Mycoscience (2007) 48:358–364
DOI 10.1007/s10267-007-0384-2

FULL PAPER

Mizuho Kusuda · Mitsuhiro Ueda · Yasuhito Konishi
Katsuji Yamanaka · Takao Terashita · Kazutaka Miyatake

Effects of carbohydrate substrate on the vegetative mycelial growth of an ectomycorrhizal mushroom, *Tricholoma matsutake*, isolated from *Quercus*

Received: January 31, 2007 / Accepted: July 2, 2007

Abstract We studied the characteristics of the utilization of carbohydrate substrates and the production of those hydrolyzing enzymes of the *Tricholoma matsutake* J-1 strain isolated from hardwood (*Quercus* sp.). In the culture medium, 5% glucose inhibited mycelial growth. The growth inhibition rate was remarkable in the Z-1 strain from softwood (*Pinus densiflora*) compared with that of the J-1 strain from hardwood. α-Amylase production varied with starches from different origins in contrast to mycelial growth. The range of the effect of 0.5%–15% soluble starch on vegetative mycelial growth was also investigated. The optimal concentration for mycelial growth was 15% for the J-1 strain but 10% for the Z-1 strain. Mycelial growth of the J-1 strain was strongly inhibited in PMML medium containing Sunpeal-CP prepared from sulfite pulp softwood waste, but that of the Z-1 strain was not inhibited by Sunpeal-CP. Moreover, mycelial growth of the J-1 strain from *Quercus* sp. dramatically decreased with the addition of CNF-HWSF (hot water-soluble fractions from corn fiber) to the PMML and PDL medium. However, inhibition by CNF-HWSF was not shown in the Z-1 strain from *P. densiflora*.

Key words Artificial cultivation · Carbohydrase · Carbohydrate utilization · Mycorrhizal fungus · *Tricholoma matsutake*

M. Kusuda · M. Ueda (✉) · K. Miyatake
Laboratory of Biocycle Engineering, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan
Tel. +81-72-254-9468; Fax +81-72-254-9468
e-mail: mueda@biochem.osakafu-u.ac.jp

Y. Konishi · T. Terashita
Laboratory of Food Microbiological Science and Biotechnology, School of Agriculture, Kin-ki University, Nara, Japan

K. Yamanaka
Kyoto Mycological Institute, Kyoto, Japan

Introduction

The ectomycorrhizal fungus *Tricholoma matsutake* (S. Ito et Imai) Singer is one of the most delicious and valuable edible mushrooms in Asia, especially in Japan, Korea, and China. The annual production of this mushroom in Japan was reported to be 12000 tons in 1941, 3509 tons in 1960, 211 tons in 1995, and dwindled to 34 tons in 2005 (Yamada 2005). The main factors for the decrease are considered to be the accumulation of humus in pine forests and the death of pine trees by attack of *Bursaphelenchus xylophilus* (Steiner et Bnhrer) Nickle, the pine wilt nematode (Futai 2003).

Tricholoma matsutake is difficult to cultivate artificially without a host plant because the growth substrates necessary for fruit-body formation are obtained from the host plant through ectomycorrhizae. This fungus has a low ability to decompose polysaccharides such as cellulose and hemicellulose for its growth substrate and grows slowly on artificial media (Ohta 1997). Therefore, only glucose and a few other monosaccharides and disaccharides can be used for the growth of this fungus. Ohta (1994) reported that *Lyophyllum shimeji* (Kawan.) Hongo forms mature fruit bodies on an artificial medium using barley grain without a host plant. He pointed out the importance of the amylase production of this fungus from the utilization of barley starch as the growth substrate.

To survey the potential for production of extracellular carbohydrases from *L. shimeji*, we examined amylase production using a barley grain medium. It was shown that glucoamylase in the medium increased markedly during fruit-body formation of *L. shimeji*. We completely purified the enzyme from this fungus and revealed its enzymatic properties, including substrate specificities, for the first time (Kusuda et al. 2004). Then, we studied carbohydrase production from *T. matsutake* on a partially modified matsutake liquid medium (PMML medium) and showed that *T. matsutake* produced α-amylase, α-glucosidase, and potent β-glucosidase activity in static culture filtrate. The three kinds of enzymes from *T. matsutake* were already purified (α-

glucosidase is a partially purified enzyme), and their enzymatic properties were characterized (Kusuda et al. 2003, 2006). These results suggest that *T. matsutake* has saprotrophic abilities (Kusuda et al. 2006).

