

## Effects of Hormones on Cultivation of *Tricholoma matsutake* Mycelia

Tokumitsu Okamura, Akiko Mohri, Yasuko Ohfuka and Masahiro Ohsugi

Department of Food Science and Nutrition,  
School of Human Environmental Sciences,

Mukogawa Women's University, 6-46, Ikebiraki-cho, Nishinomiya 663-8558, Japan.

A mixture of callus of *Akamatsu* and mycelium of *Tricholoma matsutake* was obtained from roots of it with MS medium containing  $0.01\mu\text{M}$  of 2, 4-D and  $0.1\mu\text{M}$  of kinetin. Colony, which formed polymer of mycelia of *T. matsutake*, was obtained with MS medium containing  $0.01\mu\text{M}$  of IAA and  $0.01\mu\text{M}$  of kinetin. Therefore, it may be possible to cultivate *T. matsutake* by means of using hormones such as IAA and kinetin on the sawdust medium.

### Introduction

The basidiomycete *Tricholoma matsutake* is a mushroom which has been eaten since old times in Japan. Ogawa and Hamada reported that the fruiting body primordium of *T. matsutake* could be developed by an artificial procedure<sup>1)</sup>. However, it is generally recognized that the artificial cultivation of a perfect fruiting body is very difficult with *T. matsutake*. This report deals with the formation of polymerized mycelia of *T. matsutake*.

### Materials and Methods

#### Separation, Induction and cultivation of callus and mycelium.

Current roots of *Akamatsu* (Japanese red pine) were collected from Tanba-sasayama. After washing with running tap water, the roots were surface-sterilized with 70% ethanol for 30 sec, further sterilized with 1% sodium hypochlorite aqueous containing a few drops Tween 80 for 5 min, and then rinsed three times in sterilized distilled water.

The peeled roots were inoculated Murashige and Skoog medium (MS medium)<sup>2)</sup> solidified by

$0.8\%$  agar, whose pH was adjusted to 5.8, and culture for callus induction at  $25^\circ\text{C}$  under fluorescence illumination of about 2,000 lux (16 hour/day) throughout the period. 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin were added to the culture medium in various concentrations (0, 0.01, 0.1,  $1\mu\text{M}$ ). The induced callus was subcultured in the dark at one-month intervals on the same solid medium as that for callus induction.

Mycelia of *matsutake* were cultured with MS medium containing indole-3-acetic acid (IAA) and kinetin (0, 0.01, 0.1,  $1\mu\text{M}$ ) at  $25^\circ\text{C}$  in the dark.

### Results and Discussion

#### Separation, induction and cultivation of callus and mycelium.

Figure 1 shows the roots of *Akamatsu*, which was obtained from Tanba-sasayama. Mycelium of *T. matsutake* was isolated from the root, as shown in Fig. 2. Mixture of callus of *Akamatsu* and mycelium of *T. matsutake* was obtained with MS medium containing  $0.01\mu\text{M}$  of 2,4-D and  $0.1\mu\text{M}$  of kinetin (Fig. 3(a), (b)).

And then callus of *Akamatsu* and colony of *T. matsutake* were purified from the mixture, as

shown in Fig.3(c)and(d). Figure 4 shows electronic scanning micrographs of the mixture of callus and mycelia.

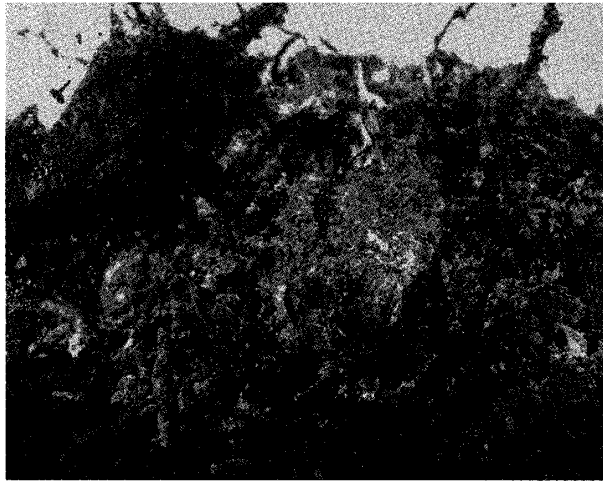


Fig. 1. Roots of *Akamatsu*.

