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Yoshinori Kobayashi · Ikuho Iida · Yuji Imamura
Ugai Watanabe

Drying and anatomical characteristics of sugi wood attacked by bacteria during pond storage

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Abstract Seven species of bacteria were isolated and identified from ponded sugi (*Cryptomeria japonica* D. Don) logs, and six species showed potent wood-degrading activities. To evaluate the effects of these isolated bacteria on the drying and anatomical characteristics of wood, small fresh blocks of sugi were immersed in water suspensions containing bacteria for 1–7 months. The permeability and drying properties were evidently improved. Most of the encrusting substances adhering to the cell lumens and the pit chambers were removed, and the pit membranes were destroyed. These anatomical changes due to bacterial activity were assumed to improve the permeability of sugi wood.

Key words Bacterial degradation · Log pond-storage · Water movement · Drying property

Introduction

Sugi (*Cryptomeria japonica* D. Don) is one of the major species in plantation forests in Japan. It is also one of the wood species refractory to drying[1] and pressure impregnation.[2] Though many attempts[3–11] have been made to improve the water movement using biological, physical, and chemical methods, practical and useful technologies have not been fully established because of high costs, long processing time, uneven effects, and the need for special treatments or expensive equipment.

Utilization of fungi,[3,12] bacteria,[4,13–15] and enzymes[5,16,17] to improve drying and pressure impregnation have been discussed from the viewpoint of biological pre-treatment. Biological treatment is assumed to be an economical and ecological method that does not require special equipment. Among the treatments, bacteria have been expected to improve water movement, and scanning electron microscopy (SEM) observations revealed that bacteria accumulated on wood long stored in pond water covers and destroys the pit membranes.[18,19]

In this paper the effects of bacterial activity on woods stored in log ponds for a long period are discussed, seeking the possibility of improving wood drying. Micro-organisms were collected from logs stored in log ponds for a long period, and the species involved in the perforation of pit membranes and the removal of substances that occlude pit apertures and cell cavities were isolated and identified. To accelerate bacterial activity in wood, the bacterial suspension was introduced into the fresh wood blocks. The effects on the permeability of wood blocks is discussed along with their anatomical characteristics.

Materials and methods

Identification and cultivation of bacteria

Sugi logs 30 cm in diameter and 3 m in length were immersed for 2 years in a log pond located in Nara at a mean temperature of 14.5°C. Small blocks of 10 (T) × 10 (R) × 10 (L) mm cut from the heartwood of the log were put on nutrient agar medium (Nissui seiyaku Co.) at 30°C to isolate the inhabiting bacteria. After the development of bacterial colonies, the agar medium was placed in sterilized water to obtain a suspension of bacteria. The suspension

Y. Kobayashi (✉)
Institute of Wood Technology, Akita Prefectural College of Agriculture, Noshiro 016-0876, Japan
Tel. +81-185-52-6985; Fax +81-185-52-6976
e-mail: koba@iwt.apca.ac.jp

I. Iida
Department of Agriculture, Kyoto Prefectural University, Kyoto 606-0823, Japan

Y. Imamura · U. Watanabe
Wood Research Institute, Kyoto University, Uji 611-0011, Japan

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was placed on another fresh agar medium, and streak cultures were repeated two times to purify the colony. The colonies were divided into seven groups depending on the features of the configurations. Bacteria were identified by the oxidation-fermentation (OF) test, Gram stain, and other enzymatic activity tests for each isolate growing on agar medium at 20°C. Each isolate was kept alive on small glass plates at 20°C.

Bacterial treatment of wood specimens

Three methods were employed for the bacterial treatment: (1) natural ponding of logs; (2) immersion of wood blocks in pond water from the log pond; and (3) immersion of wood blocks in bacterial suspensions from isolates. The natural ponding was applied to green sugi logs 30 cm in diameter and 3 m in length for 3 years in Nara. The immersions in pond water and bacterial suspensions were conducted using small wood blocks with the dimensions of 10 (T) × 10 (R) × 50 (L) mm. These wood blocks were cut from red and black heartwoods of fresh sugi logs and then sterilized with ethylene oxide gas for 2 days after seasoning. The small specimens were prepared from sapwood and heartwood from the center of the length of the log and were subjected to a test of drying properties.

