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# The Cultural Conditions Affecting the Mycelial Growth of *Grifola umbellata*

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# 저령의 균사생장에 영향을 미치는 배양조건

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**ABSTRACT: This study was carried out to obtain the basic data for artificial culture of *Grifola umbellata*. The optimal condition for the mycelial growth was obtained at 20°C and pH 4, respectively. *G. umbellata* showed the most favorable growth on the Hoppkins media. Carbon sources such as glucose, fluctose and manitol were favorable for stimulating a mycelial growth of *G. umbellata*. Valine, one of nitrogen sources also appeared to be favorable to a mycelial growth. The optimum C/N ratio was about 30:1 in case that 1% glucose as carbon source was mixed with the basal media. Lactic acid as organic acid was most favorable to the mycelial growth. Also, thiamine-Hcl as vitamin source was favorable. The mineral nutrient of $FeSO_4$ or $MgSO_4$ was most favorable to *G. umbellata*, and its optimal concentration was about 0.01% in $FeSO_4$ and 0.1% in $MgSO_4$, respectively. Among 4 different cereal extract media, polished rice extract medium which was mixed with silkworm pupae was most suitable for a favorable growth of *G. umbellata*.**

**KEYWORDS: *Grifola umbellata*, Optimal condition, Mycelial growth, Optimal concentration**

*Grifola umbellata*, one of edible fungi belongs to *Polyporaceae* of *Basidiomycetes* and is termed officially as *Polyporus umbellatus* or *Dendropolyporus umbellatus* in the other scientific name (Lee, 1988; Liu, 1978). The fruiting bodies of *G. umbellata* have been used as Chinese medicines for curing human diseases such as inflammation of the liver for a long time (Liu, 1978). Nowadays, the substances of polysaccharide such as β-glucan which was isolated from fruiting bodies of *G. umbellata* have been reported to exhibit outstanding anti-tumor effect for curing human diseases such as lung cancer, garistic cancer and cervical cancer (Sato *et al.*, 1984; Lee, 1986). Though *G. umbellata* has been considered as one of the most promising edible fungi in the global market, one of unsolved problems may remain in the difficulty of its mass production capable of corresponding to the demand of many users. Therefore, this study was carried out to find the possibility

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for an artificial cultivation of *G. umbellata* and obtain basic data for its mass production. This is the first report referring to cultural characteristics of *G. umbellata*.

## Materials and Methods

### Cultures

The strain of *G. umbellata* has been maintained at the laboratory of microbiology in the Department of Applied Biology, Dongguk University. To prepare a lot of samples which could facilitate various tests, *G. umbellata* was transferred to PDA agar plate and was incubated at 25°C untill it exhibited a full growth in the dark condition. Unless otherwise stated, all the tests which the strain was used were performed at least twice (Chang *et al.*, 1995; Chi *et al.*, 1996).

### Cultural conditions

**Effect of pH** PDA medium was used to screen pH value suitable for a favorable growth of *G. umbellata*. A 5 mm diameter plug of an inoculum was removed with cork borer from 15 days old cultures of *G. umbellata* grown on PDA medium, placed in the center of each agar plate of sterile PDA medium adjusted to the range of pH 4~9 with 1 N NaOH or HcL, and incubated for 15 days at 25°C (Chi *et al.*, 1996). The measurement of mycelial growth was performed according to the method described by Chi *et al.* (1996).

**Effect of the temperatures** To screen the temperature suitable for stimulating a favorable growth of *G. umbellata*, potato dextrose broth medium was used. A 5 mm diameter plug of an inoculum was inoculated into 50 m*l* of sterile PD broth medium and incubated for 15 days at 15°C, 20°C, 25°C and 30°C, respectively. The inocula submerged were filtrated through filter paper (Whatman No 2, dia., 9 cm), dried for 24 hours at 80°C and weighed in the balance (Chi *et al.*, 1996).

