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ORIGINAL ARTICLE

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Effects of physical properties of wood on the water activity of wood meal media for the cultivation of edible mushrooms

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Abstract The effects of physical properties of wood, including specific gravity, porosity, and water retention, on the water activity (a_w) of wood meal media for the cultivation of edible mushrooms were examined. Five species of wood, selected from an initial set of 11 species, and six species of fungi popularly cultivated in Japan were used. The water activity of each fungal species was measured using liquid media in which a_w had been reduced by adding NaCl, KCl, sucrose, or ethylene glycol. From the water activities of the media and fungi, we estimated the most suitable wood species for the cultivation of each edible mushroom in wood meal media. Suitable wood species for the wood meal cultivation of shiitake, nameko, and maitake, which had relatively high a_w, was limited to hardwoods like arakashi, konara, and irohakaede because of their higher water activities. Edible mushrooms with lower levels of a_w, like hiratake, enokitake, and bunashimeji, could be cultivated in all kinds of wood species used in this experiment, but especially in sugi.

Key words Edible mushroom · Wood species · Physical properties · Water activity

Introduction

Edible mushrooms can be commercially cultivated on a variety of wood meal media, depending on the characteristics of the mushroom. In Japan, shiitake [*Lentinula edodes* (Berk.) Pegler], nameko [*Pholiota nameko* (T. Ito)], and maitake [*Grifola frondosa* (Dicks. Ex Fr.)] are mainly cultivated in hardwood media, but hiratake [*Pleurotus ostreatus* (Jacq. Ex Fr.)], enokitake [*Flammulina velutipes* (Curt. Ex Fr.)], and bunashimeji [*Hypsizygus marmoreus* (Peck) Bigelow] are generally cultivated in previously seasoned sugi, a softwood. Mushroom producers have empirically selected the suitable wood species for the wood meal cultivation of each mushroom by checking the mycelial growth and yield of mushrooms in preliminary cultivations.

The growth of wood decay fungi is controlled by many factors including tissue structure, specific gravity, heartwood ratio, hardness, and lignin and extractives contents of wood.[1] It has been accepted that extractives are the factor most responsible for the durability of wood.[1–3] Nakajima et al.[4] reported that the ferruginol contained in the methanol extract of sugi (*Cryptomeria japonica* D. Don) inner bark has a toxic effect on the mycelial growth of shiitake. It has also been found that enokitake and hiratake could be cultivated in sugi because their growth was not inhibited by ferruginol.[4,5] However, we found that mycelial growth of shiitake in sugi could only reach about 60% of that in konara (*Quercus serrata* Thunb.), even if extract-free sugi wood meal was used.[6,7] It therefore seemed that there must be other factors significantly affecting the growth of edible mushrooms besides wood extractives.

Among the physical properties of wood, specific gravity seems to have little connection to durability.[1] However, Horisawa et al.[8] used todomatsu (*Abies sachaliensis* Masters), ezomatsu (*Picea jezonensis* Carr), and karamatsu [*Larix kaempferi* (Lamb.) Carriereas] as substrates for a garbage decomposer–extinguisher machine, they investigated the physical properties of each type of sawdust, comparing the results with those for agricultural soil. It was reported that sawdust was suitable for use as a substrate in this machine when it had a lower specific gravity, higher porosity, and better drainage than soil.

The effect of water content on the mycelial growth of shiitake in sugi wood meal media was previously investigated using extract-free wood meal to eliminate the inhibitory effects of extracts.[7] The mycelial growth of shiitake in

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sugi wood meal media increased to almost the same level of that in konara wood meal when the water content was increased from 65% to 75%. The water potential of sugi wood meal media was −14kPa and that of konara was −2kPa at 65% water content. It seemed that the mycelial growth of shiitake could be promoted even in sugi wood meal media because the water potential decreased below −2kPa by increasing the water content to 75%.

