respectively. *Bar*: 1 cm.

harbors diverse bacteria and fungi (Danell, Alström, & Ternström, 1993; Danell, 1999; Rangel-Castro, Danell, & Pfeffer, 2002) that stunt its mycelial growth on nutrient agar medium. The same phenomenon has been observed in *C. anzutake* and probably occurs in other chanterelles and *Craterellus* species. *Cantharellus anzutake* rarely produces pure cultures from basidiomata (Ogawa et al., 2019a); even *C. anzutake* cultured from basidiomata under laboratory conditions (Ogawa et al., 2019b) exhibit bacterial and fungal contamination during tissue isolation procedures (unpublished data), as has been reported in *C. cibarius* (Pilz et al., 2003). However, multispore isolation techniques developed for *C. cibarius* by Fries (1979) have shown that young, fresh basidioma can produce pure cultures. In *C. anzutake*, pure cultures have been successfully obtained from field-sampled ectomycorrhizal root tips (Ogawa et al., 2019a). *Cantharellus cibarius* cultures are not presently available in any culture collections, although *C. anzutake* cultures are available in the National Institute of Technology and Evaluation Biological Resource Center (NBRC), Japan. The whole-genome sequence of *C. anzutake* strain C-23 (NBRC no. 113265) has been published on the Joint Genome Institute (JGI) Genome Portal (https://genome.jgi.doe.gov/portal/), and its genome structure has been analyzed as representative among Cantharellales species (Miyauchi et al., 2020). The taxonomic validity of cultured yellow chanterelles is easily verified by sequencing of DNA barcoding regions such as the internal transcribed spacer 2 (ITS2) region of the rDNA operon and *tef-1* region through BLAST searching. Many contaminants of *C. cibarius*, *C. anzutake*, and likely other yellow chanterelles can be detected on nutrient agars. Some chanterelle cultures have developed fluffy aeration hyphae that fill the agar plate, suggesting contaminations. Yellow chanterelle mycelia typically exhibit clamp connections among dikaryotic hyphae and oily intrahyphal droplets with or without yellow pigments (Ogawa et

al., 2019a; Fig. 1A–D).

2.3. Recent advances in chanterelle cultivation

Early *C. anzutake* chanterelle cultivation trials identified this fungus as *C. cibarius* Fr. (Ogawa et al., 2018). Pure cultures of European *C. cibarius* were established from basidioma tissue and basidiospores (Fries, 1979; Straatsma et al., 1985; Danell & Fries 1990). Therefore, Ogawa et al. (2019a) adopted a new approach to obtain pure cultures of Japanese "*C. cibarius*" (= *C. anzutake*) from ectomycorrhizal root tips. This technique has been successfully applied in diverse fungal taxa (Yamada & Katsuya, 1995; Yamada, Ogura Degawa, & Ohmasa, 2001a; Endo et al., 2013), and has produced larger quantities of pure *C. anzutake* culture than isolation from basidioma tissue, which resulted in the establishment of multiple cultures per year. The *C. anzutake* strain C-23 was even isolated from a *Quercus crispula* mycorrhizal root tip sampled from beneath epigeous basidiomata that were wounded by mycophagous insects.

Following the acquisition of many *C. anzutake* strains, Ogawa et al. (2019b) synthesized ectomycorrhizas of this fungus *in vitro* with pine hosts, and acclimated the established ectomycorrhizal seedlings to small (250 mL) pots; successful fruiting of the fungus led to the isolation of strains EN-51 (NBRC no. 113266) and EN-61 (no. 113270) (Fig. 1E). Fruiting events occurred within approximately 1 year following fungal inoculation to the small seedlings *in vitro*, as previously reported for *Rhizopogon roseolus* (Yamada et al., 2001b, c). Although potted ectomycorrhizal seedlings grow better in organic soil than in mineral soil, fruiting was observed only in pots containing mineral soil (Ogawa et al., 2019b). Next, mushroom production was upscaled by successively transplanting the seedlings into culture pots containing 1 and 4 L mineral soil. Within a 2-y incubation period, the four tested strains (EN-51, En-61, C-2, and EN-98) grown with pine and oak hosts in 4 L pots fruited a total of 29 times (Fig. 1F–H). Such repeated fruiting demonstrates the potential for successful *C. anzutake* cultivation using this new technique. Further experiments were conducted by adding strains and increasing pot replication using pine and oak hosts. By late 2021, these cultivation trials recorded more than 200 *C. anzutake* fruiting events (unpublished data), among which C-23 was the most fertile tested strain (Fig. 1I, J). Therefore, single-spore isolates derived from strain C-23 should be tested to determine their mating types and to select next-generation populations for crossbreeding in *C. anzutake*. Therefore, I recommend strain C-23 as a candidate model fungus for ectomycorrhizal symbiosis and edible mycorrhizal mushroom production.

Cantharellus anzutake is phylogenetically closely related to *C. cibarius* (Ogawa et al. 2018). In Asia another edible yellow chanterelle, *Cantharellus yunnanensis* W. F. Chiu, had been included in *C. cibarius* s.l. (Pilz et al., 2003). Therefore, I inferred that a Chinese yellow chanterelle sold under the name of *C. yunnanensis* is conspecific to *C. anzutake*, because both species were externally quire similar, and the several known ITS2 sequences under the name of *C. yunnanensis* on GenBank showed high homology to that of *C. anzutake*. In addition, the habitats of *C. anzutake* and *C. yunnanensis* shared the same biome, i.e., the Asian temperate broadleaf and mixed forests. However, a recent taxonomic study that designated the epitype of *C. yunnanensis* clearly distinguished these two Asian yellow chanterelles based on morphological and phylogenetic (*tef-1α*) analyses (Shao, Liu, Wei, & Herrera, 2021). The high degree of phylogenetic relatedness of *C. anzutake* and *C. yunnanensis* allows them to be misidentified as conspecific (Cao, Hu, Yu, Wei, & Yuan, 2021) even in phylogenetic analyses using the large subunit (LSU) and ITS2 rDNA regions and indicates recent speciation. Thus, accumulated knowledge about *C. anzutake* cultivation can be applied to *C. yunnanensis*.

2.4 Outdoor cultivation of yellow chanterelles

Once ectomycorrhizal seedlings have been established in the laboratory, they can be outplanted for outdoor mycorrhizal cultivation. Guerin-Laguette et al. (2014) successfully cultivated *Lactarius deliciosus* in New Zealand for several years following outplantation of mycorrhizal seedlings. Although similar practices have been reported for various fungal taxa, few have been commercially viable, with the notable exception of truffles. In the truffles, commercial harvests have been conducted in Europe, Australia, New Zealand, and North America, in plantations established by outplanting of mycorrhizal seedlings (e.g., Zambonelli et al., 2016). Although 600 Scots pine seedlings inoculated with *C. cibarius* were outplanted in 24 locations in southern Sweden, the long-term results have not been reported (Pilz et al., 2003).

At the KOA Corporation field near the Faculty of Agriculture of Shinshu University, *C. anzutake* cultivation trials have been conducted since 2014, comparing forest edge ($N = 12$) and inner forest plots ($N = 12$). In 2020, outplanted pine seedlings inoculated with several strains of *C. anzutake* (EN-51, EN-52 [NBRC no. 113267], EN-53 [no. 113268], EN-60 [no. 113269], EN-61, and C-2) grew to a height of 2–3 m at the forest edge; tree root systems showed increased *C. anzutake* biomass, as identified through microscopy and ITS sequencing (unpublished data). However, no fruiting was observed. Growing basidiomycetous ectomycorrhizal biomass is anticipated to fruit in response to induction signals, as shown in saffron milk cap and porcini mushrooms (De la Varga et al., 2013; Guerin-Laguette et al., 2014). If fruiting events are observed among the inoculated *C. anzutake* strains at the experimental site, large-scale plantations will likely be established, as occurred for *C. cibarius* in Sweden (Pilz et al., 2003).

3. Matsutake cultivation

3.1. In vitro ectomycorrhization of Tricholoma matsutake for mushroom cultivation

The first trial of ectomycorrhization of *Tricholoma matsutake* with a pine host was conducted by Masui (1927); however, that experiment was later deemed invalid because its microscopic observations of inoculated hyphal cells suggested *Umbelopsis* species (or "*Mortierella*"; Ogawa, 1978), which is a common contaminant during *T. matsutake* tissue isolation, rather than *T. matsutake* itself (Yamada et al., 2001a). Hamada (1974) recalled having first established *T. matsutake* cultures in 1940; these exhibited slow growth rates and white to pale cream colonies on nutrient agar medium. This description is adequate for the current identification of *T. matsutake* colonies through DNA barcoding. The taxonomic identity and ectomycorrhizal ability of strain NBRC no. 6933 (Institute for Fermentation, Osaka [IFO] no. 6933), which was originally established in 1952 (Ogawa & Hamada, 1975), was recently examined (Yamada, Kobayashi, & Murata, 2003). In the 1950s and 1960s, various approaches for increasing or recovering *T. matsutake* harvests in pine forests have been tested in Japan (Matsutake Research Association, 1964; Ogawa, 1978; Hosford et al., 1997), including *in vitro* ectomycorrhizal synthesis. However, very few data from these trials were reported. Wang, Hall, & Evans (1997) tested *in vitro* ectomycorrhization of *T. matsutake* with pine hosts and discussed their unique plant–fungus interactions, e.g., matsutake is involved in a symbiosis–saprobiosis–pathogenesis continuum. During this

time, *T. matsutake* mycorrhizae were considered atypical ectomycorrhizae *in situ*, due to the absence of Hartig net development at the pine root cortex (Ogawa, 1978; Hosford et al., 1997), although this finding was technically incorrect according to the microscopy results. However, Yamada, Kanekawa, & Ohmasa (1999a) reported continuous Hartig net development, which was confirmed shortly thereafter by another research group (Gill et al., 1999; Gill, Guerin-Laguette, Lapeyrie, & Suzuki, 2000). Yamada et al. (1999b) conducted *in vitro* mycorrhizal synthesis using *T. matsutake* and *Pinus densiflora* and demonstrated ectomycorrhizal development, as was later confirmed (Gill et al., 2000; Guerin-Laguette et al., 2000; Vaario, Guerin-Laguette, Gill, Lapeyrie, & Suzuki, 2000). The understanding how we characterize *T. matsutake* mycorrhizae has been revolutionized within the past 30 y as described above, leading to the adoption of the concept of ectomycorrhizal symbiosis in mycorrhizal studies of this fungus. The establishment and outplantation of ectomycorrhizal seedlings has subsequently been conducted in matsutake cultivation research (Yamada, Maeda, Kobayashi, & Murata, 2006; Kobayashi et al., 2007; Yamanaka et al., 2014, 2020).

