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ORIGINAL ARTICLE

Wood decaying properties of the termite mushroom *Termitomyces eurrhizus*

Kazuko Ono[1] · Toshimitsu Hata[1] · Tsuyoshi Yoshimura[1] · Kazuhiko Kinjo[2]

Abstract Four strains of the termite mushroom *Termitomyces eurrhizus* collected in Japan were surveyed for their wood decaying properties in three softwood and two hardwood species, in comparison with the white-rot fungus *Trametes versicolor* and the brown-rot fungus *Fomitopsis palustris*. All strains of *T. eurrhizus* degraded only the surfaces of the wood samples, and differences in mass-loss rates between heartwood and sapwood were generally not significant. Higher mass-loss rates were generally obtained in softwood than in hardwood. The results of chemical analyses of decayed wood samples indicated that *T. eurrhizus* does not have high lignin-degradation ability, even though it is categorized as a white-rot fungus. These results clearly suggest the unique physiological characteristics of *T. eurrhizus*.

Keywords Chemical analysis · Scanning electron microscope (SEM) · Termite mushroom · Wood decay test

Introduction

The fungi *Termitomyces* are known to have a unique life cycle. These fungi are distributed in tropical and subtropical areas from Africa to Southeast Asia [1], and are found only in nests of Macrotermitinae termites in the wild. They live in a medium called the fungus comb, located in a special chamber, fungus garden, inside the nest. The termites collect plant materials from outside the nest to maintain the fungus combs, which they later eat [2]. The function of this symbiotic relation is still not clear, but some tentative theories have been proposed [3]: (1) *Termitomyces* is an additional protein-rich food source for the termites; (2) *Termitomyces* has a role in lignin degradation for the termites, facilitating their access to cellulose; (3) *Termitomyces* decreases the C/N ratio of foraged plant materials by metabolizing carbohydrates; (4) *Termitomyces* provides cellulases and xylanases to work synergistically and/or complementarily with endogenous termite enzymes.

In Japan, two *Termitomyces* fungi have been reported from a region of Okinawa Prefecture, the southernmost tip of Japan [4–6]. These mushrooms have fruit bodies during a brief period at the end of the rainy season, and are not only edible but actually prized for their taste [7]. Thus, *Termitomyces* mushrooms have a potential market value in Japan, where wide variety of mushrooms is incorporated into the traditional cuisine, and where mushrooms are artificially cultivated. If artificial cultivation methods could be developed for these tasty mushroom species, they would likely find an appreciative audience in Japan. However, because of the symbiotic relationship with termites, there has been no report of the successful cultivation of these mushrooms under artificial conditions.

The only known host termite of *Termitomyces* fungi in Japan is *Odontotermes formosanus* [8, 9]. This subterranean termite is known as an important pest for agricultural crops, forest and timber constructions in Asia [10]. It is believed that *O. formosanus* collects various plant residues in the field to prepare the fungal media. Therefore, we consider that various plant materials can be used for the media of *Termitomyces* fungi.

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In this study, we surveyed the wood-decaying characteristics of four strains of *Termitomyces eurrhizus*. These strains were selected from twenty-seven strains collected in Okinawa Prefecture, Japan, for their rapid growth and easy handling [11]. The major aim of this study was to evaluate wood decaying ability of *T. eurrhizus* with some strains under the same culture condition. In addition, the feasibility of wood materials to artificial media was also discussed. It would also reveal some unknown relationships between host termites and *Termitomyces*. Among the five wood species used in the present study, *Pinus densiflora* is generally preferred by termites. *Cryptomeria japonica* and *Chamaecyparis obtusa* are dominant softwood plantation trees in Japan. *Fagus crenata* is commonly used in artificial cultivation of mushrooms in Japan. Finally, *Quercus miyagii* is a hardwood species distributed in the southern islands of Japan, where *T. eurrhizus* and *O. formosanus* are also present [12].

Materials and methods

Fungal strains

Four strains of *Termitomyces eurrhizus* (T3, T11, T25 and T26) were used in this study. They were collected in Okinawa Prefecture, Japan in 2004 and 2005, and isolated from fruit bodies. As controls, a white-rot fungus, *Trametes versicolor* (FFPRI 1030), and a brown-rot fungus, *Fomitopsis palustris* (FFPRI 0507), were also employed. These two strains were designated as decay test fungi according to the Japan Industrial Standard [13] (JIS K 1571-2010).

Wood samples

Heartwood and sapwood of three softwood species, *Pinus densiflora*, *Cryptomeria japonica*, and *Chamaecyparis obtusa* and one hardwood species, *Fagus crenata*, were employed for an eight-week decay test. *C. obtusa* is highest decay durability, followed by *C. japonica*, *P. densiflora* and *F. crenata* [14]. For the twelve-week decay test, heartwood and sapwood of the above four wood species and the hardwood *Quercus miyagii* were used. Wood species was same, but samples for the eight-week test were taken from different logs than samples for twelve-week test. The sample size was 10 mm (R) × 10 mm (T) × 5 mm (L). This small size of samples was determined by the slow growth of *T. eurrhizus*, 1.1–1.2 mm/day on cellulose containing medium [15]. They were dried at 60 °C for 48 hours to determine the oven-dried weight before the tests according to the methods of JIS K 1571-2010 [13].

Decay tests

Fungal inoculums were prepared with potato dextrose agar (PDA; Nissui, Tokyo, Japan) plates. A seven-mm-diameter inoculum was taken from the plate, and was inoculated on 100 ml of agar medium [glucose 4 % (w/v), malt extract 1.5 % (w/v), peptone 0.3 % (w/v), agar 2 % (w/v)] in a 450-ml screw cap glass bottle. After the mycelia grew over the media surface, three test wood blocks were set on the mycelial mat. The bottle-caps were tightly sealed because *T. eurrhizus* is extremely sensitive to contamination, and then kept for eight or twelve weeks at 26 °C in the dark. After exposure, the mycelia that covered the samples were removed gently, and the samples were dried at 60 °C for 48 hours and weighed to determine the mass loss. The numbers of repetitions were six and nine for the eight- and twelve-week trials, respectively.