In this article, the effects of physical properties of woods including specific gravities, porosities, and water retentions on the water activity (a_w) of wood meal media for the cultivation of edible mushrooms were investigated. Five species of wood, selected from an initial set of 11 species, and six species of fungi popularly cultivated in Japan were used. The water activities of edible mushrooms were measured with liquid media, the a_w of which had been reduced with the addition of ionizing and nonionizing solutes. We tried to estimate the suitable wood species for the cultivation of each edible mushroom in wood meal media, from the water activities of media and fungi.

Materials and methods

Organisms

Six kinds of edible mushrooms, commercial dikaryotic strains of shiitake (*Lentinula edodes*) Mori 465, maitake (*Grifola frondosa*) G-125, nameko (*Pholiota nameko*) MO-056, bunashimeji (*Hypsizygus marmoreus*) Z-092, enokitake (*Flammulina velutipes*) OMI-0046, and hiratake (*Pleurotus ostreatus*) OMI-0027, maintained at 5°C on potato dextrose agar slants were used in the present study.

Wood samples

Eleven species of wood were supplied by Miyazaki University Forest in Tano: Konara (*Quercus serrata*), arakashi (*Quercus glauca* Thunb.), irohakaede (*Acer palmatum* Thunb.), kojii [*Castanopsis cuspidata* (Thunb.) Schottky], kunugi (*Quercus acutissima* Carruth.), beimatsu [*Pseudotsuga taxifolia* (Poir.) Britt], kuromatsu (*Pinus thunbergii* Parl.), hinoki (*Chamaecyparis obtusa* Sieb. et Zucc.), inuside (*Carpinus tschonoskii* Maxim.), sugi (*Cryptomeria japonica*), and kiri (*Paulownia tomentosa* Steud.). Wood chips and wood meals were prepared from logs with a chain saw and a Wiley mill. Wood chips were sieved to a size of about 1cm.[3] Wood meals were sieved to a grain size of 0.355–1.000mm. Wood chips and wood meals were extracted with methanol for 6h and then extracted with hot water for 6h.

Culture methods

A 25-g portion of the wood meal medium (wood meal/rice bran = 3:1, water content: 65% w/w) was placed in a petri dish. After autoclaving (120°C, 1.2atm, 1h), the medium was inoculated with an agar disk (about 6mm in diameter) from stock culture and incubated for 8 days at 25°C and 60% relative humidity in a dark room. The mycelial weight was estimated based on the glucosamine content in the wood meal medium. The glucosamine content was determined by a method similar to that described by Tokimoto and Fukuda[9] and others.[10,11] The edible mushroom mycelial growth in each wood meal medium is presented in relation to the values for konara wood meal medium.

Determination of specific gravity and water retention

Specific gravity was measured with wood chips extracted previously with methanol and hot water, using the same method of the previous study.[7] Porosity of wood was calculated from specific gravity.

Water retention was determined according to the method of the previous study.[7] Wood meal (approximately 1g) was soaked in water for 1h with evacuation. Saturated wood meal was put into a 1G1 glass filter and centrifuged at 60*g* for 1min; water retention was calculated as previously described.[7]

Determination of water activities of wood meal media and edible mushrooms

Water activity in wood meal media was determined using a water activity instrument (AW meter WA-360, Shibaura) after autoclaving. It was calibrated with five samples of each wood meal medium.

The water activity of each edible mushroom was measured using liquid medium. Sodium chloride, potassium chloride, sucrose, and ethylene glycol were used to prepare water solutions with a_w levels of 0.995, 0.990, 0.980, 0.960, and 0.940, respectively. Glucose (20g) and polypepton (5g) were added to 1000ml of each solution in order to prepare the liquid media. A series of 100-ml Erlenmeyer flasks containing 5ml of the liquid medium were each inoculated with an agar disk (about 6mm in diameter) from stock culture after autoclaving (120°C, 1.2atm, 0.5h) and were incubated at 25°C and 60% relative humidity in a dark room until complete colonization of the control liquid media. After autoclaving, a_w values of liquid media were determined using the water activity instrument.