### Screening of favorable culture media

Fourteen different culture media were used to investigate a favorable growth of *G. umbellata*, and adjusted to pH 6.0 before a high-pressure sterilization (Table 1). After a total of 14 different culture media were autoclaved for 15 minutes at 121°C (15 psi pressure), 20 ml of each agar solution melted was aseptically poured into a petri-dish. A 5 mm diameter plug of an inoculum was removed with cork borer from 15 days old cultures of *G. umbellata* grown on PDA medium (potato 200 g, Dextrose 20 g and agar 20 g/distilled water 1 L), placed in the center of each agar plate of 14 diffferent culture media and incubated for 15 days at 25°C (Chi *et al.*, 1996).

After 15 days of incubation, the mycelial growth and density were observed on 14 different culture media.

### Screening of favorable nutrient sources

**Effect of carbon sources** To screen favorable carbon source capable of stimulating mycelial growth of *G. umbellata*, the basal medium used was composed of $MgSO_4$ 0.05 g, $KH_2PO_4$ 0.46 g, $K_2HPO_4$ 1.0 g, Thiamine-Hcl 120 μg, agar 20 g, and distilled water 1 L (Sung *et al.*, 1993). Based on each molecular weight of 19 different carbon sources including glucose, each carbon source was added to the basal medium at the concentration of 0.1 M per 1 L and mixed throughly (Chi *et al.*, 1996). The basal medium was adjusted to pH 6.0 before high-pressure sterilization, and autoclaved for 15 minutes at 121°C (15 psi pressure). With the basal medium containing each carbon source, all the other processes including the inoculation, incubation and measurement of mycelial density of *G. umbellata* were performed according to the method described by Chi *et al.* (1996).

**Effect of nitrogen sources** Except for the addition of 2% glucose as carbon source per 1 L of the medium, the basal medium which was

**Table 1.** Composition of the media used in this experiment

| | Media and composion (g/l) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hamada | Czapekdox | YM | Hennerberg | Lilly | Modified Lutz | Hopkins | PDA | Glucose Peptone | Glucose triptone | Mushroom complete | Ebiose | PD (M) |
| Dextrose | 10 | | 10 | | | | | 20 | | | | | 20 |
| Ebiose | 5 | | | | | | | | | | | 5 | |
| Yeast ext. | 3 | | 3 | | | | | | 10 | 3 | 2 | | |
| Hyponex | 3 | | | | | | | | | | | | |
| Sucrose | | 30 | | | | | | | | | | | |
| Peptone | | | 5 | | | | | | 10 | | 2 | | |
| Malt ext. | | | 3 | | | 10 | | | 15 | | | | 5 |
| Glucose | | | | 50 | | | 10 | | 10 | 5 | 20 | | |
| Maltose | | | | | 10 | | | | | | | | |
| Asparagine | | | | | 2 | | | | | | | | |
| Potatoes | | | | | | | | 200 | | | | | 200 |
| Tryptone | | | | | | | | | | 10 | | | |
| $NaNO_3$ | | 3 | | 2 | | | | | | | | | |
| $K_2HPO_4$ | | 1 | | | | 1 | | | | | 1 | | |
| $MgSO_4$ | | 0.5 | | 0.5 | 0.5 | 0.1 | 0.5 | | | | 0.5 | | |
| KcL | | 0.5 | | | | | | | | | | | |
| $FeSO_4$ | | 0.01 | | | | | | | | | | | |
| $CaCl_2$ | | | | 0.1 | | | | | | | | | |
| $KH_2PO_4$ | | | | 1 | 1 | | 0.1 | | | | 0.5 | | |
| $KNO_3$ | | | | 2 | | | 2 | | | | | | |
| $NH_4NO_3$ | | | | | | 1 | | | | | | | |
| $(NH_4)_2HPO_4$ | | | | | | 1 | | | | | | | |
| $MnSO_4$ | | | | | | 0.025 | | | | | | | |