Before immersion of the wood blocks, they were autoclaved for 15 min at 121°C and then dipped in 500 cc of distilled water. Bacterial suspensions of 100 cc, each containing different species of bacteria, were mixed into the sterilized water. The wood blocks were then stored at 20°–21°C for periods ranging from 1 to 7 months.

SEM observations

The anatomical changes in the sugi wood attacked by mixed or single species of bacteria were observed by SEM (Hitachi S450). Small specimens of 10 (T) × 10 (R) × 5 (L) mm were cut from three kinds of sugi wood treated by bacteria for the SEM observations. (1) One set of specimens was prepared from logs stored in the pond for 2 and 3 years. Other sets were cut from small specimens immersed in (2) pond water or (3) separate suspensions of seven kinds of bacteria at 20°–21°C. The specimens were dried by the solvent exchange method using an alcoholic series, acetone, and pentane at room conditions before the SEM observations.

Test method for drying properties

For the drying tests, two sets of specimens of 10 (T) × 10 (R) × 50 (L) mm were prepared in the same way as for SEM observations from (1) the 2- and 3-year ponded logs and (2) the wood blocks treated by solutions of the various species of bacteria. The samples were left under conditions of 60°C drying temperature and 65% relative humidity until an equilibrium moisture content of 10% was attained.

Results and discussion

Identification and characterization of bacteria isolated from ponded logs

Seven configurations of bacterial colonies were found in logs stored in the log pond. They were distinguished and identified as *Pseudomonas* sp. (bacillus-type, A, B, C, D1, D2, D3) and *Staphylococcus* sp. (coccus-type, A1) as shown in Table 1.

When observed by SEM, a large number of bacteria were detected in the lumens of tracheids and ray parenchyma cells of the logs stored in the pond for 2 and 3 years and immersed in the bacterial suspensions. Figure 1 shows bacillus-type bacteria (D1) accumulating around the bordered pit aperture in the tracheid of heartwood stored in the suspensions of the different species of bacteria. The average values of their lengths and diameters were 1–2 μm and 0.3–0.5 μm, respectively. Figure 2 shows the coccus-type bacteria (A1) adhering to the cross-field pit membrane in a tracheid of heartwood stored in a suspension of the various species of bacteria. The coccus-type bacteria had an average diameter of about 0.5–1.0 μm, which was the same dimension as the coccus-type bacteria previously reported for loblolly pine.[20] Incidentally, the sizes of the pit aperture and the pore of margo of the bordered pit membrane in sugi wood were described to be 2–6 μm and 0.01–1.0 μm, respectively.[21] Therefore the bacteria detected in this study are assumed to be able to move freely through the large pores of the pit apertures and pass through the interstices of the unaspirated pit membranes of sapwood.

When the wood blocks of 10 (T) × 10 (R) × 50 (L) mm were immersed in the water suspensions containing

Table 1. Species of bacteria isolated from ponded logs

Test	Bacteria isolated			
	A,B,C	D1,D3	D2	A1
Gram stain	N	N	N	P
OF test	O	–	–	F
Motility test	+	ND	ND	–
Growth in anaerobic condition	–	–	–	+
Growth in aerobic condition	ND	ND	ND	+
Growth on MacConkey's agar	+	+	+	ND
Oxidase test	++	+	–	–
Citrate test	+	+	+	ND
Maltose test	–	–	–	ND
Xylose test	+	–	–	ND
Nitrate reduction test	–	+	+	ND
DNase test	–	–	–	ND
Amylacidase test	–	+	+	ND
Alginine test	+	+	+	ND
Endospore formation	ND	ND	ND	–
Acid-fast stain test	ND	ND	ND	–
Catalase test	ND	ND	ND	+
VP test	ND	ND	ND	+

ND, not determined; N, negative; P, positive; OF, oxidation-fermentation.
A1, *Staphylococcus* sp. (coccus); A, B, C, D1, D2, D3, *Pseudomonas* sp. (bacillus); D2, possibly *Achromobacter*