Results

Effects of physical properties on water retention of wood

A total of eleven species of wood were used in this experiment. Specific gravities and water retentions were measured using wood chips extracted by methanol and hot water. The relationship of specific gravities and porosities to water retentions of various different woods is shown in Fig. 1. The water retentions of different woods tend to increase linearly as the specific gravities decrease and porosities increase, in

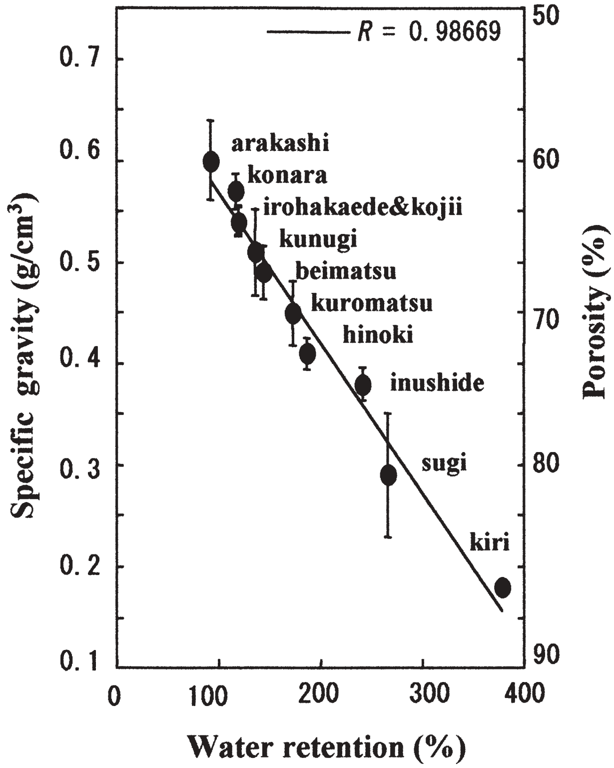

Fig. 1. Relationship of specific gravities and porosities to water retention of woods

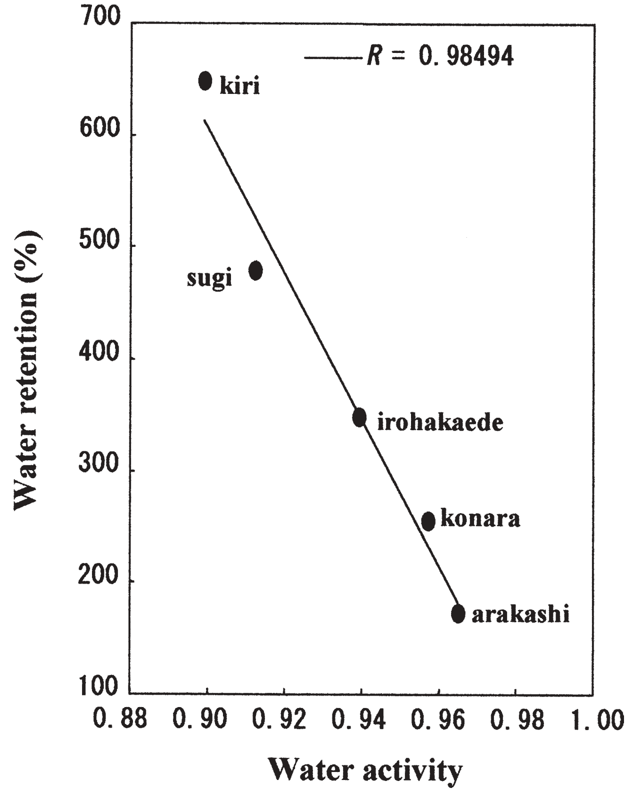

Fig. 2. Relationship of water retention of woods to water activities of wood meal media

the order of arakashi, konara, irohakaede, kojii, kunugi, beimatsu, kuromatsu, hinoki, inushide, sugi, and kiri. We found that the specific gravity and porosity of wood, which are fundamental physical properties of wood, significantly affect its water retention.

Because the relationship of specific gravity to water retention of various woods was approximately linear, five species of wood, arakashi, konara, irohakaede, sugi, and kiri, were selected from each end of the spectrum to investigate the effects of the specific gravity of wood on the water activity of wood meal media.