used to screen nitrogen source suitable for stimulating mycelial growth of *G. umbellata* was made of the same additives as those described by Sung *et al.* (1993). Based on each molecular weight of 17 different nitrogen sources including alanine, each nitrogen source was added to the basal medium at the concentration of 0.02 M (Park, *et al.* 1995; Chi *et al.*, 1996). The basal medium was adjusted to pH 6.0 before high-pressure sterilization, and autoclaved for 15 minutes at 121°C (15 psi pressure). With the basal medium containing each nitorgen source, all the other processes including the inoculation, incubation and measurement of mycelial density of *G. umbellata* were performed according to the method described by Chi *et al.* (1996).

**Effect of C/N ratio** The basal media which D-glucose as carbon source was mixed at the rate of 1, 2, 3 and 4% (w/v) were continually added with $NaNO_3$ as nitrogen source. Finally, the ratios of $NaNO_3$ versus D-glucose in each basal medium were adjusted to the C/N ratio of 10:1, 20:1, 30:1 and 40:1, respectively. The basal media were adjusted to pH 6.0, autoclaved for 15 minutes at 121°C (15 psi pressure) and poured into a peri-dish. Also, all the other processes including the inoculation, incubation and measurement of mycelial density of *G. umbellata* were performed according to the method described by Chi *et al.* (1996).

**Effect of organic acids** After the addition of 2% glucose as carbon source and 0.25% alginine as nitrogen source per 1 L of the medi-

um, the basal medium was mixed with each organic acid. Each of 9 different organic acids used was added at the rate of 0.1% per 1 L of basal medium (Kim *et al.* 1994; Kang *et al.* 1994; Chi *et al.* 1996). The basal media were adjusted to pH 6.0, and autoclaved for 15 minutes at 121°C (15 psi pressure). All the other processes were performed according to the method described by Chi *et al.* (1996).

**Effect of vitamins** To screen the vitamin suitable for stimulating a mycelial growth of *G. umbellata,* YM liquid medium which was composed of peptone 5 g, yeast extract 3 g, malt extract 3 g, dextrose 10 g and distilled water 1 L was used. The YM liquid medium was autoclaved for 15 minutes at 121°C (15 psi pressure) and used as the basal medium for testing an effectiveness of a total of 5 different vitamins (Chi *et al.*, 1996). Each vitamin was mixed with a sterile water, filtrated through metrical membrane filter (0.2 μm pore dia., Nucleopore Corp., U.S.A.) and added at the concentration of 0.5 ppm per 50 m*l* of YM liquid medium. A 5 mm diameter plug of an inoculum was inoculated into 50 m*l* of YM liquid medium,and incubated for 20 days at 25°C. After 20 days of the incubation, the inocula submerged were filtrated through filter paper (Whatman No 2, dia., 9 cm), dried for 24 hours at 80°C and weighed in the balance (Chi *et al.*, 1996).

**Effect of mineral nutrients** To screen optimal concentrations of 5 different mineral nutrients necessary to stimulating a favorable growth of *G. umbellata,* YM agar medium which was composed of peptone 5 g, malt extract 3 g, dextrose 10 g, agar 20 g and distilled water 1 L was used as the basal medium. Each of four different nutrients including potassium phosphate ($KH_2PO_4$) was added to the basal medium at intervals of 0.02 g in the range of 0.02~0.1 g, whereas magnesium sulfate ($MgSO_4$) was added to the basal medium at intervals of 0.2 g in the range of 0.2~1.0 g. YM agar medium was adjusted to pH 6.0, and autoclaved for 15 minutes at 121°C (15 psi pressure). All the other processes were performed according to the method described by Chi *et al.* (1996).

