Mycoscience 35: 83–87, 1994

Some cultural characteristics of mycelia of a mycorrhizal fungus, *Lyophyllum shimeji**

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Accepted for publication 6 January 1994

Cultural characteristics of 45 strains of *Lyophyllum shimeji* and 10 strains of three related species were determined. The average optimum temperature for mycelial growth of *L. shimeji* on a medium consisting of rye grains was 24.9°C, slightly higher than those for *L. fumosum* and *L. decastes*. The average mycelial growth rate of the *L. shimeji* strains, each at its optimum temperature, was 2.0 mm/day, almost the same as that of *L. decastes* and 2 times greater than those of *L. semitale* and *L. fumosum*. All strains of *L. shimeji* could grow on beech and pine sawdust, but none could significantly decompose beech wood blocks. Of the 45 strains of *L. shimeji*, 3 strains had ability to form primordia on the rye grain medium without the host plant.

Key Words——cultural characteristics; *Lyophyllum shimeji*; mycelial growth; primordium; wood decay capability.

Introduction

Two mycorrhizal mushrooms, *Tricholoma matsutake* (S. Ito et Imai) Singer and *Lyophyllum shimeji* (Kawamura) Hongo, are typical of edible wild mushrooms in Japan. Cultural conditions and cultural characteristics of *T. matsutake* mycelia have been intensively studied since the first scientific report by Mimura (1909), as summarized by Ogawa (1978), but available data for *L. shimeji* are few and sporadic (Kiuchi, 1983; Clémençon and Moncalvo, 1990; Ohta, 1990). *Lyophyllum shimeji* is a mycorrhizal fungus that can be expected to produce fruitbodies artificially, because its mycelia can grow on a wide variety of culture media including those for saprophytic fungi such as potato dextrose agar, and they grow as rapidly as wood-rotting fungi. This fungus is also an interesting material to study the mechanism of mycorrhiza formation because of its ability to form mycorrhiza with both broad-leaf and coniferous trees, in contrast to *T. matsutake*, which forms mycorrhiza only with pinaceae plants (Ogawa, 1978).

This paper reports on cultural characteristics of *L. shimeji* mycelia, especially on variations in the characteristics among the strains tested. Two mycorrhizal fungi, *L. fumosum* (Persoon: Fr.) P. D. Orton and *L. semitale* (Fr.) Kuhn., and a wood-rotting fungus, *L. decastes* (Fr.: Fr.) Singer, were used for comparison in some experiments. The capability for primordium formation was briefly discussed in relation to the growth characteristics on wood meal.

* This study was supported in part by the Forestry Agency, Japan.

Materials and Methods

Cultivation of mycelia Mycelial growth rates of *L. shimeji* and the reference species were determined using 18 mm (i. d.) × 180 mm test tubes containing a rye grain medium prepared as follows: 8 g of rye grains and 12 ml of water were placed in the test tube, allowed to stand at 5°C overnight to allow the grains to absorb water, then autoclaved for 20 min at 120°C. Mycelial agar blocks from the stock cultures were inoculated on the surface of the medium, and the cultures were incubated at 21, 23, 25, and 27°C. Six test tubes were used for each strain at each temperature. Linear growth of the mycelia from the inoculum was measured every second day, and the growth rate was calculated on linear part of the growth curve plotted against incubation time. Optimum temperatures for the mycelial growth of the test strains and the maximal growth rates at their optimum temperatures were estimated by curve-fitting treatment from the data of growth rates obtained at four temperatures using a computer program, Systat version 5.2.1 (Systat Inc, Illinois, U.S.A.).

Mycelial growth on wood meal was examined in test tubes containing sawdust at 65% moisture content on a wet basis. Sawdusts of beech wood, *Fagus crenata* Blume, and pine wood, *Pinus densiflora* Sieb. et Zucc. were used. Test tubes packed with sawdust to about 10 cm depth were autoclaved for 20 min at 120°C, then inoculated as above. The cultures were incubated at 23°C. Measurement of mycelial growth and determination of mycelial growth rate were performed as described above.

Wood decay capability of the test fungi was determined for 10 strains of *L. shimeji* and 2 strains each of the other species of mushrooms by the JIS Z 2119

Table 1. Derivation of *Lyophyllum* species and their mycelial growth on the rye grain medium and sawdust.

