THE KOREAN JOURNAL OF MYCOLOGY
Vol. 26, No 3, p361-364 September 1998

Printed in S. KOREA

Effect of Complex Nitrogen Source on Mycelial Growth of *Tricholoma matsutake* DGUM 26001

Eung-Gi Min[1], Kwang-Kyo Chung, and Yeong-Hwan Han*
[1]*Department of Applied Biology, Graduate School, Dongguk University*
**Department of Biology, College of Natural Science, Dongguk University, Kyongju 780-714, Korea*

송이(*Tricholoma matsutake* DGUM 26001) 균사의 생육에 미치는 복합 질소원의 영향

민응기[1] · 정광교 · 한영환*
[1]*동국대학교 대학원 응용생물학과*
**동국대학교 자연과학대학 생물학과*

ABSTRACT: Among the organic sources of nitrogen tested, yeast extract and soytone were excellent for the mycelial growth of *Tricholoma matsutake* DGUM 26001. The mycelial growth was enhanced, when yeast extract at the concentration up to 1.0% was added to the starch-pyridoxine medium. After 30-day cultivation of the mycelia at 24°C in the medium supplemented with yeast extract, 518 mg/50 m*l* of dry mycelia could be harvested.

KEYWORDS: ***Tricholoma matsutake*, Nitrogen source, Mycelia, Culture media**

The *Tricholoma matsutake* has been of importance to be an edible mushroom mainly due to its strong volatile flavors such as 1-octen-3-ol, 2-octanol, 1-octene, and 4-methyl cinnamate (Ahn and Lee, 1986; Ohta, 1983). It is harvested from the communities of several species of needle-leaf plants such as *Pinus, Tsuga, Picea*, and *Abies* (Ogawa, 1976a, 1976b, 1977, 1981; Ogawa and Ohara, 1978). The *T. matsutake* were known as an ectomycorrhizal fungus (Ogawa and Ohara, 1978; Ogawa *et al.*, 1980). Although a lot of studies have been reported mainly on ecology, artificial reproduction, morphology, and genetics (Hwang and Kim, 1995; Iwase *et al.* 1987; Lee, 1991; Lee and Sung 1997; Ogawa, 1976, 1977, 1981; Song and Min, 1991; Ito, 1981; Kawai and Ogawa, 1977; Lee *et al.* 1984; Ogawa and Hamada, 1975; Ogawa *et al.* 1978; Okazawa, 1978; Ryoo *et al.* 1980; Shimazono, 1979; Yokoyama and Yamada, 1987), little information has been available except for culture conditions of mycelia (Lee *et al.* 1997; Ohta, 1990). In order to produce higher mycelial mass of *T. matsutake* in a limited culture time, sources and concentration of organic nitrogen as a source of nitrogen were determined in this study.

The complex nitrogen sources for preparing media such as yeast extract, malt extract and peptone etc. were obtained from Difco Co. The isolated *T. matsutake* DGUM 26001, whose morphological and cultural characteristics for carbon and vitamin sources previously mentioned (Lee *et al.* 1997), was used. The mycelia grown on the *Tricholoma matsutake* agar medium (composition of TMM: 2.0% of glucose, 0.15% of soytone, and 0.15% yeast extract, and 1.5% of agar, pH 5.2) were collected by using a cork borer (dia, 8

*Corresponding author

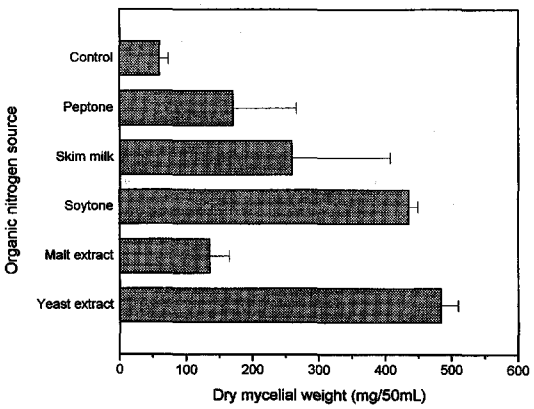

Fig. 1. Effect of organic nitrogen source on mycelial growth in *Tricholoma matsutake* DGUM 26001. The mycelia were cultivated at 24°C for 30 days with shaking (120 rpm). The organic nitrogen (0.3%) was added to 50 m*l* of starch-pyridoxine medium (pH 5.2) in 250 m*l* flask.

