4
The Significance of Mycorrhizae in Forest Ecosystems

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4.1 Introduction

In forests, various organisms live by interaction with other species. Fungi, in particular, have various modes of life such as those of saprophytes, parasites, and symbionts. For example, the honey mushroom (*Armillaria* spp.) is known as "a fungus shrouded in a mystery," because it goes through various modes of life. It was recognized as the largest organism in the world by *Nature* in 1992 (Smith et al. 1992; Suzuki 1996). In recent years, it has been revealed that ectomycorrhizal fungi play a significant role in production of substances in forests. For example, the current amount of ectomycorrhizal fungi in the forest biomass of a 180-year-old fir stand in the United States is only 0.3%, but when rootlets are included, the ratio of ectomycorrhizal fungi to net primary production (NPP) goes up to 75%. In the case of a 50-year-old Douglas fir stand, the ratio is estimated to be 50% (Fogel and Hunt 1979; Vogt et al. 1982). Research on fir stands in Japan has shown that the ratio of mycorrhizal biomass to nonmycorrhizal biomass (rootlets) is 4:6 (Nara et al. 1992). Based on the results of these studies, it can be observed how significant a role ectomycorrhizal fungi play in forest ecosystems. It is expected that active methods based on symbiosis with mycorrhizae will be established for enhancement of forest functions in the future.

Species of the pine family such as pines, firs, spruces, and Douglas fir are dominant components of the forests of the northern hemisphere. The origin of the pine trees can be traced to the Bering Strait in the Mesozoic era. They expanded their area of distribution widely into the northern hemisphere to become the circumpolar plant species of the Tertiary period of the Cenozoic era, adapting to various environments around the world, and formed forests (Suzuki 1991). Recently, forest decline has become increasingly apparent in the United States and in European countries, and it

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has emerged as a serious issue (Fukuda et al. 1997). This forest decline can be attributed to complex interplays of biotic and abiotic factors. Therefore, the development and preservation of healthy forests, which, to a high degree, can fulfill their functions of producing substances and establishing an environment, are of critical importance in taking measures against global-scale environmental changes, such as global warming, which are expected to occur in the future. Of particular importance are most of the species of Pinaceae dominant in the northern hemisphere that form ectomycorrhiza. Their ability to produce substances is enhanced by symbiosis with ectomycorrhiza.

As described above, measures for allowing forests to fulfill their functions of producing substances and preserving environments will be of critical importance in coping with problematic environmental issues such as declining forests, changes in forest environments, and the worldwide spread of epidemic diseases such as a pine wilt disease. In particular, measures for enhancing the functions of pine forests and making effective use of them are critical to forests not only in Asia, but also in the northern hemisphere. This is because pine trees are the most important tree species for forest formation and the timber industry, and they are also vital environmental resources in Japan as well as in the northern hemisphere.

It can be considered that the symbiotic relationships between trees and fungi play an important role in the mechanism of maintaining forest ecosystems. Achievement of the following objectives is important to understand forest ecosystems: (1) elucidation of physiological and ecological characteristics of the symbiotic system between trees and ectomycorrhizal fungi, (2) evaluation of the function of trees in resisting environmental stress, which is based on the use of the symbiotic relationships, (3) elucidation of the symbiotic function of ectomycorrhizal fungi such as *Tricholoma matsutake*, and (4) contribution to the preservation of pine forests through effective use of the symbiotic function.

This chapter clarifies the phenomenon of symbiosis of ectomycorrhizal fungi such as *T. matsutake* by focusing on their structure and functions. First, a method for identifying strains of *T. matsutake* and its closely related species around the world is established by determining their genetic characteristics via polymorphic DNA analysis. Next, the morphogenesis of *Pinus densiflora* trees and matsutake mycorrhizal roots is examined. The following methods and technologies are developed: (1) methods for synthesizing artificial mycorrhizae, which can be used as ectomycorrhizal fungi such as *T. matsutake*, (2) methods for encouraging development of artificial shiros of *T. matsutake*, and (3) techniques for enabling *T. matsutake* to colonize and become established. Attempts were then made to make effective use of pine forests via the use of ectomycorrhizal fungi.

One of the features of this research is its focus on interactions between trees and fungi in forest ecosystems, a topic not fully addressed until now, and the study associated experimental systems in vitro with the ones in the field. The results of this research were presented at international symposiums such as *Ectomycorrhizal Ecophysiology and Its Applications in Pine Forests* (Tange et al. 1999; University of Tokyo 2001; Aga et al. 2004).