Scanning electron microscopic (SEM) observation

The eight-week decay samples were subjected to microscopic observation. The samples were dehydrated using a solvent displacement method by soaking in an ethanol series [10, 20, 40, 60, 80, 90 and 100 % (v/v)], acetone and pentane for 10 minutes, respectively. Each sample was sliced with a fresh razor and coated with gold for observation under a SEM (JSM-5310; JEOL, Tokyo, Japan) at 10 kV. Both surfaces and core portions of the samples were observed (Fig. 1).

Chemical analyses

For chemical analyses, twelve-week decay samples were ground into 40-mesh-pass size by a high-speed blender (VM0113; Vitamix, Cleveland, OH). Lignin content was determined by the Klason method [16]. Using the filtrates from the Klason lignin analysis, holocellulose was quantitated as a total sugar by the phenol–sulfuric acid method
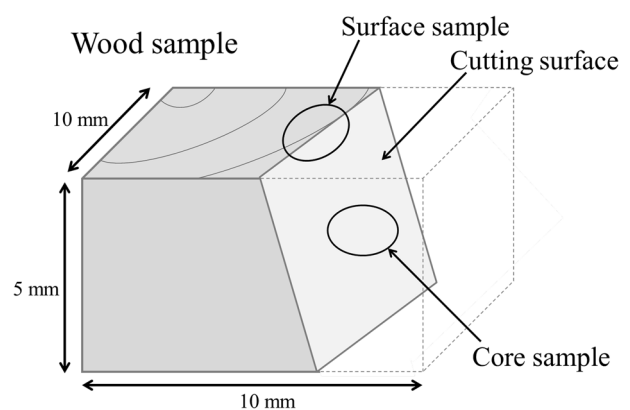

Fig. 1 Objects of SEM observation

[17]. Glucose was used as a standard. The lignin decrease rates (LDRs) and total sugar decrease rates (SDRs) were calculated from these results using the following pair of equations:

$$\text{LDR} = [L_1 - (100 - \text{MLR})/100 \times L_2]/L_1 \times 100$$

where L_1 is the average lignin content of sound wood samples ($n = 3$), L_2 is the lignin content of the individual decayed wood sample, and MLR is the mass-loss rate of the sample, and

$$\text{SDR} = [S_1 - (100 - \text{MLR})/100 \times S_2]/S_1 \times 100$$

where S_1 is the average total sugar content of sound wood samples ($n = 3$), S_2 is the total sugar content of the individual decayed wood sample, and MLR is the mass-loss rate of the sample.

Statistical analysis

The mass-loss rates (MLRs) of the decay tests, LDRs and SDRs were analyzed using analysis of variance (ANOVA) and Tukey–Kramer tests ($p < 0.05$).

Results

Eight-week decay test

The MLRs of the wood samples after the 8-week decay tests are displayed in Table 1.

In the heartwood of *P. densiflora*, there were no significant difference in MLRs between the strains of *T. eurrhizus* and the control strain *T. versicolor*, with the exception of T25. T25 showed lower MLRs in both heartwood and sapwood than those of the other *T. eurrhizus* strains. In all the *T. eurrhizus* strains, the MLRs of sapwood (8.7, 7.6, 6.0 and 8.3 %) were significantly higher than those of heartwood (6.7, 6.6, 3.5 and 6.8 %).

There was a wide variety in the strains of *T. eurrhizus* regarding decay in the heartwood of *C. japonica*. The MLR by T11 (10.1 %) was not significantly different from that of *T. versicolor*, which had the highest rate (11.5 %). Meanwhile, T25 showed the lowest MLR (4.4 %), and those of T3 and T26 were not significantly different. The MLR of T26 (5.6 %) was not significantly different from that of the brown-rot fungus *F. palustris* (8.0 %). In sapwood, *T. eurrhizus* exhibited lower MLRs than those of *T. versicolor* and *F. palustris*. In addition, T11 and T25 did not display a significant difference in MLRs between heartwood and sapwood. However, T3 and T26 showed lower MLRs in heartwood than in sapwood.

The various strains of *Termitomyces eurrhizus* also showed a variety of MLRs for heartwood of *C. obtusa*. T11

had the highest MLR (10.2 %), while T25 had the lowest MLR (5.2 %). There was no significant difference between the MLRs of T25 and *F. palustris* (5.2 %), but both were significantly lower than the MLRs of the other 3 strains. In sapwood, two strains (T3 and T26; 11.7 and 12.0 %, respectively) showed no significant difference from *T. versicolor* (14.5 %) and other 2 strains (T11 and T25; 8.7 and 7.2 %) showed lower than *T. versicolor*. All the strains had lower MLRs than that of *F. palustris* (21.1 %).

In the case of the hardwood *F. crenata*, all strains of *T. eurrhizus* showed unexpected results. This wood is known to be susceptible to decay fungi [18], yet the MLRs of the 4 strains were 2.3, 2.3, 0.9 and 2.4 % in heartwood, and 5.3, 3.7, 3.2 and 4.7 % in sapwood, respectively. These values were significantly lower than those of the two control fungi, *T. versicolor* and *F. palustris*, in both heartwood (15.3 and 8.5 %) and sapwood (17.9 and 15.2 %).

In this test, strain T25 showed the lowest MLRs in all wood samples with the exception of *P. densiflora* sapwood. In many cases, the MLRs of *T. eurrhizus* in sapwood were higher than those of heartwood, and in some cases, there was no significant difference in MLRs between heartwood and sapwood.

Twelve-week decay test

The MLRs in twelve-week decay tests are also shown in Table 1. In all test bottles, the mycelia of strain T25 died before finishing the test period; therefore, the results in T25 were omitted.

For *P. densiflora*, all fungi showed similar MLRs in the heartwood; in the sapwood; only T11 had lower MLR (4.8 %) than the others. T11 had a lower MLR in sapwood than in heartwood, but the others showed no significant difference between heartwood and sapwood.

For *C. japonica* heartwood, T11 had a significantly lower MLR (1.1 %) than the other 2 strains of *T. eurrhizus* (2.2 and 4.9 %). All *T. eurrhizus* strains showed lower MLRs than that of *F. palustris* (9.4 %), but there was no significant difference from that of *T. versicolor*. T26 (8.4 %) showed no significant difference from *F. palustris* (9.9 %) in sapwood, and both were higher than those of the other strains. The MLRs of T3 and T11 were not significantly different between heartwood and sapwood, but the MLR of T26 in heartwood was significantly lower than that in sapwood.