Species	Strains	Locality collected	Date isolated	Growth on rye grain medium		Growth on sawdust (mm/day)	
				Optimum temperature (°C)	Growth rate (mm/day)	Beech	Pine
L. shimeji	AK1L	Akita Pref.	Oct. 8, 1990	25.3	2.10	0.93	1.04
	AK2L		Oct. 8, 1990	25.8	2.27	1.63	1.52
	NG1L	Niigata Pref.	Oct. 18, 1989	25.0	2.55	1.51	1.49
	NG2L		Oct. 19, 1991	23.8	2.12	1.46	1.38
	SF-Ls4	Kyoto Pref.	Oct. 29, 1987	25.1	1.53	0.93	0.83
	NF1501	Nara Pref.	Oct. 16, 1986	24.6	1.87	1.51	0.85
	NF1502		Oct. 16, 1986	23.9	1.93	0.98	0.92
	NF1505		Oct. 20, 1987	26.1	2.02	1.28	1.28
	NF1511		Oct. 18, 1988	23.9	1.71	1.61	1.16
	NF1514		?	24.8	2.28	1.24	1.31
	NF1515		?	23.6	2.16	1.14	1.21
	KY1L	Kyoto Pref.	?	26.0	1.91	0.93	0.69
	KY2L		?	23.6	1.88	1.46	1.40
	KY3L		?	22.4	1.37	1.06	0.79
	KY-LS-1		?	25.2	2.19	1.81	1.48
	KY-LS-4		?	25.4	2.39	1.38	1.14
	HY1L	Hyogo Pref.	Nov. 5, 1990	26.1	2.03	1.34	1.43
	HY2L		Oct. 19, 1989	26.0	2.01	1.15	1.39
	HY3L		Nov. 6, 1989	26.5	2.00	1.57	1.03
	HY4L		Nov. 4, 1986	23.2	2.05	1.80	1.55
	HY5L		Oct. 22, 1987	25.1	2.39	1.45	1.42
	HY6L		Oct. 6, 1988	23.7	2.38	1.66	1.34
	W49	Wakayama Pref.	Nov. 7, 1986	25.8	2.10	1.34	1.39
	W53		Nov. 7, 1986	23.4	2.17	1.27	1.38
	W56		Nov. 14, 1986	24.4	2.42	1.40	1.44
	W131		Oct. 2, 1988	23.6	1.46	0.67	0.53
	W136		Oct. 27, 1988	25.1	1.62	1.35	1.21
	W140		Nov. 12, 1988	25.4	2.11	0.50	0.53
	W141		Oct. 24, 1988	25.7	1.98	1.41	1.37
	W142		Oct. 27, 1988	23.9	1.74	1.57	1.27
	150302-2	Ehime Pref.	Nov. 2, 1985	23.4	2.35	1.52	1.41
	150302-3		Oct. 30, 1990	25.7	1.91	1.53	1.48
	Izumo-2-4	Shimane Pref.	Nov. 6, 1986	22.6	2.28	1.61	1.26
	Izumo-2-5		Nov. 2, 1990	22.8	2.55	1.65	1.70
	YG1L	Yamaguchi Pref.	Nov. 12, 1983	24.8	2.47	1.10	1.38
	YG6L		Nov. 16, 1987	26.7	2.57	1.04	1.15
	YG8L		Oct. 13, 1986	26.3	1.85	0.93	0.68
	YG10L		Oct. 13, 1986	24.7	2.00	1.42	1.22
	YG14L		Oct. 22, 1986	26.2	2.35	1.43	1.31
	YG17L		Oct. 24, 1986	26.5	1.73	1.27	1.32
	YG19L		Oct. 27, 1986	24.9	1.61	1.37	1.40
	YG23L		Nov. 16, 1987	25.1	1.58	1.31	1.13
	YG25L		Nov. 1, 1986	26.1	2.12	1.29	1.31
	YG28L		Oct. 30, 1987	25.4	1.77	1.48	1.16
	YG30L		Nov. 30, 1987	25.4	1.73	1.16	1.00
L. semitale	J9	Chiba Pref.	Oct. 8, 1991	25.1	1.16	0.94	1.00
	YG32S	Yamaguchi Pref.	Oct. 21, 1986	24.9	0.87	1.14	0.86
L. fumosum	SF-Lf1	Shiga Pref.	Oct. 13, 1986	23.6	0.49	1.00	0.19
	KY-LF-2	Kyoto Pref.	Oct. 13, 1987	25.1	1.11	1.12	0.00
	NF1504	Nara Pref.	Oct. 16, 1986	22.8	0.84	1.04	0.00
	YG31F	Yamaguchi Pref.	Oct. 14, 1986	26.0	0.90	0.79	0.00
L. decastes	SF-Ld1	Shiga Pref.	Oct. 28, 1986	24.4	2.52	0.91	0.69
	SF-Ld5		Nov. 10, 1987	24.3	1.55	0.97	0.76
	NF1313	Nara Pref.	Nov. 7, 1988	22.4	0.98	0.61	0.00
	YG33D	Yamaguchi Pref.	Oct. 27, 1986	24.1	1.74	1.01	1.06

