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Effect of Carbon sources and Vitamins on Mycelial Growth of *Tricholoma matsutake* DGUM 26001

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송이균사(*Tricholoma matsutake* DGUM 26001)의 생육에 미치는 탄소원 및 비타민의 영향

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ABSTRACT: The mycelium was isolated from the fruiting body of *Tricholoma matsutake* collected from Mt. Namsan, Kyongju and it was named as *Tricholoma matsutake* DGUM 26001. For the mycelial growth of *T. matsutake* DGUM 26001, the complex media, yeast-malt extract medium and Czapek-Dox medium supplemented with yeast extract, were excellent. The media such as nutrient glucose medium, mushroom complex medium, and *Tricholoma matsutake* medium (TMM), were effective. However, There was no a mycelial growth in the media used for bacterial cultivation such as colombia medium, brain heart infusion medium, Luria-Bertani medium supplemented with glucose, and brucella medium. When carbohydrate as a carbon and energy source was supplemented in the TMM medium for the mycelial growth, starch as a polysaccharide was best. As a disaccharide, trehalose and maltose were excellent. Sorbitol, xylitol and glucose were excellent carbon sources of monosaccharose. When the mycelia were cultivated for 30 days at 24°C in the TMM supplemented with 2.0% starch, the dry weight of the mycelia harvested was 8.85 g/L. When organic acid was given as a carbon source, only succinic acid was utilized. As a vitamin source, coconut water and pyridoxine were excellent. After 30 day-cultivation in the TMM medium, the dry weights with coconut water and pyridoxine were 8.65 and 8.32 g/L, respectively.

KEYWORDS: ***Tricholoma matsutake*, Mycelia, Media, Carbon source, Vitamin**

The pine-mushroom, fruiting body of *Tricholoma matsutake*, has been known as a king of mushrooms in Eastern asia and usually 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).

Although its demand increased greatly due to the volatile strong flavor, little information have been known about the cultural characteristics of *T. matsutake* in Korea. The mycelia of *T. matsutake* were reported to be closely associated with the roots of *P. densiflora* as termed "ectomycorrhiza" (Ogawa and Ohara, 1978; Ogawa *et al.*, 1980). So far, many studies of *T. matsutake* have been focused mainly on ecology (Iwase *et al.* 1991; Kang *et al.* 1989; Lee, 1991; Ogawa, 1976, 1977, 1981; Song and Min, 1991) and on artificial reproduction (Ito, 1981; Kawai and

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Ogawa, 1976, 1977; Lee *et al.* 1984; Ogawa and Hamada, 1975; Ogawa and Kawai, 1976; Ogawa *et al.* 1978; Okazawa, 1978; Ryoo *et al.* 1980; Yokoyama and Yamada, 1987). However, a few article has been reported on genetics (Hwang and Kim, 1995; Iwase *et al.* 1987; Lee and Sung, 1997), storage (Cho *et al.* 1984), morphology (Shimazono, 1979) and flavors (Ahn and Lee, 1986; Ohta, 1983, Tsuruta and Kawai, 1979).

As well known, slow growth and little production of the mycelial mass hinder the application of mycelia to agricultural and industrial uses. Although there were a few reports on effects of physicochemical culture conditions such as pH, temperature and vitamins (Ohta, 1986a, 1986b, 1988, 1990), no article referring to the other carbon source except glucose was shown. In order to shorten cultivation time and to enhance the mycelial mass production of *T. matsutake*, the effect of carbon source on mycelial growth was investigated in this experiment.

Materials and Methods

Chemicals and media

The chemicals used in this experiment were purchased from Sigma Chemical Co. The compounds for preparation of complex media such as yeast extract, malt extract and peptone etc. were obtained from Difco Co.

