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Note

Growth Promotion of Mycelia of the Matsutake Mushroom *Tricholoma matsutake* by D-Isoleucine

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Mycelial growth of the Matsutake mushroom (*Tricholama matsutake*) was much slower than that of the other mushroom species. We found that the addition of D-isoleucine to the culture medium strikingly promoted mycelia growth. The other amino acids tested had no effect on this growth promotion.

Key words: mycelium; growth promotion; D-isoleucine; Matsutake mushroom; *Tricholoma matsutake*

Matsutake (*Tricholoma matsutake*) is the most popular and expensive mushroom in Japan. However, cultivating fruiting bodies from mycelia of this fungus on an artificial culture medium has proved impossible. There are two main reasons for this difficulty. First, it is generally difficult to produce fruiting bodies from mycorrhizal fungi, and second, the growth of this fungus is much slower than that of other higher fungi.

As the first step of artificially growing the fruiting bodies, the purpose of this study is to find additives to the culture medium which can promote the growth of the mycelia. We added various plants to the culture medium of the fungus and examined the effects of these on the mycelial growth. As a result, we found that Chinese yam (*Dioscorea batatas*, Nagaimo in Japanese) promoted the mycelia growth (Sugawara F. and Tanaka O., unpublished result) and tried to isolate the promoting principle(s). Although isolation of the principle(s) has not yet been completed, a fraction containing isoleucine, leucine and some unidentified substances exhibited activity (data not shown). Therefore, commercially available L- and D-leucine and L- and D-isoleucine were tested.

The strain of *T. matsutake* used in this experiment was TM-01 from the storage strains of IBI Co., Ltd. This strain was first grown on potato dextrose agar (Difco) in Petri dishes of 90 mm internal diameter for 30 days at 25 °C in a dark chamber. The components of the basal medium (PCMY medium) were as follows: 1% Bacto Peptone (Difco), 0.2% Daigo casamino acids (Nihon Pharmaceutical), 1% malt extract (SIGMA), 0.4% yeast extract (Difco), and 0.4% starch wheat (Wako Pure Chemical) had been in distilled water. After the pH of the basal medium in 100-ml Erlenmeyer flasks was adjusted to 6.0 with 1 N HCl, 45 ml of distilled water (control) or D-Isoleucine (Wako Pure Chemical) at various concentrations was added to 40 g of the PCMY medium. These vessels were capped with cotton plugs and autoclaved at 121 °C for 20 min. The pre-incubated mycelia on the Petri dishes were punched by a 10-mm internal diameter cork borer, inoculated at the center of the basal medium in each flask, and incubated in the dark chamber. Growth of the mycelia began after about 20 days of incubation (data not shown). As shown in Fig. 1, D-isoleucine promoted the growth of the fungus at concentrations up to 60 μg/40 g of medium. A remarkable difference in growth depending on the content of the amino acid could be observed after about 35 days of the incubation (Fig. 1A). The effect of the amino acid was evaluated by comparing the diameter of each colony (Table 1). As well as increasing the colony diameter, the colony height was increased by the addition of the amino acid (Fig. 1). The other amino acids, L- and D-leucine and L-isoleucine, did not have any effect on the growth (data not shown). This observation was reproduced by the other strain of *T. matsutake*, ATM30, that was kindly supplied by Mr. Ohta of Shiga Prefecture Forest Technical Center (data not shown).

There are some reports concerning promotion of the growth of *T. matsutake*.[1–3)] Thiamine, nicotinic acid, folic acid, and riboflavin increased the growth of the mycelia, a synergistic effect being found between thiamine and nicotinic acid. Nucleosides or nucleotides were also effective in stimulating growth. Cyclic 3′,5′-AMP stimulated the growth slightly, its effect being enhanced by theophylline, but not by morphol. Phytohormones did not show any special effect.[1)] Acetic,