The basic biology of matsutake has also been clarified. *T. matsutake* is distributed in both Asia and Europe (Bergius & Danell, 2000; Matsushita et al., 2005), and extant *T. matsutake* associations with coniferous hosts have been found to have evolved from an ancestral fungus that may have been associated with oak hosts, based on retrotransposon DNA analyses of matsutake genomes (Murata et al., 2013b). *In situ T. matsutake* colonies can form fairy rings comprising basidiomata in a "shiro" structure including several genets (Murata, Ohta, Yamada, Narimatsu, & Futamura, 2005; Lian, Narimatsu, Nara, & Hogetsu, 2006). This is because basidiospores of *T. matsutake* contribute to subsequent generations within the present geographic range (Amend, Garbelotto, Fang, & Keeley, 2010). *Tricholoma matsutake* associates with oaks as well as pines at the foot of the Tibetan Plateau in China (Yamanaka, Aimi, Wan, Cao, & Chen, 2011), and its inoculation promotes the growth of pine seedlings *in vitro* (Guerin-Laguette et al., 2004). Cultured *T. matsutake* mycelia colonize arbuscular mycorrhizal plants, forming "root endophytes" *in vitro* (Murata et al., 2013a, 2014a, b). All of these findings have informed later cultivation studies.

3.2. Mycorrhizal seedlings as a Tricholoma matsutake cultivation bottleneck

In vitro ectomycorrhizal synthesis of *T. matsutake* with a pine host requires approximately 6 mo from inoculum preparation (cultured mycelium) and seed germination to data sampling of mycorrhizal properties, i.e, morphology and anatomy, and the quantitative data of fungal colonization and the host growth (Yamada et al., 1999b, 2006). Once the inoculated mycelium has attached to the lateral root surface of the host pine, Hartig net hyphae develop at the root cortex within only a few weeks (Vaario et al., 2000). However, using large culture vessels (e.g., 1L) for large mycorrhizal seedlings increases the total incubation period to 1 y or longer. As *T. matsutake* mycelia grow slowly (1–2 cm/mo), they require longer periods than chanterelles to expand through the soil and develop ectomycorrhizae. Throughout the incubation period, light, temperature, soil water content, and ambient CO_2 and O_2 conditions should be controlled to prevent microbial contamination, which can influence fungal and plant growth. Soil nutrient composition and physiochemistry (Saito et al., 2018), as well as genetic properties of the matsutake strains (Yamada et al., 2010) and host plants (Yamada, Endo, Murata, Ohta, & Fukuda, 2014) can affect experimental outcomes. No studies have yet clarified the dominant nitrogen form adsorbed by *T. matsutake* from soil *in situ* (Vaario, Sah, Norisada, Narimatsu, & Matsushita, 2019; Yamanaka et al., 2020), although adding dried yeast to soil has been highly effective as a nitrogen source for mycorrhizal synthesis *in vitro* (Yamada et al., 2006). To optimize these factors for further ectomycorrhizal synthesis experiments, relevant soil, fungal strain, and plant cell line (somatic plant strain) standards must be determined for the study objectives.

It remains difficult to acclimate *T. matsutake* mycorrhizae synthesized *in vitro* to open and non-axenic conditions. In many ectomycorrhizal fungal taxa such as *Laccaria*, *Suillus*, *Rhizopogon*, *Hebeloma*, *Paxillus*, *Pisolithus*, *Lyophyllum*, *Amanita*, *Lactarius*, *Boletus*, and *Cantharellus*, ectomycorrhizal seedlings synthesized *in vitro* are easily acclimated to non-axenic pot soil conditions (Cairney & Champers, 1999; Yamada et al., 2001b, c; Endo et al., 2013, 2014; Ogawa et al., 2019b; Guerin-Laguette, 2021). Even *Tricholoma* species such as *T. portentosum*, *T. flavovirens*, *T. saponaceum*, and *T. terreum* show similar properties (Yamada et al., 2001b, c; Yamada, Kobayashi, Ogura, & Fukada, 2007). However, *T. matsutake* ectomycorrhizal root tips disappear easily from the host root system when the shiro structure, i.e., mycelium–soil aggregates associated with plant host roots, are damaged upon seedling transplantation from an *in vitro* system to pots containing soil. The effects on *T. matsutake* macroscopic structure in mycorrhizal acclimation require careful manipulation during the establishment of large mycorrhizal seedlings for outplanting. The instability of *T. matsutake* on ectomycorrhizal root tips during seedling acclimation to open pot soil remains unexplained enough. Previous studies have reported that *T. matsutake* has significantly smaller shoot/root ratios *in vitro* than *R. roseolus* or *C. anzutake* (Yamada et al., 2010; Ogawa et al., 2019b), which suggests that the colonized fungus imposes a cost on host plant growth. It is generally thought that late-stage ectomycorrhizal fungi represent a cost to symbiont seedlings during forest succession (Deacon & Fleming, 1992; Smith & Read, 2008). Therefore, studies on ectomycorrhizal synthesis for the ecological group are limited, and mycorrhizal acclimations for those fungi are not always easy (e.g., Cairney & Chambers, 1999). In a well-known late-stage fungus, *Boletus edulis*, however, ectomycorrhizae synthesized *in vitro* can be acclimated in open pot soil even by washing the root system prior to transplantation (Endo et al., 2014). These experimental data highlight that *T. matsutake* has unique mechanisms associated with shiro structures that promote their survival and growth *in situ*.

Due to the difficulty of manipulating fungus–plant associations in *T. matsutake*, field trials have produced only limited or preliminary data. Outplanted pine seedlings synthesizing *T. matsutake* ectomycorrhizae *in vitro* in a soil volume of 1 L were found to sustain mycorrhizal status and shiro structures for 2 y (Kobayashi, Terasaki, & Yamada, 2015). However, the shiro did not expand in the area, and extended pine roots were colonized by native ectomycorrhizal fungi such as suilloids. Direct inoculation of cultured *T. matsutake* mycelia to non-ectomycorrhizal roots of adult pine trees *in situ* led to successful ectomycorrhization, but mycorrhizal status did not persist beyond 1 y (Guerin-Laguette, Matsushita, Lapeyrie, Shindo, & Suzuki, 2005).

3.3. Role of shiro structures in Tricholoma matsutake cultivation

The term shiro originated from the soil area (meaning probably in the color or the territory in Japanese) in pine forests where *T. matsutake* mycelia inhabit (occupy) and from which basidiomata of this fungus occur (Hamada, 1974; Ogawa, 1978). A small fairy

ring of epigeous *T. matsutake* basidiomata ranging a few meters in diam is thought to be provided from the margin of a single shiro structure (single mycelial colony) (Hamada, 1953; Murata et al., 2005; Lian et al., 2006). Thus, shiro structures and fairy rings are often considered equivalent (e.g., Narimatsu et al., 2015); however, *T. matsutake* shiro structures consist of macroscopic (visible) mycelium–soil aggregates that develop from ectomycorrhizal root tips. Shiro mycelium is nutritionally supported by the host plant via connected ectomycorrhizal root tips; thus, even if a shiro structure does not produce a fruiting body, it can survive and grow in pine forest soil or in experimental pots for long periods, such that it is distinct in the meaning from a fairy ring.

Shiro research has taken a soil microbiological perspective because the dilution plating of shiro soil has led to the discovery of unique microbial arrays, with very low microbial detection, particularly beneath the basidioma stipe base, compared to soils sampled outside of the shiro (Ohara, 1966; Ohara & Hamada, 1967; Ogawa, 1978). Shiro antibiotic activity against soil microbes has been inferred from halo formations observed in soil bacteria from shiro soils spread on agar nutrient plates (Ohara, 1966). Although the nature of these antibiotic effects remain to be elucidated completely, Nishino et al. (2017) clarified that the main compound involved is aluminum oxalate, which was detected in *T. matsutake* shiro, and that this compound produced distinct halos in soil bacteria under natural concentrations. This insoluble aluminum compound has been suggested to be synthesized through a reaction of oxalate exuded from *T. matsutake* hyphae and soil aluminum phosphate, followed by absorption of the released phosphates by *T. matsutake* hyphae. This aluminum oxalate formation process provides yet another advantage in the nutrient-poor, acidic granite soils preferred by *T. matsutake*. Dissoluble aluminum ions, which are toxic to various organisms including plants, released from aluminum hydroxide under acid conditions are also converted into aluminum oxalate. Therefore, it is really the "killing three birds with one stone" effect (Hirai & Nishino, 2019). Due to this complicated chemical system, the shiro structure acts as the functional unit conferring ecological fitness to *T. matsutake* for *in situ* survival. These phenomena are also studied by Vaario et al. (2015) from the perspective of the effects of minerals produced by rock weathering on ectomycorrhizal fungi (Landeweert, Hoffland, Finlay, Kuyper, & van Breemen, 2001; Hoffland et al., 2004).

Single *T. matsutake* shiro structures can consist of several individuals (genets) of vegetative mycelia *in situ* (Murata et al., 2005; Lian et al., 2006). Since shiro formations are mainly studied in terms of epigeous basidiomata along with limited data available about shiro mycelia in the soil (Lian et al., 2006; Horimai et al., 2021), more comprehensive study is required for the structure of genetic heterogeneity and functional roles of sympatric genets to improve *T. matsutake* cultivation.