In *C. obtsa* heartwood, T26 showed a significantly higher MLR (10.2 %) than all the other test fungi. There was no significant difference in the MLRs of T3 (7.0 %) and T11 (7.7 %), T11 and *F. palustris* (8.6 %), respectively. In sapwood, T26 had a higher MLR (10.0 %) than the other *T. eurrhizus* strains (7.9 and 6.4 %). In addition, T26 and the control fungi did not have significantly

Table 1 Mass-loss rates of 4 wood samples after eight-week decay tests and five wood samples after twelve-week decay tests

Strains	The mass loss rates of decay samples															
	P. densiflora								*C. japonica*							
	Heartwood				Sapwood				Heartwood				Sapwood			
	8-week	SD	12-week	SD	8-week	SD	12-week	SD	8-week	SD	12-week	SD	8-week	SD	12-week	SD
T3	6.7	0.4B	8.2	1.5A*	8.7	0.2A	7.3	0.6A	4.7	1.8D	2.2	3.5B*	8.8	2.2CD	1.8	2.2B
T11	6.6	0.7B	7.6	1.2A	7.6	0.1AB	4.8	1.0B	10.1	1.6AB*	1.1	2.8C*	7.5	3.0CD	4.4	1.8B
T25	3.5	0.4C			6.0	0.3B			4.4	1.3D*			5.5	1.5D		
T26	6.8	0.5B	8.3	0.9A*	8.3	0.4A	7.9	0.7A	5.6	0.7CD	4.9	2.2B	10.8	2.6C	8.4	0.7A
T. versicolor	8.3	0.8B*	8.5	2.9A*	7.0	1.3AB	8.0	2.0A	11.5	1.1A	−5.2	9.7BC*	16.7	0.6B	3.7	2.7B
F. palustris	16.3	2.0A	9.0	1.3A*	5.5	2.0B	8.1	0.8A	8.0	1.4BC	9.4	1.8A*	22.7	0.7A	9.9	1.1A

Strains	The mass loss rates of decay samples															
	C. obtusa								*F. crenata*							
	Heartwood				Sapwood				Heartwood				Sapwood			
	8-week	SD	12-week	SD	8-week	SD	12-week	SD	8-week	SD	12-week	SD	8-week	SD	12-week	SD
T3	7.8	0.5B	7.0	1.0C*	11.7	0.4BC	7.9	0.9B	2.3	0.2C	4.4	0.3B*	5.3	0.2B	4.2	0.4B
T11	10.2	0.4A*	7.7	0.7BC*	8.7	1.2C	6.4	1.6B	2.3	0.5C	1.4	1.0C*	3.7	0.3B	0.9	1.7C
T25	5.2	1.1C			7.2	0.8D			0.9	0.5C			3.2	0.5B		
T26	9.4	0.8AB	10.2	0.5A*	12.0	0.8B	10.0	0.6A	2.4	0.4C	4.2	0.3B*	4.7	0.2B	4.8	0.4B
T. versicolor	7.8	1.5B	−7.8	3.9D	14.5	2.2B	6.5	6.8AB	15.3	3.2A*	17.9	5.0A*	17.9	3.6A	26.1	12.3A
F. palustris	5.2	1.1C	8.6	0.4B	21.1	2.2A	10.0	0.5A	8.5	6.6B*	5.2	0.5B	15.2	1.0A	4.5	0.1B

Strains	The mass loss rates of decay samples							
	Q. miyagii							
	Heartwood				Sapwood			
	8-week	SD	12-week	SD	8-week	SD	12-week	SD
T3			0.5	0.3B			2.1	0.7C
T11			1.2	0.6AB			2.4	0.2C
T25								
T26			1.0	0.3A			3.0	0.2B
T. versicolor			1.1	2.4ABC			5.1	1.9A
F. palustris			−0.3	0.2C			2.2	0.3C

The same characters indicate no significant difference by Tukey–Kramer test ($p > 0.05$)

The samples for the 8-week test were taken from different logs with samples for 12-week test

The mass-loss rates for 8-week decayed samples cannot be compared with those for 12-week decayed ones

SD standard deviation of mass-loss rate

* There was no significant difference between heartwood and sapwood (*t*-test $p > 0.05$)

different MLRs. No *T. eurrhizus* strains showed a significant different in MLRs between heartwood and sapwood.

In the heartwood and sapwood of *F. crenata*, the MLRs in T3 (4.4, 4.2 %) and T26 (4.2, 4.8 %) were significantly lower than those of *T. versicolor* (17.9, 26.1 %), but they were not significantly different from those of *F. palustris* (5.2, 4.5 %), while the MLRs of T11 (1.4, 0.9 %) were significantly lower than those of the other fungi. There was no significant difference between heartwood and sapwood MLRs for any *T. eurrhizus* strain.

All the fungi showed the lower MLRs for the heartwood of *Q. miyagii* than for other wood species. Three *T. eurrhizus* strains showed low MLRs (0.5, 1.2 and 1.0 %), without any significant difference from that of *T. versicolor* (1.1 %). In contrast, for sapwood, *T. versicolor* had a higher MLR (5.1 %) than those of the *T. eurrhizus* strains (2.1, 2.4 and 3.0 %). All *T. eurrhizus* strains displayed significantly lower MLRs in heartwood than in sapwood.

SEM observation

In this observation, wood samples decayed by the white-rot fungus *T. versicolor* were employed as comparative control, because *Termitomyces* fungi are classified as white-rot fungi [19]. The observation positions in the sample are indicated in Fig. 1. The results of SEM observations are shown in Figs. 2, 3, 4, 5.