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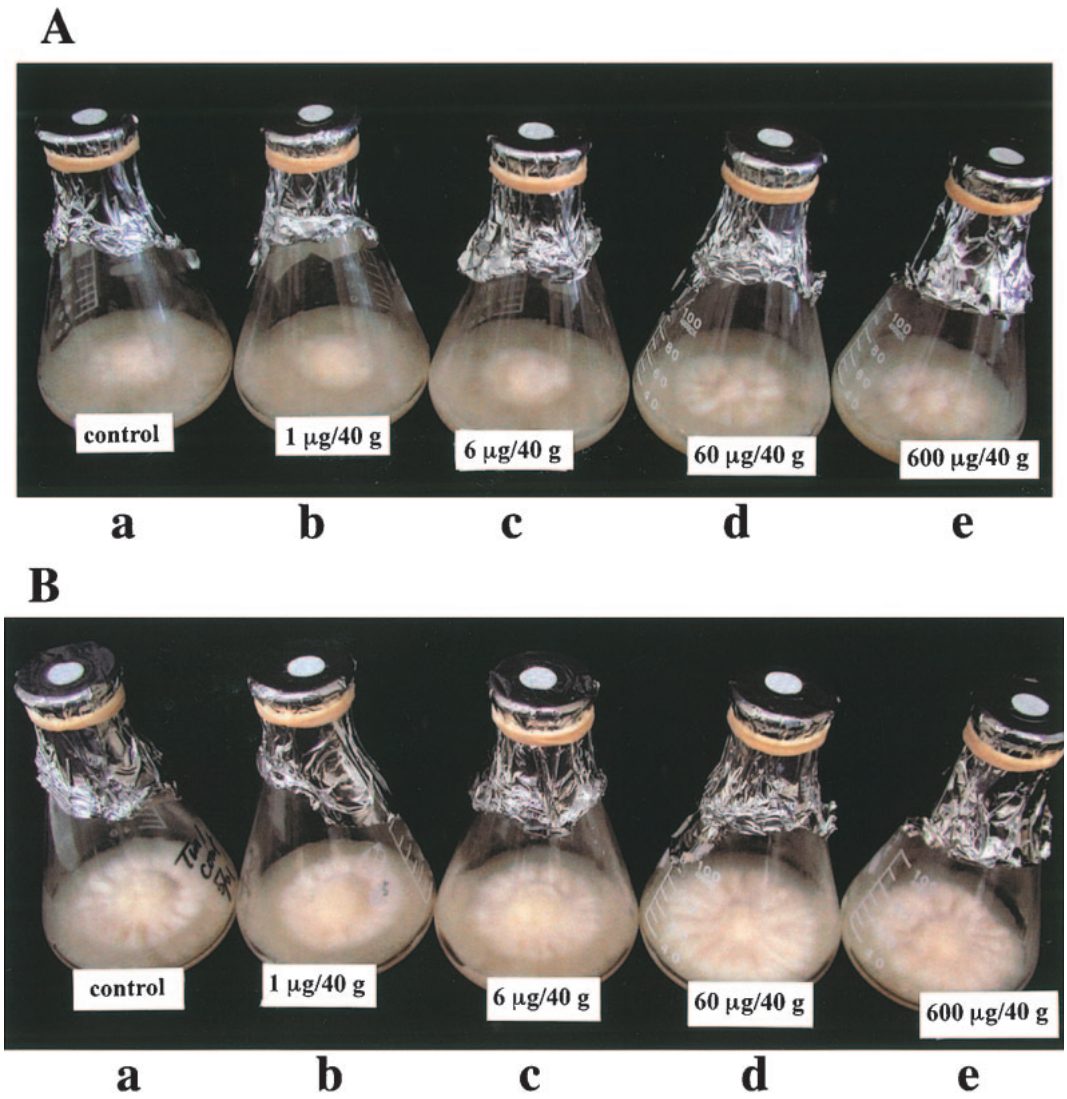

Fig. 1. Effect of D-Isoleucine on the Mycelial Growth of *T. matsutake*.
A, after 35 days of incubation; B, after 50 days of incubation. Control (**a**) contained 40 g of the basal medium; **c** to **e** respectively contained 1, 6, 60, and 600 μg of D-isoleucine in 40 g of the basal medium.

Table 1. Effect of D-Isoleucine on the Mycelial Growth of *T. matsutake*

D-Isoleucine content (μg/40 g of medium)	Diameter of the mycelial colony (mm)*	
	35 days after inoculation	50 days after inoculation
0 (control)	30	45
	31	44
1	29	41
	28	40
6	29	43
	30	42
60	38	51
	39	50
600	36	49
	35	48

* Duplicated results for each condition.

propionic, and *n*-butyric acid induced swelling of the basidiospores, but only *n*-butyric acid induced germination. The germination rate reached 8–23% upon the addition of 0.005% *n*-butyric acid to the agar medium.[2] The addition of surfactants (Tween 80 and Tween 40) to the liquid medium stimulated the mycelial growth. Such growth stimulation was associated with a sharp increase in protein and β-glucosidase excretion by hyphae in the culture filtrate.[3]

D-Isoleucine accelerated the growth of *T. matsutake* hyphae at a very low concentration (60 μg/40 g of medium). This result suggests that the amino acid did not act as a mere nutritional element and might act as a signal for the beginning of growth. This issue remains under investigation. In addition, all isoleucine in the active fraction from Nagaimo was of L form by an HPLC chiral column analysis (data not shown). The isolation of the "real" active principle in the fraction is now in progress.

To the best of our knowledge, this is the first report of growth promotion of fungi by a D-amino acid. This amino acid will become a useful probe for investigating the molecular mechanism for the mycelial growth of *T. matsutake*.

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