3.4. Shiro manipulation under controlled environmental conditions

Mycorrhizal synthesis experiments *in vitro* generally use small culture vessels containing 100–200 mL soil or other substrate with or without added nutrients (Yamada et al., 2006; Yamanaka, Maruyama, Yamada, Miyazaki, & Kikuchi, 2012; Murata et al., 2013a; Yamanaka et al., 2014; Endo et al., 2015; Saito et al., 2018; Horimai et al., 2020, 2021), which limit the volume of fully developed shiro structures in symbiosis with hosts to approximately 50 mL. Ectomycorrhizal systems can be established in even smaller culture vessels such as test tubes or Petri dishes to shorten the incubation period to a few mo; these are unsuitable for estimating shiro development despite the presence of measurable mycorrhizal root tips. Shiro structures can be increased in size by one order of magnitude using larger culture vessels (~500 mL) or bottles containing 4–5 L soil, which require 2–3 y of incubation (Guerin-Laguette, 2021; Horimai et al., 2021). Horimai et al. (2021) produced large shiro structures by transplanting an ectomycorrhizal seedling synthesized *in vitro* (~200 mL) into a larger culture vessel filled with 1 L soil under axenic conditions, and then into a culture bottle containing 4 L soil under non-axenic laboratory conditions. Kobayashi et al. (2007) synthesized ectomycorrhizae *in vitro* in culture vessels containing 1 L soil, with a ca. 1-y period from mycelium inoculation to mycorrhizal status estimation.

Mycorrhizal synthesis experiments typically use a single dikaryotic strain as inoculum for a single host seedling, such that the resulting shiro structure consists of a single fungal individual. It remains unknown whether such single-individual shiro development occurs naturally *in situ*. Matsutake mushrooms can be considered to have *k*- or C-selective survival strategies *in situ* (Yamada et al., 2014; Horimai et al., 2020), because they sustain a shiro structures on the meter-orders in area and are thought to live for decades (Hamada, 1953). Therefore, an established dikaryotic shiro mycelial system should encounter intraspecific spores on mycelial surfaces *in situ*, such that dikaryon–monokaryon mating will occur, producing fine-scale (millimeter- or centimeter-order) shiro mycelium heterogeneity. Such a process would explain the genetic mosaics observed among shiro structures *in situ* (Murata et al., 2005). Therefore, I hypothesize that mycelial genetic heterogeneity in shiro confers a functional advantage and can be produced experimentally through spore inoculation to an established shiro mycelium.

To test the this hypothesis (functional significance), I established a line of sibling strains from a single *T. matsutake* basidioma using the multispore isolation method, and then selected nine strains from among 100 tested strains based on their hyphal growth rates on modified Norkrans's C (MNC) agar medium, including rapid, moderate, and slow growth groups (N = 3 per group). The selected strains showed distinct physiological variation in response to changes in carbon and nitrogen levels in the MNC medium (Yamada et al., 2019). Therefore, I tested the mycelial growth patterns of the strains to evaluate their mycorrhizal symbiosis properties. Carbon/nitrogen balance significantly influences symbiosis efficiency in response to fungal nitrogen acquisition from soil, plant carbon fixation via photosynthesis, and interactive exchange at the interface of the Hartig net (Smith & Read, 2008). The nine sibling strains exhibited significant variation in ectomycorrhization in response to soil nitrogen levels (Horimai et al., 2020). The strain with the largest ectomycorrhizal biomass (#84) exhibited slow mycelial growth on MNC agar (Yamada et al., 2019), which suggests that preliminary ectomycorrhizal synthesis experiments are more important for identifying suitable fungal symbionts for cultivation than measuring mycelial growth rates on nutrient agar (Table 1). Next, I conducted a fungal combination experiment using three strains with different ectomycorrhization levels: #52 (moderate), #84 (high), and #99 (low) (Horimai et al., 2020). Paired inoculation of #52 and #99 led to higher ectomycorrhization levels than sin-

Table 1. Comparison of three sibling strains of *T. matsutake* in their mycelial properties

Mycelial properties	Relative comparison of three strains in their abilities
Growth rate on MNC agar	#52 > #99 > #84
Mycorrhization level	#84 > #52 > #99

This is a summarized result of Horimai *et al.* (2020)

gle-strain inoculation (Table 2). In a triple inoculation experiment with these strains in a single vessel, paired inocula (#52/#84, #52/#99, and #84/#99) showed higher ectomycorrhization levels than single inoculation with each strain (#52, #84, and #99) (Fig. 2). These results suggest the existence of a mechanism by which some combinations of sibling strains increase ectomycorrhizal biomass compared to a given single strain. Therefore, a new fungal strategy, competitive mycelium activation, was proposed such that the coexistence of genetically different intraspecific individuals (mycelia) increase their biomass relative to that of single individuals under ectomycorrhizal symbiosis (Horimai et al., 2020). When strains #52 and #99 were combined, strain #52 was activated by the presence of strain #99, as shown in polymerase chain reaction (PCR) detection results. Such a mechanism would have a significant impact on mycelium heterogeneity in *T. matsutake* shiro. By contrast, in shiro mycelium consisting of a single genet for many years, biomass could gradually decrease due to a lack of competitive activation.

Table 2. Comparison of ectomycorrhization level (number of ectomycorrhizal root tips) between paired strain inoculations and single strain inoculation to a single host.

Paired strain inoculations		Single strain inoculation
#52/#84	≈	Means of #52 and #84
#52/#99	>	Means of #52 and #99
#84/#99	≈	Means of #84 and #99

This is a summarized result of Horimai *et al.* (2020)

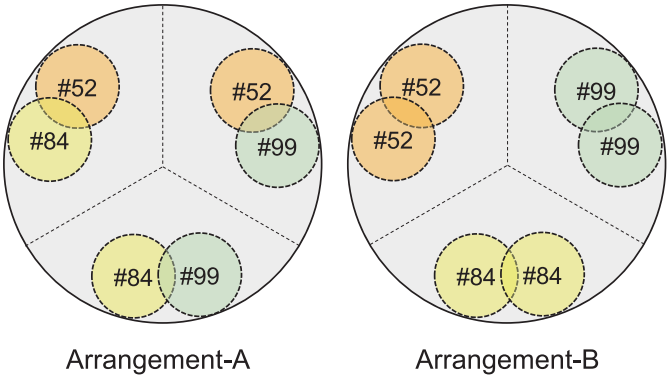

Fig. 2–Inoculation locations of *Tricholoma matsutake* mycelium in the triple inoculation experiment viewed from the top of the culture vessel (Horimai et al., 2020). Two configurations (arrangement-A, arrangement-B) were set up in this experiment, where three cultured strains were inoculated in a culture vessel as three pairs (arrangement-A) or singly (arrangement-B). The number in each circle of dashed line indicates the inoculated strain. Dashed straight lines show where the soil was separated, when the root system was measured, and the root tips were sampled for fungal DNA analysis (typing of fugal genet). As a result, the arrangement-A configuration showed significantly higher ectomycorrhizal biomass than that of the arrangement-B configuration. This figure is redrawn from Fig. 1 of Horimai et al. (2020).

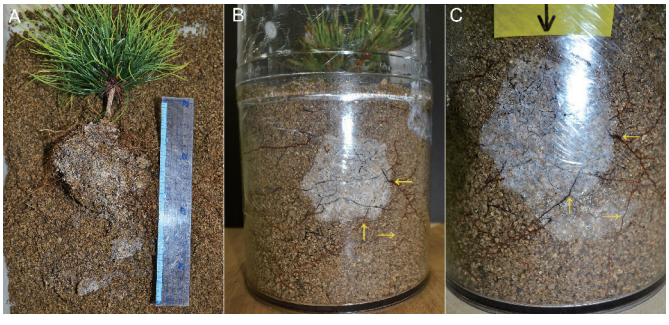

Fig. 3–*Tricholoma matsutake*. A: A large shiro development by the spore inoculation to the previously established ectomycorrhizae from a dikaryotic mycelial strain #84 in the 4 L pot soil, photographed Dec 2015. Please see Horimai et al. (2021) in the details of experiment. B, C: Shiro development of strain #84 in the 4 L pot soil, photographed Nov 2017 (B) and May 2018 (C). Arrows with the same directions in B and C indicating the same point in the pot soil show the shiro development in the lower area of pot soil throughout the six months observation.

To confirm another hypothesis (cause of the mycelial heterogeneity), I inoculated basidiospores onto established shiro structures following a previous study (Horimai et al., 2020). The spores colonized the shiro and developed new genets through ectomycorrhizal association with the host. Spore inoculation increased the amount of ectomycorrhizal biomass (Fig. 3A), supporting the competitive activation hypothesis and presenting a mechanism for the shiro genetic mosaic of shiro *in situ*. Thus, spore inoculation into ectomycorrhizal seedlings is a practical method for the effective production of large *T. matsutake* shiro structures on plant host root systems and to increase the genetic diversity of shiro mycelia (Horimai et al., 2021). This method apparently has a synergistic effect that stimulates competitive activation under environmental conditions.

3.5. Key gaps in our understanding of T. matsutake *biology*

Dr. Minoru Hamada spent a long time observing and recording the phenology of autumn *T. matsutake* fruiting in the pine forests of Kyoto, Japan (Hamada, 1974). His ideas and knowledge in the biology of this fungus were studied deeply and widely by his disciples. Dr. Makoto Ogawa is one of them, who published a textbook "The biology of matsutake" (Ogawa, 1978). Subsequently, these ideas have been applied in studies conducted throughout Japan (Ito & Iwase, 1997; Saito, 2020) as well as in Oregon, USA (Hosford et al., 1997), New Zealand (Wang et al., 1997), and other regions worldwide. Despite huge knowledge of matsutake mushrooms obtained in the academic world, it may be a very small part of the real nature of matsutake (Satsuka, 2019). I pick up here only four aspects in the paucity of our scientific knowledges in the biology of matsutake described below.

Among the poorly understood aspects of *T. matsutake* biology, the reason for the broad variation in *T. matsutake* basidioma production among forest sites remains unknown, that is high or less productivity is found even within the same mountain range, where vegetation, topography, soil structure, and forest use history are very similar. In Japan, matsutake habitats generally occur on mountain slopes, often rocky or steep, with relatively dry, shallow, and poor organic soil layers where minerals are weathered (B layer) on parent rocks (C layer) under humid and high-precipitation climate conditions (Ogawa, 1978; Vaario, Yang, & Yamada, 2017). In addition, *T. matsutake* prefers habitats with acidic or neutral parent rock such as granite, sedimentary rocks, or volcanic ash and avoids regions with mafic, basic, and ultrabasic parent rocks. Multivariate statistical analyses of soil elements and other environmental factors have shown that *T. matsutake* exhibits specific environmental preferences; however, it remains difficult to clarify the effects of these soil elements on *T. matsutake* mycelial growth, as well as its colonization patterns in forests. In Japan, forest management for *T. matsutake* production typically begins with historical records of past *T. matsutake* harvests, which indicate the suitability of the topography and soil structure of the site for this purpose. Accordingly, geographic patterns suitable for production of *T. matsutake* and other matsutake mushrooms can have important impacts on local economies. Annual *T. matsutake* harvests fluctuate significantly with the climate (Furukawa et al., 2016; Vaario et al., 2017), such that ongoing global climate change is also an immedi-

ate concern.