Isolation and identification

The agar plate of *Tricholoma matsutake*

Table 1. Composition of complex media used in this study

Compound	Composition (g/L) of complex medium									
	TMM	MMM	MCM	MLM	YMM	CDY	NGM	GPM	LBG	PM
Peptone			2.0		5.0		5.0			
Yeast extract	1.5		2.0		3.0	1.0		3.0	5.0	
Malt extract					3.0					
Soytone	1.5									
Beef extract							3.0			
Tryptone									10.0	
Glucose	20	20	20	10	10	30	20	30	20	15
$CaCO_3$										0.314
$NaNO_3$						3.0				
K_2HPO_4		1.0	1.0	1.5		1.0		10.0		1.584
KH_2PO_4		0.46	0.46							
$MgSO_4$		0.5	0.5	2.0		0.5		0.7		8.138
KCl						0.5				
NaCl									10.0	
$FeSO_4$				0.02		0.01				0.02
$ZnSO_4$				0.02						0.03
$MnSO_4$				0.02						0.02
$CaCl_2$				0.33						
$(NH_4)_2SO_4$										3.06
Serine				2.0						
Thimine				0.001						
Asparagine		2.0								
Arginine										3.484

*Commercially available brucella medium (BM), colombia medium (CM), and brain heart infusion medium (BHI) were obtained from Difco Co.

medium (TMM) containing antibiotics (50 μg/ml of streptomycin, 60 μg/ml of ampicillin) was used to isolate the mycelia in fruiting body of *T. matsutake* collected from Mt. Namsan, Kyongju (Table 1, Fig. 1) and identified based on morphology of the colony and mycelia comparison with those of strains (FRI 91010, FRI 91024) of Forest Research Institute in Seoul.

Inoculation and cultivation

The mycelia grown on the TMM agar plate were collected by using a cork borer (dia, 8 mm) and inoculated into 100 ml of TMM broth in 250-ml flask. Then, they were cultivated for 30 days at 24°C with shaking (120 rpm). For mycelial growth, the mycelia were homogenized with a homogenizer (Braun Co., model MR-500-MCA) and then inoculated to the TMM broth.

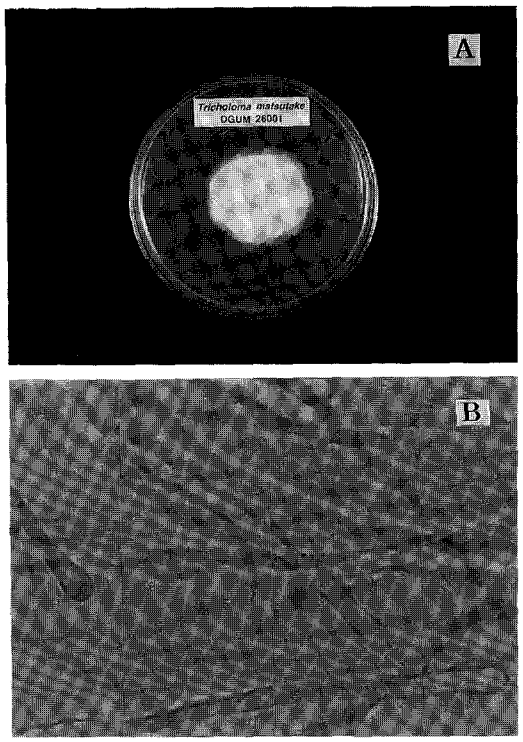

Fig. 1. Morphology for colony (A) and for mycelia (B) in *Tricholoma matsutake* DGUM 26001.

Determination of mycelial growth

After 30 day-cultivation, the culture broth was filtered with a filter paper (Toyo No. 2) and washed 3 times with distilled water. 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.

Complex media

The composition of complex media used for a mycelial growth are listed in Table 1 and they are mushroom minimal medium (MMM), mushroom complex medium (MCM), glucose-peptone medium (GPM), Macaya-Lizano medium (MLM), yeast-malt extract media (YMM), *Tricholoma matsutake* medium (TMM), Czapek-Dox yeast extract medium (CDY), Park medium (PM), nutrient glucose medium (NGM), Luria-Bertani glucose medium (LBG), brucella medium (BM), columbia medium (CM), and brain heart infusion medium (BHI). The pH of medium was adjusted to 5.2 with 1.0 N HCl.

Sources of carbon and vitamin

To determine the effect of carbohydrates on mycelial growth, various sources of carbohydrate and organic acid were supplemented to the TMM broth at the final concentration of 2.0%. Using a membrane filter (pore size, 0.2 μm), various sources of vitamin were amended to the TMM broth at the final concentration of 0.01 mg/ml except for 1.0% (v/v) of coconut water.