**Effect of cereal extract media** Four different cereal extract media were used to screen cereal extract medium suitable for stimulating a favorable growth of *G. umbellata.* Each of four different cereal extract media was made by mixing 20 g of agar with 1 L of extract solution which was extracted from 40 g of cereal powder. The cereal extract medium was mixed with nutrient sources including silkworm pupae whose additive percentages were added to the cereal extract medium in the range of 10~30% (w/v), adjusted to pH 6.0 and autoclaved for 15 minutes at 121°C (15 psi pressure). A 5 mm diameter plug of an inoculum was placed in the center of each agar plate and incubated for 20 days at 25°C. The measurement of mycelial growth was carried out according to the method described by Chi *et al.* (1996).

## Results and Discussions

### Cultural conditions of *G. umbellata*

**Effect of pH** To screen pH value suitable for a favorable growth of *G. umbellata*, the pH values in the PDA medium were adjusted to intervals of pH 1.0 in the range of pH 4~9. The mycelial growth of *G. umbellata* was most favorable at pH 4, and most unfavorable at pH 9 (Fig. 1). Shoji (1996) suggested that pH range suitable for a favorable growth of Genera *Grifola* was from pH 4.4 to pH 4.9. Lee (1986) reported that pH value suitable for a favorable growth of *G. umbellata* could be obtained in the range of pH 4.2~5.8. According to the result shown in Fig. 1, pH range suitable for *G. umbellata* appears to be lower than pH 5.0. The mycelial growth of *G. umbellata* appeared to be suppressed in proportion to the rise of pH.

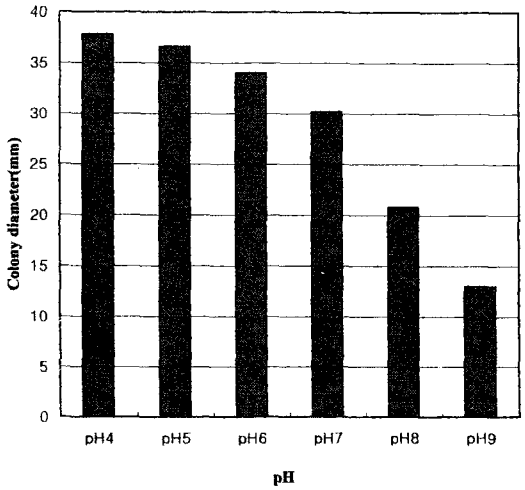


**Fig. 1.** Mycelial growth of *Grifola umbellata* in the potato dextrose agar at different pHs for 15 days at 25°C.

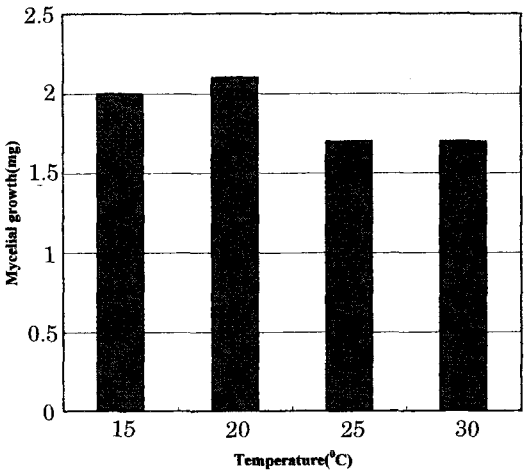


**Fig. 2.** Mycelial growth of *Grifola umbellata* on the PD broth for 15 days at different temperature.

**Effect of culture temperatures** Based on *G. umbellata* which was cultured for 15 days at 4 different temperatures, the mycelial growth was most favorable in the temperature of 20°C. Shoji (1996) clarified that a favorable growth of *G. frondosa* and its allied species could be obtained in the range of 24~27°C. However, the mycelial growth of *G. umbellata* was most favorable at 20°C. When the temperature was raised above 20°C, the mycelial growth of *G. umbellata* appeared to be suppressed in potato dextrose broth (Fig. 2).