Effects of physical properties of woods on water activities of various wood meal media

The relationship between the water retention of each wood and the water activity of its wood meal medium is shown in Fig. 2. The water activities of wood meal media tend to increase as the water retentions decrease, in the order of kiri, sugi, irohakaede, konara, and arakashi. The water activity in kiri wood meal medium was the lowest of the five kinds of wood meal media. It was found that a higher specific gravity and a lower level of porosity in wood tended to produce a wood meal medium with a higher water activity.

Effects of water activity of wood meal media on mycelial growth of edible mushrooms

The effects of water activities in the wood meal media prepared from five wood species on the mycelial growth of edible mushrooms were investigated. The results are shown in Fig. 3. The mycelial growth of shiitake, maitake, and nameko, which grew poorly in sugi wood meal medium, tended to decrease with decreased water activity of wood meal media in the order of arakashi, konara, irohakaede, sugi, and kiri. On the other hand, enokitake, hiratake, and bunashimeji, which are generally cultivated in sugi wood meal medium, showed better mycelial growth than shiitake, maitake, and nameko in all of the five wood meal media, and were promoted with lower levels of water activity in wood meal media. Hiratake and bunashimeji showed maximum growth in sugi wood meal medium, but their mycelial growth decreased in kiri wood meal medium, which has a lower water activity than that of sugi. It was found that one group of edible mushrooms could grow in wood meal media with lower water activities, while the other group could grow only in the media with higher activities. The mycelial growth of edible mushrooms was significantly affected by the water activity of the wood meal media, suggesting that the two groups of edible mushroom had different levels of a_w.

Water activity of edible mushrooms

Liquid media with water activities that were reduced by the addition of NaCl, KCl, sucrose, or ethylene glycol, were used to investigate the a_w of edible mushrooms. The results in liquid media containing NaCl and KCl are shown in Fig. 4. In the media with NaCl, the mycelia could not completely grow at levels of $a_w < 0.935$ for shiitake and nameko, $a_w <$

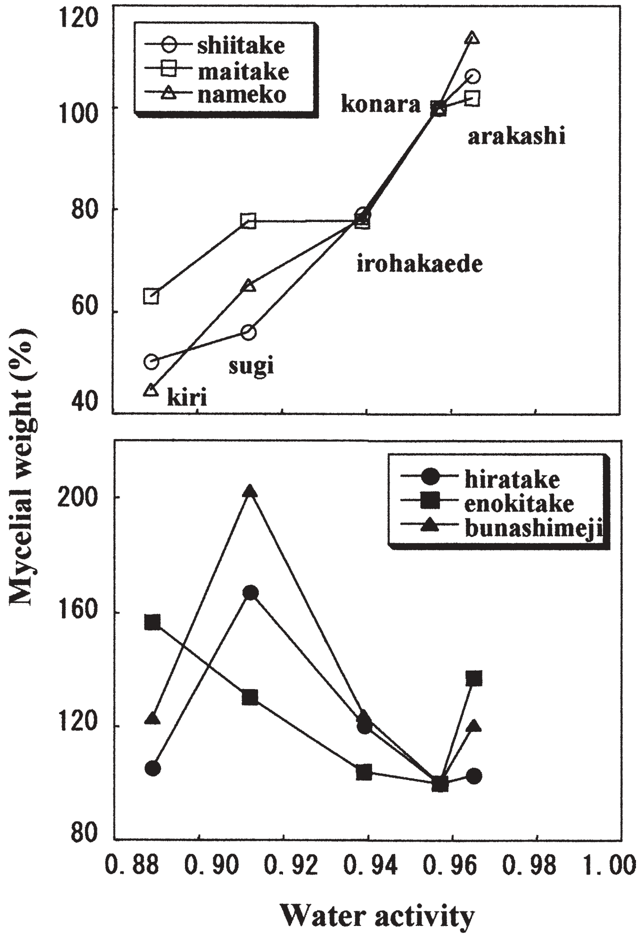

Fig. 3. Effect of water activity of wood meal media on mycelial growth of edible mushrooms