Results and Discussion

Isolation and identification

By using the method of Terashita and Kono (1989), the mycelia of *T. matsutake* was isolated from lamella of the fruiting body of *T. matsutake* collected from Mt. Namsan, Kyongju in October, 1996. According to the

morphology of colony on agar plate and that of mycelia in broth, it was identified and named as *Tricholoma matsutake* DGUM 26001 (Fig. 1). The morphology of colony on the TMM agar plate was similar to that of *T. matsutake* reported by Shimazono (1979) and Lee & Sung (1997). The mycelia of *T. matsutake* DGUM 26001 formed white thick uphead colony with deep radial wrinkled hollows and coarse subulate bundles of aerial hyphae. On the agar plate, semi-transparent mycelia were shown in the edge of colony and compact substratum mycelia were abundant. The septa of mycelia were rarely found, whereas no clamp connection was shown. These characteristics of morphology were similar to those of *T. matsutake* strains (FRI 91010, FRI 91024) obtained from Forest Research Institute in Seoul. The mycelial culture on agar plate and in broth smells the typical flavor of 1-octene-3-ol, which is usually known to be the major flavor from fruiting body of *T. matsutake* (Ahn and Lee, 1986; Ohta, 1983).

Complex media for mycelial growth

When the mycelia were cultivated for 30 days at 24°C, the mycelial growth in YM and CDY (Czapek-Dox medium supplemented with 0.1% of yeast extract) were excellent (Fig. 2). The media of NGM, MCM, and TMM were comparatively effective. There was no mycelial growth in the media for bacterial growth such as CB, BHI, LBG, and BB medium. Moreover, there was no mycelial growth in MMM medium (minimal mushroom medium contains carbon and mineral sources plus asparagine as a nitrogen source). There was no mycelial growth in the CD medium, which consist of carbon, inorganic nitrogen and minerals. When organic nitrogen sources of yeast extract, malt extract and soytone were supplemented, the mycelial growth was enhanced. These results suggest that sources of nitrogen and vitamin in yeast extract, malt extract and soytone might be very important for enhancing the mycelial growth of *T. matsutake* DGUM 26001. The sources and the concentrations of nitrogen sources will be further studied to optimize the composition of media.

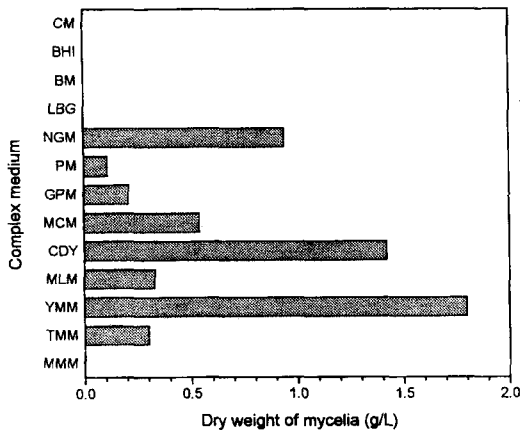

Fig. 2. Effect of complex media on mycelial growth in *Tricholoma matsutake* DGUM 26001. Inoculum size was 1.0%.

Effect of carbon source on mycelial growth

The TMM medium was used to determine the effect of carbon source on mycelial growth, because the composition of TMM medium was comparatively simple and showed relatively high mycelial production. Glucose as a carbon source in the TMM medium was replaced with various carbon sources such as monosaccharose, disaccharide, and polysaccharide. The mycelia were cultivated for 30 days at 24°C and then the mycelial dry weight was measured. As shown in Fig. 3, starch as a polysaccharide was best for mycelial growth because mycelial dry weight recorded 8.85 g/L in the TMM medium supplemented with starch. Also, trehalose and maltose as a disaccharide were excellent. Among monosaccharoses tested, sorbitol, xylitol, and glucose were very good carbon sources. When the carbon source with higher

