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Improvement of penetrability of sugi wood by impregnation of bacteria using sap-flow method

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Abstract The sap flow method of wood impregnation was conducted to aid the movement of bacteria through the living tree, thereby accelerating their distribution through wood within a short time. When log-pond water containing mixed species of bacteria were introduced in the living trees by butt-end dipping and then laid horizontally for 6 months, bacteria could be delivered by sap flow vertically through the sapwood tracheids up to the high portions from the butt-end of trees; they could be detected in the ray parenchymal cells. The sap-flow method was assumed to deliver the bacteria to sapwood and heartwood at high levels of standing sugi (*Cryptomeria japonica* D. Don) trees. Degradation of the pit membranes was observed even at more than 3 m upward from the butt-end after the treatment in sapwood, as well as around the butt-end of the trees. The uptake of the aqueous dye solutions in sapwood of the treated logs were about eight times more than those of control specimens after 8 h.

Key words Sugi (*Cryptomeria japonica*) · Sap-flow method · Bacterial degradation · Penetrability

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Introduction

Sugi (*Cryptomeria japonica* D. Don) wood is difficult to dry, as it is impervious in the intermediate zone and the heartwood[1] and has high moisture in both sapwood and heartwood in the green condition.[2] Drying sugi wood of full size is required, as the wood is used in large sizes for construction because of its low strength.

Ponding, a traditional predrying treatment to accelerate water movement, has been applied to impervious wood species in large logs.[3] With this treatment the bacterial attacks were expected to degrade and perforate the wood cells and improve the water movement in wood. In a previous study[4] we determined that some species of bacteria could effectively improve water movement owing to their ability to perforate pit membranes and decompose the extractive substances on pit chambers and in ray parenchymal cells.

Bacteria cannot move for a long distance by themselves, so water flow is needed for their penetration and distribution within the wood. As the result, it generally takes a long time for the bacteria to be delivered from cell to cell, or from sapwood to heartwood, so they can attack the pit membranes. Ponding is needed long term such that it cannot play an important role as a useful processing method for pretreatment of drying. From these results it seems to be important to accelerate the distribution of bacteria into wood.

On the other hand, the sap-flow method has been attempted as an effective, high-speed staining method in standing trees.[5] When using the sap flow in living trees, the bacteria are assumed to be easily transported for a long distance in the wood within a short time. The method employing bacteria for standing trees is considered to be the most rational and energy-saving way to enhance water movement in wood if the problem of a long treatment period is to be overcome.

In this paper, the sap-flow method was used to increase the rapid distribution of bacteria, shorten the time needed for ponding, and accelerate perforation of the pit mem-

branes. As long-term treatment by bacteria would cause degradation of the wood structure and a reduction in wood strength,[6] it is important to deliver bacteria rapidly and to shorten the term of the treatment. From this point of view, we attempted to apply the sap-flow method as an effective way to transport bacteria into the living tree.

Materials and methods

Sap-flow method for bacterial impregnation

Impregnation of bacteria with the help of the sap-flow method was conducted in six standing sugi trees with a diameter of about 20 cm at breast height. The standing trees were cut at the butt-end to install a water container in a standing position without cutting the branches and leaves, and 20 l of log-pond water containing the mixed species of microorganisms was introduced into each tree through their butt-end in June 1994. The water was collected from the depth 1.5 m in a log pond immediately before the operation. To confirm the flow of the pond water, a 1% solution of patent blue was introduced into another three standing trees by the same method. The dye solution reached the crown by sap flow within 3–7 days. The inoculated trees were then laid horizontally for 6 months (until December 1994).

Preparation for scanning electron microscopy

To evaluate anatomical characteristics, small fresh blocks of 10 (T) × 10 (R)× 5 (L) mm were cut from the sapwood, intermediate wood, and heartwood at 0, 1, 2, 3, 4, 5, and 6 m away from the butt-end of the treated logs. These blocks were then prepared for scanning electron microscopy (SEM) (Hitachi S450). The specimens were dried by the solvent exchange drying method using an alcohol series, acetone, and pentane at room conditions before SEM observations.

Test method of penetrability

The small specimens, with dimension of 20 (T) × 20 (R) × 180 (L) mm, were prepared from sapwood, intermediate wood, and heartwood at 0, 1, 2, 3, 4, 5, and 6 m away from the butt-end of the untreated (control specimens) and treated logs for the measurement of water penetrability after drying. The specimens from sapwood, heartwood, and intermediate wood were divided on the basis of their color. Some specimens from intermediate wood contained portions of sapwood or heartwood, or both. Before the tests, the side surfaces of the wood blocks were coated with silicon grease to prevent the aqueous dye solutions from penetrating along the grain on the side surfaces. The butt end of the wood blocks cut along the edge of the grain were immersed in aqueous dye solutions (0.5% acid fuchsin) for 24 h at room temperature, and then the amounts of solution uptake were measured over time to evaluate water movement. Each of two standing trees treated or untreated by the pond water were subjected to the penetrability test.