In all samples exposed to *T. versicolor*, there were many mycelia in almost all tracheids and vessels, with serious decay symptoms (*P. densiflora*, Fig. 2 A-B; *C. japonica*, Fig. 3a, b; *C. obtusa*, Fig. 4a, b; and *F. crenata*, Fig. 5a, b). However, mycelia of *T. eurrhizus* were rarely found in the tracheids of softwood (*P. densiflora*, Fig. 2c, d; *C. japonica*, Fig. 3c, d; and *C. obtusa*, Fig. 4c, d). Additionally, there were no decay symptoms around the mycelia at all. In *F. crenata*, the mycelia of *T. eurrhizus* were found inside vessels in the hardwood more frequency than tracheids in softwood (Fig. 5c, d), but no decay symptoms were observed. In addition, there were no mycelia in other woody tissues, such as xylem rays and fibers. There were many *T. eurrhizus* mycelia covering the sample surfaces and edges (*P. densiflora*, Fig. 2e, f; *C. japonica*, Fig. 3e, f; *C. obtusa*, Fig. 4e, f; and *F. crenata*, Fig. 5e, f).

Component analyses

LDRs and SDRs of 3 *T. eurrhizus* strains (T3, T11 and T26), *T. versicolor* and *F. palustris* are shown in Tables 2 and 3, respectively.

There was no significant difference among all fungal strains in either the LDRs or the SDRs of heartwood and sapwood for *P. densiflora* (Tables 2, 3).

In *C. japonica*, none of the strains showed any significant difference in LDRs between the heartwood and sapwood samples (Table 2). They also showed no difference in SDRs in the heartwood of *C. japonica*. In the sapwood, there was no significant difference in SDRs among the three *T. eurrhizus* strains and *T. versicolor*. T3 exhibited a significantly lower SDR than that of *F. palustris*, but the other two strains showed no significant difference from *F. palustris* (Table 3).

Interestingly, all the fungi exhibited negative LDRs in heartwood of *C. obtusa*, and T3 and T11 also showed negative LRDs in sapwood (Table 2). LDRs of all the fungal strains were not significantly different in sapwood. It is suggested that *T. eurrhizus* has low lignin degradation ability in *C. obtusa*. In heartwood, the SDRs of T11 and T26 were not significantly different from that of *F. palustris*; in sapwood, only the SDR of T26 was not significantly different from that of *F. palustris* (Table 3). With respect to the LDRs and the SDRs of *C. obtusa*, there was no significant difference among *T. eurrhizus* strains.

In the heartwood of *F. crenata*, the LDRs of T3 and T11 were significantly lower than that of *T. versicolor*, while the LDR of T26 was not significantly different. On the other hand, the LDRs of all the *T. eurrhizus* strains were significantly lower than that of *T. versicolor* in sapwood. The SDRs in heartwood of all fungal strains were not significantly different. But, in sapwood, the SDR of T11 was significantly lower than that of *F. palustris*.

Lignin degradation of *Q. miyagii* was not significantly different among all the fungal strains in heartwood, and in sapwood, the only significant difference was significantly lower LDR in T11 compared to *T. versicolor*. In heartwood, no fungal strains had significantly different SDRs. T26 did exhibit a significantly higher SDR than those of other *T. eurrhizus* strains in sapwood, but it was not significantly different from that of *F. palustris*.

Discussion

Regardless of wood species and sample location, all *T. eurrhizus* strains showed similar mass losses after eight-week exposure. The results suggested that *T. eurrhizus* can degrade heartwood as well as sapwood. Heartwood generally exhibits higher durability than that of sapwood, because heartwood has more extractives than that in sapwood. *T. eurrhizus* may have some resistance to extractives. We propose that a mixture of heartwood and sapwood may be used as a medium for artificial cultivation of *T. eurrhizus*, e.g., a whole small log produced by forest thinning.

From the results of eight-week decay test, T25 showed the lowest values of MLRs in all wood species both heartwood and sapwood. This strain has low degradation potential of wood. On the other hand, T26 showed the highest MLRs both decay tests in most of wood species. T26 is comparatively high wood degradation ability among four strains.

Meanwhile, the LDRs of *T. eurrhizus* were not significantly different from that of the brown-rot fungus *F. palustris*. Since brown-rot fungi generally decompose little lignin [20], this might indicate that *T. eurrhizus* has low lignin degradation ability. *Termitomyces* fungi are symbionts of Macrotermitinae termites, and one of the roles of the fungi is thought to be decomposition of lignin for termite digestion [21]. Other scientists have reported that the
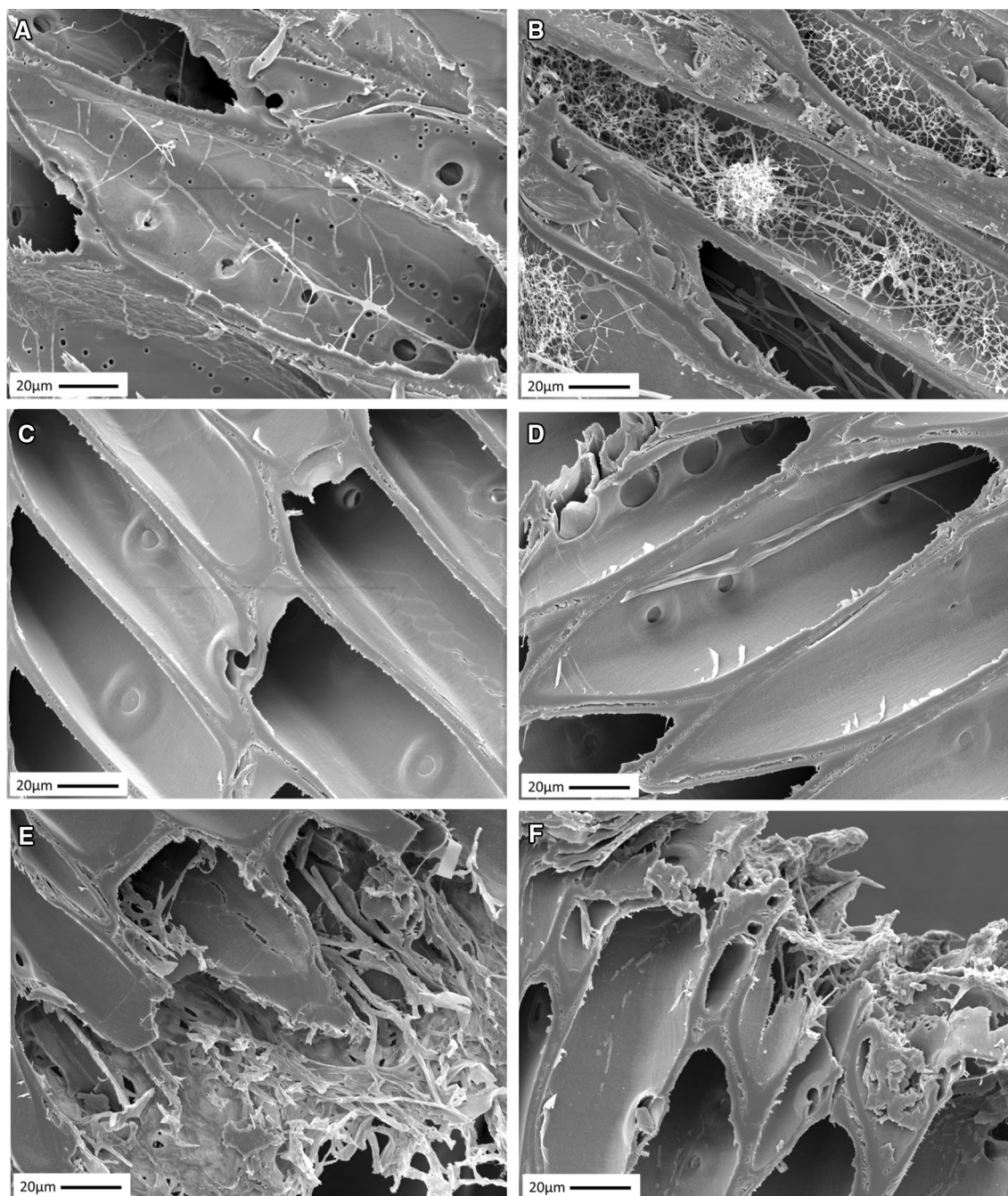
Fig. 2 SEM images of decayed wood samples of *Pinus densiflora*. **a** Heartwood core sample decayed by *T. versicolor* (FFPRI1030). **b** Sapwood core sample decayed by *T. versicolor* (FFPRI1030). **c** Heartwood core sample decayed by *T. eurrhizus* (T3). **d** Sapwood core sample decayed by *T. eurrhizus* (T26). **e** The surface of a heartwood sample decayed by *T. eurrhizus* (T11). **f** The surface of a sapwood sample decayed by *T. eurrhizus* (T3)

