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Original contribution

MRI visualization of shiitake mycelium growing in woodchip blocks used for shiitake mushroom cultivation

Kuniyasu Ogawa[a,*], Takeshi Yashima[b]

[a] Keio University, Department of Mechanical Engineering, 3-14-1 Hiyoshi, Kouhoku-ku, Yokohama, Kanagawa 223-8522, Japan
[b] Ishikawa Agriculture and Forestry Research Center, 1-1 Mizuho, Noto-chou, Housu-gun, Ishikawa 927-0311, Japan

A B S T R A C T

In order to eliminate woodchip blocks where unwanted fungi have grown and select only blocks where shiitake mycelium are growing well, there is a need to develop a visualization technique for shiitake mycelium growing in woodchip blocks, and MRI is an obvious candidate technique. From the results of measurements of the woodchip bed in a small bottle (26 mm inside diameter) where shiitake mycelium was growing, the T_1 relaxation time constant immediately after inoculation was 77.9 ± 5.5 ms, and the value after about 10 to 20 days increased to 135.0 ± 9.8 ms (the increase rate was 73%). The T_1 maps of the wood-chip block (130 mm length, 75 mm height and 55 mm thickness) in which shiitake mycelium grew were calculated from T_1 weighted images measured by changing *TR* from 28 to 400 ms. From the T_1 maps of time series, it was found that the shiitake mycelium extended from the right-hand side to the left-hand side of the woodchip block in a planar manner. Furthermore, in a woodchip block in which penicillium was generated, since the T_1 relaxation time constant of only the shiitake mycelium became longer, it was possible to visualize the shiitake mycelium distinctly from penicillium.

1. Introduction

Shiitake (*Lentinula edodes*) is a common edible mushroom, and 68,000 tons of shiitake are produced every year in Japan. Approximately 90% of the shiitake production in Japan is cultivated using blocks made from solidified woodchips [1]. In order to harvest a lot of good quality shiitakes, it is necessary to widely spread a high density of the shiitake mycelium throughout a whole woodchip block. However, different fungi such as penicillium often grow in the woodchip block and inhibit the growth of shiitake mycelium. Elimination of blocks where unwanted fungi have grown and selecting only those where shiitake mycelium are widely spread can minimize the number of blocks placed in a narrow cultivation room and contributes to the improvement of the management efficiency of shiitake cultivation. The development of a visualization technique for shiitake mycelium growing in a woodchip block is useful to select blocks that can produce a lot of good quality shiitakes.

There are several reports that visualize mushrooms and mycelium growing in a woodchip block with MRI [2–4]. In one report, the spatial distribution of water and the T_2 relaxation time constant depending on the static magnetic field strength in the mushroom were measured by MRI, but the mycelium growing in a woodchip block was not visualized [2]. In other reports, the mycelium growing in a woodchip block inoculated with Maitake (*Grifola frondosa*) mycelium was visualized by a T_1 weighted method which is one of the MRI visualization techniques [3,4]. In these papers, Maitake mycelium grew in a woodchip block at intervals like tree branches, and the shape of the mycelium was radial around the inoculation position with its central part being extremely long.

On the other hand, there is no report showing a visualized image of shiitake mycelium growing in a woodchip block. Since a large number of fruiting bodies of shiitake are generated from all sides of a block, it is presumed that the shape of shiitake mycelium spreading in the block is different from that of Maitake mycelium. Furthermore, when penicillium is generated in the block, a measurement technique that can visualize shiitake mycelium distinctly from penicillium is required.

In this study, we have found a measurement technique to visualize shiitake mycelium growing in a woodchip block by MRI utilizing the phenomena that the T_1 relaxation time constant of the shiitake mycelium is longer than that of the block. Using this method, the spatial distributions of shiitake mycelium growing in the block were obtained in time series. Furthermore, T_1 and T_2 relaxation time constants of shiitake mycelium growing in a small block were measured. In addition, an MR image showing that the penicillium generated in the block suppresses the growth of the shiitake mycelium was obtained.

* Corresponding author.
E-mail addresses: ogawa@mech.keio.ac.jp (K. Ogawa), yashima2@pref.ishikawa.lg.jp (T. Yashima).

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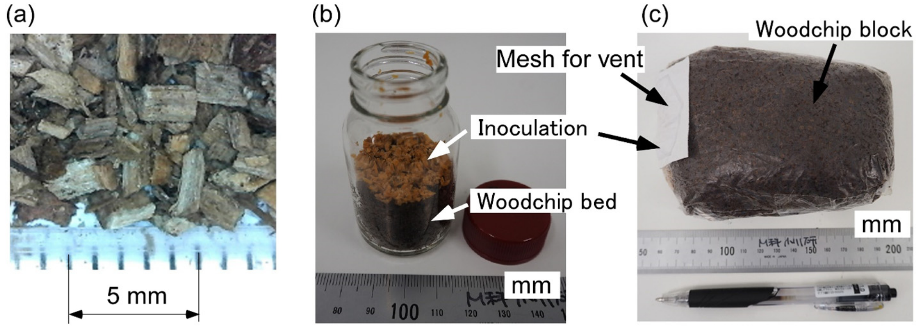

Fig. 1. (a) Dry woodchips, (b) Woodchip bed in the small bottle, (c) Woodchip block in the polypropylene bag.