4.2 The Puzzle of Ectomycorrhizal Fungus *Tricholoma matsutake* (matsutake)

The Japanese authoritative dictionary of Kojien (Shinmura 1991) explains that matsutake (*Tricholoma matsutake*) is parasitic on *Pinus densiflora* trees and grows wild on the ground of *P. densiflora* forests in the autumn months, and sometimes in cold regions it grows in Yezo spruce and hemlock forests. It has a fragrant aroma and is delicious. Stories related to matsutake date back to the Yayoi Era (300 BC–300 AD). As for comprehensive reference materials, there are several documents available. In addition to the documents, reports on recent trends have been published by Wang et al. (1997).

Matsutake have been produced as a special forest product in Japan. The total production volume reached the record-breaking level of 12 000 tons in 1941. Since then, production had hovered around 3000 to 6000 tons/year, but it declined to a level of about 1000 tons/year in the 1960s. Since the 1970s, production continued to fall to the level of several hundred tons per year (Onodera and Suzuki 1998). Matsutake produced in its season in Japan is expensive. Usually, a piece of domestically produced *T. matsutake* is priced at several hundreds US$, and it trades at an average price of around 400 US$/kg (five to six pieces). With the aim of increasing the production of edible mycorrhizal mushroom as a special forest product, the Japan Forestry Agency has been engaged in the following experimental research projects on improvement and utilization of forest resources: (1) Development of cultivation techniques for edible mycorrhizal mushroom (1986–1990), which did not include matsutake; (2) Development of artificial inoculation techniques for mycorrhizal fungi (1991–1995), which included matsutake; and (3) Development of stable production techniques for mycorrhizal mushroom (1996–2003). At present, imports from China and South Korea account for more than 90% of Japan's total consumption of matsutake, and the country is estimated to consume about 3000 tons/year. Based on these facts, it is estimated that matsutake production has the potential to create a billion US$ market. In the mushroom markets of the world, the sales of mycorrhizal mushrooms such as *Tuber melanosporum* called "truffles," *Boletus edulis* called "porcini," and *Cantharellus cibarius* called "girolle" or "chanterelle" exceed 3 billion US dollars (Wang et al. 1997).

Research on mycorrhizae has made little progress due to poor objectivity of experimental results, even though the symbiosis between the roots of plants and fungi has been known for more than 100 years. The preface of *Methods and Principles of Mycorrhizal Research* (Schenck 1982), which was the first book on mycorrhizal research published by the American Phytopathological Society, states:The mycorrhizal research has made progress for the past 15 years thanks to the efforts by researchers in many study fields. At present, however, there is no comprehensive education on mycorrhizae, and most of the past research is not handed down to younger scientists. Given this fact, the American Phytopathological Society publishes this book as the first textbook on mycorrhizae for the purpose of providing reliable knowledge on it."

Almost a decade later, *Techniques for the Study of Mycorrhiza* (Norris et al. 1991) was published. By that time, the whole situation had changed drastically as the preface of the book states:

> Ten years have passed since the publication of *Methods and Principles of Mycorrhizal Research*. During the period, the interest in the study of mycorrhizae has increased explosively. And mycorrhizae have been recognized as a common phenomenon in the natural world by experts in a wide range of fields such as plant physiology, ecology, and plant–parasitic interactions. In addition, new sophisticated scientific techniques such as the ones based on NMR, RFLP, and DNA have been widely applied to research on mycorrhizae.

As described above, the clarification of the symbiotic relationship, which is based on the mycorrhizal symbiosis, required the advent of new scientific techniques that could overcome the problems of handing down research results to a new generation of researchers on an individual basis. Even so, Read (2002) commented, "We have just started understanding the effects of mycorrhizae on the functions of plants and the rhizosphere in the natural world."

As for the study of matsutake, which is famous for its mystery, there are a series of unsolved issues. They are as follows: (1) identification among *T. matsutake* and its closely related species, and intraspecific variations; (2) physiological and biological characteristics based on the differences in the mode of the nutrient system, such as saprophytes, parasites, and symbionts; and (3) issues related to applied studies of artificial cultivation of *T. matsutake*, such as morphogenesis of matsutake mycorrhizae, dynamics of matsutake shiro, and fruit body formation. The findings obtained regarding these unresolved issues are discussed.