In 2002, Yamanaka et al. (2003) found a fruit body of *T. matsutake* from the *Quercus* sp. forest in eastern Tibet (Jiulong, Garze Tibetan Nationality Autonomous Prefecture, Sichuan), China, and discovered that the fungus formed mycorrhizae into *Quercus pannosa* or *Q. guyavaefolia*. Also, it was shown that the fungus strain from the broadleaf forest was identical to *T. matsutake* from pine trees by molecular phylogenetic analysis. However, the physiological and biological properties of this fungus isolated from broadleaf forests have not yet been investigated.

In this article, we have examined the characteristics of carbohydrate substrate utilization and enzyme production of the *T. matsutake* strain from the mycorrhizae of trees of the genus *Quercus*.

Materials and methods

Microorganisms

Tricholoma matsutake J-1 and Z-1 strains were used in these experiments. The J-1 strain was isolated from the fruit bodies of *Quercus pannosa* or *Q. guyavaefolia* from a broadleaf forest in eastern Tibet, China, in 2002 by Yamanaka et al. (2003). The Z-1 strain was isolated from the fruit bodies of *T. matsutake* in a pine tree forest (*Pinus densiflora*) in 1985 by Inaba et al. (1995). Mycelial growth rates and carbohydrase production of the *T. matsutake* Z-1 strain showed the average values of about 20 kinds of strains stored in our laboratory. Both strains (J-1 and Z-1) were stored on Hamada matsutake agar medium (HMA medium) (Terashita and Kono 1987) at a low temperature (4°C).

Medium composition and culture conditions

For experiments of the utilization of saccharides, a mycelial block (5 × 5 × 6 mm) was cut from a plate culture that had grown on HMA medium (2.0% agar) for 40 days at 24°C in a Petri dish (diameter, 90 mm). It was inoculated in an Erlenmeyer flask (100 ml) containing 20 ml Kawai synthetic liquid medium (KSL medium) (Kawai and Abe 1976) after sterilization at 121°C for 15 min. After inoculation, it was static cultured at 24°C for 30–60 days in the light (about 200 lux).

Potato dextrose liquid medium (PDL medium) consists of potato extract (200 g boiled in 500 ml distilled water), 15 g glucose, and 1.0 mg thiamine hydrochloride per liter of distilled water. PMML medium was prepared according to the method of Terashita et al. (2000). Hamada matsutake liquid medium (HML medium) consists of 20 g glucose, 10 g dried beer yeast (Wako Pure Chemical; the extract from dried beer yeast was prepared in 100 ml tap water at 80°C for 30 min), and 1.6 ml 1 N HCl per liter of distilled water. Sterilization was accomplished at 121°C for 15 min.

Each medium (HML, PMML, and PDL) was supplemented with 0.5% and 2.5% of hot water-soluble fractions from corn fiber (CNF-HWSF), dispensed in 20-ml aliquots in 100-ml Erlenmeyer flasks, and autoclaved at 121°C for 15 min.

Preparation of CNF-HWSF

Corn fiber (CNF) (100 g) was mixed with 1 liter of distilled water and extracted for 3 h at 80°C. After the residue was removed by centrifugation at 10000 *g* (0°C, 10 min), the supernatant solution (hot water-soluble fractions, HWSF) (Arai et al. 2003) was concentrated at 40°C to 100 ml by a rotary evaporator.

Effect of glucose and starch concentrations and starches from different origins on mycelial growth

Tricholoma matsutake J-1 and Z-1 strains were cultured in KSL medium with glucose (0%–5%) and starch (0%–15%) at 24°C for 60 days in the light (about 200 lux), respectively. Moreover, both strains were also cultured in various starches (2%) such as soluble starch from potato, sweet potato, corn, waxy corn, rice powder, tapioca, palm, and flour containing gluten at 24°C for 40 days in the light, and the dry weight of the mycelial growth was compared. Data represent averages of triplicate experiments with five flasks per experiment.

Effect of starches from different origins on α-amylase activity

Tricholoma matsutake J-1 and Z-1 strains were cultured in KSL medium with the addition of starches (2%) from different origins at 24°C for 60 days in the light.

Extraction of crude enzyme and determination of mycelial growth

On the 60th day after incubation, the culture medium was filtered through No. 2 filter paper (Toyo ADVANTEC, Tokyo, Japan) for mycelial removal. The culture filtrate was used as the crude enzyme solution. The mycelia were removed from the flask and washed with distilled water five times. Mycelial dry weight was measured after drying in a forced-air oven for 3 h at 110°C.

Measurements of α-amylase and β-glucosidase activity

Alpha-amylase activity was assayed using soluble starch solution as a substrate. The enzyme reaction took place at 50°C for 180 min. After the reaction, residual starch was stained by an iodine-potassium iodide solution (Terashita et al. 2000). One unit of α-amylase activity was defined as the activity that caused lower absorbency for 0.01–1.0 ml of the crude enzyme solution at 50°C for 1 min. The β-glucosidase

activity was assayed using ρ-nitrophenyl β-glucopyranoside (ρ-NβG) as a substrate. The enzyme reaction was done at 37°C for 30 min. One unit of β-glucosidase activity was defined as the activity that forms 1 μmol ρ-nitrophenol in 1.0 ml crude enzyme solution at 37°C for 1 min (Kusuda et al. 2006).