roles of symbiotic fungi are unclear and differ depending on their host termites [22, 23]. The host termite *Odontotermes formosanus* in Japan might not depend on high lignin degradation by *T. eurrhizus*. The lignin contents of the fungus comb collected in Japan were 3–10 % [24], significantly lower than those of plants. *Odontotermes formosanus* may collect lignin-decomposed material for the comb. Alternatively, *Xylaria* fungi are known to settle

in fungus combs of Macrotermitinae termites [7, 25, 26]. *Xylaria* species have a solitary habit and the ability to degrade lignin [27]. Hence, the lignin of the comb may be decomposed by these *Xylaria* fungi. The termites are also known to feed on the fungal nodules, balls of *Termitomyces* mycelia, on the fungus comb [3]. *T. eurrhizus* is likely to be a source of protein for *O. formosanus*. Although we found wide variation in the SDRs, those of *T. eurrhizus* and
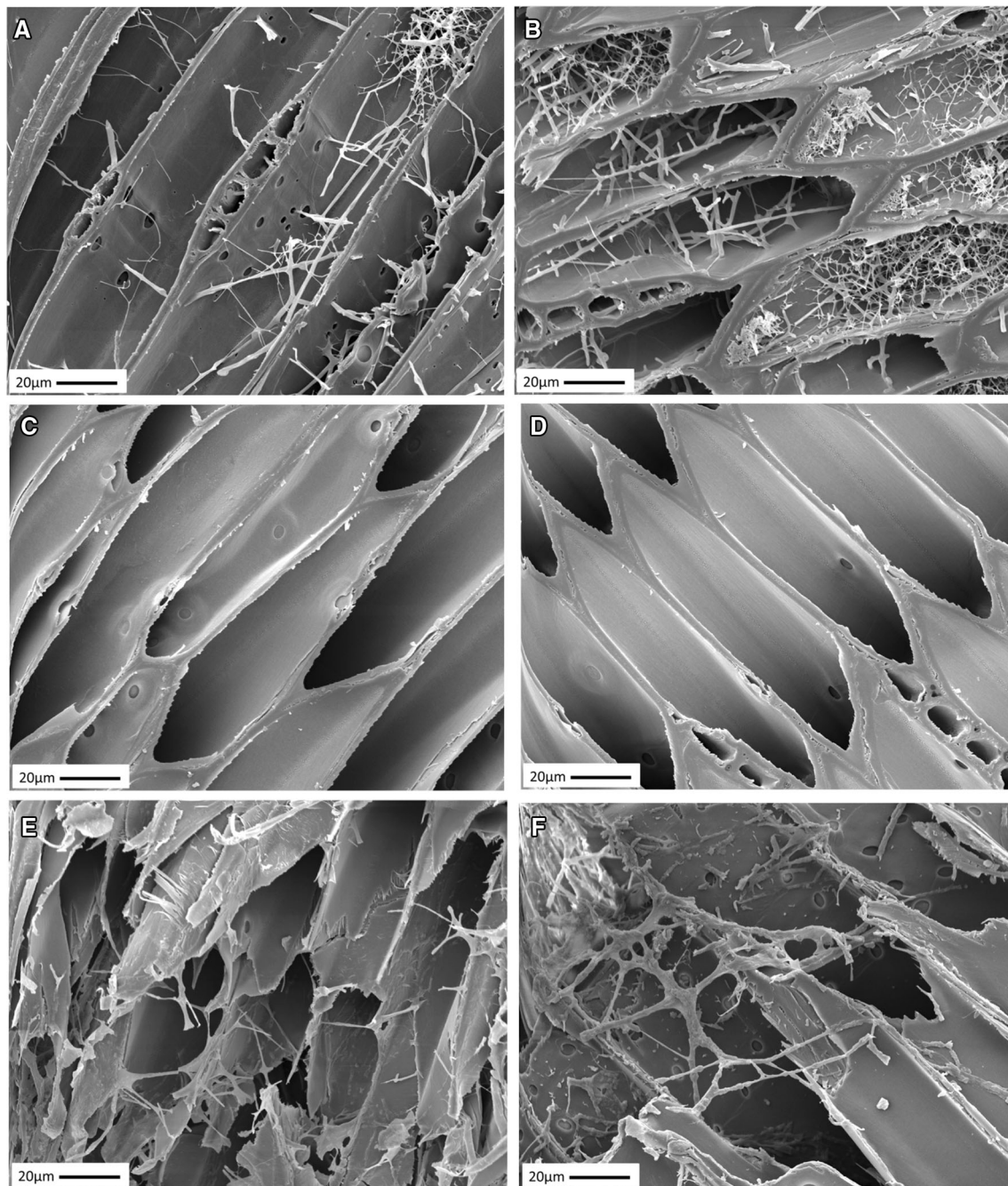
Fig. 3 SEM images of decayed wood samples of *Cryptomeria japonica*. **a** Heartwood core sample decayed by *T. versicolor* (FFPRI1030). **b** Sapwood core sample decayed by *T. versicolor* (FFPRI1030). **c** Heartwood core sample decayed by *T. eurrhizus* (T3). **d** Sapwood core sample decayed by *T. eurrhizus* (T11). **e** The surface of a heartwood sample decayed by *T. eurrhizus* (T26). **f** The surface of a sapwood sample decayed by *T. eurrhizus* (T25)