4.3 Ectomycorrhizal Ecophysiology of Matsutake

4.3.1 Diversity of *Tricholoma matsutake*: Establishment of a Method for Identification

Species related to *Tricholoma matsutake* in Japan include *Tricholoma robustum* in *Pinus densiflora* forests, as well as *Tricholoma fulvocastaneum* and *Tricholoma bakamatsutake*, which exist in broadleaf forests of beech such as *Quercus serrata*. *Tricholoma robustum* and *T. fulvocastameum* have no aroma like that of *T. matsutake*. *Tricholoma bakamatsutake* does have the aroma, but it is small in size. Therefore, distinguishing the three species is considered to be easy achieved by examining the morphological characteristics of their fruiting bodies. In other parts of the world, *Tricholoma magnivelare* is distributed widely in North America, and *Tricholoma caligatum* grows widely in Europe and North Africa. In addition, there is a species of mushroom in Europe called *Tricholoma nauseosum* that bears a close resemblance to *T. matsutake*. Their similarities and differences have been discussed for the sake of categorization (Kytovuori 1988; Bergius and Danell 2000). Similarly, in

order to study *T. matsutake*, it is necessary to identify species related to it. Furthermore, clarification of intraspecific variation of *T. matsutake* is necessary for the elucidation of its physiological characteristics and the establishment of culture techniques.

Remarkable progress has been made in the recent development of molecular biological techniques, which are used for analysis of genetic variation of fungi and have also been adopted as definitive methods for determining various fungi. rRNA coding regions (rDNA) comprise a single unit by sandwiching noncoding regions called the internal transcribed spacer (ITS) and the intergenic spacer (IGS). About 200 copies of the unit exist on a genome and these have been used frequently for molecular phylogenetic systematics by selecting relevant regions. Generally, intraspecific variation is smaller in the ITS regions, so the regions are useful for determining fungi at the species level (Gardes and Bruns 1993). On the other hand, the variation is larger in the IGS regions. Therefore, the IGS regions are useful for examining intraspecific variation (Bruns et al. 1991).

In general, when a method for identifying species based on DNA analysis is used, it is often difficult to perform direct sequence analysis, which reads base sequences directly. In the case of mycorrhizae or mycelia in soil, DNAs derived from various organisms such as other plants and microorganisms in the soil can be present in a test sample. Thereupon, a primer specific to *T. matsutake* was designed based on the results of the examination of the ITS regions of its rDNA. Using the primer, polymerase chain reaction (PCR) analysis was performed on cultured mycelium of 30 strains of 16 species of ectomycorrhizal fungi, including *T. matsutake*, *T. robustum*, *T. fulvocastaneum*, *T. bakamatsutake*, *T. magnivelare*, and *T. caligatum*. As the result, amplification products were found only in *T. matsutake*. In addition, amplification products were also discovered in the DNAs extracted from fruiting bodies of *T. matsutake*, which were collected from *Pinus densiflora* forests, as well as from the ectomycorrhizal fungi and the soil in *T. matsutake* shiro (soil located directly below fruiting bodies) (Kikuchi et al. 2000). These findings proved that this primer specific to *T. matsutake* was effective for analysis of samples that were collected from forests and fields. At present, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) is a commonly used method for identifying species of ectomycorrhizal fungi through DNA analysis. In the case of determining whether a sample is the species being searched for, identification by PCR using a specifically designed primer makes it easier to interpret analysis results and does not require restriction enzyme processing, which is a cumbersome and time-consuming operation. Therefore, it is considered that PCR analysis is superior to PCR-RFLP in the case of species identification. Based on these findings, it can be concluded that the primer specific to *T. matsutake*, which is designed on the basis of the base sequences of the ITS regions, is the best tool for identifying the species related to *T. matsutake*, because it allows its users to reliably determine whether matsutake mycelia exist by analyzing a small amount of a sample in a quantity of less than several milligrams.

For the purpose of clarifying the intraspecific variation of *T. matsutake* in Japan, 84 strains collected from all over the country were compared by analyzing the IGS1 regions of their rDNA. The results indicated no variation among the strains, because

all of their molecular lengths were identified as about 460 bp. The RFLP patterns obtained by analysis of restriction enzyme Cfr131 in the amplified IGS1 regions were categorized into eight types from A to H (Guerin-Laguette et al. 2002). It was also revealed that *T. matsutake* belonging to RFLP type A is widely dominant throughout Japan. In addition, it was proved that *T. matsutake* that grows in East Asia and Europe is identical to the type A species, which is dominant in Japan.