Results

Effect of glucose concentration on vegetative mycelial growth

The effects of glucose concentration on vegetative mycelial growth of the *T. matsutake* J-1 strain isolated from hardwood (broadleaf wood) and the Z-1 strain from softwood (conifer) were studied (Fig. 1). When the different kinds of strains were cultured in 2% glucose medium at 24°C for 60 days, the mycelial growth increased by 1.79 (J-1 strain) and 1.36 (Z-1 strain) times that of the control (1.0% glucose concentration). However, 5% glucose in the culture medium inhibited the mycelial growth of both strains. The growth inhibition rate was remarkable in the case of the Z-1 strain from softwood compared with that of the J-1 strain from hardwood.

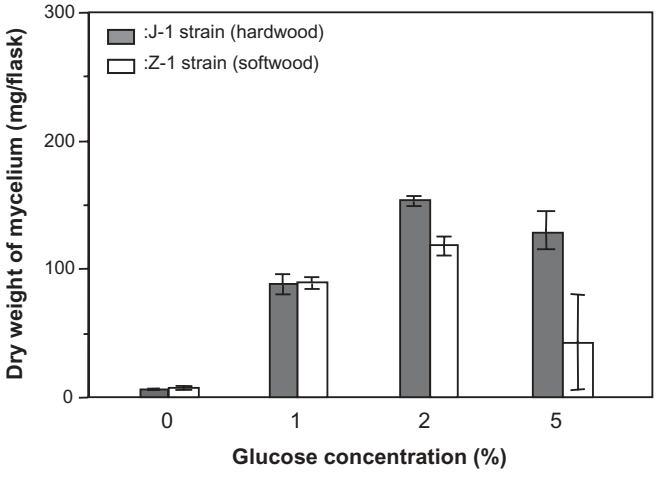

Fig. 1. Effect of glucose concentration on vegetative mycelial growth of *Tricholoma matsutake* isolated from hardwood (broadleaf wood, J-1 strain) and softwood (conifer, Z-1 strain). After inoculation, each was static cultured at 24°C for 60 days in the light. Data represent averages of triplicate experiments with five flasks per experiment. *Vertical bars* indicate SD

Effect of different origins of starches on mycelial growth

We examined the effect of different origins of starches (seven kinds of starches) on the vegetative mycelial growth of both strains of *T. matsutake* (Table 1). The starch concentration of the medium was tested at 2.0% (w/w). However, no remarkable difference in vegetative mycelial growth was recognized between the J-1 and Z-1 strains.

Effect of different origins of starches on α-amylase production

To reveal the ability to utilize starch in both strains of *T. matsutake*, production of extracellular α-amylase was studied in the artificial liquid media using starches of different origins (Fig. 2). The α-amylase of the *T. matsutake* Z-1 strain from the pine tree showed considerably higher activity levels when cornstarch (11.5 U/ml), rice powder (13.8 U/ml), and flour containing gluten (12.5 U/ml) were used as growth substrates. On the other hand, the activity of J-1 from *Quercus* showed high values in the cases of tapioca (8.1 U/ml) and palm starch (12.1 U/ml). In addition, these α-amylase activities varied with starches from different origins, in contrast to the vegetative mycelial growth.

Effect of starch concentrations on mycelial growth

After inoculation, both strains (J-1 and Z-1) were static cultured at 24°C for 60 days in the light. The effect of soluble

Table 1. Effect of different origins of starches on vegetative mycelial growth of *Tricholoma matsutake* isolated from hardwood (broadleaf wood, J-1 strain) and softwood (conifer, Z-1 strain)

Origins	J-1 strain from hardwood	Z-1 strain from softwood
	Mycelial dry weight (average ± SD, mg/flask)	Mycelial dry weight (average ± SD, mg/flask)
Soluble starch from potato	36.9 ± 2.1	33.1 ± 3.8
Sweet potato	39.5 ± 0.7	40.1 ± 1.9
Corn	38.3 ± 0.5	38.4 ± 1.2
Waxy corn	36.5 ± 1.1	36.7 ± 0.4
Rice powder	38.4 ± 1.7	41.5 ± 5.7
Tapioca	38.9 ± 1.0	37.1 ± 0.1
Palm	38.9 ± 1.9	36.5 ± 1.1
Flour containing gluten	38.5 ± 0.2	39.4 ± 0.4

