THE ECTOMYCORRHIZAL FUNGUS *TRICHOLOMA MATSUTAKE* IS CAPABLE OF FACULTATIVE SAPROTROPHY

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ABSTRACT

We studied carbon acquisition in *Tricholoma matsutake* by combining morphological, chemical and enzymatic experiments conducted both in the laboratory and natural setting. Associations between host plants and isolates of *T. matsutake* from Finland (2) and Japan (1) were confirmed via *in vitro* formation of ectomycorrhizae (ECM). Chemical properties and enzyme-activity rates were determined for samples of mycelia-soil aggregation (shiro) collected from sites of sporocarp formation and nearby control spots. Annual growth and seasonal changes in tissue and ECM health were monitored in a natural population of matsutake. Finally, several organic substrates were evaluated as the sole carbon source for *T. matsutake* growing *in vitro* and according to the most active enzymes in the shiro.

Matsutake formed typical ECM with the conifers *Pinus sylvestris* and *Picea abies* but did not form associations with Silver Birch (*Betula pendula*). Finnish isolates formed ECM on both conifers but the Japanese strain was less compatible, with only a partial Hartig net being observed in *P. sylvestris*. Saprotrophic feeding of the Japanese isolate was observed in culture with *P. abies.* Preferred organic carbon sources and enzyme activities *in vitro* corresponded to those observed in the shiro. Enzyme assays confirmed the presence and increased production of organic carbon degradation related enzymes during sporocarp formation, when ECM root tips were necrotic. Mycelial growth on culture media consisting of complex polysaccharides was similar to that composed of simple sugars (e.g., glucose). In addition to its typical life strategy as an ECM symbiont, results suggest that *T. matsutake* can exist as a saprotroph.

Key words: Matsutake; Mycorrhization; Saprophytic potential; Fungal ecology

INTRODUCTION

Tricholoma matsutake (S. Ito *et Imai)* is an ectomycorrhizal (ECM) fungus found in pine and spruce forests in the Northern hemisphere [11, 20, 18]. The fungus produces commercially valuable mushrooms that have been revered in Japan for their flavour, medicinal properties and iconic significance for centuries. Over the past 50–60 years, these edible fungi have become increasingly rare in Japan where the annual yield of matsutake has decreased from 12,000 tons in the 1940s to a few hundred tons today. One of the reasons for this reduction may be the introduction and spread of the pine nematode (*Bursaphelencus lignicolus*) in Japanese forests. Nearly 3000 tons of *T. matsutake* or closely related species are exported to Japan annually, with a retail value of approximately one billion US dollars [13]. This mushroom was known as *T.*

nauseosum in Nordic countries until recently when molecular techniques revealed its conspecificity with *T. matsutake* [2]. Matsutake mushrooms are distributed patchily throughout Finland [8], where they became a commercially harvested mushroom in 2007. The new and growing value of this non-woody product has received increasing attention in Nordic countries.

Many studies have been focusing on improving sporocarp formation in nature, e.g., by outplanting the mycorrhizal seedlings, and cultivating matsutake under controlled conditions. However, efforts to cultivate this species have not been successful, and knowledge of sporocarp formation in the shiro remains in its infancy. The shiro is a unique and massive aggregate of mycorrhizae, mycelium, host plant roots and soil particles [6, 12].

The objectives of this study are to confirm the relationships between *T. matsutake* and the major forest tree species in Finland, and to evaluate the saprotrophic potential of *T. matsutake* both *in vitro* and *in vivo*. We combined morphological observations, enzyme activity measurements and physico-chemical profiling of soil dominated by *T. matsutake*. We tested the following hypotheses: (1) *T. matsutake* is a typical ectomycorrhizal fungus but can exist as a facultative saprotroph, and (2) sporocarp formation is related to the amount of available organic carbon and other degradation products in the shiro.

MATERIALS AND METHODS

Three isolates of *T. matsutake* (S. Ito et Imai) Sing. were screened in this study: Japanese isolate (JA) (Tm 0945, [10]), Finnish eastern isolate (EF) (GQ904716, [18]) and Finnish southern isolate (SF) (JF346748). The *in vivo* study was conducted in Nuuksio national park (60°18'16"N, 24°31'10" E) in southern Finland, which supports a mixed forest of Scots Pine (*Pinus sylvestris* L.), Norway Spruce (*Picea abies* [L.] H. Karst.) and Silver Birch (*Betula pendula* Roth). At this site, sporocarps of *T. matsutakae* were found continuously during the study period (2008–2010).