control fungi were not significantly different. It might indicate the similar degradation abilities of *T. eurrhizus* to holocellulose. Our previous study indicated that *T. eurrhizus* could grow sufficiently on cellulose-containing medium as a carbon-source [15].

In general, *F. crenata* is known to be susceptible to wood-decaying fungi, and is used in the cultivation of

mushrooms in Japan [28]. But our *T. eurrhizus* strains induced little decay in either the 8- or the 12-week exposure. In addition, these strains also could not degrade *Q. miyagii*. These two species belong to the family Fagaceae. Fagaceae has tannin (0.2–8.8 %) in wood [29], and tannin was reported to inhibit fungal decomposition [30]. Tannin could inhibit wood degradation of *T. eurrhizus*. Timbers of
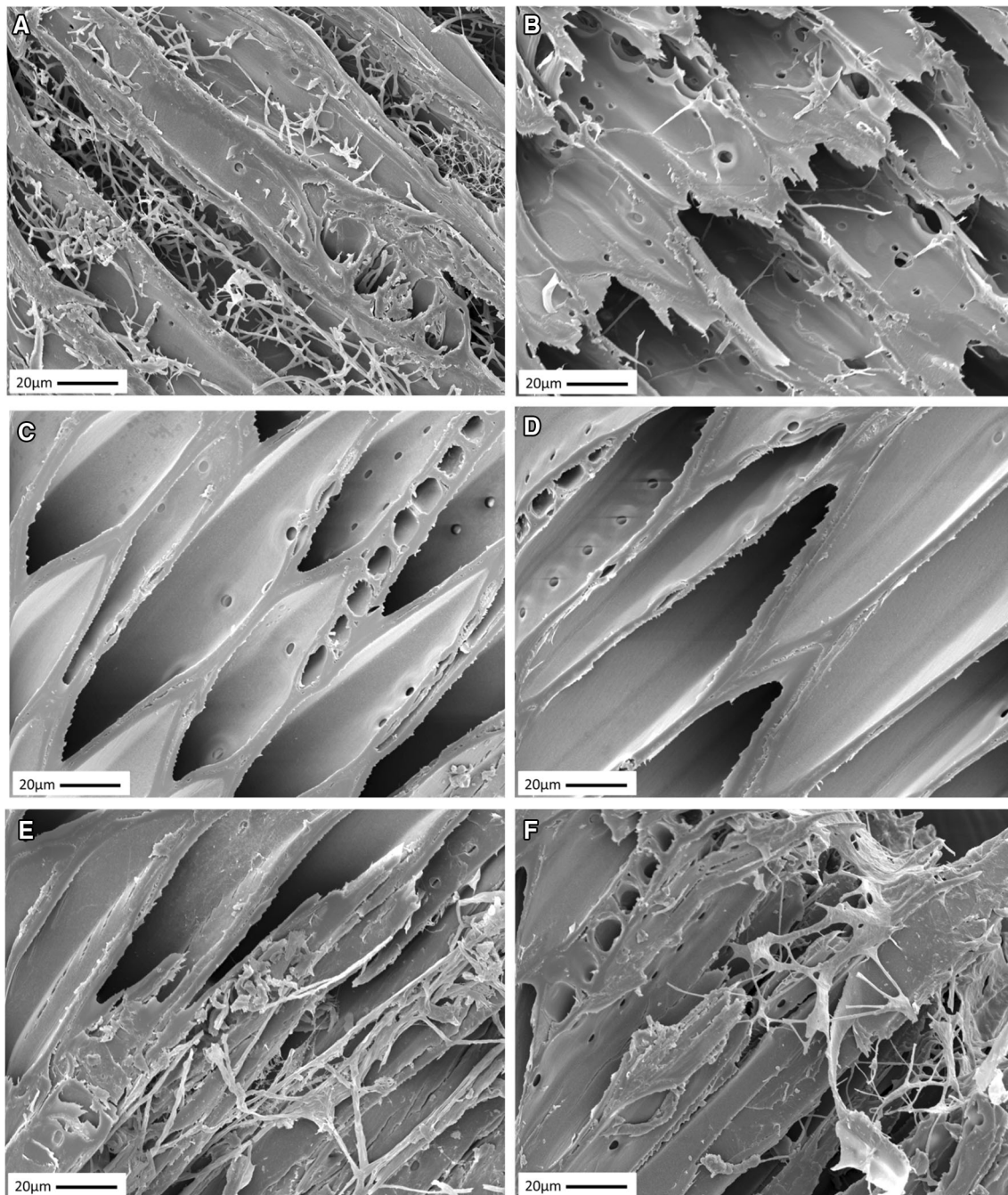
Fig. 4 SEM images of decayed wood samples of *Chamaecyparis obtusa*. **a** Heartwood core sample decayed by *T. versicolor* (FFPRI1030). **b** Sapwood core sample decayed by *T. versicolor* (FFPRI1030). **c** Heartwood core sample decayed by *T. eurrhizus* (T25). **d** Sapwood core sample decayed by *T. eurrhizus* (T26). **e** The surface of a heartwood sample decayed by *T. eurrhizus* (T11). **f** The surface of a sapwood sample decayed by *T. eurrhizus* (T26)

Q. miyagii have been known as hard and durable materials since early times [31, 32]; the heartwood is especially known for its high decay durability.