2. Method for making woodchip blocks for shiitake cultivation

2.1. Materials and processing procedures

The procedure for making the woodchip blocks for shiitake cultivation is described below. Woods such as quercus (*Quercus serrata*) were pulverized to sizes of about 2 to 3 mm and became wood chips. A photograph of the dry woodchips is shown in Fig. 1(a). Wood chips, rice bran and distilled water were well mixed in a bowl at the ratio of 45: 5: 50 wt%. The mixed woodchips materials were placed in a small bottle and a polypropylene bag as shown in Fig. 1(b) and (c).

The mixed woodchips material of 10 g mass was placed in a small bottle with an inner diameter of 26 mm. We shall designate this as the wood-chip "bed" in the small bottle. The height of this bed was about 23 mm. On the other hand, mixed woodchips material of 300 g mass was placed in a polypropylene bag, referred to here as a woodchip "block". The dimensions of the block were 130 mm in length, 75 mm in height and 55 mm in thickness. The polypropylene bag has a mesh for a vent, and carbon dioxide can be discharged from this mesh. The bed and the block were placed in a steam sterilizer and sterilized at 121 °C for 2.5 h. Thereafter, the woodchip bed and block were returned to room temperature in a steam sterilizer over a period of 12 h.

Shiitake mycelium (XR1, Mori & Company, Limited.) inoculum of 1 g mass was inoculated on the top of the woodchip bed as shown in Fig. 1(b). The small bottle containing the bed was covered with a lid. Shiitake mycelium inoculum of 10 g mass was inoculated at the left end (below the mesh) of the woodchip block in the polypropylene bag shown in Fig. 1(c). The block was placed in an acrylic cylindrical holder. By using this holder, the MRI measurements were always taken at the same position each time.

The bed and the block were placed in a thermostatic chamber with a humidity of 90% and a temperature of 21 °C. Shiitake mycelium were grown for about 1 month under these conditions.

2.2. T_1 and T_2 relaxation time constant measurements of the woodchip bed

The time required for the growth of shiitake mycelium over the whole of the woodchip block in the polypropylene bag is about one month. On the other hand, in the case of the woodchip bed in the small bottle, the period during which the mycelium spreads throughout the whole bottle is 8 to 10 days due to the small size. Therefore, since the mycelium heterogeneity in the bed is lower than that of the block, the bed in the small bottle is suitable for measuring the T_1 and T_2 relaxation time constants of the whole bed. Furthermore, the T_1 and T_2 relaxation time constants of an un-inoculated woodchip bed were measured as a control sample.

3. MRI system and measurement methods

3.1. MRI system

The MRI system utilizing a permanent magnet was used to visualize shiitake mycelium in the woodchip blocks. The magnetic field strength of the permanent magnet was 0.35 T, and the air gap of the magnet was 140 mm. The magnetic resonance frequency of ^{1}H in this magnet is about 13.34 MHz. A pair of disc-shape gradient magnetic field coils was attached inside the magnet. The magnetic field gradient conversion efficiencies of the gradient magnetic field coil were 0.177 G/cm A at G_X and G_Y, 0.280 G/cm A at G_Z. The magnet and magnetic field gradient coils were manufactured by NEOMAX ENGINEERING Co. Ltd.

The MRI console consists of a detector, a modulator, a power supply to the gradient magnetic field coils, an oscillator, and a control PC with an analog-digital converter (ADC) and a digital signal processor (DSP) as shown in Fig. 2. The MRI console and measuring software were produced by MR Technology Inc.

The RF probe consisted of a parallel resonance circuit composed of a solenoid coil and a variable capacitor. The solenoid coil was an elliptical shape with a height of 118 mm, a lateral width of 78 mm and a length of 120 mm. The maximum size of the sample that can be inserted into the RF probe was 108 mm high, 68 mm wide and 150 mm long. The RF probe was manufactured in our laboratory.

3.2. Sequences of T_1 and T_2 measurement

The sequence used to measure the T_1 relaxation time constant of a bed has an inversion pulse added to a simple spin echo sequence. The sequence is illustrated in Fig. 3. The time width of the 90° excitation pulse was 200 μs, and the time width of the 180° excitation pulse was 400 μs. The outline shape of two excitation pulses was a rectangle and is expressed as a hard pulse. A spin echo signal is induced by a set of a second 90° pulse and the third 180° pulse. The echo time *TE* was 10 ms. In order to remove the FID signal appearing after two excitation pulses, a gradient magnetic field was applied for 1.5 ms.