0.92 for maitake, enokitake, and bunashimeji, and $a_w < 0.89$ for hiratake (Fig. 4). In KCl media, mycelial growth of shiitake, maitake, and nameko was not observed at $a_w <$ 0.93, while enokitake and bunashimeji grew actively at $a_w =$ 0.93, with mycelial weights about 40%–80% of that in the control media. However, their mycelial growth was not observed at $a_w < 0.89$. Hiratake was able to grow until a_w decreased to 0.87 (Fig. 4). From these results, water activities of shiitake, maitake, and nameko tend to be higher than those of hiratake, enokitake, and bunashimeji in liquid media in which a_w is reduced by ionizing solutes.

The results for liquid media to which sucrose and ethylene glycol were added are shown in Fig. 4. In sucrose media, the mycelial growth of edible mushrooms was significantly promoted, but as the solute concentration increased and a_w decreased, it became depressed. Mycelial growth was not observed at $a_w < 0.94$ for maitake and nameko, and $a_w <$ 0.92 for shiitake. Hiratake and bunashimeji could not grow at $a_w < 0.90$. The mycelial growth of enokitake was observed at a_w as low as 0.90 (Fig. 4). In ethylene glycol media, the mycelial growth was not observed at $a_w < 0.94$ for maitake and nameko, and $a_w < 0.93$ for shiitake. Hiratake and bunashimeji showed no mycelial growth at $a_w < 0.91$. Enokitake could not grow at $a_w = 0.89$ (Fig. 4). Shiitake,