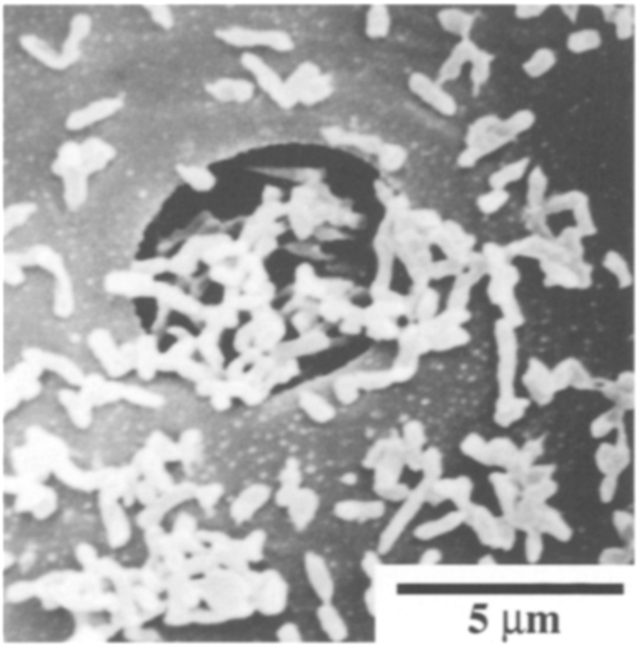

Fig. 1. Bacillus-type bacteria (D1) accumulating around the bordered pit aperture in a tracheid of sugi heartwood stored in a suspension of different species of bacteria

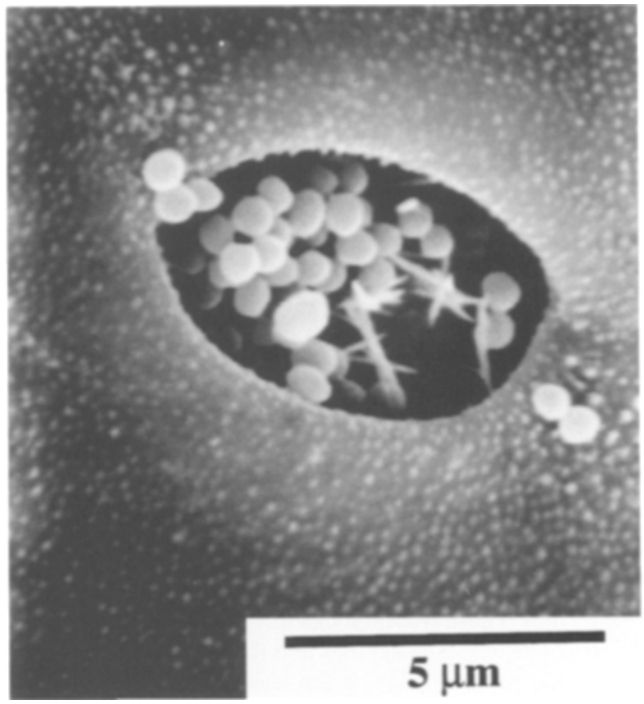

Fig. 2. Coccus-type bacteria (A1) adhering to the cross-field pit membrane of sugi heartwood stored in a suspension of the various species of bacteria

the various species of the identified bacteria, occluding substances in the tracheids and ray parenchyma cells disappeared within 7 months due to the bacterial activity.

An interesting observation was that the bacillus-type bacteria tended to concentrate in the tracheids and the coccus-type bacteria in the ray parenchyma cells. It is possible that bacteria move with the flow of the solvent introduced by the drying procedure, but the same features were detected in specimens prepared with and without the solvent exchange method. This finding indicates that the anatomical changes of ponded wood depend on the species of the colonized bacteria, possibly because each species can attack the specific extractives or portions of the wood. Johnson and Gjovik reported that the half-bordered pit membranes and the ray parenchyma cells of loblolly pine were completely destroyed by coccus-type bacteria.[20]

Effect of bacterial activities on drying properties and anatomical characteristics of wood

The opening of the pits of tracheids or ray cells by bacteria should contribute to the improvement of wood drying especially at the stage of free water movement. Figure 3 shows the drying curves of the sapwood and the heartwood treated by ponding. Sugi wood is one of the species refractory to drying because of its heavy encrustation and the existence of extractives around the pit membranes and cell lumens. From Fig. 3, it was assumed that the longer the ponding, the faster the drying speed of heartwood compared to that of sapwood, in which the drying was easy and fast originally. The accelerated drying speed might depend on improved permeability due to storing in water.[22]