In situ growth patterns of *T. matsutake* mycelia are yet to be documented by the quantitative approach. The real-time quantitative PCR (qPCR) is expected for the documentation because the quantity of shiro mycelium probably determine the productivity of fruiting bodies. To date, qPCR data are mostly limited to the *in vitro* experimental systems (e.g., Yamaguchi et al., 2016; Fig. 3B, C). The qPCR, however, may not precisely mirror the growth pattern for hyphal cell mass. Because each hyphal cell can increase the biomass without cell division prior to the start of a growth phase such as sexual reproduction (basidioma morphogenesis). The variation in qPCR conversion factors among growth conditions has also been described as a weakness of this method (Wallander et al., 2013). The start of active cell division is preceded by biosynthetic events including nutrient accumulation in the cell, i.e., biomass growth (Jomura et al., 2020). Therefore, there may be a time lag between sequential patterns in actual biomass accumulation and those estimated by qPCR. Due to almost no alternative techniques that specifically measure quantity of the shiro mycelium *in situ*, the qPCR approach is needed technical progress that allow reliable measurement for the shiro biomass under heterogeneous and changeable environmental conditions.

Metagenomic analyses of microbial communities in shiro soils have allowed us to visualize general patterns of species composition (Kataoka et al., 2012; Kim et al., 2013, 2014; Oh, Fong, Park, & Lim, 2016); however, because most of the key microbes detected in such studies appear not to be culturable through general microbial culture methods, we cannot easily apply these findings to *T. matsutake* cultivation, particularly in seedling experiments. Although there are technical limitations to our understanding of *in situ* mycelial growth patterns in *T. matsutake*, it is evident that its shiro biomass is mainly controlled by the photosynthetic activity of hosts and photosynthate allocation to the root system for ectomycorrhizal symbiosis. Other environmental parameters also affect shiro biomass growth patterns in *T. matsutake*. Therefore, it is important to determine which factors alter *in situ* mycelial growth patterns of *T. matsutake* to identify the mechanisms underlying shiro structures, as has been conducted for other edible ectomycorrhizal mushrooms (Iotti et al., 2014; Iotti, Leonardi, Vitali, & Zambonelli, 2018; De la Varga et al., 2013; Castaño et al., 2017; Parladé, Martínez-Peña, & Pera, 2017).

To date, data supporting the molecular mechanisms of matsutake–pine interactions are rare. Genome data for matsutake mushrooms and pine trees are available; however, it remains difficult to test specific genetic mechanisms using suitable mutants. Murata et al. (2019) used *T. matsutake* mutants to demonstrate a unique fungus–plant interaction described as a conversion from mutualism to parasitism. Although this concept is based only on data from *in vitro* testing, we may infer that the tight relationship of ectomycorrhizal symbiosis is anchored by an important genetic function. A recent study reported taxon-specific expression of small secreted proteins (SSPs) that likely function as effectors in fungus–plant interactions in *Suillus*, which may explain the association between the host of this fungal taxon and Pinaceae (Lofgren et al., 2021). Even in saffron milk caps, specific SSPs bearing LysM have been reported (Lebreton et al., 2022). The identification of specific SSPs that determine the conifer hosts of *T. matsutake* and control gene expressions to influence host colonization levels would dramatically facilitate *T. matsutake* cultivation. The unique mycorrhizal structure of *T. matsutake* on its pine host, i.e., slender ectomycorrhizal root tips with a thin fungal mantle, carbonized root cortex in older ectomycorrhizae, elaborate branching that forms mycorrhizal clusters known as witch's brooms, and the macroscopic shiro structure require elucidation at the molecular level (Yamada et al., 1999a, 2006; Horimai et al., 2021).

4. Conclusion

When I first discovered a large cluster of *Cantharellus anzutake* basidiomata as a child, I wondered why it grew in that specific pine forest. I next encountered a large cluster of this fungus in a mixed oak–pine forest with my students, whose work opened new avenues toward *C. anzutake* cultivation. During my study of *T. matsutake* ecology as a postdoctoral researcher, the elderly owner of a pine forest producing *T. matsutake* basidiomata (*matsutake-yama*) told me that although her father had clear-cut the trees several decades ago, *T. matsutake* returned to the site once the pine forest had recovered. This story further inspired me to investigate the nature of this fungus. Another elderly *matstake-yama* owner recently mentioned "breath of soil" referring to the flow of air from deep soil to the forest floor during the summer rainy season, which was a familiar notion to me, as I had experienced it in another matsutake habitat. Yet another elderly *matstake-yama* owner told me that he had lectured Dr. Makoto Ogawa in the 1970s in a pine–hemlock forest where *T. matsutake* mushrooms were harvested, a mere 50 m from his own house. These voices of *matsutake-yama* owners suggest that the current scientific understanding of *T. matsutake* represents only a fragment of the human knowledge about its cultivation. The key to successfully cultivating a variety of edible ectomycorrhizal mushrooms, including chanterelles and matsutakes, in association with their hosts will likely depend on how much research time can be allocated in the habitat of these fungi. The appropriate manipulation of different types of fungal cells, i.e., spores, monokaryotic and dikaryotic hyphae, symbiotic mycorrhizal hyphae, at specific experimental stages should lead to mushroom production within active mycorrhizal systems. Key points in the experimental cultivation of *C. anzutake* and *T. matsutake* mushrooms have been summarized in flowcharts (Figs. 4, 5), which will be useful for designing future experiments and cultivation systems.

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References

Amend, A., Garbelotto, M., Fang, Z., & Keeley, S. (2010). Isolation by landscape in populations of a prized edible mushroom *Tricholoma matsutake*. *Conservation Genetics*, *11*, 795–802. https://doi.org/10.1007/s10592-009-9894-0

Aoki, W., Bergius, N., Kozlan, S., Fukuzawa, F., Okuda, H., Murata, H., Takahide A. Ishida, T. A., Vaario, L. M., Kobayashi, H., Kalmiş, E., Fukiharu, T., Gisusi, S., Matsushima, K., Terashima, Y., Narimatsu, M., Matsushita, N., Ka, K.H., Yu, F., Yamanaka, T., Fukuda, M., & Yamada, A. (2022). New findings on the fungal species *Tricholoma matsutake* from Ukraine, and revision of its taxonomy and biogeography based on multilocus phylogenetic analyses. *Mycoscience, 63* (in

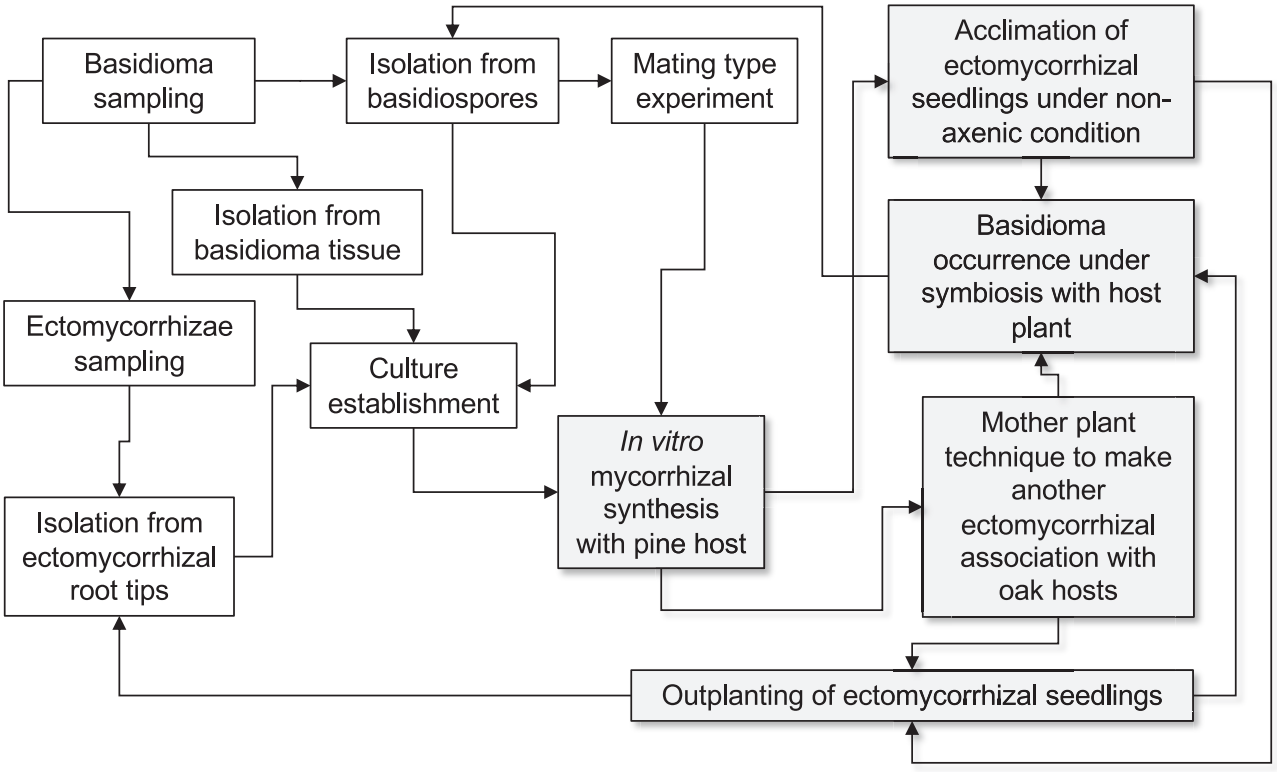

Fig. 4–Flowchart of experimental approach for the cultivation of *Cantharellus anzutake* with host associations. The highlight of pale gray color with shadow indicates the process under mycorrhizal symbiotic state. As *C. anzutake* fruits under experimental conditions, crossbreeding experiment can be conducted as routine works.