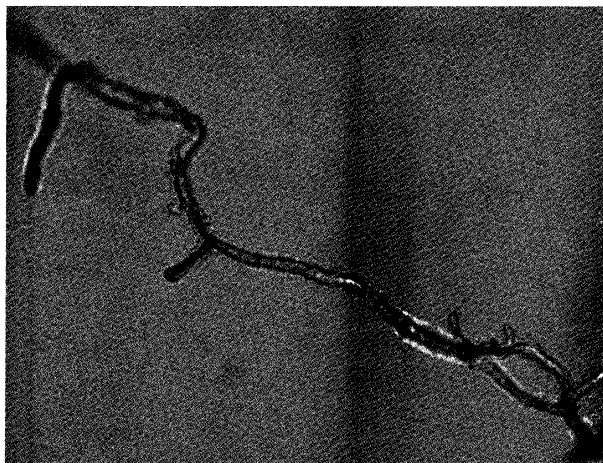


Fig. 2. Mycelium of *T. matsutake*.

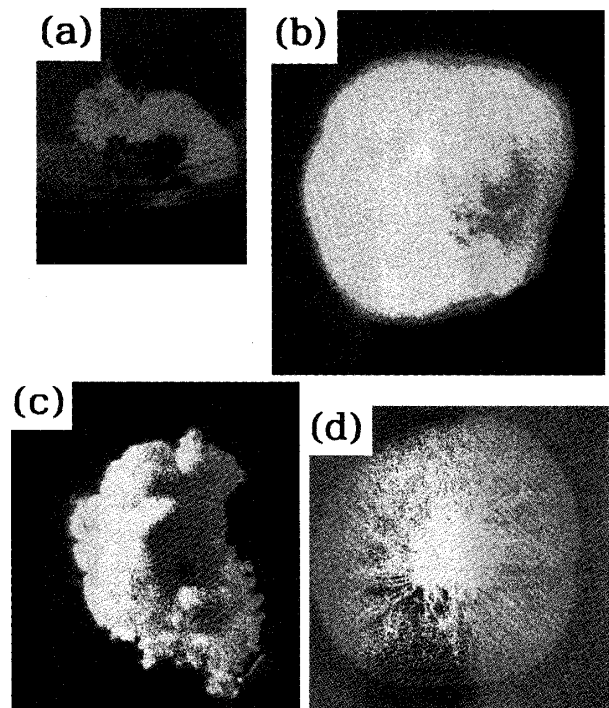


Fig. 3. Callus of *Akamatsu* and colony of *T. matsutake*.

(a) Callus of *Akamatsu* and mycelia of *T. matsutake* (side view).

(b) Callus of *Akamatsu* and mycelia of *T. matsutake* (upside view).

(c) Callus of *Akamatsu*.

(d) Colony of *T. matsutake*.

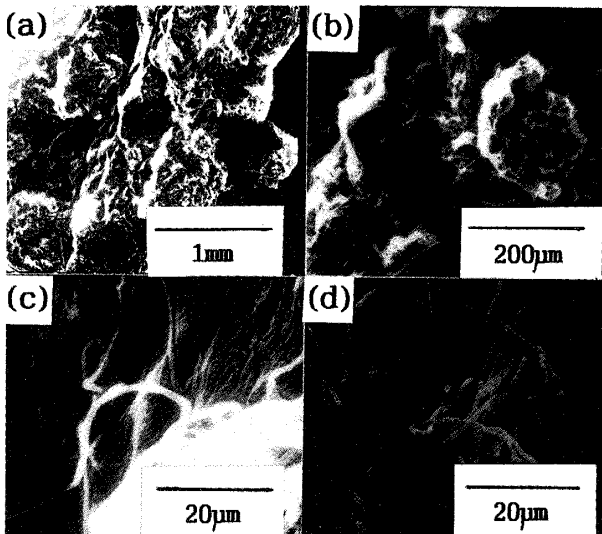


Fig. 4. Electronic scanning micrographs of callus of *Akamatsu* and mycelia of *T. matsutake*(a, b, c, d).