After inoculation, each was static cultured at 24°C for 40 days in synthetic liquid medium in the light
Starch concentration of the medium: 2% (w/w)
Data represent averages of triplicate experiments with five flasks per experiment

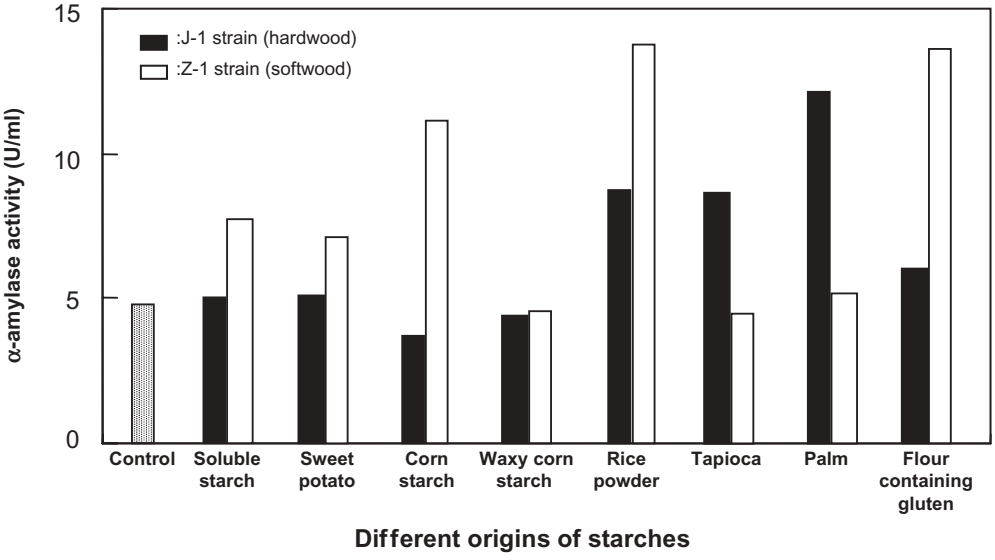

Fig. 2. Effect of different origins of starches on α-amylase production of *T. matsutake* from hardwood (broadleaf wood, J-1 strain) and softwood (conifer, Z-1 strain). Control: J-1 strain, 2% soluble starch from potato. Enzyme activity was measured by the iodine-potassium iodine method. Data represent averages of triplicate experiments with five flasks per experiment

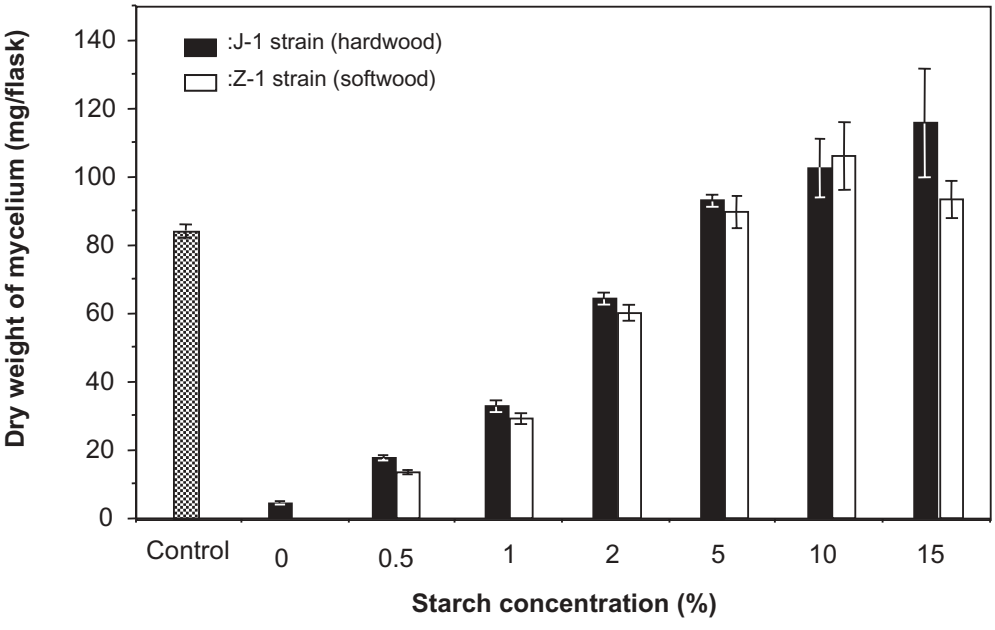

Fig. 3. Effect of soluble starch (potato) concentration on vegetative mycelial growth of *T. matsutake* isolated from hardwood (broadleaf wood, J-1 strain) and softwood (conifer, Z-1 strain). Control: J-1 strain, 2% glucose medium. After inoculation, each was static cultured at 24°C for 60 days in the light. Data represent averages of triplicate experiments with five flasks per experiments. *Vertical bars* indicate SD

starch (potato) concentration (0.5%–15%) on vegetative mycelial growth was investigated in a liquid medium. The dry weight of mycelia of the J-1 and Z-1 strains increased linearly with increasing soluble starch concentration (Fig. 3). The highest growth value of the J-1 strain from the broadleaf tree (112.3 mg/flask) was obtained with 15% starch concentration, but that of the Z-1 strain from the pine tree (105.2 mg/flask) was obtained with 10% starch.