RESULT AND DISCUSSION

Formation of ectomycorrhizae between *Tricholoma matsutake* and the main forest tree species in Finland. We tested the extent to which a local Finnish isolate (GQ904716, [18]) and a Japanese isolate (Tm945, [10]) formed ectomycorrhizae with the three most common tree species in Finland; *P. sylvestris*, *P. abies* and *B. pendula*. Under laboratory conditions, *T. matsutake* formed typical ectomycorrhizae with *P. sylvestris* (Fig. 1a & 1b from [18]) and *P. abies* but not *B. pendula*. Germinated seedlings of *P. sylvestris* and *P. abies* were inoculated with either isolate, and after eight months the Finnish isolates had formed a sheath and Hartig net on both host species. Inoculation with the Japanese isolate resulted in an initial Hartig net-like structure in *P. sylvestris* but not in *P. Abies*, but a fully formed Hartig net was not observed in either. Ectomycorrhizal *P. sylvestris* seedlings inoculated with the Finnish isolates showed the same shoot height and dry mass as controls, whereas those of *P. abies* had similar shoot height but slightly less dry mass than control seedlings. For both tree species, inoculation with the Finnish isolate resulted in reduced total nitrogen content per seedling but carbon content was unaffected. Seedlings of both species inoculated with the Japanese isolate showed significantly reduced growth, dry mass, nitrogen and carbon content per seedling and shoot height (in spruce) compared to the controls. These results document and describe the *in vitro* ectomycorrhization between *T. matsutake* and *P. sylvestris* and *P. abies* and the variable mycorrhizal structures that strains of matsutake can form [18].

Interestingly, in addition to the apparent preference shown by the Japanese isolate for *P. sylvestris*, we observed its saprotrophic behaviour in co-culture with *P. abies*. It should also be

mentioned that although we did not find any Hartig net or similar structure in inoculated seedlings of *Betula pentula*, root tips exhibited signs of necrosis (Fig. 1c & 1d, unpublished data). Host specificity or host preference of *T. matsutake* remains an ongoing study in our group.

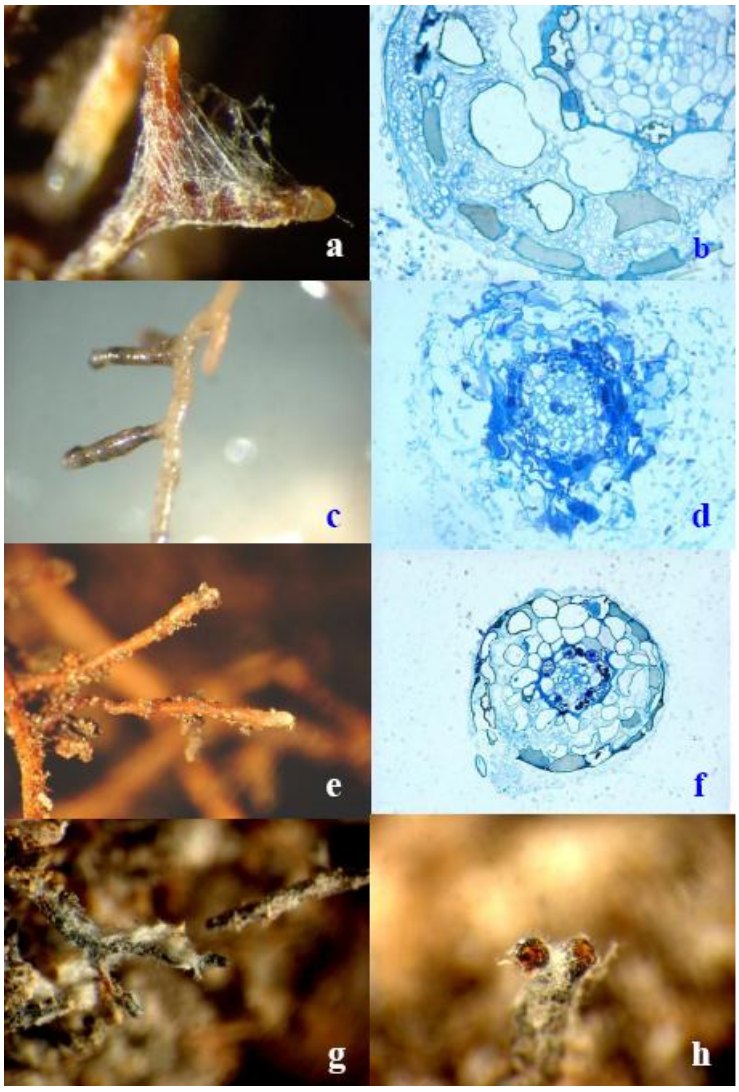

Figure 1: a-b, External morphology and light micrographs of fungus-inoculated root system of *Pinus sylvestris* eight months post-inoculation by the Finnish isolate (GQ904716): (a) the dichotomous lateral root is colonized by dense fungal mycelium; (b) transverse section of ectomycorrhizal root showing multiseriate Hartig net development within the cortex, between cortical cells, tannin cells present in cortex.
c-d, External morphology and light micrographs of fungus-inoculated root system of *Betula pentula* two months post-inoculation by the Finnish isolate (GQ904716): (c) the monopodial lateral root with light necroses; (d) transverse section of root tip showing no Hartig net formation within the cortex.
e-f, External morphology and light micrographs of root tips from *T. matsutake* shiro spot in early summer of 2009: (e) the monopodial lateral root with a loose external mycelium; (f) transverse section of root tip showing the well-developed Hartig net structure within the cortex.
g-h, External morphology of root tips from *T. matsutake* shiro spot in autumn of 2009: (g) many mycorrhizal root tips were necrotic; (h) or clearly suffering.