In sugi wood the microfibrillar structure of the margo in the bordered pit membrane is so densely and heavily encrusted with extractives, especially around heartwood, that materials such as preservatives can hardly be impregnated into practical dimension lumbers when using the conventional pressure method. Figure 4 shows the untreated bordered pit membrane with no visible openings due to the encrustation with heartwood extractives. These encrustations were found to be more intense in black heartwood than red heartwood. Therefore, to improve the water movement in sugi heartwood, decomposition of these encrusting substances or destruction of the pit membranes (or both) are necessary.

After immersing the small wood blocks in the bacterial suspension for 1 month or the practical-size logs for more than 2 years, bacteria were observed to penetrate the inner part of the wood, suggesting that they could move through the tracheids, pit chambers, and ray parenchyma cells. Figure 5 shows the bacillus-type bacteria adhering to the bordered pit membranes of the small sugi heartwood specimens stored in the log pond for 2 years. The microfibrillar spaces in margo were enlarged; and the extractive substances, which should encrust the membrane before treatment, disappeared completely. In the ray parenchyma cells, only accumulation of the coccus-type bacteria was detected, and the cell contents were assumed to be decomposed.

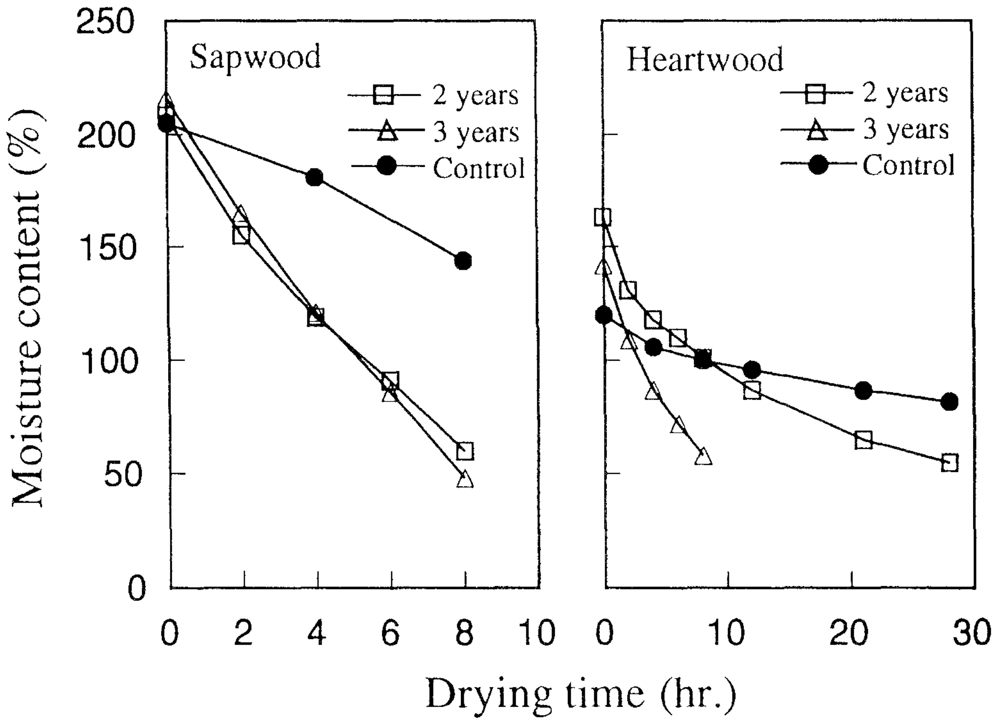

Fig. 3. Drying curves of sugi sapwood and heartwood stored in the log pond for 2 and 3 years

