

## The Artificial Cultivation of *Collybia* spp. W0003

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We report the artificial cultivation of W0003, which is an edible fungus easy to cultivate because of rapid growth. The W0003 mushrooms has been grown on sterile sawdust with good yields in small and large scale experiments. The temperature at 15°C appeared to be optimal for the fruiting, whereas the mycelium grew well up to 30°C. The pH range of 7 to 10 was the most favorable for the growth of the mycelium. Total yield obtained was 48.1% on the moistened media on the beech wood sawdusts supplemented with wheat bran.

### Introduction

Commercial cultivation of *P. ostreatus* (Jacq. ex Fr.) Kummer has been practiced in Japan for a long time. Some European countries, Asiatic countries and African countries also have already started commercial cultivation of the mushroom such as *Pholiota nameko*, *Flammulina velutipes*, *Lyophyllum ulmarium*, *Agricus bisporus*, *Lentinus edodes*<sup>(1-4)</sup>. However, almost edible or medicinal basidiomycetes have not been artificially cultivated yet.

Thus, we tested a number of different edible, wood-destroying basidiomycetes for their growth and fructification on fortified sawdust media.

Herein we examined the cultivation of basidiomycetes to clarify whether W0003 can be grown on such materials as sawdust supplemented with rice bran and wheat bran. We also describe the morphological and cultural characteristics of W0003.

### Materials and Methods

#### Organisms

W0003 was a wild type basidiomycetes isolated from Kinugasa, Kyoto. Cultures of W0003 were obtained by aseptic inoculation of the tissue from developing fruit bodies onto the media.

#### Culture experiments

The morphological characters were mainly based on the fruit bodies developed under artificial cultivation in the laboratory. Cultivation of W0003 was initially attempted in 300 ml Erlenmyer flasks with 100 ml of 2% malt extract medium. The sawdust medium was produced in the mixture of either sawdust of beech or cedar : agricultural waste of rice bran or wheat bran = 3:1 ratio in bottle and kept up in 70% moisture. Thirty five grams (small scale) or 350g (large scale) of the medium was put in a plastic bottle which was covered with aluminum sheets to prevent contamination and to retain moisture, and then the bottle was sterilized in an autoclave, for 30 minutes at 15 pounds of

pressure. The autoclaved bottles on cooling were inoculated with a master culture of W0003 grown in 300 ml Erlenmyer flasks and incubated at 25°C. When the fungus was spread to cover all of the substrate, enough water was added to each bottle and kept overnight. On the next day, excess water was drained off completely. The bottles were kept in a room maintained at 15°C and 90% relative humidity. After about 7 days the first fruit bodies appeared on the surface of the culture, and several days later they were large enough for harvesting. The bottom portion of the stems was cut off, and the weight of the fruit bodies was recorded.

## Results and Discussion

### Morphological characters

The pileus was yellow to brown-colored. The flesh was almost white, the gills were white or light cream colored. The pileus has a diameter of 10~30mm, the yellow to dark brown stipe is 20~70mm in length and 2~6 mm in diameter. The sporeprint was white (Fig. 1). W0003 is characterized by its shape, size and yellow to brown-colored pileus. Thus, W0003 belongs to *Collybia* spp.

### Physical conditions for growth

Table 1 shows the effect of temperature on the growth of the mycelium of W0003. No growth occurred at 35°C. The mycelium grew very well up to 30°C. The temperature at 15°C appeared to be optimal for the fruiting.

No growth occurred at an initial pH of 3.0. The mycelial growth occurred in a pH range of 4.0 to 12.0, and the initial pH range of 7 to 10 was the most favorable for the mycelium of W0003, as shown in Table 2.

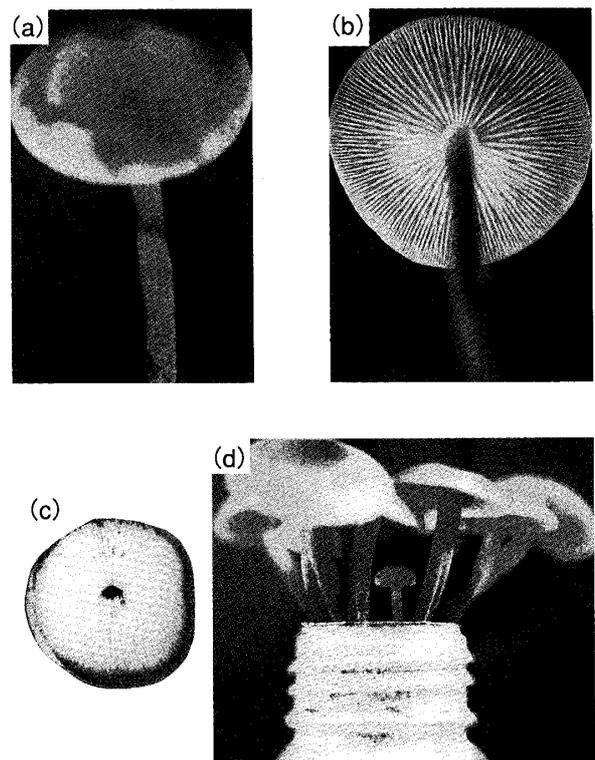


Fig.1. W0003 (laboratory cultivation)