Mycorrhizal root tips showed signs of necrosis in the shiro. In our study site, live mycorrhizal root tips and Hartig net (or similar) structures were observed in the early summer (Fig. 1e & 1f, unpublished data). However, we found that many mycorrhizal root tips were necrotic (Fig. 1g) or clearly suffering (Fig. 1h) during the fruiting season. This phenomenon has also been reported elsewhere [5]. A breakdown in the association between matsutake and host plant during the reproductive season may be connected to its facultative saprotrophy.

Saprotrophic potential of *Tricholoma matsutake*. In this study, eight enzymes were assayed in soil samples dominated by *T. matsutake* (shiro+) or nearby control (PCR-negative: shiro-) spots. We assayed the same enzymes in an *in vitro* culture system in which mycelium of *T. matsutake* was used to inoculate bark chips of *P. sylvestris*. Results indicated higher activities of cellulose and hemicellulose degradation related enzymes in shiro+ than in shiro- soil immediately following sporocarp harvesting (unpublished data). It should be emphasized that many root tips in shiro+ samples were necrotic [11, 5].

In a *T. matsutake*–sawdust culture system, Vaario et al. [17] observed mycelial growth in xylem tissue and reported earlier that *T. matsutake* produced mainly β-glucosidase when pine bark was used as the substrate *in vitro* [16]. Subsequently, Kusuda et al. [7] purified and characterized β-glucosidase in wild matsutake. These findings suggest that *T. matsutake* can secrete cellulolytic and hemicellulolytic enzymes to acquire carbon from its environment, and results from the bark culture experiment confirmed this (unpublished data). Furthermore, enzyme activity between day 20 and day 40 were similar, which suggests the capacity to utilize this form of organic carbon (i.e., bark chips) is stable. Hyphae continued growing throughout the two-month study period.

We provided polysaccharides as the sole carbon source in a liquid medium to measure the growth capacity of *T. matsutake*. After a 60-day incubation period, a similar net increase of mycelial biomass was observed in culture media containing either simple sugar (e.g., glucose) or polysaccharides. The *in vitro* culture data suggest that *T. matsutake* can secrete a cocktail of enzymes suitable for bark as well as a medium containing simple sugars. This finding suggests that *T. matsutake* has, at least, the chemical means to feed saprotrophically.

Because *T. matsutake* can use organic carbon and secrete related cellulolytic and hemicellulolytic enzymes, we suggest three possible mechanisms to explain how *T. matsutake* gains extra energy for shiro and fruitbody formation. Firstly, different kinds of organic carbon compounds exist in the litter layer that could be leached to the mineral layer where most of the mycelium is found. Such nutrient leaching would be especially important if most of the mycorrhizal root tips are necrotic and the symbiotic association is no longer in place. Secondly, *in vitro* enzyme profiles suggest that *T. matsutake* can produce certain cellulose degradation related enzymes, which could facilitate the degradation of cellulose and make its products available, e.g., cellobiose [9]. Finally, our earlier work showed that several saprotrophic and mycorrhizal/litter-decay fungi (e.g., *Trichoderma viride*) in the litter layer above the shiro were positively correlated with the presence of matsutake [19]. It seems possible that the degradation carried out by these fungal associates could provide the available carbon source leached to the mineral layer for *T. matsutake* uptake.

Deacon and Fleming [4] reviewed the succession of ECM fungal guilds and suggested that species may be classified as early (low sugar requirements, small and ephemeral sporacarps with easily germinated spores) or late (high sugar requirements, large and persistent sporocaps with spores that are difficult to germinate) stage. According to this classification, *T. matsutake* conforms to a late stage species. Whether high sugar requirement is the limiting factor in shiro and sporocarp formation needs to be examined *in vivo*. Recent studies on the functional and ecological significance of ECM symbiosis have emphasized its importance in the ecosystem [1, 3]. Increasing attention is being given to the functional diversity of ECM fungi [14], and how

they may occupy a position along the biotrophy-saprotrophy continuum [15]. A flexible trophic ecology would be a considerable advantage for *T. matsutake* in shiros where disconnection from host plants occurs, and may be a necessary stage of the life cycle.

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