One of the species of *Tricholoma* that grow in other countries, Swedish *T. nauseosum*, is very similar to Japanese *T. matsutake*. It is reported that the degree of similarity of base sequences between them is 99% to 100%, based on comparison of the ITS regions of their rDNA (Bergius and Danell 2000). Consequently, the ITS regions of *T. matsutake* and *T. nauseosum* in Asia and Europe were compared. As the result, it was found that the degree of similarity of base sequences between the species of *T. matsutake* in the *Pinus densiflora* forests of Japan and South Korea and that in the *Quercus* spp. and *Castanopsis orthacantha* forests of China was 99.7% to 100%. It was also revealed that the degree of similarity of base sequences among the species of *T. nauseosum* in the *Picea abies* forests of Switzerland, the *Pinus sylvestris* forests of Sweden, and the *Picea abies* and *Castanea setiva* forests of Italy was 98.4% to 100% (Matsushita et al. 2005). The degree of similarity of base sequences between *T. matsutake* in Japan and *T. nauseosum* was 98.1 to 100% (Matsushita et al. 2005). Judging from these results, it can be concluded that according to the DNA-based classification, *T. matsutake* in the coniferous forests of Japan and South Korea can be included in the same species *as T. matsutake* in the broadleaf forests of China, and that *T. nauseosum* in the coniferous forests and the broadleaf forests of Europe can also be categorized as the same species. However, even if the hypothesis that *T. matsutake* and *T. nauseosum* are the same species cannot be denied by DNA investigations, in order to verify the identification it is necessary to examine them from the aspect of biological species (Suzuki 1996). Until these points are clarified experimentally, it is reasonable to consider that *T. matsutake* is still shrouded in mystery (*Tricholoma matsutake* sensu lato).

4.3.2 Is *Tricholoma matsutake* an Ectomycorrhizal Fungus?: Morphogenesis of *Tricholoma matsutake* Mycorrhizae on *Pinus densiflora*

In response to the basic question "Is *T. matsutake* an ectomycorrhizal fungus?," photos of the mycorrhizae have been presented in a number of studies; however, the anatomical details of *T. matsutake* have not been properly revealed because the photos have been unclear and illegible (Masui 1927; Ogawa 1975; Wang et al. 1997). Determination of whether *T. matsutake* is an ectomycorrhizal fungus is essential in clarifying its nutritional mode throughout its entire life cycle and establishing artificial culture.

Examination of various mycorrhizae of *T. matsutake* on *Pinus densiflora*, which were collected from forests and fields, under stereoscopic microscope and by chlorazol black E (CBE) staining revealed their following characteristics: (1) while the amount

of mycelia in the fungal sheaths declines during the growing period, the amount of phenolic compounds increases, and then their color turns black, which suggests that the fungal sheaths go through many changes (Gill and Suzuki 2000); and (2) a Hartig net that spreads to the endodermis has multibranched hyphal structures, which are characteristic of the *T. matsutake* ectomycorrhizal fungus (Gill et al. 1999, 2000). Examination of the mycorrhizae by electron microscopy confirmed that cell walls and mycelia cell walls existed apart from each other on the contact surface between host cells and Hartig nets. In some cases, examination confirmed the existence of progressive inclusion layers, which were buried in the matrix of the layers between cells, and the existence of mature inclusion layers that were formed while cell walls and mycelia cell walls were in contact and merging gradually into each other (Gill et al. 2000). All of the samples were proved to be *T. matsutake* by DNA analysis. As stated above, although the mycorrhizae of *T. matsutake* on *P. densiflora* grow through various morphological stages, they have Hartig nets, which are typical structures of ectomycorrhizal fungi. It was also revealed that there were two structures for the contact surface between host cells of *P. densiflora* and the mycelia of *T. matsutake*: progressive and mature.

To clarify the physiological significance of the *T. matsutake* mycorrhizae, the distribution and localization of ATPase, which is involved in the active transportation of ions between cells, was examined. The ATPase of the mycelia grown in Hartig nets is located on both sides of the septum of mycelia cells. It was revealed that ATPase is highly active in the cell membranes of host cells and mycelia cells on the contact surface between the two kinds of cells, especially on the areas that Hartig nets invaginate and are considered as regions that nutrition moves through. On the other hand, activation of ATPase was not observed in the mycelia of the fungal sheaths. Therefore, it can be assumed that nutrition is not exchanged between mycelia. Based on these findings, it was revealed that transfer of nutrition, which is one of the basic functions of *T. matsutake* mycorrhizae, is actively carried out between *P. densiflora* and *T. matsutake*.

