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Screening of wood preservatives and natural compounds against 2 isolates of *Loweporus tephroporus* from severely damaged ekki wooden bridges in Japan

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Ekki wood (*Lophira alata* Banks) is a western African species having high density (*d* 1.07) and is widely used in European countries from its naturally large durability (classified "durable species" in EN 350-2). However, many decay damages were reported when it is used in Japan. In this paper, we describe the isolation and characteristics of two fungi from severely decayed bridges in Tokyo and Kyushu and screening the preservatives against them. These fungi were identified as *Loweporus tephroporus* having the optimal growth temperatures around 35℃. From the hyphal growth test on the PDA medium containing various preservatives or natural components with different concentrations, minimum inhibitory concentrations increased as follows; AAC (alkyl ammonium compound) and hinokitiol < NCu (copper naphthenate), NZn (zinc naphthenate), $CuSO_4$, $ZnCl_2$, coumarin < boric acid against both the fungal strains. Natural plant extractives or persimmon tannin had no suppression effect. The suitable application of above preservatives may afford a long service life of Ekki wood in Japan.
Kew words: Durability, Ekki (*Lophira alata*), *Loweporous tephroporus*, Wood preservatives, Antifungal activity, Screening

1. INTRODUCTION

Ekki wood (*Lophira alata* Banks) is a western African species with high density (*d* 1.07) and large mechanical properties which are modulus of elasticity (17,000 MPa) and compression strength parallel to grain (87 MPa)[1),2)]. The most unique character is the naturally high durability. It is classified "durable species" in an EN standard with the service life of 20-30 years of heartwood[3)]. From these reasons, ekki wood is widely used without preservative treatment for outdoor construction or marine use in Europe. However, many decay damages were reported when it is used in Japan. The most surprised accident was the fall down of a decayed ekki bride from decay damage in Ehime prefecture[4),5)].

The differences in climate conditions and the decay fungi are assumed to affect the durability of ekki wood. The performance on the durability by a fungus cellar test agreed with the European evaluation[6)]. We must consider the reason of decay of ekki wood in Japan, especially the specific fungi responsible for it[7)].

This paper deals with the characteristics of two fungal strains isolated from severely decayed ekki bridges in Tokyo and Kyushu as well as the minimum inhibitory concentrations of some preservatives and wood extractives against their hyphal growth.

2.EXPERIMENTAL

2.1 Isolation of decay fungi by a continuous-inoculation technique

The decay fungi were isolated from two ekki bridges located at Tokyo (built in 1990 and inspected in 2002) and Kyushu (built in 1995 and inspected in 2000) respectively.

Decayed wood specimens were obtained from the heavily wounded and soften portion of decayed ekki bridges by a chisel sterilized with ethanol[8)]. Decay fungi

were purified from the decayed wood specimens by a continuous-inoculation technique using the potato dextrose agar (PDA; 25 ml of 0.4 % potato extract, 2% glucose, 1.5% agar) medium in a Petri dish (8 cm in diameter). Three pieces of decayed wood specimen were cut to the suitable size and placed on the PDA medium. After incubation a week at 25℃, the small edge mycelium from the wood specimens was taken off and transferred into the PDA medium. This procedure was repeated several times until the decay fungi seemed to be completely purified without mold or bacteria.

2.2 Identification and the paring test

The isolated fungi were identified by microscopic observation of their hyphae, by determination of optimal growth temperatures, and by morphological observation of fruit bodies collected from the decay bridges. The paring test was done by using a dual culture technique on a PDA plate. Each inoculum was transferred from the active growing margin of the mycelium. The inoculated plate was incubated at 25℃, then judged whether the growth mycelium were harmonized or not at 25℃.