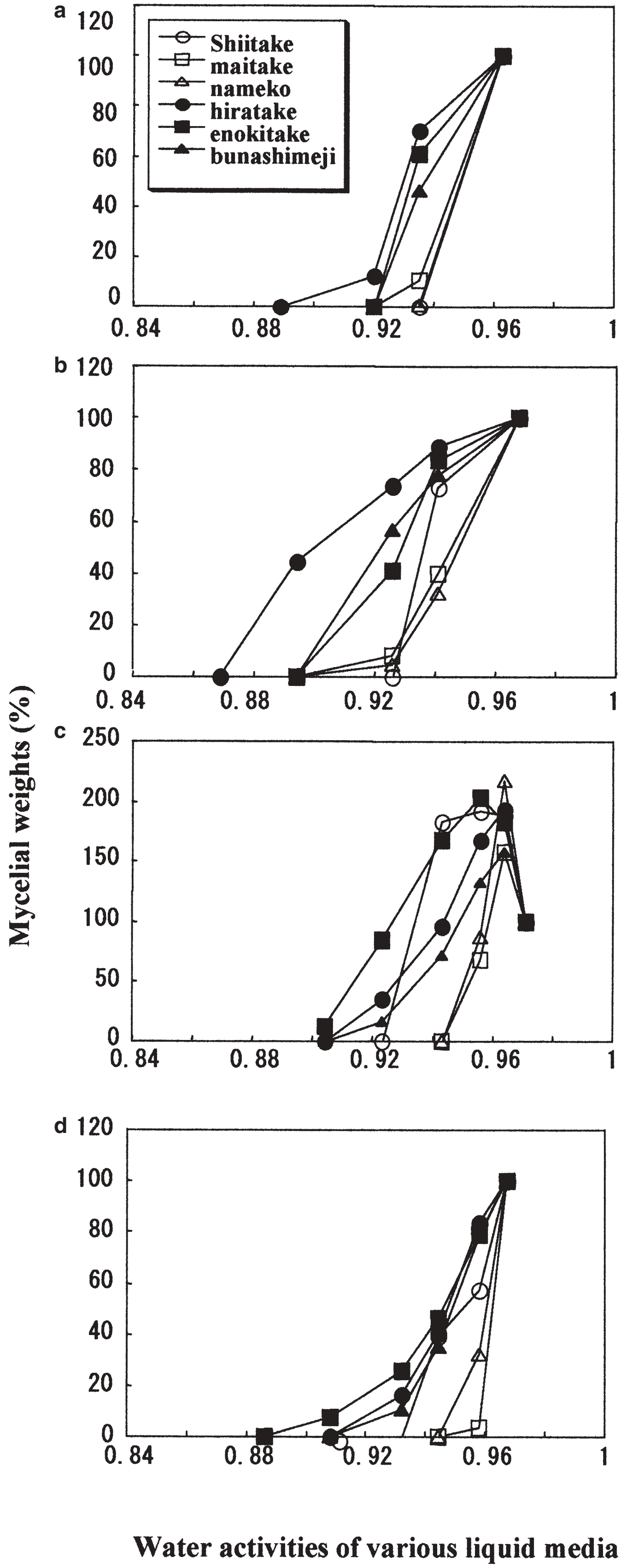

Fig. 4a–d. Effects of water activity on mycelial growth of edible mushrooms in liquid media with added solutes. **a** NaCl, **b** KCl, **c** sucrose, **d** ethylene glycol

maitake, and nameko had higher a_w than that of hiratake, enokitake, and bunashimeji in liquid media in which a_w was reduced by nonionizing solutes, the same tendency as that observed in ionizing solutes.

Discussion

The water activity of foodstuff is a very important aspect of food preservation because the growth of various microorganisms is controlled by the level of a_w.[12] The water activity of a food is not the same thing as its water content, but refers to free, unbound, or active water that can support the growth of bacteria, yeast, and fungi.[12,13] The water activity simply represents the ratio of the water vapor pressure (P) in any food system to the water vapor pressure of pure water (P_0) under the same condition.[12]

$$a_w = P/P_0$$

Water activity is therefore defined as the equilibrium relative humidity (ERH) divided by 100 and the scale of a_w extends from 0 (completely dry) to 1.0 (pure water).[12] The water potential ψ could be calculated from the water activity as follows:[14]

$$\Psi = RT/M \times \ln(P/P_0)$$

where R is the gas constant (8.31 J/mol K), T is the absolute temperature, M is the molecular mass of water, and P/P_0 is the water activity.

In a previous study, the water potentials of wood meal media before autoclaving were determined by the centrifugal method.[7] In media with 65% water content, the water potential of sugi wood meal medium was about −12.4 kPa and that of konara wood meal medium was about −2.1 kPa. In this experiment, the water potentials of wood meal media after autoclaving were determined using the water activity instrument. The water potentials of sugi and konara wood meal medium were about −12.7 MPa and −6.1 MPa, respectively. It was found that the water potential in wood meal media after autoclaving was approximately 1000 times higher than that before autoclaving, suggesting that free water sufficiently penetrated into the depths of wood meal swollen from autoclaving. Ohga[15] investigated the changes of water potentials in buna [*Fagus crenata* Bl.] wood meal media during both the vegetative growth and the fruit body formation periods of shiitake. It was reported that the water potential of wood meal media after autoclaving was about −4 MPa, which was approximately the same level as the results obtained in this study.

Water activity has its most useful application in predicting the growth of bacteria, yeast, and fungi. It has been established that most bacteria do not grow at water activities below 0.91, and most fungi cease to grow at water activities below 0.80.[12] This means that the water activity of the medium determines the lower limit of available water for microbial growth.

In this study, the water activities of six species of edible mushrooms were determined in liquid media in which the water activity had been reduced by the addition of NaCl, KCl, sucrose, and ethylene glycol. The effects of water activities on mycelial growth varied slightly according to the type of solute. On the whole, however, the water activities of hiratake, enokitake, and bunashimeji tended to be lower than those of shiitake, maitake, and nameko in liquid media. Specifically, shiitake, nameko, and maitake could grow only at $a_w > 0.92$, approximately the same level as most bacteria, but hiratake, enokitake, and bunashimeji could grow at a_w as low as 0.88–0.92.