- (a) 10-days old fruit body, face view
- (b) 10-days old fruit body, reverse view
- (c) Sporeprint
- (d) 10-days old fruit body on the sawdust medium

Table 1. Effect of temperature on vegetative growth

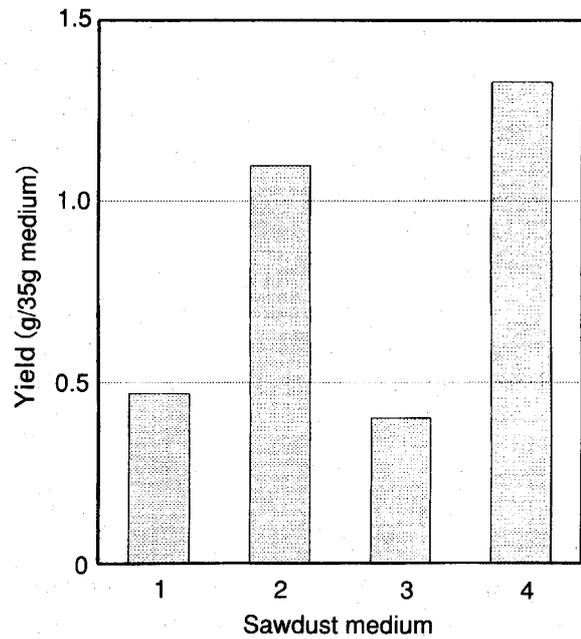
Temperature (°C)	Average diameter of Colonies*(cm)
15	5.5
20	7.1
25	8.5
30	8.5
35	0

\*After 10 days, the extent of growth was measured on a 2% malt extract agar plate.

**Table 2.** Effect of pH on vegetative growth

Initial pH	Final pH	Average Diameter of Colonies*(cm)	
		7 days	10 days
3.0	-	0	0
4.0	-	6.3	8.5
5.0	4.9	6.9	8.5
6.0	5.6	7.0	8.5
7.0	5.9	7.3	8.5
8.0	6.3	7.3	8.5
9.0	6.5	7.4	8.5
10.0	6.9	7.5	8.5
11.0	7.4	7.0	8.5
12.0	8.7	3.8	5.9

\*After 7 and 10 days at 25°C, the extent of growth was measured on a 2% malt extract agar plate.



**Fig.2.** Effect of different substrates on the yield of W0003

Lanes 1 and 2; the cedar sawdust [the cedar sawdust+rice bran(medium 1), the cedar sawdust+wheat bran(medium 2)], lanes 3 and 4; the beech sawdust [the beech sawdust+rice bran(medium 3), the beech sawdust+wheat bran(medium 4)].

**Table.3.** Yield of W0003 mushroom grown on sawdust medium

Flush	Time (days)	Weight (g)
1	10	126.0
2	10	39.1
3	10	2.9
4	10	0.5
Total		168.5

**Growth and fruiting**

When the mycelium of W0003 spread through sawdust medium, the aluminum sheets were re-

moved and water was sprayed as necessary to keep the material moist but not wet. Usually, 3 days later the cover was removed, the fruit bodies began to appear as tiny coralline heads on the surface of the media and the first crop was picked 3 days after the appearance of the tiny heads.

The yield was about 0.40g on the beech wood sawdust supplemented with rice bran (medium 3), 0.47g on the cedar wood sawdust supplemented with rice bran (medium 1), 1.33g on the beech wood sawdust supplemented with wheat bran (medium 4), and 1.10g on the cedar wood sawdust supplemented with wheat bran (medium 2) as spawning to first yield for a period of 10 days, as shown in Fig. 2. As shown in Table 3, the fruit bodies of W0003 were harvested 4 times as spawning to first, second, third and fourth yield for every period of 10 days from the sawdust medium (medium 4) of 350g and total product was 168.5g fresh mushrooms. The yield obtained was 48.1% of the moistened medium on the beech wood sawdust supplemented with wheat bran, and the yield and quality of fresh mushroom was satisfactory.

This is the first report on the artificial cultivation of W0003, which is an edible fungus, and easy to cultivate because of rapid growth.

### References

- 1) K. Bech, *Mushroom Journal*, **513**, 17-18 (1992).
- 2) A. Eicker, *Mushroom Journal*, **513**, 19-21 (1992).
- 3) T. Elliot, *Mushroom Journal*, **513**, 14-16 (1992).
- 4) P. Flegg, *Mushroom Journal*, **513**, 16-17 (1992).