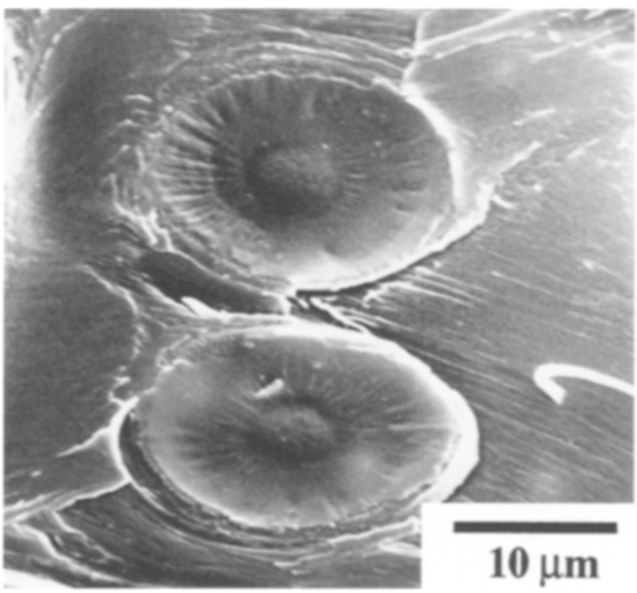

Fig. 4. Heavy encrustation of the pit membranes in the heartwood of untreated sugi wood

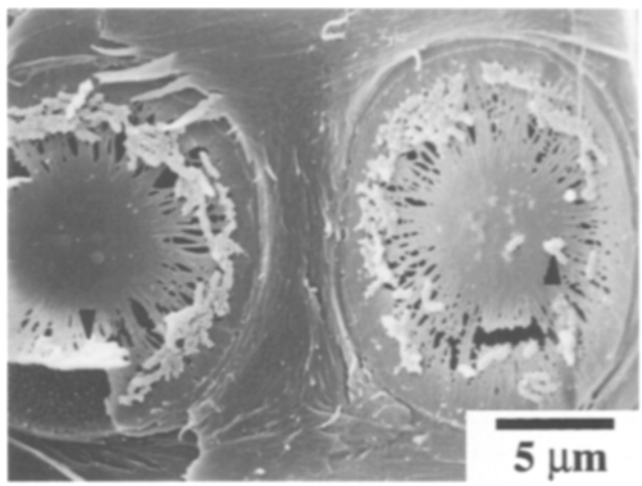

Fig. 5. Broken pit membranes and bacillus-type bacteria (*arrowhead*) adhering to the membranes of sugi heartwood stored in the log pond for 2 years

Effect of isolated bacteria on improvement of drying properties

The bacteria isolated from the stored wood showed uneven effectiveness on wood drying. Figure 6 shows the drying curves of the specimens treated by the isolated seven species of the bacteria for the same period. Hardly any differences in effectiveness were detected between coccus- and bacillus-type bacteria and among the species employed, although one species (coded C) showed small acceleration effects on drying as described above in the SEM observations.

Figure 7 shows the improved drying rate of heartwood treated by bacteria coded D1. In the stage of free-water movement, the drying speed of the treated specimen was extensively: increased up to twofold that of the untreated specimen. An improvement in water movement during drying was observed, especially in black heartwood, which contained heavy encrustations in the cell lumens and pit chambers usually resulting in hard, slow drying.

After the small black heartwood blocks were immersed in the water suspensions containing various species of bacteria, destruction of the pit membranes and decomposition of the encrustation were observed with six of the bacterial species, as was seen in the ponded logs. The exception was the bacteria coded C (Table 1).