Effect of medium composition on vegetative mycelial growth

To survey the physiological characteristics marking the difference between the J-1 strain from the *Quercus* tree and the Z-1 strain from the pine tree, vegetative mycelial growth on HML, PMML, and PDL media for 30 and 60 days after inoculation was investigated (Table 2). Slow mycelial growth was recognized with the J-1 strain at an early stage of cultivation (30 days after inoculation) compared with that of the Z-1 strain. However, vegetative mycelial growth in the J-1 strain was markedly enhanced on the HML medium. On the other hand, mycelial growth of the J-1 strain was strongly inhibited (19.2 mg/flask, 60 days after inoculation) in PMML medium containing Sunpearl-CP (commercial name) (Inaba et al. 1993) prepared from sulfite pulp softwood waste. However, the Z-1 strain was not inhibited (120.1 mg/flask, 60 days after inoculation) by Sunpearl-CP. The greatest amount of mycelial growth was shown by the Z-1 strain on PMML medium 60 days after incubation.

Table 2. Effect of medium composition on vegetative mycelial growth of *T. matsutake* isolated from hardwood (broadleaf wood, J-1 strain) and softwood (conifer, Z-1 strain)

Strains	Dry weight of mycelium (mg/flask)					
	Incubation days					
	Hamada matsutake liquid medium		Partially modified matsutake liquid medium		Potato dextrose liquid medium	
	30	60	30	60	30	60
J-1	8.7 ± 3.4	41.8 ± 18.4	11.5 ± 1.1	19.2 ± 0.3	4.3 ± 3.0	88.7 ± 1.4
Z-1	13.5 ± 3.1	22.0 ± 1.0	23.2 ± 4.5	120.1 ± 13.1	21.5 ± 8.5	84.5 ± 6.1

After inoculation, each was static cultured at 24°C for 30 and 60 days in the light
J-1 strain was isolated from hardwood
Z-1 strain was isolated from softwood
Data represent averages of triplicate experiments with five flasks per experiment

Table 3. Effect of hot water-soluble fractions from corn fiber (CNF-HWSF) on vegetative mycelial growth of *T. matsutake* isolated from hardwood (broadleaf wood, J-1 strain) and softwood (conifer, Z-1 strain)

Strains	Dry weight of mycelium grown in different concentrations of CNF-HWSF (%)								
	Hamada matsutake liquid medium			Partially modified matsutake liquid medium			Potato dextrose liquid medium		
	0	0.5	2.5	0	0.5	2.5	0	0.5	2.5
J-1	32.5 ± 23.6	66.2 ± 1.8	74.6 ± 6.6	18.3 ± 0.5	17.2 ± 1.6	8.5 ± 2.7	98.5 ± 1.5	7.6 ± 1.3	7.4 ± 1.8
Z-1	21.3 ± 1.0	59.2 ± 4.8	67.2 ± 7.1	125.4 ± 13.2	120.5 ± 13.3	114.3 ± 7.8	99.7 ± 7.6	93.8 ± 4.8	100.2 ± 15.1

After inoculation, each was stationary cultured at 24°C for 60 days in the light
J-1 strain was isolated from hardwood
Z-1 strain was isolated from softwood
Data represent averages of triplicate experiments with five flasks per experiment

Table 4. Production of α-amylase and β-glucosidase of *T. matsutake* isolated from hardwood (J-1 strain) and softwood (Z-1 strain)

	Enzyme activity		
	Hamada matsutake liquid medium	Partially modified matsutake liquid medium	Potato dextrose liquid medium
α-Amylase (U/ml)			
J-1	1.10	0.35	0.84
Z-1	2.85	1.41	4.37
β-Glucosidase (mU/ml)			
J-1	10.21	135.12	2.78
Z-1	3.74	244.63	58.33

After inoculation, each was static cultured at 24° for 60 days in the light
J-1 strain was isolated from hardwood; Z-1 strain was isolated from softwood
Data represent averages of triplicate experiments with five flasks per experiment

Effect of CNF-HWSF on vegetative mycelial growth of both strains of *T. matsutake*

The effect of CNF-HWSF on the vegetative mycelial growth of *T. matsutake* J-1 and Z-1 strains was compared using the three kinds of liquid media already mentioned (HML, PMML, and PDL) (Table 3). The vegetative mycelial growth of both strains was increased (J-1 strain: 2.0–2.3 times that of the control; Z-1 strain: 2.8–3.2 times that of the control) with the addition of CNF-HWSF (0.5%–2.5%) to the HML medium. However, mycelial growth of the J-1 strain from *Quercus* sp. dramatically decreased with the addition of CNF-HWSF to the PMML and PDL media. These inhibitions by CNF-HWSF were not observed in the Z-1 strain from the pine tree.