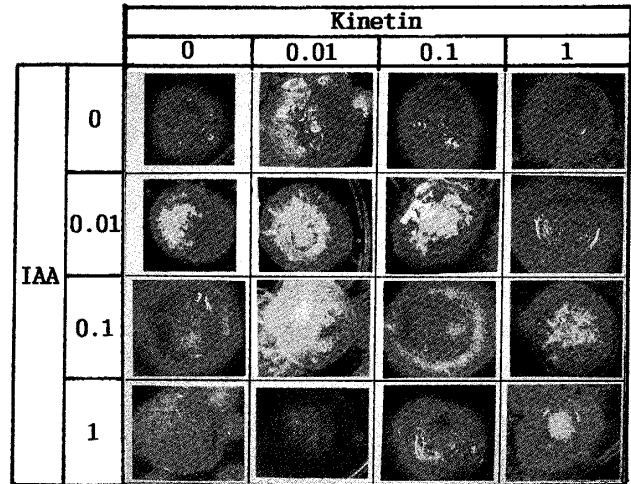


Fig. 5. Effects of IAA and kinetin on the formation of polymerized mycelia of *T. matsutake*.

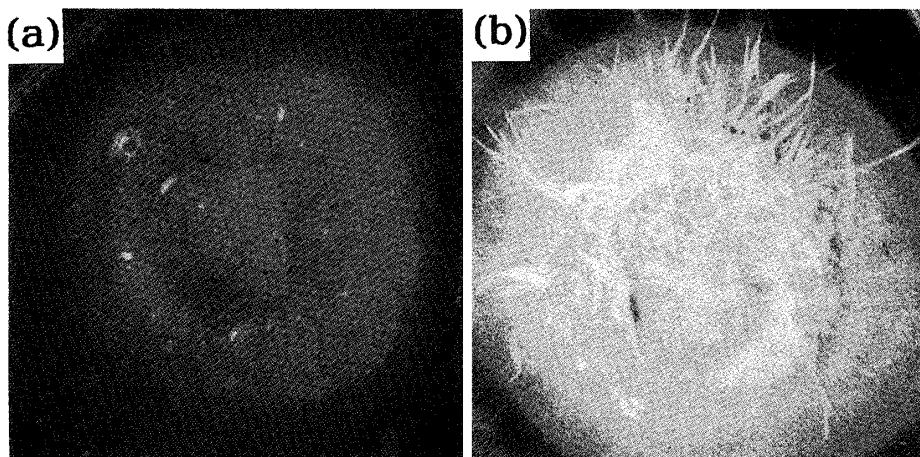


Fig. 6. Formation of polymerized mycelia of *T. matsutake* with hormones.  
 (a) Colony was grown without hormone.  
 (b) Colony was grown with  $0.01\mu\text{M}$  of IAA and  $0.01\mu\text{M}$  of kinetin.

**Effects of IAA and kinetin on the formation of polymerized mycelia of *T. matsutake*.**

Figure 5 shows the effects of IAA and kinetin on the formation of polymerized mycelia of *T. matsutake*. Colony, which formed polymer of mycelia, was obtained with MS medium containing  $0.01\mu\text{M}$  of IAA and  $0.01\mu\text{M}$  of kinetin (Fig.6). On the other hand, polymer of mycelia was not obtained with MS medium without hormone. It is suggested that hormones such as IAA and kinetin promote to polymerize mycelia of *T. matsutake*. Polymer of mycelia, which seems like to be fruit body, was obtained with MS medium containing  $0.01\mu\text{M}$  of IAA and  $0.01\mu\text{M}$  of kinetin. Therefore, it may be possible to cultivate *T. matsutake* by means of using hormones such as IAA and kinetin on the sawdust medium.

**References**

- 1) Ogawa, M. and Hamada, M., *Trans. Mycol. Soc. Jpn.*, **16**, 406(1975).
- 2) Murashige, T. and Skoog, F., *Physiol. Plant.*, **15**, 473-497(1962).