method (Japanese industrial standard committee, 1977) with modification, as follows: Fungal mycelia were grown at 23°C for 21 days in a 500-ml culture bottle containing 200 ml of sea sand and 70 ml of glucose-yeast extract medium (glucose 20 g, yeast extract 2 g, and water 1000 ml, adjusted to pH 5.4 with 1 M HCl). Beech wood blocks of 2×2×2 cm stood in grass petri dishes were autoclaved for 30 min at 120°C, then placed on the mycelial colonies grown in the bottles. Three wood blocks were placed into each culture bottle, and each strain was tested with four replicates. The cultures were incubated at 23°C for 60 days, then the blocks were weighed after drying them at 105°C for 24 h. Wood decay capability was indicated as the percentage reduction of dry weight as compared to those for the control blocks without fungal inoculation.

The capability of primordium formation of *L. shimeji* strains was examined with the cultures used for the mycelial growth tests on rye grain medium. When the mycelia of *L. shimeji* had grown over the surface of the medium in most of the test tubes (70 day after inoculation), the incubation was continued after lowering the temperature from 23°C to 15°C. Primordium formation was observed with the naked eye.

Fungal strains The strains of *L. shimeji* were collected in 9 prefectures of Honshu and Shikoku, Japan between 1983 and 1991, as shown in Table 1. *Lyophyllum semitale, L. fumosum* and *L. decastes* were also collected in 5 prefectures in the same islands. Mycelia isolated from fruit-bodies were stocked at 5°C until use after incubating them on a glucose-yeast extract medium of 1/10 concentrations with 1.5% agar.

Results

Optimum temperature and mycelial growth rate on rye grain medium Several strains of *L. shimeji* were each preliminarily incubated on five kinds of media, as follows: Rye grains, rice grains, vermiculite containing glucose-yeast extract medium, peat containing glucose-yeast extract medium, and a mixture of beech sawdust and rice bran. Since the rye grain medium gave the most rapid growth and thick mycelia, this medium was used in the following experiments.

Table 1 shows optimum temperatures for mycelial growth of the test strains and growth rates at their optimum temperatures on the rye grain medium. Optimum temperatures for *L. shimeji* strains ranged between 22.4°C and 26.7°C and their average was 24.9°C, which was similar to that for *L. semitale*, and a little higher than those for *L. fumosum* and *L. decastes*. Figure 1 shows the distribution of the optimum temperatures for *L. shimeji* strains. Mycelial growth rates of *L. shimeji* strains were 1.4–2.6 mm/day, and their average 2.0 mm/day, which was similar to that of *L. decastes* and about 2 times greater than those of *L. fumosum* and *L. semitale*.

Mycelial growth on wood substrates Mycelial growth rates of the different strains of *Lyophyllum* species on the sawdust medium are also shown in Table 1. Mycelia of all strains of *L. shimeji* grown on the sawdust media were very thin in comparison with those on the rye grain medium. The growth rates on the beech sawdust medium were 0.5–1.8 mm/day and their average 1.3 mm/day, and those on pine sawdust were 0.5–1.7 mm/day and their average 1.2 mm/day. A significant correlation was observed between the growth rates on beech sawdust and pine sawdust as shown in Fig. 2. The mycelial growth rates of *L. shimeji* strains did not differ from those of the other *Lyophyllum* species, although *L. fumosum* showed no or slight growth on the pine sawdust.

Table 2 shows the result of the modified JIS wood decay test. All tested strains of *L. shimeji* caused a slight decrease in weight of wood blocks after 60 days incubation; *L. decastes* caused a greater decrease. Among these test cultures, KY3L formed a primordium in one of four bottles.

Primordium formation capability In the tests of primordium formation, only 3 strains, W136, W141, and HY1L, formed primordia within 35 days after the change of temperature to induce fruiting. The proportions of the test tubes in which primordia were formed were 3/6 for W136, 5/6 for W141, and 6/6 for HY1L. Typical primordia of strains W136 and W141 are shown in Figs. 3a and