**Screening of favorable culture medium** Fourteen different culture media were used to investigate a favorable growth of *G. umbellata* (Table 1). It was observed that *G. umbellata* brought out an outstanding growth on the Hoppkins medium (Table 2). Even if the mycelial density of *G. umbellata* was somewhat thin on the Hoppkins medium, the size of its mycelial growth seemed to countervail the handicap of density appearance. The mycelial growth of 14 different culture media was observed in the range of 18.4-54.6 mm.

**Effect of carbon sources** The mycelial growth of *G. umbellata* was favorable on 9 different culture media which were supplemented with each of 9 carbon sources including glucose as compared with the control (Table 3). The mycelial growth of *G. umbellata* was most fa-

**Table 2.** Mycelial growth of *Grifola umbellata* on different culture media

| Culture media | Colony diameter[b] (mm) | |
|---|---|---|
| | *G. umbellata* | |
| Hamada | 39.2 ab[a] | C[c] |
| Czapek | 44.4 ab | T |
| YM | 31.0 ab | C |
| Hennerberg | 19.8 b | ST |
| Lilly | 23.8 ab | ST |
| Modified Lutz | 43.0 ab | ST |
| Hoppkins | 54.6 a | ST |
| PDA | 35.6 ab | SC |
| Glucose peptone | 27.8 ab | C |
| Glucose tryptone | 28.8 ab | SC |
| Mushroom complete | 34.2 ab | SC |
| Ebiose | 41.0 ab | SC |
| PD(M) | 30.2 ab | C |
| MEA | 18.4 b | C |

[a]The different letters are significantly different at p=0.05 according to Duncan's new multiple range test.

[b]The colony diameter was measured after 15 days of incubation.

[c]Mycelial density: C, Compact; S, Somewhat compact: ST, Somewhat thin; T, Thin.

vorable on the culture media which were supplemented with glucose, and recorded colony diameter of 53.6 mm (Table 3). The result referring to glucose, one of monosaccharides was similar to that of Ishikawa (1981). There was no a mycelial growth on the culture media which were contained with salicin. Hong *et al.* (1986) clarified that cellobiose, one of disaccharides was most favorable for stimulating a mycelial growth of *Ganoderma lucidum*. However, Chang *et al.* (1995) reported that cellobiose was most unfavorable for *Fomitella fraxinea*. Though *G. lucidum* exhibited a favorable growth on the culture media containing cellobiose, the mycelial growth of *G. umbellata* was poor and dissimilar to that of *G. lucidum*.

**Table 3.** Effect of carbon sources for mycelial growth of *Grifola umbellata* in the basal medium

| Carbon source[e] | Colony diameter[d] (mm) | |
|---|---|---|
| | *G. umbellata* | |
| Glucose | 53.6 a[a] | T[b] |
| Galactose | 51.0 a-c | T |
| Fructose | 53.2 a | T |
| Xylose | 22.0 d | T |
| Arabinose | 42.8 bc | T |
| Mannose | 50.6 a-c | T |
| Sorbitol | 52.0 a-c | T |
| Rhamnose | 46.8 a-c | T |
| Ribose | 44.2 a-c | T |
| Sucrose | 42.2 c | T |
| Manitol | 52.6 ab | T |
| Cellobiose | 17.8 d | T |
| Lactose | 21.0 d | T |
| Raffinose | 48.6 a-c | T |
| Dextrin | 50.8 a-c | T |
| Salicin | 0 e | - |
| Adonitol | 48.0 a-d | T |
| Trehalose | 50.0 a-c | T |
| Maltose | 52.0 a-c | T |
| Control | 49.6 a-d | T |

[a]The different letters are significantly different at p=0.01 according to Duncan's new multiple range test.

[b]Mycelial density: ST, Somewhat thin; T, Thin.

[c]This was composed of $MgSO_4 \cdot 7H_2O$ 0.05 g, $KH_2PO_4$ 0.46 g, $K_2HPO_4$ 1.0 g, Thiamine-Hcl 120 μg, agar 20 g, and distilled water 1000 m*l*.