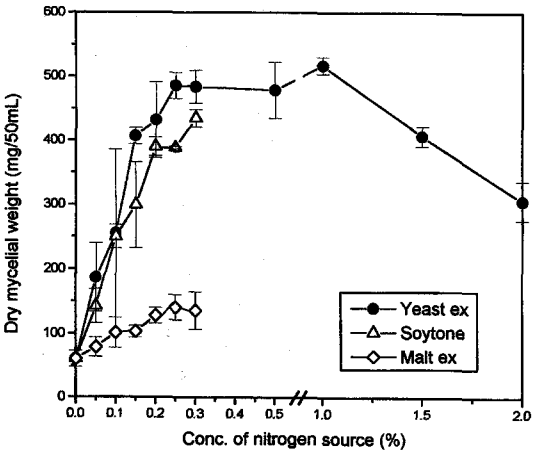

Fig. 2. Effect of organic nitrogen source on mycelial growth in *Tricholoma matsutake* DGUM 26001. The mycelia were cultivated for 30 days at 24°C with shaking (120 rpm).

mm) and inoculated into 100 m*l* of TMM broth in 250 m*l* flask. Then, they were cultivated at 24°C for 30 days with shaking (120 rpm). The mycelia were homogenized with a homogenizer (Braun Co., model MR-500-MCA) and then inoculated. Determination of mycelial growth was performed by using the method (Lee *et al.* 1997). After 30 day-cultivation at 24°C in 50 m*l* of 250 m*l* flask, the culture broth was filtered with a filter paper (Toyo No. 2), washed 3 times with distilled water and then, the mycelia were dried for 24 h at 105°C. The dry weight of mycelia was determined by subtracting the dry weight of a filter paper from the total dry weight. In order to determine the effect of organic nitrogens on mycelial growth, various sources of organic nitrogen were supplemented to starch-pyridoxine broth (2.0% of starch supplemented with 0.01 mg/m*l* of pyridoxine). Pyridoxine was added to the medium by using the method of membrane filtration (pore size, 0.2 um).

Results and Discussion

In order to determine organic nitrogen source for enhanced mycelial growth of *T. matsutake* DGUM 26001, 0.3% of various sorts of organic nitrogen were added to starch-pyridoxine medium (composition: 2.0% of soluble starch, 0.01 mg/m*l* of pyridoxine). Yeast extract and soytone were excellent sources of organic nitrogen and skim milk and peptone were very effective. However, a comparatively little mycelial weight was produced with malt extract. After 30-day cultivation with 0.3% of yeast extract as a nitrogen source, the dry weight of mycelia was 480 mg/50 m*l*. The dry mycelial weight of *T. matsutake* DGUM 26001 obtained in this study was very excellent, compared with that of ca. 424 mg/50 m*l* (Lee *et al.* 1997) with TMM medium and that of ca. 4.5 mg/50 m*l* in *T. matsutake* tm3 and tm30 (Ohta, 1990) after 15 day-cultivation in the *synthetic* medium. When yeast extract, soytone and malt extract were used, up to 0.3% of each organic nitrogen source enhanced the mycelial growth. Although the mycelial growth was enhanced with yeast extract at concentrations upto 1.0%, it was declined at concentrations over 1.0%.

적 요

시험한 유기 질소원 중 yeast extract와 soytone이 *Tricholoma matsutake* DGUM 26001의 균사생장에 가장 우수한 질소원이었다. Yeast extract를 starch-pyridoxine 배지에 1.0% 농도까지 첨가하였을 때, 균사생장이 증가하였다. 24°C에서 30일간 yeast extract가 첨가된 배지에서 배양하였을 때, 50 m*l* 배양액에서 518 mg의 건조 균사체를 얻을 수 있었다.

References

Ahn, J. S. and Lee, K. H. 1986. Studies on the volatile aroma components of edible mushroom (*Tricholoma matsutake*) of Korea. *J. Kor. Soc. Food Nutr.* **15**(3): 253-257.

Hwang, S. K. and Kim, J.-G. 1995. Nucleotide sequence analysis of the 5S ribosomal RNA gene of the mushroom *Tricholoma matsutake*. *J. Microbiol.* **33**(2): 136-141.

Ito, T. 1981. The present status of matsutake production technique in Japan. *Kor. J. Mycol.* **9**(4): 219-222.

Iwase, K., Matsui, S., Taniguchi, T. and Obayashi, A. 1987. Behavior of cell nuclei during basidiospore formation in *Tricholoma matsutake*. *Trans. Mycol. Soc., Japan* **28**: 63-67.

Kawai, M. and Ogawa, M. 1977. Studies on the artificial reproduction of *Tricholoma matsutake* (S. Ito et Imai) Sing. V. Effects of some chemicals on the growth of mycorrhizal and non-mycorrhizal soil fungi. *Trans. Mycol. Soc., Japan* **18**: 391-398.

Ko, H. G., Kim, D.-M. and Park, W.-M. 1997. Composition of a new medium for mycelial growth of *Hericium erinaceus*. *Kor. J. Mycol.* **25**: 369-376.