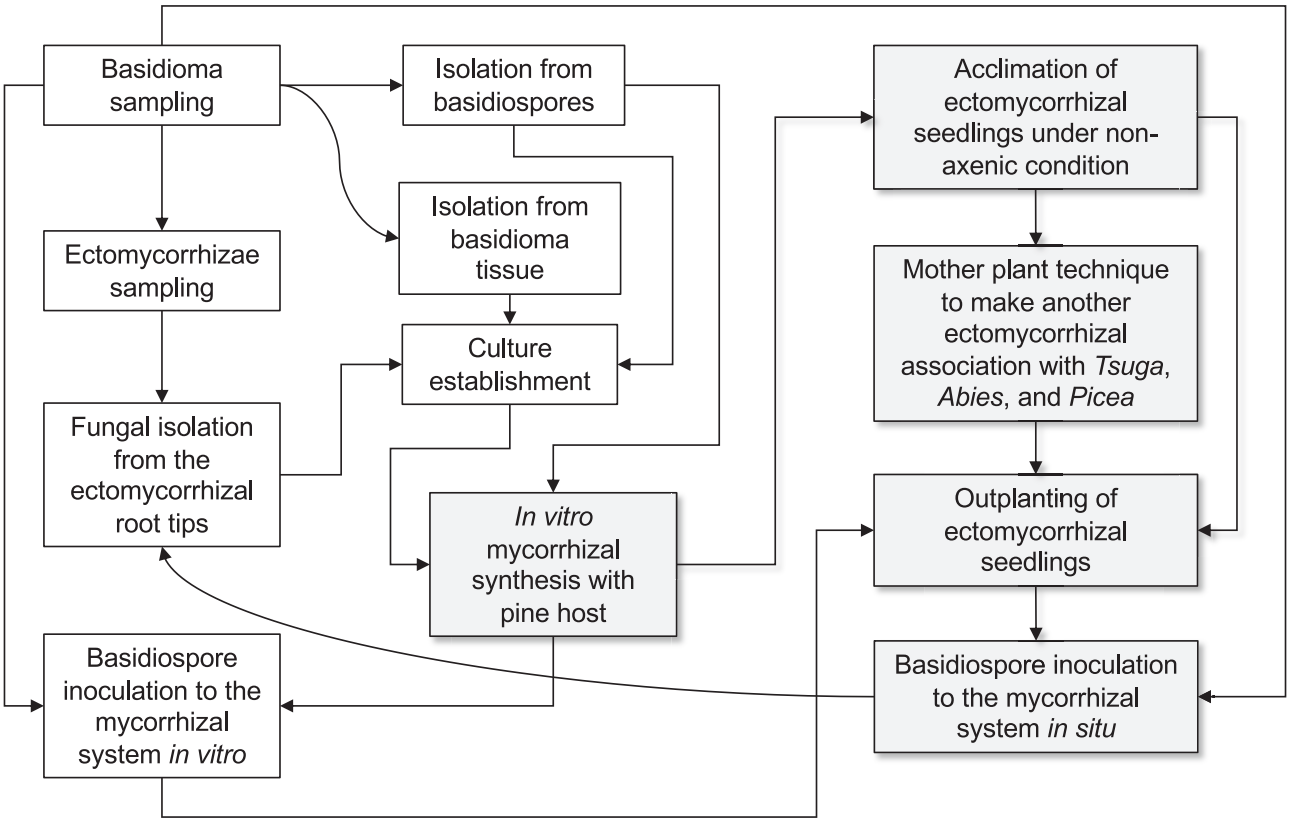

Fig. 5–Flowchart of experimental approach for the cultivation of *Tricholoma matsutake* with host associations. The highlight of pale gray color with shadow indicates the process under mycorrhizal symbiotic state. Although *T. matsutake* cannot be applicable crossbreeding experiments due to not fruiting under experimental condition, the spore inoculation to the mycorrhizal system substitutes in part the crossbreeding experiments.