The first 180° excitation pulse is an inversion pulse, which is the same as the third 180° excitation pulse. The time interval τ between the inversion pulse and the 90° excitation pulse was sequentially changed and acquired the spin echo signal. The time interval τ was a value obtained by dividing the interval time between 2 and 1000 ms by 20 points. The repetition time *TR* was 10,000 ms. The number of averages was set, *Nex*, to 1. The frequency width of the low pass filter, *LPF*, at the time of acquiring an echo signal was 20 kHz.

The spin echo signal was acquired in the ADC at 20 μs interval. The echo signal intensity was calculated from the average value of eleven points including the echo center of the echo signal. By approximating the relationship between the acquired echo signal intensity and the time interval τ with an exponential function, the T_1 relaxation time constant of the wood-chip bed in which shiitake mycelium grew was obtained.

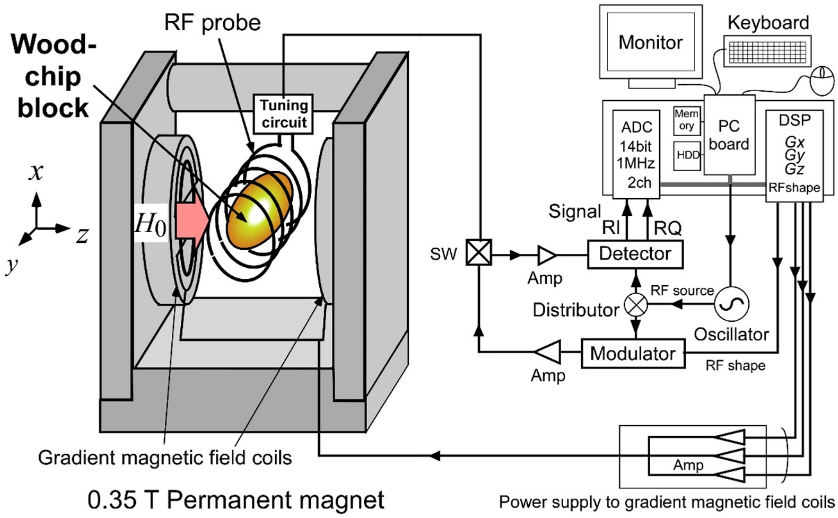

Fig. 2. MRI system for visualization of shiitake mycelium growing in a woodchip block.

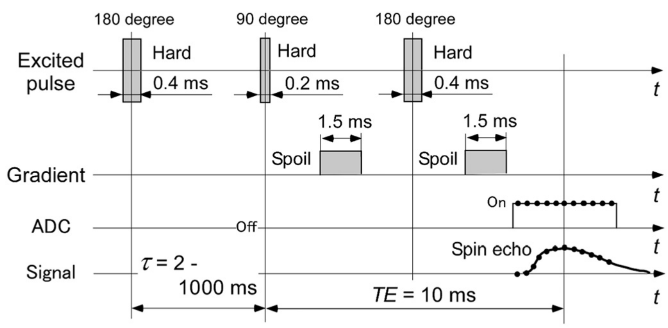

Fig. 3. Sequence to measure T_1 relaxation time constant of the woodchip bed in which shiitake mycelium grew.

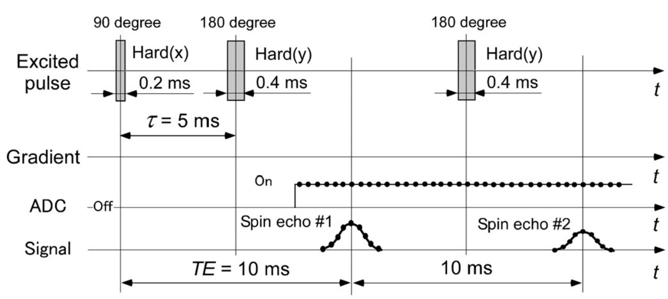

Fig. 4. Sequence to measure T_2 relaxation time constant of the woodchip bed in which shiitake mycelium grew.

The sequence used to measure the T_2 relaxation time constant of the bed was a CPMG (Carr-Purcell Meiboom-Gill sequence [5]. The sequence is illustrated in Fig. 4. The 90° excitation pulse was a hard pulse of 200 µs time width, and the 180° excitation pulse was a hard pulse of 400 µs time width. Multiple 180° excitation pulses were repeatedly irradiated at the interval of 10 ms. *TR* was 10,000 ms. *Nex* was set to 2. The *LPF* was 20 kHz.

Multiple spin echo signals were acquired in the ADC at 20 µs intervals. The echo signal intensity was calculated from the average value of eleven points including the echo center of the echo signal. By approximating the relationship between the acquired even-numbered echo signal intensities and the elapsed time from the 90° excitation pulse by an exponential function, the T_2 relaxation time constant of the wood-chip bed on which shiitake mycelium grew was obtained.