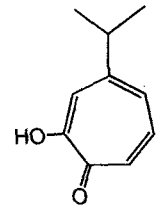
2.3 Fungal growth test at different temperatures

The inoculum of each test fungus was incubated in a PDA plate. When the fungal mycelium fully grew on the plate, filter discs (Ø=8 mm) were put onto the mycelial mat. After the filter discs were covered with the mycelium, they were taken out and put onto the center of a new PDA plate. Fungal growth was determined at different temperatures, 17℃, 20℃, 25℃, 31℃, 35℃, and 41℃ according to the method of Suzuki *et al.* [9] The diameter of fungal growth was measured by a slide caliper every two or three days, and the mean values of the perpendicular lines were calculated. Three replications were carried out.

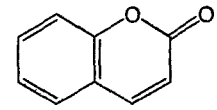
2.4 Antifungal activities of wood preservatives and natural products against two isolates

An alkyl ammonium compound (AAC, didecyl dodecyl ammonium chloride), copper naphthenate (NCu, copper salt of naphthenic acid $C_nH_{2n-1}COOH$), zinc naphthenate (NZn) and boric acid were used as test chemicals. $CuSO_4$ and $ZnCl_2$ are also tested as references. In addition to these, several antifungal natural components[10)-13)], hinokitiol (β-thujaplicin), coumarine, persimmon tannin, and a vegetation extract (a commercial essential oil) were tested.

Antifungal activities of preservatives were determined as the minimum inhibitory concentration (MIC). For obtaining MIC, the fungal growth test was carried out on the PDA medium containing each preservative and natural product (hereafter, compound) with different concentration at 25℃ for a week. The medium for this test was prepared by mixing the each compound in 500μl of water (for AAC, boric acid, $CuSO_4$, $ZnCl_2$, persimmon tannin, a vegetation extract) or acetone (for NCu, NZn, hinokitiol, coumarin).



Hinokitiol
(β-thujaplicin)



Coumarin

3. RESULT

3.1 Identification of the isolates and the relationship between their hyphal growth and incubation temperatures

We could successfully isolated two strains from the Tokyo and Kyushu bridges by the continuous inoculation technique. These isolates were named as Tokyo-S and Kyushu-S respectively. These were identified as *Loweporus tephroporus* (Mont.) Ryv. from the existence of clamp connection of their hyphae by microscopic observation and elaborate observation of fruit bodies collected from the decay bridges. Tokyo-S and Kyushu-S were found to be the different strains by the paring test.

Fig.1 and 2 showed the relationships between hyphal growths and incubation temperatures of Tokyo-S and Kyushu-S, respectively. In addition, *Trametes versicolor* FFPRI 1030, a white rot fungus designated in Japanese industrial standard[14)] was used as reference (Fig.3). The fastest growth of two isolates were shown at around 35℃ and still fairly fast at 41℃ while the growth of *T. versicolor* was fastest from 25 to 31℃ and completely suppressed at 41 ℃. The high optimal growth

temperatures are accorded with those for *L. tephroporus*[15] reported by Doi *et al.*