press).
Arora, D. (2008). The houses that matsutake built. *Economic Botany, 62*, 278–290. https://doi.org/10.1007/s12231-008-9048-1
Bergius, N., & Danell, E. (2000). The Swedish matsutake (*Tricholoma nauseosum* syn. *T. matsutake*): Distribution, abundance and ecology. *Scandinavian Journal of Forest Research, 15*, 318–325. https://doi.org/10.1080/028275800447940
Cairney, J.W.G., & Chambers, S. M. (1999). *Ectomycorrhizal Fungi Key Genera in Profile*. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-06827-4_1
Cao, T., Hu, Y-P, Yu, J-R., Wei, T-Z., & Hai-Sheng Yuan, H-S. (2021). A phylogenetic overview of the *Hydnaceae* (*Cantharellales*, *Basidiomycota*) with new taxa from China. *Studies in Mycology, 99*, 100121. https://doi.org/10.1016/j.simyco.2021.100121
Castaño, C., Alday, J.G., Parladé, J., Pera, J., de Aragón, J.M., & Bonet, J.A. (2017). Seasonal dynamics of the ectomycorrhizal fungus *Lactarius vinosus* are altered by changes in soil moisture and temperature. *Soil Biology and Biochemistry, 115*, 253–260. https://doi.org/10.1016/j.soilbio.2017.08.021
Danell, E. (1994). Formation and growth of the ectomycorrhiza of *Cantharellus cibarius*. *Mycorrhiza 5*, 89–97. https://doi.org/10.1007/BF00202339
Danell, E. (1999). Cantharellus. In: Cairney, J.W.G., & Chambers, S.M. (eds) *Ectomycorrhizal Fungi Key Genera in Profile*. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-06827-4_10
Danell, E., Alström, S., & Ternström, A. (1993). *Pseudomonas fluorescens* in association with fruit bodies of the ectomycorrhizal mushroom *Cantharellus cibarius*. *Mycological Research, 97*, 1148–1152. https://doi.org/10.1016/S0953-7562(09)80519-4
Danell, E., Camacho, F. (1997). Successful cultivation of the golden chanterelle. *Nature, 385*, 303. https://doi.org/10.1038/385303a0
Danell, E., & Fries, N. (1990). Methods for isolation of *Cantharellus species*, and the synthesis of ectomycorrhizae with *Picea abies*. *Mycotaxon, 38*, 141–148.
Deacon, J. W., & Fleming, L. V. (1992). Interactions of ectomycorrhizal fungi. *In: Allen, M.F. (ed.) Mycorrhizal functioning: an integrative plant-fungal process.* London: Chapman and Hall, pp. 249–300.
De la Varga, H., Águeda, B., Ágreda, T., Martínez-Peña, F., Parladé, J., & Pera, J. (2013). Seasonal dynamics of *Boletus edulis* and *Lactarius deliciosus* extraradical mycelium in pine forests of central Spain. *Mycorrhiza, 23*, 391–402. https://doi.org/10.1007/s00572-013-0481-3
Dunham, S. M., Kretzer, A., & Pfrender, M. E. (2003a) Characterization of Pacific golden chanterelle (*Cantharellus formosus*) genet size using co-dominant microsatellite markers. *Molecular Ecology, 12*, 1607–1618. https://doi.org/10.1046/j.1365-294X.2003.01837.x
Dunham, S. M., O'Dell, T. E., & Molina, R. (2003b). Analysis of nrDNA sequences and microsatellite allele frequencies reveals a cryptic chanterelle species *Cantharellus cascadensis* sp. nov. from the American Pacific Northwest. *Mycological Research*, *107*, 1163–1177. https://doi.org/10.1017/S0953756203008475
Endo, N., Gisusi, S., Fukuda, M., & Yamada, A. (2013). In vitro mycorrhization and acclimatization of *Amanita caesareoides* and its relatives on *Pinus densiflora*. *Mycorrhiza, 23*, 303–315. https://doi.org/10.1007/s00572-012-0471-x
Endo, N., Kawamura, F., Kitahara, R., Sakuma, D., Fukuda, M., & Yamada, A. (2014). Synthesis of Japanese *Boletus edulis* ectomycorrhizae with Japanese red pine. *Mycoscience, 55*, 405–416. https://doi.org/10.1016/j.myc.2013.11.008
Endo, N., Dokmai, P., Suwannasai, N., Phosri, C., Horimai, Y., Hirai, N., Fukuda, M., & Yamada, A. (2015). Ectomycorrhization of *Tricholoma matsutake* with *Abies veitchii* and *Tsuga diversifolia* in the subalpine forests of Japan. *Mycoscience*, *56*, 402–412. https://doi.org/10.1016/j.myc.2014.12.004
Fries, N. (1979) Germination of spores of *Cantharellus cibarius*. *Mycologia, 71*, 216–219. https://doi.org/10.1080/00275514.1979.12021003
Furukawa, H., Masuno, K., & Takeuchi, Y. (2016). Forest management of matsutake productive sites for the optimization to global warming. *Annual Reports of Nagano Prefecture Forestry Research Center*, *30*, 87–100. https://agriknowledge.affrc.go.jp/RN/2010902230.pdf
Gill, W., Lapeyrie, F., Gomi, T., & Suzuki, K. (1999) *Tricholoma matsutake*– an assessment of in situ and in vitro infection by observing cleared and stained whole roots. *Mycorrhiza, 9*, 227–231. https://doi.org/10.1007/s005720050271
Gill, W., M., Guerin-Laguette, A., Lapeyrie, F., & Suzuki, K. (2000). Matsutake – morphological evidence of ectomycorrhiza formation between *Tricholoma matsutake* and host roots in a pure *Pinus densiflora* forest stand. *New Phytologist, 147*, 381–388. https://doi.org/10.1046/j.1469-8137.2000.00707.x
Guerin-Laguette, A. (2021). Successes and challenges in the sustainable cultivation of edible mycorrhizal fungi – furthering the dream. *Mycoscience, 62*, 10–28. https://doi.org/10.47371/mycosci.2020.11.007
Guerin-Laguette, A., Vaario, L-M., Gill, W. M., Lapeyrie, F., Matsushita, N., & Suzuki, K. (2000). Rapid in vitro ectomycorrhizal infection on *Pinus densiflora* roots by *Tricholoma matsutake*. *Mycoscience, 41*, 389–393. https://doi.org/10.1007/BF02463952
Guerin-Laguette, A., Shindo, K., Matsushita, N., Suzuki, K., & Lapeyrie, F. (2004). The mycorrhizal fungus *Tricholoma matsutake* stimulates *Pinus densiflora* seedling growth in vitro. *Mycorrhiza, 14*, 397–400. https://doi.org/10.1007/s00572-004-0322-5
Guerin-Laguette, A., Matsushita, N., Lapeyrie, F., Shindo, K., & Suzuki, K. (2005). Successful inoculation of mature pine with *Tricholoma matsutake*. *Mycorrhiza, 15*, 301–305. https://doi.org/10.1007/s00572-005-0355-4
Guerin-Laguette, A., Cummings, N., Butler, R.C., Willows, A., Hesom-Williams, N., L,i S., & Wang, Y. (2014). *Lactarius deliciosus* and *Pinus radiata* in New Zealand: towards the development of innovative gourmet mushroom orchards. *Mycorrhiza, 24*, 511–523. https://doi.org/10.1007/s00572-014-0570-y
Hamada M. (1953). Matsutake. *Shizen, 8*, 56–64.
Hamada, M. (1974). Matsutake nikki (diaries of matsutake). Kyoto University, Kyoto (in Japanese)
Herrera, M., Wang, R., Zhang, P., & Yu, F-Q. (2022). The ectomycorrhizal association of *Tricholoma matsutake* and two allied species, *T. bakamatsutake* and *T. fulvocastaneum*, with native hosts in subtropical China. *Mycologia, 114*, 303–318. https://doi.org/10.1080/00275514.2022.2025563
Hibbett, D.S., R. Binder, B.M., Giachini, A. J., Hosaka, K., Justo, A., Larsson, E., Larsson, K. H., Lawrey, J. D., Miettinen, O., Nagy, L. G., Nilsson, R. H., Weiss, M., & Thorn, R. G. (2014). 14 Agaricomycetes. In: McLaughlin, D., & Spatafora, J. (eds) *The Mycota, vol 7A: Systematics and Evolution*. Berlin Heidelberg: Springer. https://doi.org/10.1007/978-3-642-55318-9_14
Hirai, N., & Nishino, K. (2019). The antimicrobial metal complex protecting the shiro of *Tricholoma matsutake* from soil microorganisms. *Regulation of Plant Growth & Development, 54*, 54–59. https://doi.org/10.18978/jscrp.54.1_54
Hoffland, E., Kuyper, T.W., Wallander, H., Plassard, C., Gorbushina, A. A., Haselwandter, K., Holmström, S., Landeweert, R., Lundström, U.S., Rosling, A., Sen, R., Smits, M. M., van Hees, P. A. W., & van Breemen, N. (2004). The role of fungi in weathering. *Frontiers in Ecology and the Environment, 2*, 258–264. https://doi.org/10.1890/1540-9295(2004)002[0258:TROFIW]2.0.CO;2
Horimai, Y., Misawa, H., Suzuki, K., Fukuda, M., Furukawa, H., Masuno, K., Yamanaka, T., & Yamada, A. (2020). Sibling spore isolates of *Tricholoma matsutake* vary significantly in their ectomycorrhizal colonization abilities on pine hosts in vitro and form multiple intimate associations in single ectomycorrhizal roots. *Fungal Ecology, 43*, 100874. https://doi.org/10.1016/j.funeco.2019.100874
Horimai, Y., Misawa, H., Suzuki, K., Tateishi, Y., Furukawa, H., Yamanaka, T., Yamashita, S., Takayama, T., Fukuda, M., & Yamada, A. (2021). Spore germination and ectomycorrhizae formation of *Tricholoma matsutake* on pine root systems with previously established ectomycorrhizae from a dikaryotic mycelial isolate of *T. matsutake*. *Mycorrhiza, 31*, 335–347. https://doi.org/10.1007/s00572-021-01028-3
Hosford, D., Pilz, D., Molina, R., & Amaranthus, M. (1997). Ecology and management of the commercially harvested American matsutake mushroom. Gen. Tech. Rep. PNW-GTR-412. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 68 p.
Iotti, M., Leonardi, M., Lancellotti, E., Salerni, E., Oddis, M., Leonardi, P., Perini, C., Pacioni, G., & Zambonelli, A. (2014) Spatio-temporal dynamic of *Tuber magnatum* mycelium in natural truffle grounds. *PLoS ONE, 9*, e115921. https://doi.org/10.1371/ journal.pone.0115921
Iotti, M., Leonardi, P., Vitali, G., & Zambonelli, A. (2018). Effect of summer soil moisture and temperature on the vertical distribution of *Tuber magnatum* mycelium in soil. *Biology and Fertility of Soils, 54*, 707–716. https://doi.org/10.1007/s00374-018-1296-3
Ito, T., & Iwase, G. (1997). *Matsutake mushrooms–Grow and nurture like an orchard*. Tokyo: Nobunkyo.
Jeon, S-M., & Ka, K-H. (2016) Korean *Tricholoma matsutake* strains that promote mycorrhization and growth of *Pinus densiflora* seedlings. *Korean Journal of Mycology, 44*, 155–165. http://dx.doi.org/10.4489/KJM.2016.44.3.155
Jomura, M., Kuwayama, T., Soma, Y., Yamaguchi, M., Komatsu, M., & Maruyama, Y. (2020). Mycelial biomass estimation and metabolic quotient of *Lentinula edodes* using species-specific qPCR. *PLoS ONE, 15*, e0232049. https://doi.org/10.1371/journal.pone.0232049
Kataoka, R., Siddiqui, Z.A., Kikuchi, J., Ando, Sriwati, R., Nozaki, A., & Futai, K. (2012). Detecting nonculturable bacteria in the active mycorrhizal zone of the pine mushroom *Tricholoma matsutake*. *The Journal of Microbiology, 50*, 199–206. https://doi.org/10.1007/s12275-012-1371-7
Kim, M., Yoon, H., You, Y. H, Kim, Y. E., Woo, J. R., Seo, Y., Lee, G. M., Kim, Y.J., Kong, W. S., & Kim, J. G. (2013). Metagenomic analysis of fungal communities inhabiting the fairy ring zone of *Tricholoma matsutake*. *Journal of Microbiology and Biotechnology, 23*, 1347–1356. http://dx.doi.org/10.4014/jmb.1306.06068
Kim, M., Yoon, H., Kim, Y. E., Kim, Y. J., Kong, W. S., & Kim, J. G. (2014). Comparative analysis of bacterial diversity and communities inhabiting the fairy ring of

Tricholoma matsutake by barcoded pyrosequencing. *Journal of Applied Microbiology, 117*, 699–710. https://doi.org/10.1111/jam.12572

Kobayashi, H., Watahiki, T., Kuramochi, M., Onose, S., & Yamada, A. (2007). Production of pine seedlings with the shiro-like structure of the matsutake mushroom (*Trichotoma matsutake* (S.Ito et Imai) Sing.) in a large culture bottle. *Mushroom Science and Biotechnology, 15*, 151–155. https://doi.org/10.24465/msb.15.3_151

Kobayashi, H., Terasaki, M., Yamada, A. (2015) Two-year survival of *Tricholoma matsutake* ectomycorrhizas on *Pinus densiflora* seedlings after outplanting to a pine forest. *Mushroom Science and Biotechnology, 23*, 108-113. https://www.jstage.jst.go.jp/article/msb/23/3/23_KJ00010232484/_pdf

Landeweert, R., Hoffland, E., Finlay,R.D., Kuyper, T. W., & van Breemen, N. (2001). Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends in Ecology and Evolution, 16*, 248–254. https://doi.org/10.1016/S0169-5347(01)02122-X

Lebreton, A., Tang, N., Kuo, A., LaButti, K., Andreopoulos, W., Drula, E., Miyauchi, S., Barry, K., Clum, A., Lipzen, A., Mousain, D., Ng, V., Wang, R., Dai, Y., Henrissat, B., Grigoriev, I.V., Guerin-Laguette, A., Yu, F., & Martin, F. M. (2022). Comparative genomics reveals a dynamic genome evolution in the ectomycorrhizal milk-cap (*Lactarius*) mushrooms. *New Phytologist* (online version). https://doi.org/10.1111/nph.18143

Lian, C., Narimatsu, M., Nara, K., & Hogetsu, T. (2006). *Tricholoma matsutake* in a natural *Pinus densiflora* forest: correspondence between above- and below-ground genets, association with multiple host trees and alteration of existing ectomycorrhizal communities. *New Phytologist, 171*, 825–836. https://doi.org/10.1111/j.1469-8137.2006.01801.x

Lofgren, L.A., Nguyen, N.H., Vilgalys, R., Ruytinx, J., Liao, H.L., Branco, S., Kuo, A., LaButti, K., Lipzen, A., Andreopoulos, W., Pangilinan, J., Riley, R., Hundley, H., Na, H., Barry, K., Grigoriev, I.V., Stajich, J.E., & Kennedy, P.G. (2021). Comparative genomics reveals dynamic genome evolution in host specialist ectomycorrhizal fungi. *New Phytologist, 230*, 774–792. https://doi.org/10.1111/nph.17160

Masui, K. (1927). A study of the ectotrophic mycorrhizas of woody plants. *Memoirs of the College of science, Kyoto Imperial University, Series B III, 2*, 152–279.

Matsushita, N., Kikuchi, K., Sasaki, Y., Guerin-Laguette, A., Vaario, L.-M., Suzuki, K., Lapeyrie, F., & Intini, M. (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. https://doi.org/10.1007/S10267-004-0220-X

Matsutake Research Association (1964). *Matsutake (Tricholoma matsutake Singer) – Its fundamental studies and economic production of the fruit body–*. Matsutake Research Association, Kyoto.