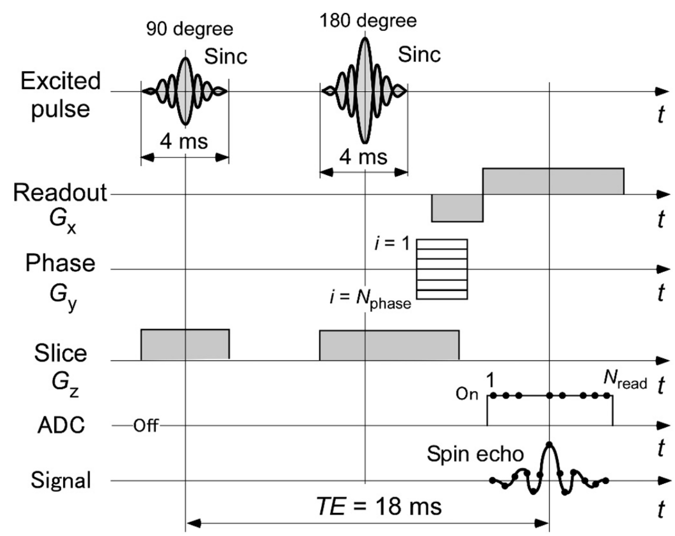

Fig. 5. Multiple slice sequence of spin echo to visualize shiitake mycelium growing in the woodchip block.

3.3. Multi-slice spin echo method

A multi-slice spin echo sequence was used to visualize shiitake mycelium growing in the woodchip block. The sequence is illustrated in Fig. 5. The shape of the 90 and 180° excitation pulses was a sinc-shaped waveform of 4 ms time width. The echo time *TE* was 18 ms. In order to acquire T_1 weighted images, *TR* was measured between 28 and 400 ms. The measurement parameters for this simple multiple slice sequence are shown in Table 1.

The spin echo signal was acquired in the ADC at 20 µs intervals. The *LPF* was 20 kHz. The number of pixels of the MR image, $N_{read} * N_{phase}$, was 256 ∗ 256 and the field of View (FOV) was 160 mm ∗ 150 mm. The slice thickness was 4 mm.

The T_1 relaxation time constant of each pixel can be calculated from

Table 1
Measurement parameters for multi-slice spin echo sequence.

Repetition time, *TR*	400 ms	200 ms	100 ms	54 ms	28 ms
Number of addition, *Nex*	8	8	16	32	64
Number of slice cross sections, N_s	7	7	3	2	1
Required measurement time, T_m	13.5 min	7 min	7 min	7 min	7 min

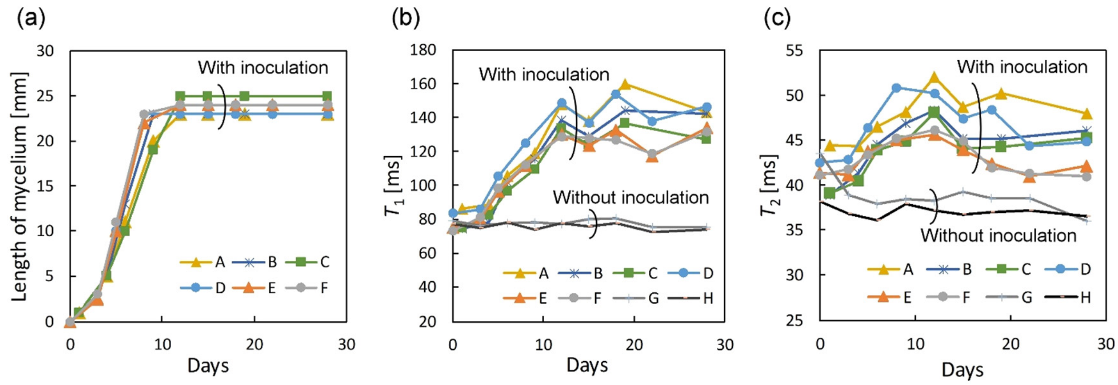

Fig. 6. Time-change of (a) Length of mycelium, (b) T_1 and (c) T_2 relaxation time constants of the woodchip bed in a small bottle. The symbols A to F are for woodchip beds that were inoculated, while symbols G and H are woodchip beds without inoculation.

a plurality of MR images acquired at different *TR*. By approximating the relationship between the signal intensity at a position in the MR image and the repetition time *TR* with an exponential function, the T_1 relaxation time constant of the wood-chips block in which shiitake mycelium grew was obtained.