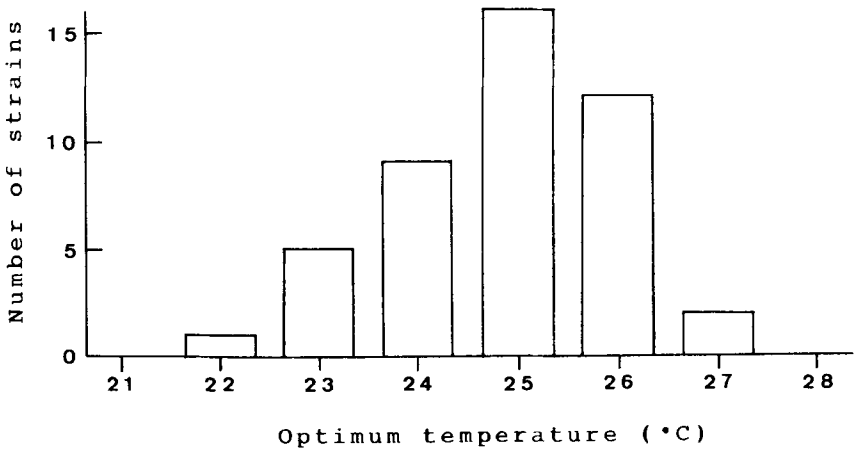

Fig. 1. Variation of the optimum temperature for mycelial growth of *Lyophyllum shimeji* strains.

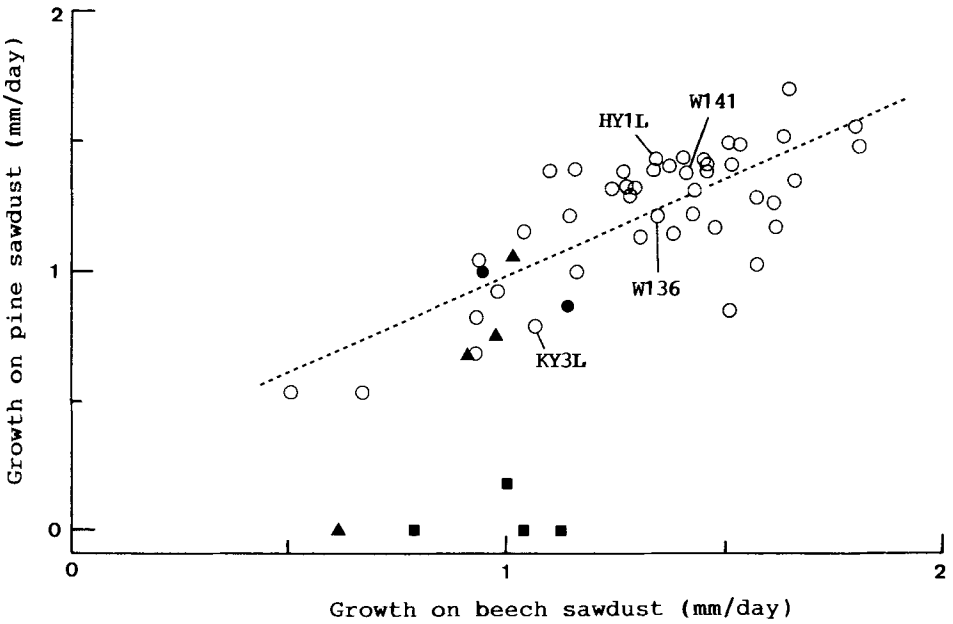

Fig. 2. Relationship between mycelial growth rates on beech and pine sawdusts. ○, *Lyophyllum shimeji*; ▲, *L. decastes*; ■, *L. fumosum*; ●, *L. semitale*. Broken line indicates the result of simple regression analysis for *L. shimeji* strains; Y=0.24+0.74X (r=0.75, significant at 1% level). HY1L, W136, W141 and KY3L: strains capable of forming primordia.

Table 2. Weight loss of beech wood block caused by *Lyophyllum* species in a modified JIS wood decay test[a].

Species	Weight loss (%)[b]	
	Average	Range
L. decastes	1.32	1.31-1.33
L. fumosum	0.42	0.29-0.55
L. semitale	0.74	0.69-0.79
L. shimeji	0.70	0.31-1.44

[a] Test procedure is described in the text.
[b] Determined for 10 strains of *L. shimeji* and 2 strains each of *L. decastes*, *L. fumosum*, and *L. semitale* using 12 wood blocks for each strain.

3b. One primordium of strain HY1L developed into a fruit-body-like organ with a small pileus and a stipe about 20 mm long 42 days after lowering the temperature (Fig. 3c).