A variety of discussions have been conducted on whether *T. matsutake* is an ectomycorrhizal fungus. Wang et al. (1997) proposed that *T. matsutake* can be positioned as a species that goes through the triangle of saprophyte, parasite, and symbiont, and each of the characteristics become more dominant or less dominant as the season changes. The reasons why the basic question of the mode of nutrition of *T. matsutake* has not been answered are a lack of scientific data and absence of concomitant use of molecular biology methods. In this research on the structure of *T. matsutake* mycorrhizae, multibranched hyphal structures, which are the distinctive features of *T. matsutake* ectomycorrhizal fungus, were observed. Therefore, decisive anatomical evidence was obtained. Based on the distribution mode of ATPase, it was confirmed that *P. densiflora* and *T. matsutake* form mycorrhizae that have a distinctive function of symbiosis, and it was proved for the first time that *T. matsutake* is a typical ectomycorrhizal fungus.

Given that *T. matsutake* was identified as a typical ectomycorrhizal fungus, attempts were made to synthesize the mycorrhizae between *T. matsutake* and *P. densiflora*. As a result, a method for rapidly synthesizing the *T. matsutake* mycor-

rhizae was established forming which the mycorrhizae formed without fail within 1 to 2 weeks after inoculation using seedlings of *P. densiflora* on artificial substrates. The method was based on techniques that addressed the following requirements: (1) induction of the growth of vigorous mycelia, (2) preparation of inoculum of mycelia, and (3) effective inoculation of mycelia (Guerin-Laguette et al. 2000; Vaario et al. 2000; Suzuki et al. 2001). A similar case of successful research, in which Hartig nets were formed on *P. densiflora* seedlings 3 months after their inoculation, was reported by Yamada et al. (1999).

The effect of *T. matsutake* infection on its host, *P. densiflora*, was examined. It was found that 10 weeks after inoculation of *T. matsutake* mycelia, the weight of *P. densiflora* seedlings increased by 71%–98% (Guerin-Laguette et al. 2004). Previously, it had been believed that *T. matsutake* was parasitic to young seedlings of the host plant (Wang et al. 1997), even though scientific evidence was lacking. This idea was disproved experimentally, and it was shown that *T. matsutake* is a typical symbiotic fungus.

4.3.3 Shiro of *Tricholoma matsutake*: Dynamics of Shiros and Its Artificial Formation

To produce fruiting bodies of *Tricholoma matsutake* under the condition of in vitro culture, it is necessary to quantitatively examine its shiros in the field. Accordingly, to clarify the dynamics of *T. matsutake* shiro, the subterranean part was examined by using the root window technique (Egli and Kalin 1991), which enables nondestructive constant observation. In addition, research on the development of fruiting bodies, which has been conducted for a long time, is also of value. This research was carried out after confirming that the mycelia in shiro soil were the same species as *T. matsutake* by DNA analysis (RFLP of the IGS1 regions) (Kikuchi et al. 2000). The mycelia in *T. matsutake* shiro and the roots of *Pinus densiflora* in the *P. densiflora* natural forests were also examined in a case study. The findings were as follows: (1) the mycelia and the roots grew actively during the period from May to July; (2) the color of the mycelia in *T. matsutake* shiro started to turn from white to light brown from August; and (3) the roots started to grow again in September; and the shiros expanded by about 10 cm width within a year (Suzuki 2005). The major axis of the shiros measured 4.5 m, and their minor axis was 3.7 m. At the site where fruiting bodies had produced over 4 years, the areas where they sprouted moved outward by 10–15 cm/year. Although the results of observation of the dynamics of *T. matsutake* mycelia based on the root window technique vary in some degree, they are almost consistent with the expansion speed of the shiros, which is measured by the conventional method based on the movement of the sites where fruiting bodies sprout. The expansion speed of shiros is not calculated based on the direct measurement of the growth of mycelia in soil. However, although the results obtained by recording the movement of the sites where fruiting bodies sprout were not constant, they showed an expansion of 10–15 cm/year on an average, which was consistent with the estimated length (Ogawa 1978). Based on these findings, it can be concluded that in

general the active site of *T. matsutake* shiros has a ring-like structure that is 10–15 cm wide and 10 cm deep.