Lee, C. Y., Hong, O.-P., Jung, M.-J. and Han, Y.-H. 1997. Effect of carbon sources and vitamins on mycelial growth of *Tricholoma matsutake* DGUM 26001. *Kor. J. Mycol.* **25**: 226-232.

Lee, S. S. 1991. Biology of *Tricholoma matsutake* found at *Pinus densiflora* communities in the areas of Kyoung Sang Do. *Kor. J. Mycol.* **19**: 203-213.

Lee, S. S. and Sung, C.-K. 1997. The mycelia isolated from the basidiocarps of *Tricholoma matsutake* in Korea. *Kor. J. Mycol.* **25**(2): 121-129.

Lee, T. S., Park, C. J., Shim, W. S., Kim, S. H., Ju, Y. W., Oh, S. W. and Jo, J. M. 1984. Studies on the artificial cultivation and propagation of pine mushroom (II)-Increasing yield and promoting quality of pine mushroom by the cap-covering or soil-covering-. *For. Rep. For. Res. Inst. Korea* **31**: 124-132.

Ogawa, M. 1976a. Microbial ecology of 'Shiro' in *Tricholoma matsutake* (S. Ito et Imai) Sing. and its allied species. II. *Tricholoma matsutake* in *Pinus pumila* var. *yezoalpina* forest. *Trans. Mycol. Soc., Japan* **17**: 176-187.

Ogawa, M. 1976b. Microbial ecology of 'Shiro' in *Tricholoma matsutake* (S. Ito et Imai) Sing. and its allied species. III. *Tricholoma matsutake* in *Picea glehnii* and *Picea glehnii-Abies sachalinensis* forests. *Trans. Mycol. Soc., Japan* **17**: 188-198.

Ogawa, M. 1977. Microbial ecology of 'Shiro' in *Tricholoma matsutake* (S. Ito et Imai) Sing. and its allied species. IV. *Tricholoma matsutake* in *Tsuga diversifolia* forests. *Trans. Mycol. Soc., Japan* **18**: 20-33.

Ogawa, M. 1981. Mycorrhiza in the pine forest-the ecological study of matsutake as a microorganism. *Kor. J. Mycol.* **9**(4): 225-227.

Ogawa, M. and Hamada, M. 1975. Primordia formation of *Tricholoma matsutake* (S. Ito et Imai) Sing. in pure culture. *Trans. Mycol. Soc., Japan* **16**: 406-415.

Ogawa, M. and Ohara, H. 1978. Microbial ecology of 'Shiro' in *Tricholoma matsutake* (S. Ito et Imai) Sing. and its allied species. VIII. *Tricholoma bakamatsutake* Hongo in *Quercus mongoloca* var. *grosserrata* forest and *Q. serrata* forests. *Trans. Mycol. Soc., Japan* **19**: 391-405.

Ogawa, M., Takeo, U., Shuji, K. and Kisoo, Y. 1978. Cultivation method of the mycorrhizal fungus, *Tricholoma matsutake* (S. Ito et Imai) Sing. (I) Growing method of the pine saplings infected with *T. matsutake* in the field. *Japan For. Soc.* **60**: 119-128.

Ogawa. M., Ito, T., Kobayashi, T. F. and Fujita, H. 1980. On the primary stage in 'Shiro' formation of *Tricholoma matsutake*. *Trans. Mycol. Soc., Japan* **19**: 391-405.

Ohta, A. 1983. Quantitative analysis of odorous

compounds in the fruitbodies of *Tricholoma matsutake*. *Trans. Mycol. Soc., Japan* **24**: 185-190.

Ohta, A. 1990. A new medium for mycelial growth of mycorrhizal fungi. *Trans. Mycol. Soc., Japan* **31**: 323-334.

Okazawa, K. 1978. Fruit-bodies of *Tricholoma matsutake* appeared on a pot of dwarfed pines. *Trans. Mycol. Soc., Japan* **19**: 87-89.

Ryoo, C. I., Nam, S. U., Lee, J. Y. and Lee, S. K. 1980. A study on multiplication of *Tricholoma matsutake*. *Kor. J. Mycol.* **8**(1): 7-12.

Shimazono, H. 1979. Comparative studies on morphological characteristics of the colonies of *Tricholoma matsutake, T. fulvocastaneum*, and *T. bacamatsutake* on agar media. *Trans. Mycol. Soc., Japan* **20**: 176-184.

Song, H. S. and Min, K. H. 1991. Microfungal flora of *Tricholoma matsutake* producing and nonproducing sites in the forest of *Pinus densiflora*. *Kor. J. Mycol.* **19**(2): 109-119.

Yokoyama, R. and Yamada, T. 1987. In vitro cultures of *Tricholoma matsutake* and *Pinus densiflora*. *Trans. Mycol. Soc., Japan* **28**: 331-338.