Miyauchi, S., Kiss, E., Kuo, A., Drula, E., Kohler, A., Sánchez-García, M., Morin, E., Andreopoulos, B., Barry, K.W., Bonito, G., Buée, M., Carver, A., Chen, C., Cichocki, N., Clum, A., Culley, D., Crous, P.W., Fauchery, L., Girlanda, M., Hayes, R.D., Kéri, Z., LaButti, K., Lipzen, A., Lombard, V., Magnuson, J., Maillard, F., Murat, C., Nolan, M., Ohm, R.A., Pangilinan, J., de Freitas Pereira, M., Perotto, S., Peter, M., Pfister, S., Riley, R., Sitrit, Y., Stielow, J.B., Szöllősi, G., Žifčáková, L., Štursová, M., Spatafora, J.W., Tedersoo, L., Vaario, L.M., Yamada, A., Yan, M., Wang, P., Xu, J., Bruns, T., Baldrian, P., Vilgalys, R., Dunand, C., Henrissat, B., Grigoriev, I.V., Hibbett, D., Nagy, L.G., & Martin, F.M. (2020). Large-scale genome sequencing of mycorrhizal fungi provides insights into the early evolution of symbiotic traits. *Nature Communications, 11*, 5125. https://doi.org/10.1038/s41467-020-18795-w

Moncalvo, J.M., Nilsson, R.H., Koster, B., Dunham, S.M., Bernauer, T., Matheny, P.B., Porter, T.M., Margaritescu, S., Weiß, M., Garnica, S., Danell, E., Langer, G., Langer, E., Larsson, E., Larsson, K.H., & Vilgalys, R. (2006). The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. *Mycologia, 98*, 937–948. https://doi.org/10.1080/15572536.2006.11832623

Moore, L. M., Jansen, A. E., & van Griensven, L. J. L. D. (1989). Pure culture synthesis of ectomycorrhizas with *Cantharellus cibarius*. *Acta Botanica Neerlandica, 38*, 273–278. https://doi.org/10.1111/j.1438-8677.1989.tb01351.x

Murata, H., Ohta, A., Yamada, A., Narimatsu, M., & Futamura, N. (2005). Genetic mosaics in the massive persisting rhizosphere colony "shiro" of the ectomycorrhizal basidiomycete *Tricholoma matsutake*. *Mycorrhiza, 15*, 505–512. https://doi.org/10.1007/s00572-005-0358-1

Murata, H., Yamada, A., Maruyama, T., Endo, N., Yamamoto, K., Ohira, T., & Shimokawa, T. (2013a) Root endophyte interaction between ectomycorrhizal basidiomycete *Tricholoma matsutake* and arbuscular mycorrhizal tree *Cedrela odorata*, allowing in vitro synthesis of rhizospheric "shiro". *Mycorrhiza, 23,* 235–242. https://doi.org/10.1007/s00572-012-0466-7

Murata, H., Ota, Y., Yamaguchi, M., Yamada, A., Katahata, S., Otsuka, Y., Babasaki, K., & Neda, H. (2013b). Mobile DNA distributions refine the phylogeny of "matsutake" mushrooms, *Tricholoma* sect. *Caligata*. *Mycorrhiza, 23*, 447–461. https://doi.org/10.1007/s00572-013-0487-x

Murata, H., Yamada, A., Yokota, S., Maruyama, T., Endo, N., Yamamoto, K., Ohira, T., & Neda, H. (2014a). Root endophyte symbiosis in vitro between the ectomycorrhizal basidiomycete *Tricholoma matsutake* and the arbuscular mycorrhizal plant *Prunus speciosa*. *Mycorrhiza, 24*, 315–321. https://doi.org/10.1007/s00572-013-0534-7

Murata, H., Yamada, A., Maruyama, T., Endo, N., Yamamoto, K., Hayakawa, N., & Neda, H. (2014b). In vitro shiro formation between the ectomycorrhizal basidiomycete *Tricholoma matsutake* and *Cedrela herrerae* in the Mahogany family (Meliaceae). *Mycoscience, 55*, 275–279. https://doi.org/10.1016/j.myc.2013.10.005

Murata, H., Nakano, S., Yamanaka, T., Shimokawa, T., Abe, T., Ichida, H., Hayashi, Y., Tahara, K., & Ohta, A. (2019). Conversion from mutualism to parasitism: a mutant of the ectomycorrhizal agaricomycete *Tricholoma matsutake* that induces stunting, wilting, and root degeneration in seedlings of its symbiotic partner, *Pinus densiflora*, in vitro. *Botany, 97*, 463-474. https://doi.org/10.1139/cjb-2019-0060

Narimatsu, M., Koiwa, T., Masaki, T., Sakamoto, Y., Ohmori, H., & Tawaraya, K. (2015). Relationship between climate, expansion rate, and fruiting in fairy rings ('shiro') of an ectomycorrhizal fungus *Tricholoma matsutake* in a *Pinus densiflora* forest. *Fungal Ecology, 15*, 18–28. https://doi.org/10.1016/j.funeco.2015.02.001

Nishino, K., Shiro, M., Okura, R., Oizumi, K., Fujita, T., Sasamori, T., Tokitoh, N., Yamada, A., Tanaka, C., Yamaguchi, M., Hiradate, S., & Hirai, N. (2017). The (oxalato)aluminate complex as an antimicrobial substance protecting the "shiro" of *Tricholoma matsutake* from soil micro-organisms. *Bioscience, Biotechnology, and Biochemistry, 81*, 102–111. http://dx.doi.org/10.1080/09168451.2016.1238298

Ogawa, M. (1978). *The Biology of Matsutake*. Tsukiji-shokan, Tokyo.

Ogawa, M., & Hamada, M. (1975). Primordia formation of *Tricholoma matsutake* (Ito et Imai) Sing. in pure culture. *Transactions of the Mycological Society of Japan, 16,* 406–415.

Ogawa, W., Endo, N., Fukuda, M., & Yamada, A. (2018). Phylogenetic analyses of Japanese golden chanterelles and a new species description, *Cantharellus anzutake* sp. nov. *Mycoscience, 59*, 153–165. https://doi.org/10.1016/j.myc.2017.08.014

Ogawa, W., Endo, N., Takeda, Y., Kodaira, M., Fukuda, M., & Yamada, A. (2019a). Efficient establishment of pure cultures of yellow chanterelle *Cantharellus anzutake* from ectomycorrhizal root tips, and morphological characteristics of ectomycorrhizae and cultured mycelium. *Mycoscience, 60*, 45–53. https://doi.org/10.1016/j.myc.2018.08.003

Ogawa, W., Takeda, Y., Endo, N., Yamashita, S., Takayama, T., Fukuda, M., & Yamada, A. (2019b) Repeated fruiting of Japanese golden chanterelle in pot culture with host seedlings. *Mycorrhiza, 29*, 519–530. https://doi.org/10.1007/s00572-019-00908-z

Oh, S.Y., Fong, J.J., Park, M.S., & Lim, Y.W. (2016). Distinctive feature of microbial communities and bacterial functional profiles in *Tricholoma matsutake* dominant soil. *PLoS ONE, 11*, e0168573. https://doi.org/10.1371/journal. pone.0168573

Ohara, H. (1966). Antibacterial activity of mycorrhiza of *Pinus densiflora* formed by *Tricholoma matsutake*. *Proceedings of the Japan Academy, 42*, 503–506. https://doi.org/10.2183/pjab1945.42.503

Ohara, H., & Hamada, M. (1967). Disappearance of bacteria from the zone of active mycorrhizas in *Tricholoma matsutake* (S. Ito et Imai) Singer. *Nature, 213*, 528–529. https://doi.org/10.1038/213528a0

Parladé, J., Martínez-Peña, F., & Pera, J. (2017). Effects of forest management and climatic variables on the mycelium dynamics and sporocarp production of the ectomycorrhizal fungus Boletus edulis. *Forest Ecology and Management, 15*, 73–79. https://doi.org/10.1016/j.foreco.2017.01.025

Pérez-Moreno, J., Guerin-Laguette, A., Arzú, R.F., & Yu, F.Q. (2020) *Mushrooms, Humans and Nature in a Changing World*. Cham: Springer.

Pérez-Moreno, J., Guerin-Laguette, A., Rinaldi, A.C., Yu, F., Verbeken, A., Hernández-Santiago, F., & Martínez-Reyes, M. (2021). Edible mycorrhizal fungi of the world: What is their role in forest sustainability, food security, biocultural conservation and climate change? *Plants People Planet, 3*, 471–490. https://doi.org/10.1002/ppp3.10199

Pilz. D, Smith, J., Amaranthus, M., Alexander, S., Molina, R., & Luoma, D. (1999). Mushrooms and Timber: Managing commercial harvesting in the Oregon Cascades. *Journal of Forestry, 97*, 4–11. https://doi.org/10.1093/jof/97.3.4

Pilz, D., & Molina, R. (2002) Commercial harvests of edible mushrooms from the forests of the Pacific Northwest United States: issues, management, and monitoring for sustainability. *Forest Ecology and Management, 155*, 3–16. https://doi.org/10.1016/S0378-1127(01)00543-6

Pilz, D., Norvell, L., Danell, E., & Molina, R. (2003). Ecology and management of

commercially harvested chanterelle mushrooms. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station General Technical Report. PNW-GTR-576. Portland, OR, 83 p. https://doi.org/10.2737/PNW-GTR-576

Pilz, D., Molina, R., & Mayo, J. (2006) Effects of thinning young forests on chanterelle mushroom production. *Journal of Forestry, 104*, 9-14.

Rangel-Castro, J.I., Danell, E., & Pfeffer, P.E. (2002). A ^{13}C-NMR study of exudation and storage of carbohydrates and amino acids in the ectomycorrhizal edible mushroom *Cantharellus cibarius*. *Mycologia, 94*, 190–199. https://doi.org/10.1080/15572536.2003.11833224

Saito, C., Ogawa, W., Kobayashi, H., Yamanaka, T., Fukuda M., & Yamada, A. (2018). In vitro ectomycorrhization of *Tricholoma matsutake* strains is differentially affected by soil type. *Mycoscience, 59*, 89–97. https://doi.org/10.1016/j.myc.2017.09.002

Saito, M. (2020). *The world of mycorrhizae*. Tokyo: Tsukiji-shokan.

Satsuka, S. (2019) Chapter 14: Translation in the world multiple. In: Omura, E., Ohtsuki, G. J., Satsuka, S., & Morita A. (eds.), *The world multiple: The quotidian politics of knowing and generating entangled worlds*. New York: Routledge, pp. 219–232.

Shao, S.C., Liu, P. G., Wei, T. Z., & Herrera, M. (2021). New insights into the taxonomy of the genus *Cantharellus* in China: epitytication of *C. yunnanensis* W. F. Chiu and the first record of *C. cibarius* Fr. *Cryptogamie Mycologie, 42*, 25–37. https://doi.org/10.5252/cryptogamie-mycologie2021v42a3

Smith, S. E., & Read, D. J. (2008). *Mycorrhizal symbiosis (3rd edn.)*. New York: Academic Press.