4. Results and discussions

4.1. T_1 and T_2 of the woodchip bed in a small bottle

The T_1 and T_2 relaxation time constants of the wood-chip bed in a small bottle were measured by the method described in Section 3.2. Fig. 6(a) shows the time change of the length of shiitake mycelium extending from the upper part to the lower part of the bed, and (b) and (c) show the time changes of the T_1 and T_2 relaxation time constants of the bed, respectively. A total of six woodchip beds were inoculated and they are indicated by the symbols A to F in Fig. 6. As a control sample, there are two beds without inoculation, and they are indicated by the symbols G and H in Fig. 6.

From Fig. 6(a), it is found that shiitake mycelium growing in the small bottle reaches the bottom of the bottle (23 to 25 mm depth) in 8 to 10 days. After the mycelium reached the bottom of the bottle, the density of mycelium in the woodchips bed increased. From Fig. 6(b) and (c), it is found that the T_1 and T_2 relaxation time constant increases with mycelium length and becomes almost constant after mycelium reached the bottom of the bottle.

The average increase rates, $\Delta T/T_{\text{init}}$, of the T_1 and T_2 relaxation time constants are shown in Table 2. In this study, "average" means the average value of six woodchip beds in small bottles. In this table, the averaged increase, ΔT, is equal to the average value after mycelium reached the bottom, T_{myc}, minus the average of initial value, T_{init}, and the rate of averaged increase is $\Delta T/T_{\text{init}}$. From Fig. 6(b) and Table 2, it is found that the rate of the averaged increase of the T_1 relaxation time constant is as large as 73% as compared with 13% for that of the T_2 relaxation time constant.

In contrast, T_1 and T_2 relaxation time constants of beds that were not inoculated remained almost constant. As a result, shiitake mycelium growth can be detected by increase of the T_1 relaxation time constant of the bed.

The reason why the relaxation time constant of the bed where mycelium grew increased is presumed to be due to the following factors. The relaxation time constant of the water that penetrated into the hard tissue of wood chip is shorter due to interaction with the components in the wood tissue. On the other hand, since the mycelium growing in the gaps between wood chips is tubular and soft and contains a lot of water, and hence the relaxation time constant of water in the mycelium is longer. As the mycelium grows, the water in the wood chip moves to the mycelium, and the mass of water in the mycelium relatively increases. As a result, the relaxation time constant of the bed increases as mycelium grows over the whole sample.

One factor that increases the relaxation time constants is the chemical change in the wood due to the biochemical reaction of shiitake mycelium, which is a wood-rotting fungus [6]. The effects of chemical change and water movement occurring in the woodchip bed on the relaxation time constants of the woodchip bed have different time scales. The chemical change in the woodchip bed due to shiitake mycelium increases the relaxation time constant of the woodchip bed over a long period of time. On the other hand, as shown in Fig. 6, the relaxation time constants of the woodchip bed increased rapidly over roughly 10 days after inoculation. We speculate that the rapid increase of the relaxation time constants over a short period of 10 days after inoculation is due to water movement from the woodchip bed to the shiitake mycelium.

Moreover, although the relaxation time constants of the shiitake mycelium could not be measured because its NMR signal was too weak, the relaxation time constants of the fruit body (shiitake mushroom) cultivated on the fungal bed was measured instead. The T_1 and T_2 relaxation time constants of fruiting bodies were about 600 to 800 ms and 90 to 100 ms, respectively. These values were much longer than those of the woodchip beds without inoculation (the values of T_1 and T_2 were about 78 ms and 38 ms respectively as shown in Fig. 6). We believe that the long relaxation time constants of fruiting bodies is one piece of evidence that water movement is a primary factor in the increase of relaxation time constants over 10 days after inoculation.

4.2. T_1 weighted image of woodchip blocks

The wood-chip block in which 15 days have passed after inoculation was measured by MRI. Fig. 7(a) shows a photograph of the block, and (b)–(f) show T_1 weighted images measured with *TR* changed to 400,

Table 2
The rate of averaged increase of T_1 and T_2 relaxation time constants of the woodchip bed in a small bottle due to shiitake mycelium growth.

Relaxation time constant	Average of initial value, T_{init}	Average value after mycelial growth, T_{myc}	Averaged increase, $\Delta T = T_{\text{myc}} - T_{\text{init}}$	Rate of averaged increase, $\Delta T/T_{\text{init}}$
T_1	77.9 ± 5.5 ms	135.0 ± 9.8 ms	57.1 ± 5.1 ms	73%
T_2	41.2 ± 2.1 ms	46.5 ± 2.5 ms	5.3 ± 1.9 ms	13%