Since T25 did not survive through the end of the test period of twelve weeks, *T. eurrhizus* might be unfit for long-term cultivation on artificial media. This was supported by the results that the MLRs of T3, T11 and

T26 after twelve weeks were the same as those of the eight-week tests. We incubated all the bottles hermetically because *T. eurrhizus* seemed to be sensitive to bacterial and fungal contamination in our preliminary experiments (Ono, unpublished data). On the other hand, the nests of the host termites are well ventilated [33, 34]. Fungus combs have a sponge-like appearance
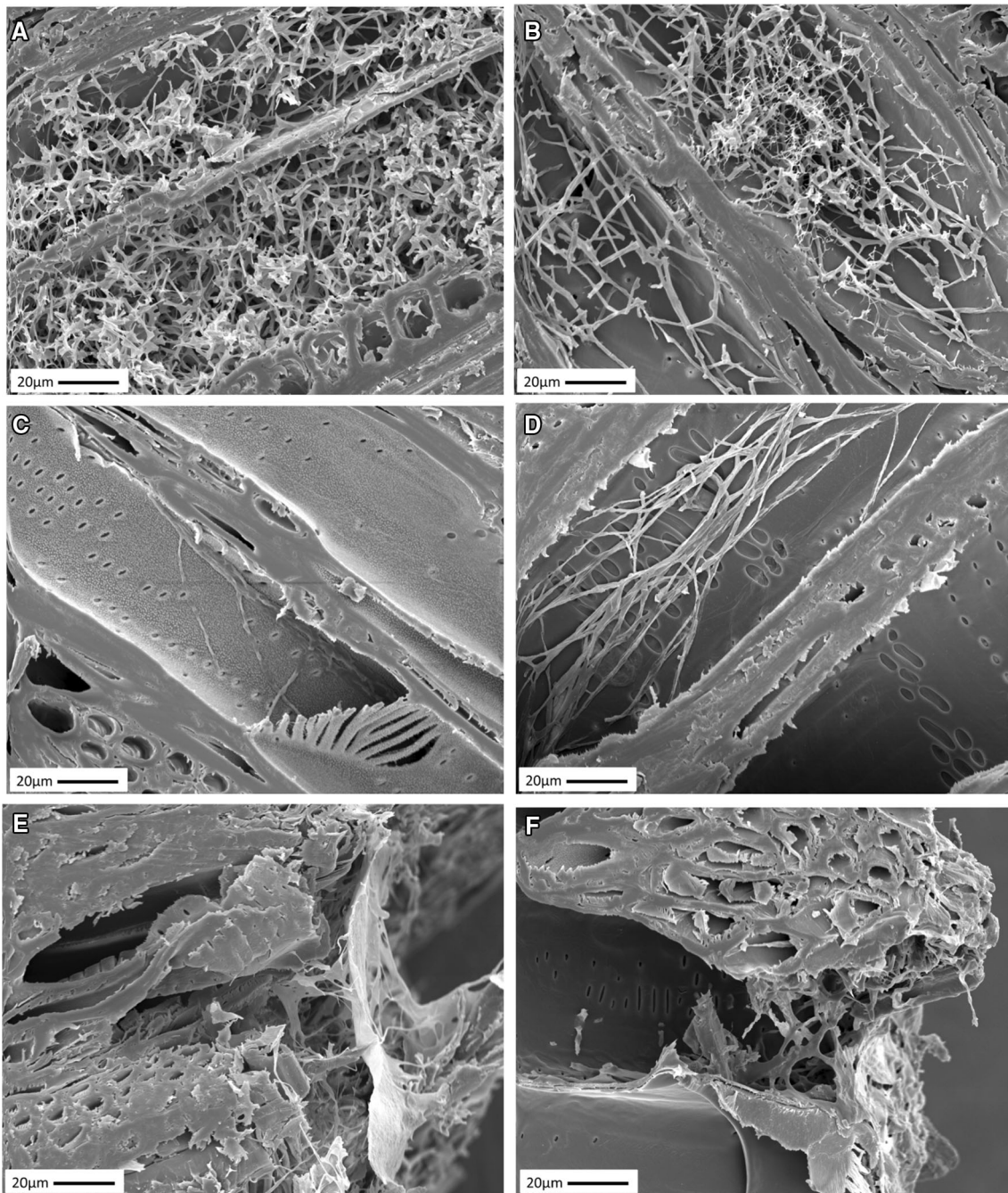
Fig. 5 SEM images of decayed wood samples of *Fagus crenata*. **a** Heartwood core sample decayed by *T. versicolor* (FFPRI1030). **b** Sapwood core sample decayed by *T. versicolor* (FFPRI1030). **c** Heartwood core sample decayed by *T. eurrhizus* (T11). **d** Sapwood core sample decayed by *T. eurrhizus* (T3). **e** The surface of a heartwood sample decayed by *T. eurrhizus* (T3). **f** The surface of a sapwood sample decayed by *T. eurrhizus* (T11)

with a large surface area, meaning that *Termitomyces* fungi may prefer a well-ventilated condition. Moreover, fungus combs are fed from the bottom, and are maintained by termites by adding fresh media [2, 35]. It is assumed that some metabolic products by the fungus might be stored in the agar media, and inhibit the growth of *T. eurrhizus*.