Straatsma, G., Konings, R. N. H., & van Griensven, L. J. L. D. (1985). A strain collection of the mycorrhizal mushroom *Cantharellus cibarius* obtained by germination of spores and culture of fruit body tissue. *Transactions of the British Mycological Society, 85*, 689–697. https://doi.org/10.1016/S0007-1536(85)80265-5.

Suwannasai, N., Dokmai, P., Yamada, A., Watling, R., & Phosri, C. (2020). First ectomycorrhizal syntheses between *Astraeus sirindhorniae* and *Dipterocarpus alatus* (Dipterocarpaceae), pure culture characteristics, and molecular detection. *Biodiversitas, 21*, 231–238. https://doi.org/10.13057/biodiv/d210130

Thorn, R. G., Kim, J.I., Lebeuf, R., & Voitk, A. (2017). The golden chanterelles of Newfoundland and Labrador: a new species, a new record for North America, and a lost species rediscovered. *Botany, 95*, 547–560. https://doi.org/10.1139/cjb-2016-0213

Tsing, A. L. (2015). *The mushroom at the end of the world: On the possibility of life in capitalist ruins*. Princeton University Press. Oxford, U. K. 331 p.

Vaario, L.-M., Guerin-Laguette, A., Gill, W.M., Lapeyrie, F., & Suzuki, K. (2000). Only two weeks are required for *Tricholoma matsutake* to differentiate ectomycorrhizal Hartig net structures in roots of *Pinus densiflora* seedlings cultivated on artificial substrate. *Journal of Forest Research, 5*, 293–297. https://doi.org/10.1007/BF02767125

Vaario, L. M., Pennanen, T., Sarjala, T., Savonen, E. M., & Heinonsalo, J. (2010). Ectomycorrhization of *Tricholoma matsutake* and two major conifers in Finland—an assessment of in vitro mycorrhiza formation. *Mycorrhiza, 20*, 511–518. https://doi.org/10.1007/s00572-010-0304-8

Vaario, L.M., Pennanen, T., Lu, J., Palmén, J., Stenman, J., Leveinen, J., Kilpeläinen, P., & Kitunen, V. (2015). *Tricholoma matsutake* can absorb and accumulate trace elements directly from rock fragments in the shiro. Mycorrhiza 25, 325–334. https://doi.org/10.1007/s00572-014-0615-2

Vaario, L.-M., Yang, X., & Yamada, A. (2017). Biogeography of the Japanese gourmet fungus, *Tricholoma matsutake*: a review of the distribution and functional ecology of Matsutake. In: Tedersoo, L. (ed.), *Biogeography of Mycorrhizal Symbiosis. Ecological Studies (Analysis and Synthesis), vol. 230*. Springer, Cham, pp. 319–344. https://doi.org/10.1007/978e3-319e56363e3_15.

Vaario, L.M., Sah, S.P., Norisada, M., Narimatsu, M., & Matsushita, N. (2019). *Tricholoma matsutake* may take more nitrogen in the organic form than other ectomycorrhizal fungi for its sporocarp development: the isotopic evidence. *Mycorrhiza, 29*, 51–59. https://doi.org/10.1007/s00572-018-0870-8

Wallander, H., Ekblad, A., Godbold, D.L., Johnson, D., Bahr, A., Baldrian, P., Björk, R.G., Kieliszewska-Rokicka, B., Kjøller, R., Kraigher, H., Plassard, C., & Rudawska, M. (2013). Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forests soils – A review. *Soil Biology and Biochemistry, 57*, 1034–1047. https://doi.org/10.1016/j.soilbio.2012.08.027

Wang, Y., Hall, I. R., & Evans, L. A. (1997). Ectomycorrhizal fungi with edible fruiting bodies. 1. *Tricholoma matsutake* and related fungi. *Economic Botany, 51*, 311–327. https://doi.org/10.1007/BF02862101

Wang, Y., & Chen, Y. L. (2014). Recent advances in cultivation of edible mycorrhizal mushrooms. In: Solaiman, Z., Abbott, L., Varma, A. (eds) *Mycorrhizal fungi: use in sustainable agriculture and land restoration. Soil Biology, vol 41*. Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-662-45370-4_23

Yamada, A., & Katsuya, K. (1995). Mycorrhizal association of isolates from sporocarps and ectomycorrhizas with *Pinus densiflora* seedlings. *Mycoscience, 36*, 315–323. https://doi.org/10.1007/BF02268607

Yamada, A., Kanekawa, S., & Ohmasa, M. (1999a). Ectomycorrhiza formation of *Tricholoma matsutake* on *Pinus densiflora*. *Mycoscience*, *40*, 193–198. https://doi.org/10.1007/BF02464298

Yamada, A., Maeda, S., & Ohmasa, M. (1999b). Ectomycorrhiza formation of *Tricholoma matsutake* isolates on *Pinus densiflora* in vitro. *Mycoscience*, *40*, 455–463. https://doi.org/10.1007/BF02461022

Yamada, A., Ogura, T., Degawa, Y., & Ohmasa, M. (2001a). Isolation of *Tricholoma matsutake* and *T. bakamatsutake* cultures from field-collected ectomycorrhizas. *Mycoscience 42*, 43–50. https://doi.org/10.1007/BF02463974

Yamada, A., Ogura, T., & Ohmasa, M. (2001b). Cultivation of mushrooms of edible ectomycorrhizal fungi associated with *Pinus densiflora* by in vitro mycorrhizal synthesis. I. Primordium and basidiocarp formation in open-pot culture. *Mycorrhiza, 11*, 59–66. https://doi.org/10.1007/s005720000092

Yamada, A., Ogura, T., & Ohmasa, M. (2001c). Cultivation of mushrooms of edible ectomycorrhizal fungi associated with *Pinus densiflora* by in vitro mycorrhizal synthesis. II. Morphology of mycorrhizas in open-pot soil. *Mycorrhiza, 11*, 67–81. https://doi.org/10.1007/s005720000093

Yamada, A., Kobayashi, H., & Murata, H. (2003). *Tricholoma matsutake* IFO6933 and IFO30604, "matsutake" isolates that have been maintained on slants and widely used in vitro for a quarter to half a century, can form ectomycorrhiza in *Pinus densiflora*. *Mycoscience, 44*, 249–251. https://doi.org/10.1007/s10267-003-0098-z

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. https://doi.org/10.1007/s00572-005-0021-x

Yamada, A., Kobayashi, H., Ogura, T., & Fukada, M. (2007). Sustainable fruit-body formation of edible mycorrhizal *Tricholoma* species for 3 years in open pot culture with pine seedling hosts. *Mycoscience, 48*, 104–108. https://doi.org/10.1007/s10267-006-0338-0

Yamada, A., Kobayashi, H., Murata, H., Kalmiş E., Kalyoncu, F., & Fukuda, M. (2010). In vitro ectomycorrhizal specificity between the Asian red pine *Pinus densiflora* and *Tricholoma matsutake* and allied species from worldwide Pinaceae and Fagaceae forests. *Mycorrhiza*, *20*, 333–339. https://doi.org/10.1007/s00572-009-0286-6

Yamada, A., Endo, N., Murata, H., Ohta, A. & Fukuda, M. (2014). *Tricholoma matsutake* Y1 strain associated with *Pinus densiflora* shows a gradient of in vitro ectomycorrhizal specificity with Pinaceae and oak hosts. *Mycoscience, 55*, 27–34. https://doi.org/10.1016/j.myc.2013.05.004

Yamada, A., Furukawa, H., & Yamanaka, T. (2017) Cultivation of edible ectomycorrhizal mushrooms in Japan. *Revista Fitotecnia Mexicana, 40*, 379–389.

Yamada, A., Hayakawa, N., Saito, C., Horimai, Y., Misawa, H., Yamanaka, T., & Fukuda, M. (2019). Physiological variation among *Tricholoma matsutake* isolates generated from basidiospores obtained from one basidioma. *Mycoscience*, *60*, 102–109. https://doi.org/10.1016/j.myc.2018.12.001

Yamaguchi, M., Narimatsu, M., Fujita, T., Kawai, M., Kobayashi, H., Ohta, A., Yamada, A., Matsushita, N., Neda, H., Shimokawa, T., & Murata, H. (2016). A qPCR assay that specifically quantifies *Tricholoma matsutake* biomass in natural soil. Mycorrhiza 26, 847–861. https://doi.org/10.1007/s00572-016-0718-z

Yamanaka, K., Aimi, T., Wan, J., Cao, H., & Chen, M. (2011). Species of host trees associated with *Tricholoma matsutake* and allies in Asia. Mushroom *Science and Biotechnology*, *19*, 79–87. https://doi.org/10.24465/msb.19.2_79

Yamanaka, T., Maruyama, T., Yamada, A., Miyazaki, Y., & Kikuchi, T. (2012). Ectomycorrhizal formation on regenerated somatic pine plants after inoculation with *Tricholoma matsutake*. *Mushroom Science and Biotechnology, 20*, 93–97.

Yamanaka, T., Ota, Y., Konno, M., Kawai, M., Ohta, A., Neda, H., Terashima, Y., & Yamada, A. (2014) The host ranges of conifer-associated *Tricholoma matsutake*, Fagaceae-associated *T. bakamatsutake* and *T. fulvocastaneum* are wider in vitro than in nature, *Mycologia, 106*, 397-406. https://doi.org/10.3852/13-197

Yamanaka, T., Yamada, A., & Furukawa, H. (2020). Advances in the cultivation of the highly-prized ectomycorrhizal mushroom *Tricholoma matsutake*. *Mycoscience*, *61*, 49–57. https://doi.org/10.1016/j.myc.2020.01.001

Zambonelli, A. & Bonito G.M. (eds) (2012). *Edible ectomycorrhizal mushrooms: Current knowledge and future prospects. Soil Biology volume 34*. Berlin Heidelberg: Springer.

Zambonelli, A., Iotti, M., & Murat, C. (2016). *True truffle (Tuber spp.) in the world: Soil ecology, systematics and biotechnology. Soil Biology volume 47*. Cham: Springer.