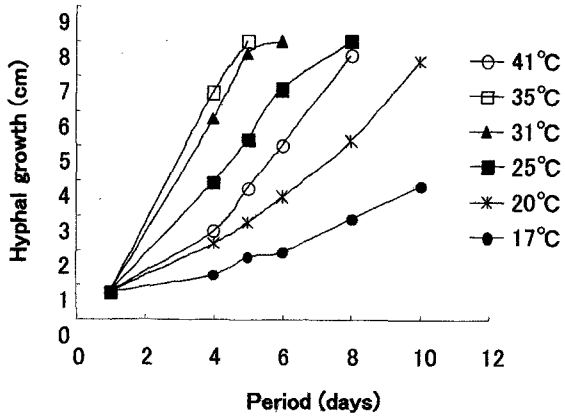


Fig.1 The hyphal growth of Tokyo-S at different temperatures

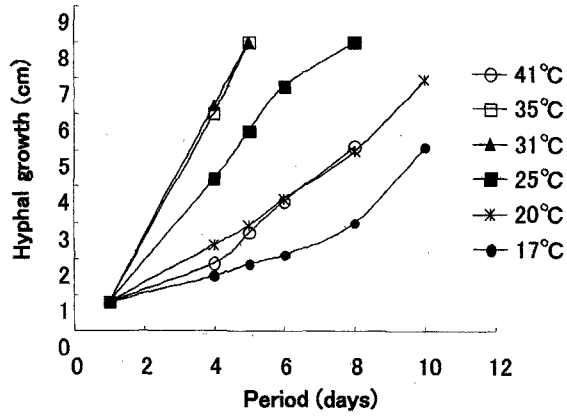


Fig.2 The hyphal growth of Kyushu-S at different temperatures

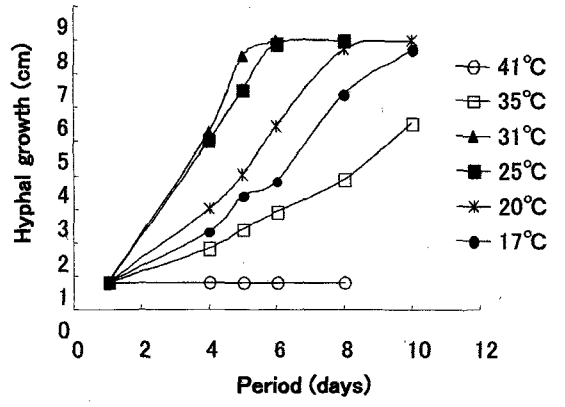


Fig.3 The hyphal growth of *T. versicolor* at different temperatures

3.3 Screening of the compounds against decay fungi

MICs of each compound against the two isolates and *T. versicolor* were shown in Table 1. The same results were obtained by the 2 strains and *T. versicolor* except AAC. The MIC increased as follows; AAC < hinokitiol < NCu, NZn, $CuSO_4$, $ZnCl_2$, coumarin < boric acid. AAC showed the largest effect as the concentration below 20 μg/ml. Boric acids showed moderate effect. Copper and zinc compounds were found to be fairly effective. The vegetation extract and persimmon tannin had no effect until 20,000 μg/ml.

Among the natural compounds, hinokitiol and coumarin were effective. Hinokitiol showed the fairly small MCIs between 20 - 50 μg/ml for both mushrooms. The acetone itself, which was used for preparing the medium, didn't inhibit the fungal growth.

TABLE 1. Antifungal activity of preservatives*

Compound	Tokyo-S	Kyushu-S	*T.versicolor*
AAC	< 20	< 20	500~2000
NCu	500~2000	500~2000	500~2000
NZn	500~2000	500~2000	500~2000
Boric acid	2000 ~20000	2000 ~20000	2000 ~20000
$CuSO_4$	500~2000	500~2000	500~2000
$ZnCl_2$	500~2000	500~2000	500~2000
Hinokitiol	20~50	20~50	20~50
Coumarin	500~2000	500~2000	500~2000
Persimmon tannin	> 20000	> 20000	> 20000
A vegetation extract	> 20000	> 20000	> 20000

*MIC (μg/ml) = minimum inhibition concentration

4. DISCUSSION

Itoh *et al.* reported that the white rot fungus, especially *L. tephroporus* was dominantly found in the several decayed ekki bridges located various area in Japan[7]. *L. tephroporus* is known as a fairly common fungus in warm temperate area of Japan, and cause the white rot of hardwoods. However, this species has not been found in European country[16),17]. Considerately, *L. tephroporus* seems to be a specific fungus that has comparatively larger decay potential to ekki wood and responsible for the short service life of the bridges.

White rot fungus generally degrades cellulose and lignin equally and brings the crucial decrease of mechanical strength of wood. In our inspection of the bridges, the fruit bodies of the *L. tephroporus* were frequently found around the connections, behind the deck boards and trusses. A distinctive feature of this damage was "internal" decay without hyphal extention and fruit body formation on wood surface.