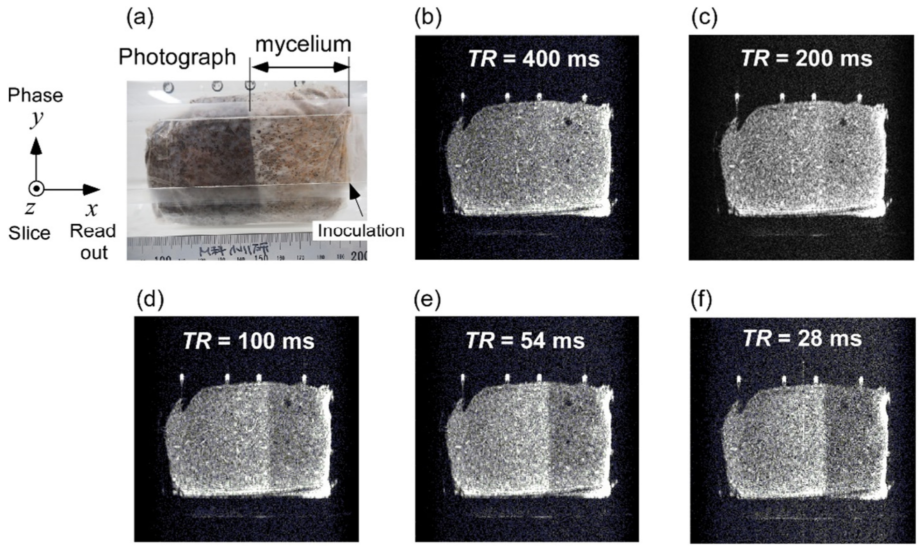

Fig. 7. Time-change of T_1 weighted images of woodchip block in which 15 days have passed after inoculation and its dependence on *TR*.

200, 100, 54 and 28 ms. The image is a cross-sectional image (*xy* plane) sliced vertically at the center of the *z* direction (slice) of the block. The shiitake mycelium is inoculated at the right-hand end of the block, and the mycelium grows to the left side as time elapses. As shown in Fig. 7(a), the region where the mycelium spread (right-hand side half) becomes white, and the region where the mycelium are not yet present (left-hand side half) is brown.

In the MR image, the region with the strong signal intensity is indicated by white, the region with weak signal intensity by black, and the middle thereof is indicated by a gray scale. From the images of Fig. 7(d)–(f), it is found that when *TR* is shortened, the signal intensity in the right-hand side region of the woodchip block decreases. Comparison of the photograph and MR images shows that the region of reduced signal intensity corresponds to the mycelium region of the block.

In the T_1 weighted image, the signal intensity of the mycelium region having a long T_1 relaxation time constant decreases when *TR* is shortened. Here we quantitatively consider the influence of *TR* on the decrease of the signal intensity of the T_1 weighted image. In general, the signal intensity, *Sig*, is expressed by the following equation as a function of *TR*, T_1 and T_2.

$$Sig(TR, T_1, T_2) = A\,\rho\left\{1 - \exp\left(-\frac{TR}{T_1}\right)\right\}\exp\left(-\frac{TE}{T_2}\right) \tag{1}$$

where *A* is the device constant and ρ is the concentration of water. The signal intensity ratio, Sig_{myc}/Sig_{wo}, is expressed as the following equation by adding subscripts of mycelium (myc) and no mycelium (wo) to T_1 and T_2 of Eq. (1).

$$\frac{Sig_{myc}(TR, T_{1myc}, T_{2myc})}{Sig_{wo}(TR, T_{1wo}, T_{2wo})} = \frac{\left\{1 - \exp\left(-\frac{TR}{T_{1myc}}\right)\right\}\exp\left(-\frac{TE}{T_{2myc}}\right)}{\left\{1 - \exp\left(-\frac{TR}{T_{1wo}}\right)\right\}\exp\left(-\frac{TE}{T_{2wo}}\right)} \tag{2}$$

We next substitute the values in Table 2 into T_1 and T_2 in Eq. (2) and substitute the value of T_{init} for T_{wo}. The signal intensity ratios, Sig_{myc}/Sig_{wo}, when *TR* is changed are indicated by blue circles in Fig. 8. From the figure, it is found that the signal intensity ratios, Sig_{myc}/Sig_{wo}, decreases when *TR* is decreased.

In order to confirm that the relational expression of Eq. (2) can be applied to the block, the signal intensity ratios, Sig_{myc}/Sig_{wo}, were calculated from the signal intensities of five MR images in Fig. 7, and are plotted as triangular symbols in Fig. 8. From this figure, it is found that the signal intensity ratios obtained from the T_1 weighted images substantially agree with Eq. (2). The deviation between them is due to the variation in the T_1 and T_2 of the woodchip block in which the mycelium grew.

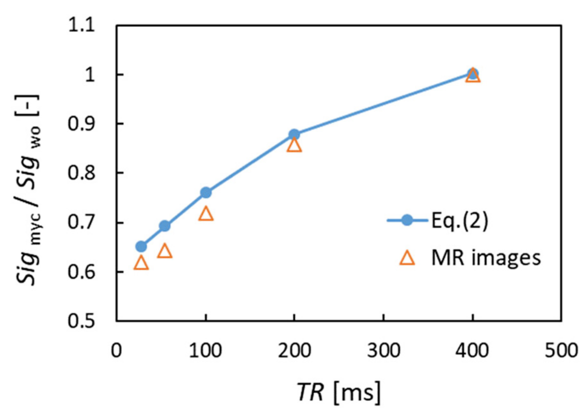

Fig. 8. The signal intensity ratios, Sig_{myc}/Sig_{wo}, of a woodchip block and its dependence on *TR*.

