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The Fruiting Body Formation of *Armillaria mellea* on Oak Sawdust Medium Covered with Ground Raw Carrots

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To produce an artificial fruiting body of *Armillaria mellea* on the oak sawdust medium, seven strains of *A. mellea* were used. The top surface of oak sawdust medium covered with ground raw carrot was inoculated with each of 7 strains and cultured for 30 days at 25°C in the dark condition until the mycelia of *A. mellea* completely colonized the medium from top to bottom. Then, the mycelia which were fully covered on the top surface of the medium were scratched slightly with a spatula and filled with tap water for 3 hours. To induce the primordial formation, the 7 strains of *A. mellea* were transferred to the growth chamber under the illumination (350 lux) of 12 hours and relative humidity of 85 ± 5% in a day and then cultured at 16 ± 1°C. Only *A. mellea* IUM 949 could form primordia on the sawdust medium, but the other strains did not make primordia at the same condition. The primordia of *A. mellea* IUM 949 were formed 10 days after complete colonization of the medium and the fruiting bodies were produced 7 days after a primordial formation. The experimental results suggested that IUM 949 strain might be a good candidate for mass production of fruiting bodies of *A. mellea*.

KEYWARDS: ***Armillaria mellea*, Fruiting bodies, Ground raw carrot, Primordium**

Armillaria mellea (Vahl. ex Fr), one of edible and medicinal mushrooms belonging to Tricholomataceae, Agaricales has been known to be inhabited as a parasite or saprophyte on many woody plant hosts such as the coniferous or broad-leaved trees from spring to Autumn, and also engaged in the symbiotic relationship with *Gastrodia elata*, one of medicinal herbs (Park and Lee, 1991). The fruiting body of *A. mellea* has been known to possess protein-polysaccharide substance, one of medicinally important chemical compounds (Kim *et al*., 1983). The protein-polysaccharide substance has exhibited good therapeutic and inhibitory effects against esophagus cancer, hypertension and paralysis (Li *et al*., 1993).

In Asian countries such as China and Japan, the researches related to *A. mellea* have been focused on an artificial production of fruiting bodies to secure precious medicinal mushroom resources (Li *et al*., 1993; Iwao and Namio, 1994, 1995; Namio, 1996). In Korea, as a part of investigating the possibility of artificial cultivation of new edible wild mushrooms, Cha (1981) tried sawdust culture for the cultivation of *A. mellea*. Kim *et al*. (1992) reported fruiting body of *A. mellea* was produced firstly on sawdust of *Quercus* spp. Although Kim *et al*. (1992) suggested the possibility of mass production of *A. mellea* in Korea, the artificial production of *A. mellea* has been not realized in Korea. Therefore, this study was initiated to realize mass production of *A. mellea*, and compared with that of Kim *et al*. (1992).

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Materials and Methods

Cultures. Seven strains of *A. mellea* were obtained from the Culture Collection of Wild Mushroom Species (CCWM) in the Department of Biology, University of Incheon (Table 1). Seven strains of *A. mellea* were transferred to PDA, incubated at 25°C in the dark condition until they exhibited a full growth, and kept at 5°C before use.

The formation of fruiting body of *A. mellea*.
The preparation of liquid inoculum: To prepare each liquid inoculum of 7 strains, a 1.2 cm plug of each inoculum was removed with cork borer from 10 days old cul-

Table 1. Strains of *Armillaria mellea* used in this study

Strain	Geographical origins
A. mellea IUM 791	Korea
A. mellea IUM 949	Korea
A. mellea IUM 535	Korea
A. mellea 184	Korea
A. mellea 185	Czechoslovakia
A. mellea 186	Czechoslovakia
A. mellea 741	Czechoslovakia

tures of *A. mellea*. Then, 6 pieces of the plug were inoculated on the 100 *ml* of sterilized PDB (potato dextrose broth + 2% malt extract, pH 6.0) within a triangular flask (250 *ml*) and incubated for 30 days at 24 ± 1°C in the shaking incubator (150 rpm/min).

The preparation of oak sawdust medium covered with the ground raw carrot.

Preparation of stimulator suitable for mycelial growth of *A. mellea*: To prepare ground raw carrots as a stimulator for favorable mycelial growth of *A. mellea* (Iwao and Namio, 1995; Iwao, 1996), the raw carrot(*Daucus carota*) was used. Raw carrots were washed with tap water, broken to several pieces, put into a blendor(Waring, U.S.A.), ground throughly for 5 minutes, mixed finally with oak sawdust containing 30% rice bran to the ratio of 50% (v/v) and kept at 5°C before use.

Preparation of oak sawdust medium: To prepare sawdust medium suitable for producing fruiting body of *A. mellea*, oak sawdust (*Quercus variabilis*) was mixed throughly with rice bran of 30% (v/v), adjusted to the moisture content of 70%, and put into the polyethylene plastic bottle (700 *ml*, 9 × 13 cm). After then, the surface of oak sawdust medium was covered with a small amount (an uniform thickness of about 1 cm) of ground raw carrots, inserted to make a hole with glass bar (diameter 1.5 cm × length 8 cm) in the center of the medium, and finally steam-sterilized for 90 minutes at 121°C.

The inoculation and culture of *A. mellea*. After each of 7 strains was incubated in the shaking incubator (150 rpm/min) for 30 days, 10 *ml* of each inoculum was removed from liquid culture medium, inoculated on the top surface of oak sawdust medium in the polyethylene plastic bottle (700 *ml*, 9 × 13 cm), and cultured for 30 days at 25°C in the dark condition until the mycelia of *A. mellea* were completely colonized from the top to bottom of the medium.