Results and discussion

Distribution of bacteria by sap-flow method

Changes in the anatomical characteristics shown in the previous report[4] suggested that the ponding treatment by the bacteria could improve water movement even in sugi wood. It takes a long time for the bacteria to penetrate equally into the sugi wood by just ponding, but if the bacteria were placed in the liquid flow in wood, it was believed they could move anywhere with the liquid.

The SEM observations revealed the existence of the bacillus-type bacteria around the bordered pit apertures and the coccus-type bacteria around the cross-field pits even in sugi heartwood tracheids of logs stored in the pond.[4] During this study using the sap-flow method, the existence of both bacillus- and coccus-type bacteria were detected in sapwood when the sap-flow method was employed in the living trees and those stored for 6 months. It is believed that when bacteria are introduced into wood with the aid of the sap-flow method, they pass through xylem cells vertically together with sap movement and then move transversely from sapwood to heartwood through ray parenchymal cells, which contain nutritious substances for microorganisms.[7] However, it was difficult to detect bacteria in the tracheids of heartwood, possibly due to the insufficient environmental conditions for the growth of microorganisms, such as the low water content. To supply water to the trees for maintenance of heartwood under adequate moisture conditions for growth of the microorganisms, modified sap flow such as the injection method[8] should be applied.

Effect of bacterial impregnation by sap-flow method

It was confirmed that the bacteria were carried up and delivered to the tracheids and the ray parenchymal cells in sapwood that contained nutrients for microorganisms[7] in a living tree by sap flow. In the previous report[4] the extractives in tracheids or ray parenchymal cells mostly disappeared after the bacterial treatment. A combination of coccus- and bacillus-type bacteria might attack the extractives in the wood cells and the microfibrils of the margo in sapwood that the bacteria could reach.

When the sap-flow method was employed on sugi trees, the SEM observations revealed destroyed bordered-pit membranes, which might have been destroyed by the attack of bacteria in sapwood even 3 m away from the butt-end (Fig. 1). They also revealed the existence of the coccus-type bacteria in the ray parenchymal cells (Fig. 2), suggesting that each species of bacteria has its own preferences for targets of attack.

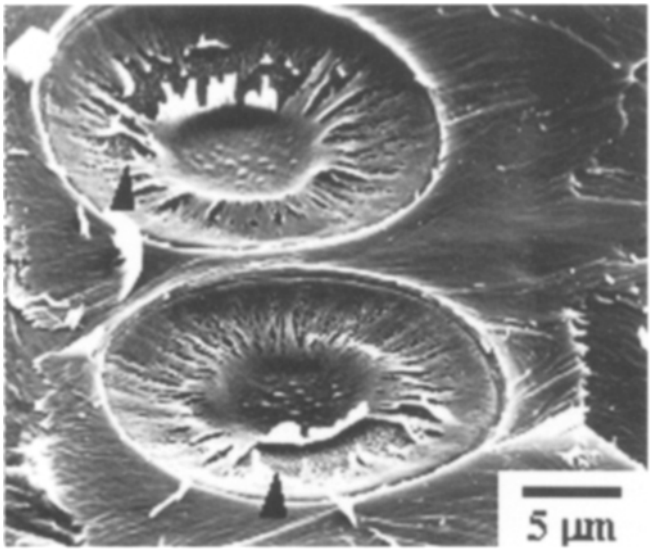

Fig. 1. Bordered-pit membranes (*arrowheads*) of sapwood 3 m above the butt of the sugi log destroyed by bacteria introduced by the sap-flow method

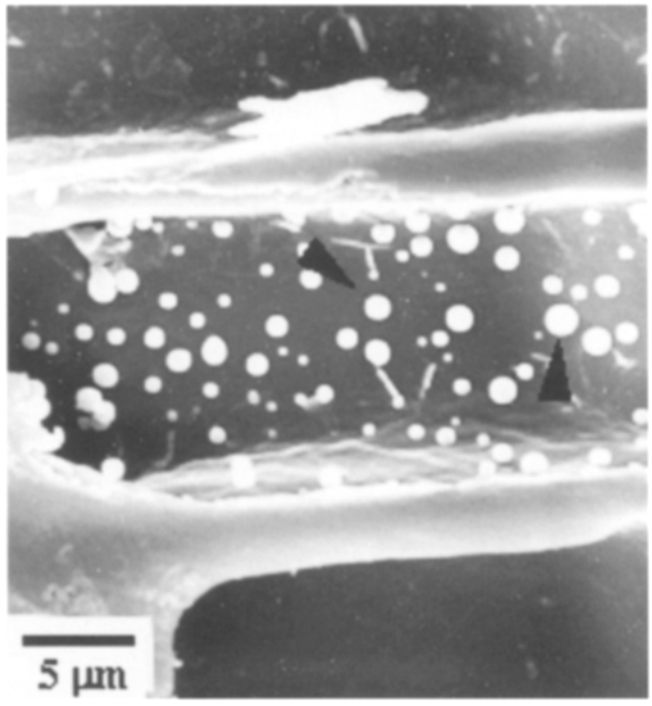

Fig. 2. Ray parenchymal cell containing the coccus-type bacteria (*arrowheads*) introduced by the sap-flow method in sugi sapwood