For the prevention of infection and hyphal extension of *L. tephroporus*, wide variety of chemicals could be available as wood preservatives[18),19)] . AAC, NCu, NZn and presumably, other preservatives including copper or zinc found to be effective from the results of the MICs obtained here. $CuSO_4$ and $ZnCl_2$, however, are not currently used as wood preservatives because their leachability. The former three preservatives are widely used for pressure treatment in the place of chromium-copper-arsenate (CCA) preservative. Among these, AAC is the best candidate compound to prevent decay of ekki wood from this fungal species. This chemical could be applicable for the temporally killing or preventing the fungi by partial injection or spraying on the surface of decayed wood.

Among the natural compounds, hinokitiol was fairly effective, which is an extractive compound in heartwood of Japanese natural durable species, *Hiba* (*Tbujopsis dolabrata* var. hondae Makino)[11,12,13)]. Thus, the durability of ekki wood may be increased by hinokitiol which is responsible for natural durability. Unfortunately, no antifungal activity was shown in pershimon tannin though it is popular for many other activities such as astringency[20)], enzyme inhibition[21),22)] and antimicrobial activities[23)] .

Considerately, it will be possible to achieve long service life of the construction made by Ekki wood in Japan with the suitable applications of the above preservatives or natural compounds in corporation with the maintenances. Further works will be done to clarify the effectiveness of these compounds when they are actually used for individual constructions in outside.

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5. REFERENCES

[1] "Wood Handbook", Ed. by USDA Forest Service, Madison (1999) pp.4-16~4-17.

[2] "Agriculture Handbook Number 607", Ed. by USDA Forest Service, Madison (1984) pp.427-434.

[3] British Standard, BS EN 350-2: 1994 (Durability of wood and wood-based products, Natural durability of wood).

[4] S. Doi *et al.*, *IRG/WP 00-10383* (2000).

[5] K. Suzuki *et al.*, *Mokuzai Kougyo* (*Wood Industry*), **55**, 78-81 (2000).

[6] K. Yamamoto *et al.*, *IRG/WP 02-20249* (2002).

[7] R. Itoh *et al.*, *Mokuzai Kougyo* (*Wood Industry*), **57**, 343-346 (2002).

[8] T. Hattori, *Nippon Kingakukai kaiho*, **41**, 197-203 (2000).

[9] K. Suzuki and K. Okada, *IRG/WP 94-10065* (1994).

[10] A. M. Taylor *et al.*, *Wood and Fiber Science*, **34**, 587-611 (2002).

[11] D. Okamura *et al.*, *Mokuzai Hozon* (*Wood Preservation*), **28**, 224-235 (2002).

[12] For example, "Jumokuno-Kao" Ed. by F. Nakatsubo, Kaiseisha press, Otsu (2002) p117.

[13] K. Fujimori *et al.*, *J. Essent. Oil Res.*, **10**, 711-712 (1998).

[14] Japanese Industrial Standard, JIS K 1571: 1998 (Qualitative standards and testing methods of wood preservative).

[15] S. Doi *et al.*, *IRG/WP 02-10444* (2002).

[16] T. Hattori , personal communication.

[17] European polypores Part 1 & 2, Ed. by L. Ryvarden & R. L. Gilbertson, Fungiflora A/S, Oslo (1993/4).

[18] H. M. Barnes, *IRG/WP 93-30018* (1992).

[19] "Wood Preservation" Ed. by Barriy A. Richardson, E & FN SPON, London (1993) pp.97-151.

[20] J. L. Goldstein and T. Swain, *Phytochemistry*, **2**, 371-383 (1963).

[21] P. P. Feeny, *ibid.*, **8**, 2119-2126 (1969).

[22] T. Ozawa *et al.*, *ibid.*, **26**, 2937-2942 (1987).

[23] J.A.Lewis and R.L.Starkey, *Soil Sci.*, **107**, 235-241 (1969).

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