Discussion

The genus *Lyophyllum* contains a wide variety of species, for instance, saprophytic and mycorrhizal, and the taxonomical positions of some species are somewhat complicated. Moncalvo and Clémençon (1992) reconsidered the taxonomy of the species and elucidated that there are 5 taxa in section Difforma. They used Japanese names for 3 taxa occurring in Japan, Honshimeji, Shaka-shimeji, and Hatake-shimeji, which are identical to the names tentatively used in the present paper, *L. shimeji, L. fumosum*, and *L. decastes*, respectively. As in their results, *L. fumosum* was excluded from *L. shimeji* and *L. decastes* by its having no or slight capability to grow on pine sawdust (Fig. 2), and no significant difference between *L. shimeji* and *L. decastes* was observed in optimum temperatures for mycelial growth and mycelial growth rates on the rye grain medium and on the sawdust medium.

It seems that the optimal temperature for mycelial growth of mycorrhizal fungi is usually lower than that of saprophytic fungi. Harley (1969) has stated that the optimum temperature for most mycorrhizal fungi is about 20°C, and Ogawa (1978) has described that those for many *Tricholoma* species are 20-23°C. The average optimum temperature for mycelial growth of *L. shimeji* on rye grain medium was 24.9°C, which is in the same range (22-35°C) as most wood-rotting fungi (Kitamoto and Suzuki, 1992).

Since *L. shimeji* strains used in the wood decay test caused some weight loss, and mycelia of all test strains

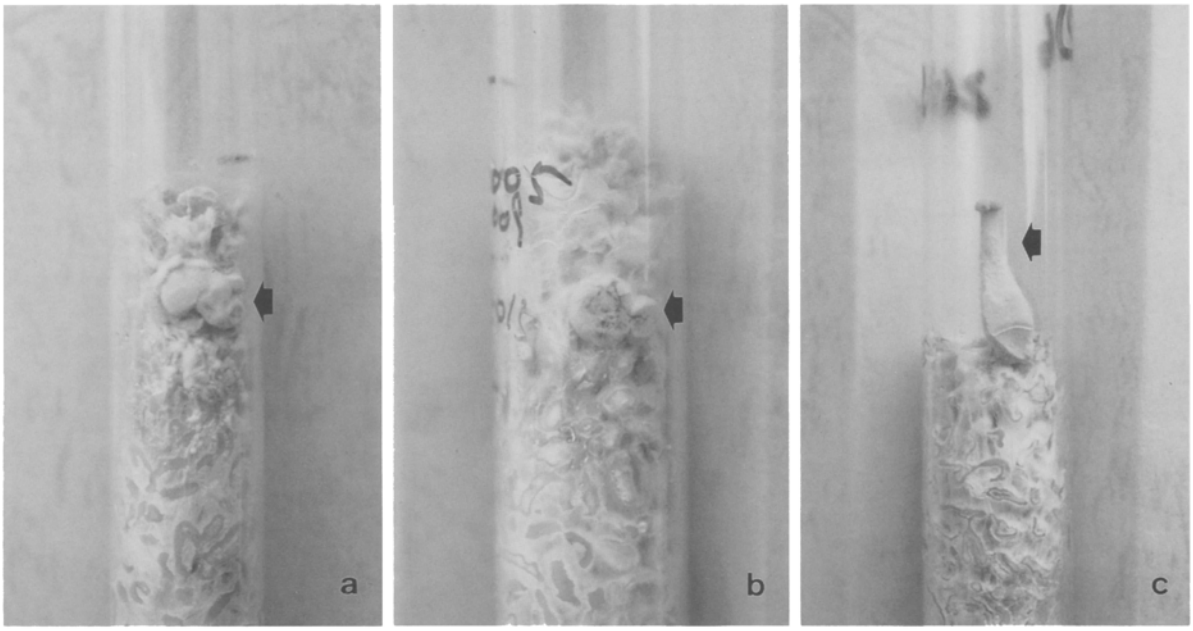

Fig. 3. Formation of primordium and fruit-body-like organ of *Lyophyllum shimeji* in the test tubes containing rye grain medium. a, strain W136; b, strain W141; c, strain HY1L.

of *L. shimeji* could grow on wood sawdusts, *L. shimeji* appears to have some capability to utilize wood components.

Among 45 strains of *L. shimeji*, 3 strains formed primordia in the test tubes. Another strain formed a primordium in the wood decay test. Fruiting capability may not relate to capability to utilize wood components, because these 4 strains showed moderate mycelial growth rates on sawdusts (Fig. 2).

One primordium of HY1L strain formed on the rye grain medium grew into a fruit-body-like organ, which suggests that further search for strains that readily form primordia and improvement of culture medium will contribute to fruit-body formation without the host plant.

Acknowledgements——I am grateful to the staffs of all forestry experiment stations in the prefectures listed in Table 1 and Dr. Kazutaka Azawa of Forest and Forestry Products Research Institute for supplying the stock cultures of the fungi. I also thank Mrs. Kazu Terazono for her technical assistance.

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