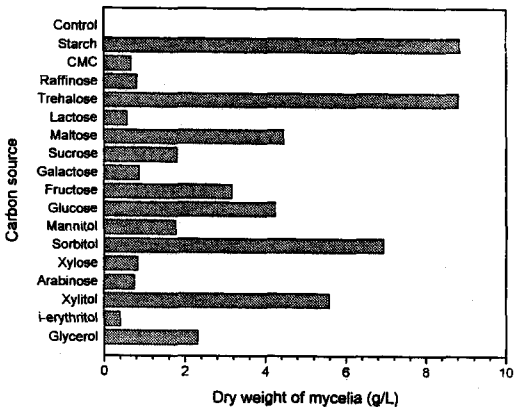

Fig. 3. Effect of carbon source on mycelial growth in *Tricholoma matsutake* DGUM 26001. The mycelia were cultivated for 30 days at 24°C with shaking (120 rpm). Glucose in the TMM was replaced by various carbon sources. The final concentration of carbon source was 2.0% (w/v). Inoculum size was 10%.

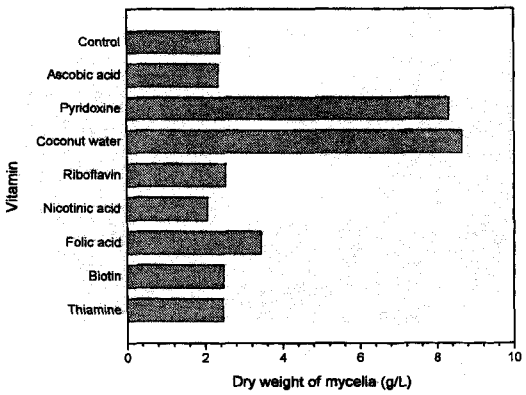

Fig. 4. Effect of vitamin on mycelial growth in *Tricholoma matsutake* DGUM 26001. The mycelia were cultivated for 30 days at 24°C in the TMM broth (pH 5.2) with shaking (120 rpm). The final concentration of vitamin was 0.01 mg/ml except 1.0% (v/v) of coconut water. Inoculum size was 10%.

mycelial production was used, the pellet was very small in size and compact in shape.

Effect of vitamin on mycelial growth

When various vitamins were added to the TMM medium, coconut water and pyridoxine were very excellent for a mycelial growth (Fig. 4). After 30 day-cultivation, the dry weights of mycelia for coconut water and pyridoxine were 8.65 and 8.32 g/L, respectively. The mycelial dry weights with vitamins were about two times larger than those without ones. Other vitamins used in this experiment showed average 2.0 to 3.5 g/L of mycelial dry weight. For optimization of media composition, the concentration of vitamin applied will be further studied.

적 요

경주시 남산으로부터 송이를 채집하여 균사를 분리하였다. 집락 및 균사의 형태, 균사의 독특한 송이향(1-octene-3-ol)을 기준으로 하여 *Tricholoma matsutake* DGUM 26001이라 명명하였다. *T. matsutake* DGUM 26001은 사용한 복합액체배지 중 yeast malt extract 및 Czapek-Dox 배지에서 가장 우수한 균사생육을 보여주었으며, nutrient glucose 배지, Macaya-Lizano 배지, 및 *Tricholoma matsutake* 배지(TMM)에서의 균사생육은 양호하였다. 세균 배양에 사용되는 colombia 배지, brain heart infusion 배지, Luria-Bertani glucose 배지 및 brucella 배지에서의 균사 생육은 매우 저조하였다. TMM 배지에 다양한 탄수화물을 탄소원으로 첨가하였을 때, 다당류의 starch, 이당류의 trehalose 및 maltose, 단당류의 sorbitol, xylitol 및 glucose를 사용하였을 때 균사생육이 양호하였다. Starch를 탄소원으로 하는 TMM 배지에서 30일간 균사를 배양하였을 경우, 8.85 g/L의 건조 균사중량을 얻을 수 있었다. 포도당을 탄소원으로 하는 TMM 액체배지에 coconut water 및 pyridoxine을 비타민으로 첨가하여 30일간 배양 후, 가장 우수한 균사 건조체를 얻을 수 있었으며 각각 8.65 및 8.32 g/L의 균사 건조량을 얻을 수 있었다.

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