4.3. T_1 map of woodchip blocks

The T_1 relaxation time constant of each pixel can be calculated by using the signal intensity of each pixel from the five T_1 weighted images shown in Fig. 7 and using Eq. (2). Fig. 9 shows the maps of the T_1 relaxation time constant of the woodchip block corresponding to the number of days passed after inoculation. In this figure, two T_1 maps in the vertical slice section (*xy* plane) and the horizontal slice section (*xz* plane) are shown with the photographs. From this figure we can see the shiitake mycelium extending from the right-hand side to the left-hand side of the block in a planar manner. The map of the T_1 relaxation time constant shows the extent of the shiitake mycelium, eliminating the non-uniformity of the signal intensity at the edge, as seen in the T_1 weighted image. This point shows the superiority of visualizing the shiitake mycelium using the map of the T_1 relaxation time constant.

4.4. Shiitake mycelium growth when penicillium competes in a woodchip block

About 10 days after inoculation, it was visually confirmed that penicillium had been generated in the center of another block. Fig. 10

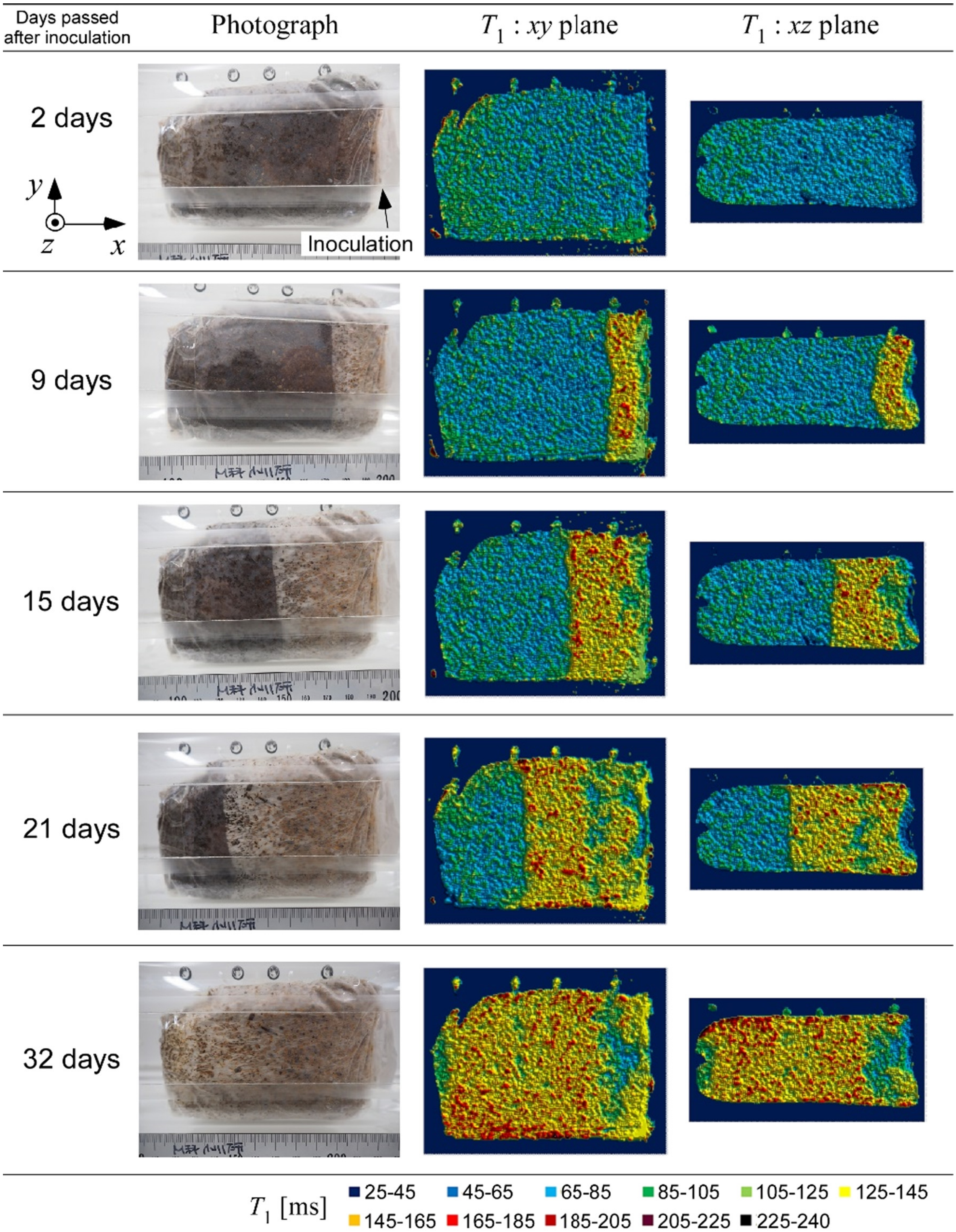

Fig. 9. T_1 maps of a woodchip block corresponding to the number of days passed after inoculation.