Effect of medium components on α-amylase and β-glucosidase production of *T. matsutake*

Using three kinds of media (HML, PMML, and PDL), we assayed α-amylase and β-glucosidase activities in the culture medium of both strains of *T. matsutake* for 60 days after incubation (Table 4). On the α-amylase assay, it was shown that enzyme activity from the Z-1 strain was higher than that of the J-1 strain in all the media (about two- to four-

fold). A similar tendency was also shown in β-glucosidase production, except for that on the HML medium.

Discussion

In 2002, Yamanaka et al. (2003) collected a fruit body of *T. matsutake* from a *Quercus* sp. forest in Eastern Tibet, Sichuan, China. It was confirmed that the mycelia of the J-1 strain form mycorrhizae within the roots of *Quercus* sp. Moreover, this strain was thought to be identical to *T. matsutake* from pine trees based on molecular phylogenetic analysis by direct sequencing of the internal transcribed spacer (ITS) region consisting of a portion of 18S rDNA (Yamanaka et al. 2003). We investigated the characteristics of the utilization of carbohydrate substrates and the hydrolyzing abilities of this strain compared to those of the *T. matsutake* Z-1 strain from a pine tree.

We found that 5% glucose of the culture medium inhibited the mycelial growth of both strains. However, the inhibitory percent was remarkable in the case of the Z-1 strain from the pine tree compared with in that of the J-1 strain from *Quercus* spp. (see Fig. 1).

Hirato and Kitamoto (1995) showed that the best growth of vegetative mycelia was obtained at 2% glucose and that it was inhibited above 3% glucose. Itaya et al. (2003) reported catabolite suppression by high concentration of glucose in an ectomycorrhizal mushroom, *Laccaria amethystea* (Bull.) Murril. Moreover, Hatayama and Ohmasa (2004) showed that many strains of the genera *Suillus* and *Boletinus* can grow well at relatively high glucose concentrations (3.33%–10%). The utilization of carbohydrates for growth substrate by *T. matsutake* in the broadleaf forest is thought to be different from *T. matsutake* in the pine tree forest. Our experiment showed a difference in the permitted limit of glucose utilization as a growth substrate.

When a mushroom forms fruit bodies, large amounts of mycelia may be needed either to store the nutrients or to transport the nutrients to the fruit bodies. However, in practice it is very difficult to cultivate large amounts of mycelia using monosaccharides in a pure culture because of the osmotic pressure in the medium (Ohta 1997).

Tricholoma spp. can grow slowly in a starch substrate when a small amount of glucose is added as a starter (Norkrans 1950). Ohta (1997) showed that strains of *T. matsutake* had the ability to utilize starch as a carbon source. For the artificial cultivation of *T. matsutake*, this ability is also very important because the fungus has previously not been known to use any polysaccharide except starch. Then, the effect of different origin of starches (seven kinds of starches) on the mycelial growth of *T. matsutake* strains obtained from a *Quercus* sp. (J-1 strain) and a pine tree (Z-1 strain) was investigated. As a result, no remarkable difference was shown between J-1 and Z-1 strains. In our previous report (Terashita et al. 2000), we showed that the addition of potato and yam to the culture medium increased the dry weight of the mycelia of *T. matsutake* 4.8–5.6 times compared to that of the control (without addition).

On the other hand, α-amylase production varied with starches from different origins in contrast to the mycelial growth (see Fig. 2). Alpha-amylase from a static culture filtrate of *T. matsutake* Z-1 was already purified and characterized in our previous report (Kusuda et al. 2003). This enzyme readily hydrolyzed the α-1,4 glucoside bond in soluble starch and amylose but did not hydrolyze the α-1,6 bond. The α-amylase seemed to be induced by cornstarch, rice powder, and flour containing gluten whereas induction of α-amylase from the J-1 strain was shown by tapioca starch and palm starch (Fig. 2).