Improvement of water movement by bacteria

The bacteria from the log pond could decompose the occluding substances in wood cells and destroy the pit membranes,[4,9] resulting in improvement of water movement. In fact, it was confirmed in the previous examination of the penetrability of the ponded and unponded specimens that the ponded specimens evenly absorbed a large amount of the solutions at the sapwood and heartwood as well as around the intermediate zone, where liquid movement is usually markedly obstructed. On the other hand, the untreated specimens could take up only a small amount of the aqueous dye solutions in sapwood except the latewood zone.[10]

In the case of the specimen treated by bacteria using the sap-flow method, the remarkable increase in uptake of the aqueous dye solutions was recognized in sapwood, intermediate wood, and heartwood, as shown in Fig. 3, in which representative data are shown. Only 1.2 g/cm^2 of the uptake of the aqueous dye solutions of sapwood was obtained 1 m above the butt-end for the control specimens, which were prepared from the tree laid down for 6 months without introduction of pond water. In contrast, the amount increased by approximately eightfold (10.2 g/cm^2) in the treated specimens 480 min after initiation of the test. The other specimen showed the same tendency but with lower values. Though the values of the water uptake decreased with increasing height along the trunk, the effect of the introduced bacteria was detected even 5 m above the butt. For the intermediate wood and heartwood, little significant effect has been detected even with the use of the sap-flow method. The bacteria were assumed to be transported up to the high portion of the living tree through sapwood tracheids, but a long time would be required for them to move

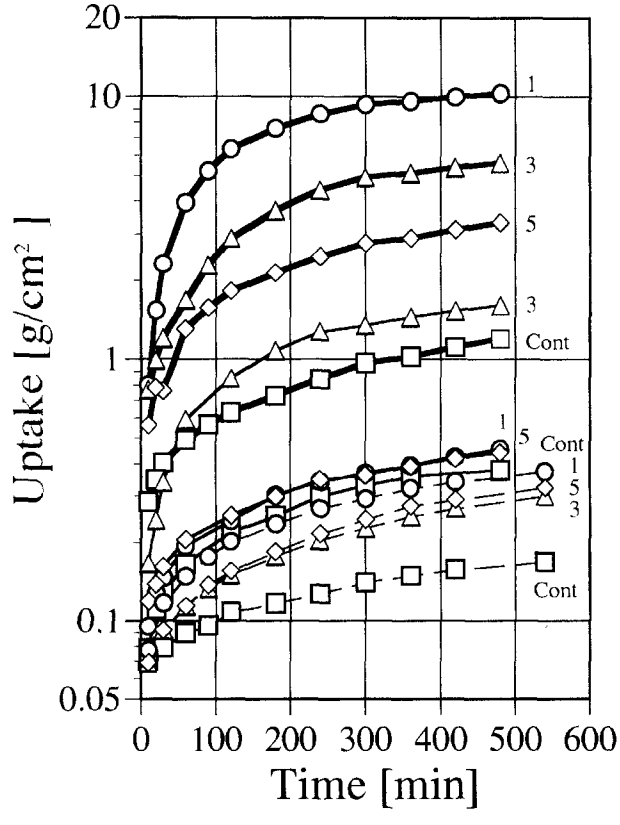

Fig. 3. Uptake of aqueous dye solutions by sugi wood treated with bacteria introduced by the sap-flow method. Numbers in the figure show the height (meters) from the butt. *Cont*, Control specimen; *heavy lines*, sapwood; *light lines*, intermediate wood; *broken lines*, heartwood

from sapwood to heartwood, possibly through ray parenchymal cells.

Conclusion

Resulting from inoculation of pond water containing mixed microorganisms into stand trees by the sap-flow method, destroyed bordered-pit membranes were observed by SEM in the tracheids in sapwood even 3m above the butt-end, which might have been destroyed by bacterial attack. The existence of bacteria in the ray parenchymal cells in sapwood was also observed. Uptake of aqueous dye solution by sapwood in the treated logs increased about eightfold more than that of the untreated logs after 8h.

Considering the anatomical changes caused by the bacteria (i.e., severe destruction of the pit membranes and decomposition of the cell substances, resulting in improved water movement in treated logs), it is suggested that the sap-flow method could deliver bacteria into the standing trees. The sap-flow method of bacterial treatment is more efficient than ponding in terms of reducting the treating time.

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