It is considered that the amount of mycelia in *T. matsutake* shiros is closely related to the amount of emerged *T. matsutake* fruiting body. Further information is necessary to understand the formation of *T. matsutake* fruiting bodies. Accordingly, the amount of ergosterol in the soil located directly below fruiting bodies was measured, and the total amount of mycelia in a *T. matsutake* shiro was also measured. The measurements showed that ergosterol was present in shiro soil at a concentration of about 64.4 μg/cm^3, and the amount of ergosterol in cultured mycelia was about 1.5 μg/mg. Therefore, the amount of mycelia in shiro soil was calculated to be about 42.9 mg/cm^3. Based on the results, the total weight of mycelia in a shiro was estimated to be 5.6–7.3 kg (Suzuki 2005). Based on the measured amount of emerged *T. matsutake* fruiting bodies in the experiment, the amount of mycelia that one fruiting body requires to grow can be estimated to be about 100 g (90–120 g). Until now, there has been no report of direct measurement of the total amount of mycelia in a shiro. The volume of a shiro was estimated by Ogawa (1978) to be 1500–2000 cm^3 based on the surface area of the soil where one fruiting body emerges (a mass of 64–86 g when calculated based on the above-mentioned amount of mycelia in the shiro soil). The results of this study revealed the details of the relationship between the dynamics of a *T. matsutake* shiro and the biomass of *T. matsutake* mycelia in the field.

It was proved that *T. matsutake* is a typical ectomycorrhizal fungus, and it is possible to artificially synthesize the mycorrhizae between *T. matsutake* and *P. densiflora*. In addition, the dynamics of the *T. matsutake* shiro were clarified. As a result, the quest to develop artificial *T. matsutake* shiros and fruiting bodies has become more interesting. The research on artificial cultivation of *T. matsutake* has been conducted for a long time in Japan. In addition to measures such as improvement cutting and land plowing (Kake et al. 2000), reports have been published on the following subjects: (1) incubation of *T. matsutake* mycelia (Inaba et al. 1993); (2) formation of primordium for *T. matsutake* fruiting bodies by using pure cultures (Ogawa and Hamada 1975); (3) promoting of seedlings infected with *T. matsutake* (Ogawa et al. 1978); (4) formation of shiros by using seedlings infected with *T. matsutake;* and (5) formation of *T. matsutake* fruiting bodies on soil collected from shiros (Inaba et al. 1995). However, it is still practically impossible to artificially develop a shiro by incubating *T. matsutake* mycelia on a culture substrate or by inoculating *T. matsutake* mycelia into the soil of a forest. Indeed there is no case where the success of artificial shiro formation has been proved scientifically by molecular biology techniques.

In general, one of the effective ways to grow a large quantity of mushrooms is to propagate their mycelia in liquid culture. However, under natural conditions it is difficult to form shiros due to the poor growth of *T. matsutake* mycelia. The poor growth is attributable to the dominant growth of saprophytic fungi in *P. densiflora* forests. Therefore, focusing on the extremely slow growth of *T. matsutake* mycelia as one of the factors that hinder artificial cultivation of *T. matsutake*, substances that stimulate the growth of the mycelia were examined. The examination of the

saprophytic ability of *T. matsutake* revealed that all of its strains had an amylolytic ability. It has also been confirmed that *P. densiflora* bark and beech sawdust can be nutrition sources for *T. matsutake* mycelia, based on research on the activities of cellulolytic enzymes (β-glucosidase, D-nitrophenyl -β-D-lactopyranosidase) and the amount of ergosterol (Vaario et al. 2002, 2003). A study of ways to stimulate the growth of *T. matsutake* mycelia revealed that the growth of *T. matsutake* mycelia could be accelerated 15 times by incubating them on culture substrates that contain surfactants such as Tween80 and Tween40, which control the cell membrane permeability of the mycelia, or natural vegetable oils such as olive oil that increase the hydrophilicity of the mycelia (Guerin-Laguette et al. 2003; Suzuki 2004). In this case, it may be considered that the growth was accelerated by the secretion of degradative enzymes from *T. matsutake* mycelia, which was induced by the increased hydrophilicity of the mycelia. Therefore, it can be said that the path toward the artificial induction of *T. matsutake* shiros, which is the first step toward the artificial cultivation of *T. matsutake*, has been paved by the rapid cultivation of large amounts of *T. matsutake* mycelia.

4.4 Concluding Remarks

The examination of the effects of ectomycorrhizal fungi on forest ecosystems has belatedly started in vitro. The existing theories on the functions of forests and trees, which were established by focusing on the aboveground part, need to be reexamined in vivo by taking into consideration the new concept of ectomycorrhizal symbiosis in the rhizosphere.

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