shows a map of the T_1 relaxation time constant of the block in which 25 days have passed after inoculation. From this figure, it was found that the T_1 relaxation time constant of the central portion where penicillium is generated does not increase and that the T_1 relaxation time constant increases only in the region of the shiitake mycelium growing on the right-hand side of the block. Comparing Fig. 9 with Fig. 10, it was found that the growth speed of shiitake mycelium is slowed and suppressed by the generation of penicillium. We continued to observe the block, but the shiitake mycelium did not grow any further. From this result, since the T_1 relaxation time constant of only the shiitake mycelium became longer, it was found that the growth region of shiitake mycelium in the block can be measured distinctly from penicillium. Therefore, by using this visualization method, it is possible to select better woodchip blocks where only shiitake mycelium has grown without penicillium growth.

5. Conclusions

In order to visualize shiitake mycelium growing in woodchip blocks, T_1 and T_2 relaxation times constants of a woodchip bed in a small bottle

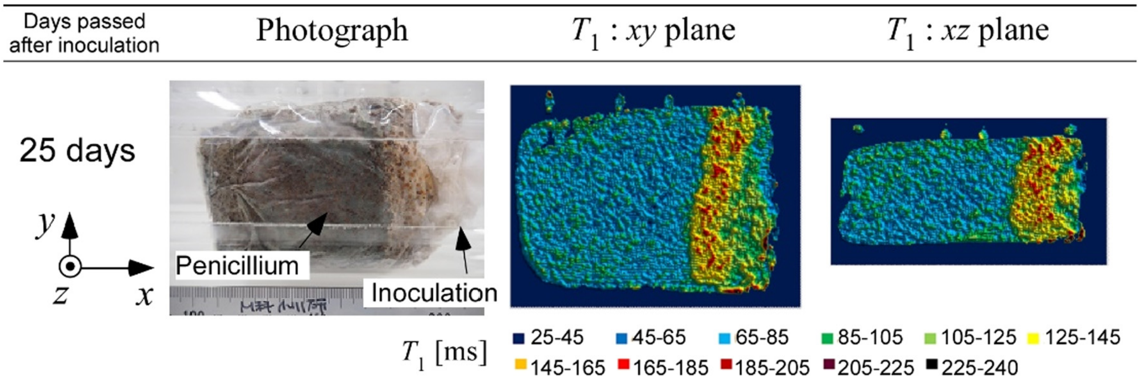

Fig. 10. T_1 maps of the woodchip block 25 days after inoculation in case that penicillium was generated in the center of the block.

and T_1 weighted images of a woodchip block in which shiitake mycelium was inoculated were measured by MRI. From the measurement results of the bed in the small bottle, the T_1 relaxation time constant immediately after inoculation was 77.9 ± 5.5 ms, and the value after about 10 to 20 days increased to 135.0 ± 9.8 ms (increase rate was 73%). The T_2 relaxation time constant immediately after inoculation was 41.2 ± 2.1 ms, and the value after about 10 to 20 days increased to 46.5 ± 2.5 ms (increase rate was 13%). The T_1 maps of the woodchip block were calculated from T_1 weighted images measured by changing *TR* from 28 to 400 ms. From the T_1 maps of time series, it was found that the shiitake mycelium extended from the right-hand side to the left-hand side of the block in a planar manner. Furthermore, in the block in which penicillium was generated, since the T_1 relaxation time constant of only the shiitake mycelium became longer, it was possible to visualize the shiitake mycelium distinct from penicillium.

Shiitake mushrooms cultivated with raw wood is known as log cultivation shiitake in Japan, and are treated as high-quality mushroom due to their attractive smell and good texture. A good quality log cultivation shiitake is very expensive in Japanese markets and is an important income source for farmers. However, the log cultivation method has a disadvantage that the cultivation period is as long as 1 year. Furthermore, it is very difficult to judge the success or failure of the shiitake cultivation until harvesting of the mushrooms because the extension of the shiitake mycelium growing in the log by overcoming other fungi cannot be visualized. We expect that the distribution of shiitake mycelium growing in a log before the actual shiitake mushrooms emerge can be visualized by the MRI method developed in this research. Our next project is to apply the MRI visualization method to the observation of shiitake mycelium growth in a log and to improve the log shiitake cultivation technique using raw wood.

Acknowledgments

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