For research on the artificial cultivation of *T. matsutake*, the ability to utilize starch as a growth substrate seems to be essential. The dry weights of the mycelia of J-1 and Z-1 strains increased linearly with increasing soluble starch concentration in the medium (see Fig. 3). The maximum yield of mycelium of the J-1 strain was obtained with 15% starch (112.3 mg/flask), but that of the Z-1 strain was at 10% (105.2 mg/flask). Thus, difference in mycelial growth between the J-1 and the Z-1 strains was shown in the response to high starch concentrations. It is considered that *T. matsutake* changed, in the process of evolution, from the broadleaf forest to the coniferous forest (Iwase 1996). Iwase (1996) mentioned that generally the broadleaf forest is rich in nutrition compared to a pine tree forest (coniferous forest).

To survey the physiological characteristics of the differences between the J-1 and the Z-1 strains, the effect of the medium composition was investigated using three kinds of medium. Slow mycelial growth was recognized with the J-1 strain at an early stage of cultivation; this is considered to be a characteristics of the J-1 strain from broadleaf trees. Also, mycelial growth of the J-1 strain was dramatically inhibited in PMML medium containing Sunpearl-CP (Inaba et al. 1993), but that of the Z-1 strain from pine trees was not inhibited. Sunpearl-CP is prepared from sulfite pulp waste of pulp manufacturing from softwood by Inaba et al. (1993). Inaba et al. (1995) had reported an instance of fruitbody formation of *T. matsutake* from a pine forest in vitro. However, this report did not lead to further information concerning artificial cultivation. Perhaps the inhibitory effect of the J-1 strain from broadleaf wood on mycelial growth is caused by the presence of some inhibiting substance contained in Sunpearl-CP from hardwood.

When CNF-HWSF was added to PDL medium, mycelial growth of the J-1 strain was dramatically inhibited. On the other hand, the addition of CNF-HWSF to the HML medium remarkably increased. These inhibitions were not recognized entirely in the Z-1 strain from the pine tree (see Table 3).

Arai et al. (2003) reported that CNF-HWSF promoted the mycelial growth of *T. matsutake* IFO 30605 from the pine forest by adding 0.5% CNF-HWSF to the PDL medium (1.53 times that of the control) and to the PMML medium (1.51 times that of the control). The main ingredient of CNF consists of 47% hemicellulose, approximately 20% cellulose and starch, and 10% crude protein. From these results, mycelial growth inhibition of the J-1 strain from broadleaf wood in the PDL medium with addition of CNF-HWSF is

thought to be caused by an inhibitory component from CNF-HWSF. Additionally, the promoting effect on the mycelial growth in the HML medium is thought to be caused by absorption of the extract from dried beer yeast, a constituent in the HML medium. However, the details are not clear.

In our previous papers (Kusuda et al. 2003, 2006), the production systems of extracellular carbohydrases were studied to reveal the ability to utilize carbohydrates, including starch, of *T. matsutake* Z-1 from the pine forest. As a result, the activities of α-amylase, α-glucosidase, and β-glucosidase were detected in a static culture medium of PMML. These enzymes have already purified and characterized.

In this report, we investigated the production of α-amylase and β-glucosidase of *T. matsutake* J-1 isolated from hardwood. In the α-amylase assay, the enzyme activity of the Z-1 strain was higher than that of the J-1 strain in all the media. A similar tendency was also detected in β-glucosidase production, except for the HML medium. The highest activity of β-glucosidase was shown with both strains in the PMML medium. The β-glucosidase from mushrooms was reported by Sengupta et al. (1991) (*Termitomyces clypeatus* Heim.), Cai et al. (1998) (*Volvariella volvacea* (Bull.: Fr.) Singer), Makkar et al. (2001) (*Lentinula edodes* (Berk.) Singer), Igarashi et al. (2003) (*Phanerochaete chrysosporium* Burds.), and Ishihara et al. (2005) (*Polyporus arcularius* (Fr.) Ames). β-Glucosidase from the *T. matsutak*e Z-1 strain (Kusuda et al. 2006) seems to be similar to CMCase IIIa (Ishihara et al. 2005) from a saprophytic mushroom, *P. arcularius*, concerning substrate specificity. It suggested that *T. matsutake* also has saprotrophic abilities from our findings about the potency of β-glucosidase (Kusuda et al. 2006). To reveal the physiological and enzymatic properties of the J-1 strain obtained from the genes of *Quercus* sp., more detailed information about the substrate specificities of this purified enzyme is needed.