The inducement of primordia and fruiting bodies of *A. mellea*. After the mycelia of *A. mellea* completely colonized the medium within the cultivation bottle, the top surface of the medium was scratched slightly with a spatula, and filled with tap water for 3 hours. After then, the tap water was removed from the bottle. To promote a good ventilation and avoid drying of the mycelial surface of the medium during primordial formation, the bottles were covered with ST cap (made of polypropylene) which were not equipped with a sponge filter and then transferred to the growth chamber (DL, DF-95G504M). The environmental condition of growth chamber was 16 ± 1°C under the illumination (350 lux) of 12 hours and relative humidity of 85 ± 5% in a day. If primordia of *A. mellea* were appeared on the surface of oak sawdust medium, ST cap was removed from the bottle.

Results and Discussions

Seven strains of *A. mellea* completely colonized the medium from top to bottom after 30 days of culture at 25°C. Then, the bottles were transferred to the growth chamber, and primordia were formed after 10 days of incubation (Fig. 2). Of 7 strains used, *A. mellea* IUM 949 was the only one strain to form primordia (Fig. 2). *A. mellea* IUM 949 strain can be used for a good candidate for mass production of fruiting body of *A. mellea* in the future.

The mature fruiting bodies of *A. mellea* IUM 949 were observed within 7 days after primordial formation (Fig. 1). The numbers of fruiting bodies obtained from the bot-

Fig. 1. The fruiting bodies of *Armillaria mellea* IUM 949 on oak sawdust media covered with ground raw carrots.

Table 2. The fruiting body of *Armillaria mellea* IUM 949 produced on oak sawdust media

	Fruiting body of *Armillaria mellea* IUM 949			
Mean[a]	Total number of fruiting body	Length of stipe (cm)	Diameter of cap (cm)	Total weight (g/bottle)
	9.0 ± 7.7	8.0 ± 2.3	3.9 ± 2.6	23.9 ± 14.5

[a]The values are the average of 24 replications and standard deviation.

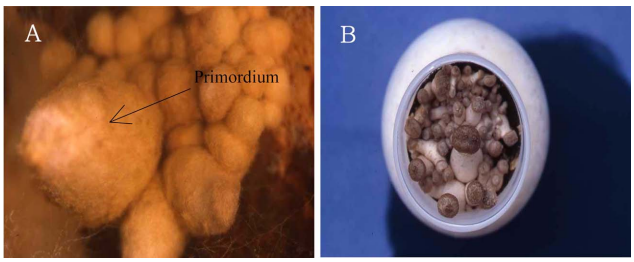

Fig. 2. The primordium of *Armillaria mellea* IUM 949 formed on oak sawdust media covered with ground raw carrots. A. The initial primordia (18 ×) of *Armillaria mellea* IUM 949 on the surface of oak sawdust medium. B. Primordia of *Armillaria mellea* IUM 949 on the surface of oak sawdust medium after 3 days of primordium formation.

tle cultivation were ranged from 1 to 26 per bottle, and the mean numbers were 9 pieces per bottle (Table 2). The total weight of fresh fruiting bodies were ranged from 2.88 to 59.15 g per bottle, and the mean weight was 23.9 g per bottle (Table 2). From this experiment, the probability to produce primordia or fruiting bodies from wild *A. mellea* seems to be very low.

Kim *et al.* (1992) reported that *A. mellea* completely colonized the medium after 70 days of culture at 24 ± 1°C and then primordia were formed 7 days after a complete colonization of medium at 15°C. When our experimental results were compared with that of Kim *et al.* (1992), we obtained much more shorten incubation period than that of Kim *et al.* (1992) by the treatment of ground raw carrot and small sawdust medium. The reason seems to be resulted from some active substances of ground raw carrots rather than medium size.

In other studies, it was suggested that some ingredient of carrot must be beneficial to the rapid growth of *A. mellea* on sawdust media (Iwao and Namio, 1994, 1995). Also, Iwao (1996) reported that the covering of ground raw carrots is needed because some ingredients of carrot might stimulate the dark mycelial coat of *A. mellea* on top surface of oak sawdust medium and then result in the formation of its primordia. Thus, it will be necessary in the future to clarify the relationship between a primodial formation of *A. mellea* and an application of ground raw carrots.

In our study, it takes 47 days from an inoculation of *A. mellea* to production of its fruiting bodies on the sawdust medium. However, Kim *et al.* (1992) reported that culture period for producing fruiting bodies of *A. mellea* was ranged from 90 to 100 days. Our results showed that 43 days of culture period in producing fruiting body of *A. mellea* was more reduced as compared with those of Kim *et al.* (1992). The additional 43 days which were taken to produce fruiting bodies in the study of Kim *et al.* (1992) might be due to the large sized mushroom culturing bags or bottles containing a large amount of oak sawdust medium. Whenever a large amount of medium is used to incubate the mushroom culture, the culture period for completely colonizing the media is increased as compared to that of a small amount of medium. In our study, fruiting bodies of *A. mellea* were produced within a small polyethylene plastic bottles (the size of 9 × 13 cm), whereas Kim *et al.* (1992) used a large polyethylene bags (the size of 13 × 30 cm) to produce its fruiting bodies.

In this study, the drastically rapid mycelial growth of *A. mellea* was achieved by adding ground raw carrots to oak sawdust medium. Consequently, the addition of ground raw carrots to oak sawdust medium shortened production period of fruiting body by almost half as compared with previous study by Kim *et al.* (1992). Also, it is known that the addition of ground raw carrots to the medium promoted a primodial formation on oak sawdust media. Therefore, the result obtained from our study can be applied to mass production of other mushrooms as well as *A. mellea*.

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