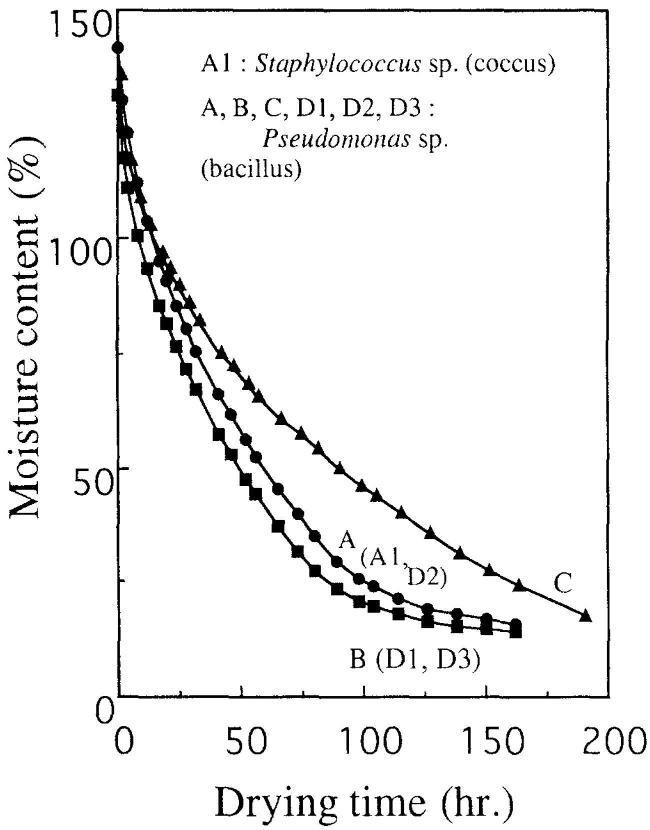

Fig. 6. Drying curves of black heartwood separately treated by seven species of bacteria.
Note that the curves of A1 and D2 showed tendencies similar to those of A, and the curves of D1 and D3 were similar to that of B

Conclusions

The effects of bacterial attacks on the permeability and drying characteristics of sugi wood were confirmed by subjecting the wood to natural ponding and to an artificial treatment using water suspensions containing bacteria. The longer the ponding, the faster was the drying rate. However, the acceleration effects on the drying rate differed depending on the species of bacteria. Seven bacterial species were isolated from the ponded logs, six of which had a powerful influence on improving the permeability and drying characteristics of sugi wood. The bacteria were observed by SEM around the pit apertures and on the pit membranes in tracheids and ray parenchyma cells. Most of the encrusting substances adhering to the cell lumens and the pit chambers were removed, and the pit membranes were destroyed after immersion in water suspensions containing the bacteria. The improved drying characteristics were observed not only in the red heartwood but also in the black heartwood of sugi wood.

It is thus confirmed that bacterial treatment improves the drying characteristics of sugi wood. Therefore it should be studied in regard to practical applications. It is also important to improve the efficiency of the treatment.

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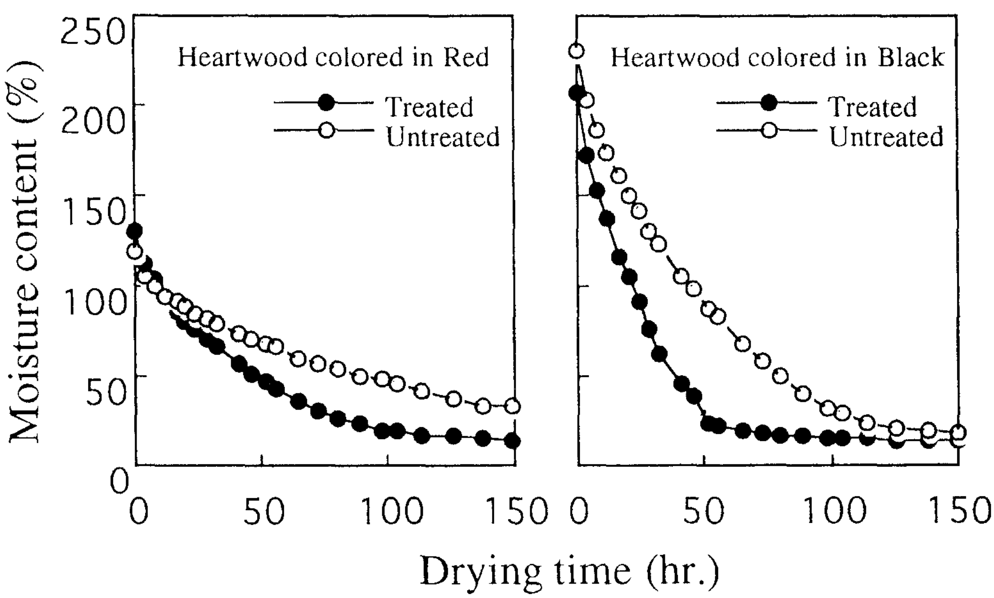
Fig. 7. Improved drying rate for two types of heartwood treated by bacteria (D1)



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