References

Arai Y, Takao M, Sakamoto R, Yoshikawa K, Terashita T (2003) Promotive effect of the hot water soluble fraction from corn fiber on vegetative mycelial growth in edible mushrooms. J Wood Sci 49:437–443

Cai YJ, Buswell JA, Chang ST (1998) α-Glucosidase components of the cellulolytic system of the edible straw mushroom, *Volvariella volvacea*. Enzyme Microb Technol 22:122–129

Futai K (2003) Died of pine tree is infectious disease of forest (in Japanese). Bun-ichi, Tokyo, pp 13–54

Hatayama T, Ohmasa M (2004) Mycelial growth characteristics in a split-plate culture of four strains of the genus *Suillus*. Mycoscience 45:188–199

Hirato H, Kitamoto Y (1995) Effect of physico-chemical characteristics of the culture substrate and nutritional conditions on mycelial growth in *Tricholoma matsutake* (in Japanese). Sci Mushroom 2:67–72

Igarashi K, Tani T, Kawai R, Samejima M (2003) Family 3-β-glucosidase from cellulose-degrading culture of the white-rot fungus *Phanerochaete chrysosporium* is a glucan 1,3-β-glucosidase. J Biosci Bioeng 95:572–576

Inaba K, Yoshida T, Mitsunaga T, Koshijima T (1993) Acceleration of the growth of *Tricholoma matsutake* mycelium by a fraction of sulfite pulping waste. Mokuzai Gakkaishi 39:710–715

Inaba K, Yoshida T, Takano Y, Mayuzumi Y, Mitsunaga T, Koshizima T (1995) An instance of fruiting-body formation of *Tricholoma matsutake*. Environ Control Biol 33:59–64

Ishihara H, Imamura K, Kita M, Aimi T, Kitamoto Y (2005) Enhancement of the viscometric endocellulase activity of *Polyporus arcularius* CMCase IIIa by cellobiose and cellooligosaccharides. Mycoscience 46:148–153

Itaya M, Hattori T, Ohta A, Shimada M (2003) Possible roles of the glyoxylate cycle enzymes in lipid and fatty acids metabolisms of the ectomycorrhizal fungus *Laccaria amethystea* (in Japanese). Abstract papers presented at the 47th Annual Meeting of the Mycological Society of Japan, Sapporo, May 31–June 1

Iwase K (1996) Evolution of mycorrhizal mushrooms (in Japanese). Trans Mycol Soc Jpn 37:65–68

Kawai M, Abe S (1976) Studies on the artificial reproduction of *Tricholoma matsutake* (S. Ito et Imai) Sing. I. Effects of carbon and nitrogen sources in media on the vegetative growth of *T. matsutake* (in Japanese). Trans Mycol Soc Jpn 17:159–167

Kusuda M, Nagai M, Hur T, Ueda M, Terashita T (2003) Purification and some properties of β-amylase from an ectomycorrhizal fungus, *Tricholoma matsutake*. Mycoscience 44:311–317

Kusuda M, Ueda M, Konishi Y, Matsuzawa K, Shirasaka N, Nakazawa M, Miyatake K, Terashita T (2004) Characterization of extracellular glucoamylase from the ectomycorrhizal mushroom *Lyophyllum shimeji*. Mycoscience 45:383–389

Kusuda M, Ueda M, Konishi Y, Araki Y, Yamanaka K, Nakazawa M, Miyatake K, Terashita T (2006) Detection of β-glucosidase as a saprotrophic ability from a ectomycorrhizal mushroom, *Tricholoma matsutake*. Mycoscience 47:184–189

Makkar RS, Tsuneda A, Tokuyasu K, Mori Y (2001) *Lentinula edodes* produces a multicomponent protein complex containing manganese (II)-dependent peroxidase, laccase and beta-glucosidase. FEMS Microbiol Lett 200:175–179

Norkrans B (1950) Studies in growth and cellulolytic enzymes of *Tricholoma*. Symb Bot Ups 11:1–126

Ohta A (1994) Production of fruit-bodies of a mycorrhizal fungus, *Lyophyllum shimeji*, in pure culture. Mycoscience 35:147–151

Ohta A (1997) Ability of ectomycorrhizal fungi to utilize starch and related substrates. Mycoscience 38:403–408

Sengupta S, Ghosh AK, Sengupta S (1991) Purification and characterization of a beta-glucosidase (cellobiase) from a mushroom *Termitomyces clypeatus*. Biochem Biophys Acta 1076:215–220

Terashita T, Kono M (1987) Purification and some properties of carboxyl proteinases from *Tricholoma matsutake* (in Japanese). Trans Mycol Soc Jpn 28:245–256

Terashita T, Kusuda M, Matsukawa S, Nagai M, Yoshikawa K, Sakai T (2000) Production of extracellular amylase from *Tricholoma matsutake* and its properties on starch hydrolysis (in Japanese). Mushroom Sci Biotechnol 8:115–120

Yamada A (2005) To the artificial cultivation: the present situation and the approach on the developmental research (in Japanese). Technol Innov 15:24–28

Yamanaka K, Tanaka T, Nakanishi J, Cao H, Chen M (2003) *Tricholoma matsutake* in East Tibet, Sichuan, China (in Japanese). 47th Annual Meeting of the Mycological Society of Japan, Sapporo, May 31–June 1