The results of SEM observation clearly showed that the mycelia of *T. eurrhizus* could not penetrate even the smaller wood blocks [1 cm (R) × 1 cm (T) × 0.5 cm (L)]. It is presumed that *T. eurrhizus* can only decay the surfaces of the samples. Wood materials of small particle size such as saw-dust are strongly recommended for the artificial media. However, mycelia of *T. eurrhizus* were often found

Table 2 Average of lignin content rates and lignin decrease rates (LDRs) of 5 wood species after twelve week decay tests

Lignin

| | P. densiflora | | | | | | C. japonica | | | | | | C. obtusa | | | | | |
| | Heart wood | | | Sap wood | | | Heart wood | | | Sap wood | | | Heart wood | | | Sap wood | | |
	Content (%)	LDR (%)	SD	Content (%)	LDR (%)	SD	Content (%)	LDR (%)	SD	Content (%)	LDR (%)	SD	Content (%)	LDR (%)	SD	Content (%)	LDR (%)	SD
Sound wood	27.0			26.7			33.9			31.1			26.4			28.9		
T3	29.3	0.6	3.2a	27.9	3.2	0.5a	35.9	−4.9	6.3a	31.2	0.8	3.7a	32.1	−11.6	3.3ab	31.8	−1.2	1.1a
T11	29.4	−1.0	2.1a	28.6	−1.1	3.6a	33.6	2.4	2.4a	31.2	3.9	2.2a	31.6	−9.9	3.6b	31.5	−1.2	1.8a
T26	29.3	1.1	5.4a	28.9	0.2	5.0a	35.0	1.5	11.2a	34.2	−0.5	7.1a	30.4	−3.1	1.7b	31.7	1.5	0.2a
T. versicolor	28.6	4.2	5.3a	28.9	0.5	1.6a	32.1	6.9	12.2a	28.8	10.3	1.2a	29.8	−22.8	8.3a	29.1	3.5	3.5a
F. palustris	34.1	−4.9	9.3a	30.4	−4.7	6.4a	44.0	−7.4	8.0a	40.3	6.5	35.6a	32.5	−10.1	1.4b	35.1	4.9	8.5a

Lignin

| | F. crenata | | | | | Q. miyagii | | | | | |
| | Heart wood | | | Sap wood | | Heart wood | | | Sap wood | | |
	Content (%)	LDR (%)	SD	Content (%)	SD	Content (%)	LDR (%)	SD	Content (%)	LDR (%)	SD
Sound wood	27.7			28.1		28.6			25.7		
T3	27.8	3.9		27.1	7.5b	29.7	−3.1	2.9a	26.4	−1.1	8.4a
T11	27.5	1.7		27.5	0.5b	30.4	−4.8	3.5a	27.1	−3.3	4.1a
T26	25.3	12.2		27.0	3.9b	30.2	−4.1	3.0a	26.7	−0.8	2.0a
T. versicolor	23.3	31.9		21.3	4.6a	28.8	−0.6	3.6a	23.5	12.3	8.6a
F. palustris	29.9	6.8		36.2	4.8b	30.3	−1.9	6.9a	27.8	6.7	2.0a

The same characters indicate no significant difference by Tukey–Kramer test ($p > 0.05$)

LDR lignin decrease rate, SD standard deviation of lignin decrease rate

Table 3 Average of total sugar component rates and total sugar decrease rates (SDRs) of 5 wood species after twelve-week decay tests

Total sugar

	P. densiflora						C. japonica						C. obtusa					
	Heart wood			Sap wood			Heart wood			Sap wood			Heart wood			Sap wood		
	Content (%)	SDR (%)	SD	Content (%)	SDR (%)	SD	Content (%)	SDR (%)	SD	Content (%)	SDR (%)	SD	Content (%)	SDR (%)	SD	Content (%)	SDR (%)	SD
Sound wood	65.0			65.7			62.9			65.2			62.0			62.4		
T3	67.9	4.5	11.6a	65.9	7.3	13.1a	67.0	−5.6	7.3a	71.2	−8.0	7.3b	66.3	1.6	1.2b	59.7	12.0	11.2b
T11	73.6	−5.1	8.9a	73.5	−5.7	5.3a	64.2	−0.5	7.1a	66.2	2.7	1.7ab	63.8	5.6	14.7ab	66.9	0.5	4.4b
T26	58.1	18.5	15.9a	62.9	11.8	6.5a	54.4	17.0	14.7a	50.3	29.6	9.6ab	60.8	12.1	9.9ab	58.4	15.9	8.2ab
T. versicolor	65.5	8.9	1.4a	67.0	6.2	2.6a	61.1	4.6	8.6a	66.5	1.3	2.2ab	61.3	−7.5	11.3b	64.5	1.1	4.6b
F. palustris	60.1	21.9	16.2a	65.6	4.3	31.4a	54.9	23.4	27.7a	55.4	36.2	30.2a	50.0	27.8	2.3a	51.9	34.8	7.1a

Total sugar

	F. crenata						Q. miyagii					
	Heart wood			Sap wood			Heart wood			Sap wood		
	Content (%)	SDR (%)	SD	Content (%)	SDR (%)	SD	Content (%)	SDR (%)	SD	Content (%)	SDR (%)	SD
Sound wood	66.6			66.7			64.1			69.0		
T3	67.1	3.6	4.8a	62.5	10.3	18.9a	68.7	−6.6	1.9a	74.3	−5.9	2.8c
T11	71.4	−6.1	12.5a	68.1	−1.2	8.1a	63.8	1.9	8.6a	72.2	−2.2	7.2c
T26	70.6	−1.7	4.2a	65.3	6.7	3.8a	60.8	6.4	2.4a	62.3	12.5	4.0ab
T. versicolor	69.9	15.1	6.4a	70.3	23.5	23.1a	64.7	−0.9	1.9a	69.4	3.9	1.3bc
F. palustris	63.3	17.3	13.6a	59.3	39.5	15.0a	71.1	−6.8	6.9a	67.5	15.8	6.5ab

The same characters indicate no significant difference by Tukey–Kramer test ($p > 0.05$)

SDR total sugar decrease rate, SD standard deviation of total sugar decrease rate

in the vessels of *F. crenata*. The size of vessels of *F. crenata* is larger than those of the softwood tracheids (Figs. 2–5). If the mycelia of *T. eurrhizus* prefer a well-ventilated condition, they might not penetrate deep into tracheids.

This study shows that *T. eurrhizus* collected in Japan does not have high lignin-degradation ability. The low lignin content wood species may be favorable to *T. eurrhizus*. In addition, the fungus can only decay the surfaces of wood block samples. There is a possibility that the fungus prefers softwood to hardwood, suggesting that in the future artificial cultivation with forest-thinning resource such as Japanese cedar (*C. japonica*) and cypress (*C. obtusa*) would be targeted. The presence of extractives might be one of factors relating to from this phenomenon.

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