From this study, it was found that the mycelial growth of edible mushrooms was significantly affected by the water activity of wood meal media, and that each species of edible mushroom had a different water activity. It seemed that suitable wood species for the cultivation each edible mushroom in wood media could be estimated by the relationship of water activity between the media and the fungi. As shown in Fig. 5, shiitake, nameko, and maitake, which had

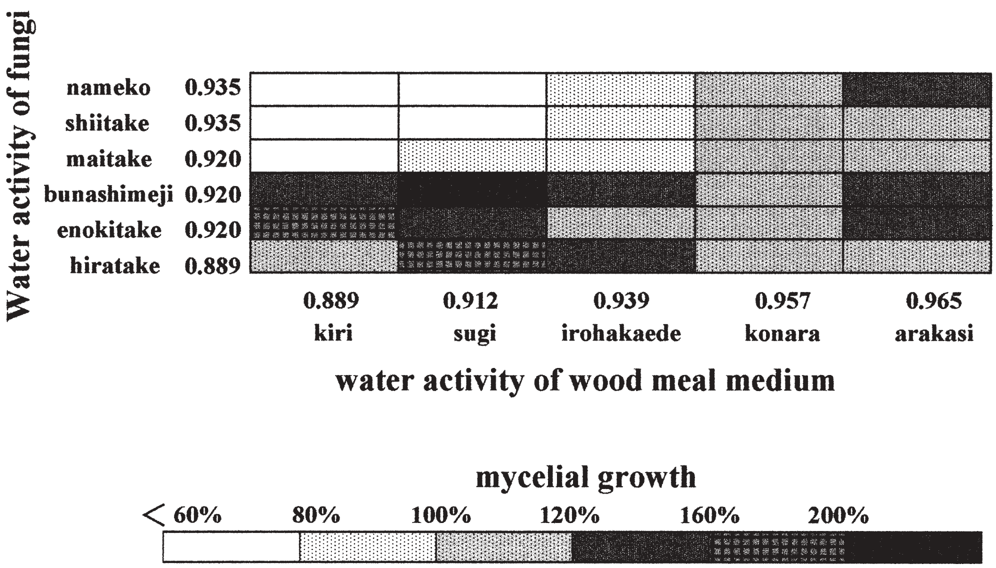

Fig. 5. Effect of the water activities of media and fungi on mycelial growth of edible mushrooms in wood meal media. The water activity of fungi was measured in liquid media with added NaCl as solute

relatively high levels of water activity, could grow at only 60% in the wood meal media with lower water activities like sugi and kiri, compared with that in konara media as a control, suggesting that these fungi should not be cultivated in sugi or kiri wood meal media. However, the cultivations of these mushrooms improved to 80% in irohakaede media, which has a higher water activity than sugi. Konara and arakashi seemed to be the most suitable wood species for the cultivation of shiitake, nameko, and maitake, because these mushrooms could grow at 100%–120% of the control in those wood meal media with $a_w > 0.94$. On the other hand, hiratake, enokitake, and bunashimeji, with relatively low water activities, had higher mycelial weights in all the wood meal media used in this experiment than that in konara media as a control, and could grow at over 120%, even in sugi and kiri media.

From the results obtained in this experiment, the suitable wood species for the wood meal cultivation of edible mushrooms like shiitake, nameko, and maitake with relatively high water activities, should be limited to hardwoods like arakashi, konara, and irohakaede because their media have higher water activities. On the other hand, edible mushrooms that have lower water activities, like hiratake, enokitake, and bunashimeji could be cultivated in all kinds of wood meal used in this experiment, however, sugi, with lower water activities, would be more suitable for cultivation of these. Kiri was also found to be effective but is not popular as a substrate wood for edible mushroom cultivation. As described above, mushroom producers have empirically selected the suitable wood species for the wood meal cultivation of each mushroom. This study obtained similar recommendations by clarifying the theoretical mechanism involved.

Our previous study showed that an ordinal seasoning treatment consisting of sunlight and rain easily eliminated almost all inhibitory substances contained in sugi wood meal.[6] Therefore, when selecting the suitable wood species for mushroom cultivation in wood meal media, physical properties including specific gravity and porosity of wood should be taken into account along with wood extractives.

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