[d]The colony diameter was measured after 15 days of incubation.

[e]Each cardon source was addeded to the basal medium at the concentration of 0.1 M.

**Effect of nitrogen sources** It was observed that valine, one of organic nitrogens was most suitable for stimulating a favorable growth of *G. umbellata* on the culture media (Table 4). There was no mycelial growth on the culture media which were supplemented

**Table 4.** Effect of nitrogen sources for mycelial growth of *Grifola umbellata* in the basal medium[c]

| Nitrogen source[e] | Colony diameter[d] (mm) | |
|---|---|---|
| | *G. umbellata* | |
| Asparagine | 17.8 cd[a] | ST[b] |
| Glutamic acid | 35.6 b | ST |
| Alanine | 6.2 gh | T |
| Phenylalanine | 11.5 dg | T |
| Valine | 42.0 a | SC |
| Methionine | 37.0 b | ST |
| Alginine | 39.2 b | ST |
| Glycine | 15.4 c-e | ST |
| Glutamine | 0 h | - |
| Urea | 0 h | - |
| Histidine | 13.6 d-f | ST |
| Ammonium acetate | 8.2 fg | T |
| Ammonium oxalate | 20.0 c | T |
| Ammonium phosphate | 39.6 b | ST |
| Calcium nitrate | 8.4 fg | SC |
| Potasium nitrate | 0 h | - |
| Sodium nitrate | 11.0 e-g | SC |
| Control | 40.0 a | T |

[a]The different letters are significantly different at p=0.01 according to Duncan's new multiple range test.

[b]Mycelial density: C, Compact; SC, Somewhat compact; ST, Somewhat thin; T, Thin.

[c]This was composed of $MgSO_4 \cdot 7H_2O$ 0.05 g, $KH_2PO_4$ 0.46 g, $K_2HPO_4$ 1.0 g, Thiamine-Hcl 120 μg, agar 20 g, and distilled water 1000 m*l*.

[d]The colony diameter was measured after 15 days of incubation.

[e]Each nitrogen source was added to the basal medium at the concentration of 0.02 M.

with nitrogen source such as glutamine, urea and potassium nitrate. Generally, it was confirmed that most of 17 different nitrogen sources couldn't exercise a distinct influence upon favorable growth of *G. umbellata* (Table 4). Kim *et al.* (1994) clarified that the mycelial growth of *Lentinus lepideus* was more favorable on the culture media containing organic nitrogen than inorganic nitrogen. As described on Table 4, it was difficult to testify the fact that the mycelial growth of *G. umbellata* was more favorable on the culture media containing organic nitrogen than inorganic nitrogen. Whether the culture media were mixed with organic nitrogen or not, *G. umbellata* seems to exhibit various colony diameters.

**Optimum C/N ratio** Optimum C/N ratio suitable for a favorable growth of *G. umbellata* was observed on the culture media which were adjusted to the ratio of thirty (carbon) to one (nitrogen). On the culture media which were mixed with 1% glucose as carbon source and then adjusted to the C/N ratio of 30:1, the most favorable growth of *G. umbellata* was not more than colony diameter of 39.0 mm (Table 5). Generally, a gradual rise of carbon concentration such as 1%, 2%, 3% and 4% glucose seemed to suppress a favorable growth of *G. umbellata* on the culture media. Song and Cho (1986) suggested that optimum C/N ratio suitable for a favorable growth of *Lentinula edodes* was observed in the ratio of 30:1. Also, it was considered that our result was similar to that of Song and Cho (Table 5).

**Effect of organic acids** The most favorable growth of *G. umbellata* was obtained in the culture media which were supplemented with lactic acid, and recorded colony diameter of 50.6 mm (Table 6). However, there was no a mycelial growth on the culture media which were supplemented with propionic acid. Chi *et al.* (1996) clarified that *Phellinus linteus* couldn't exhibit a mycelial growth on the culture media containing propionic acid. It was

**Table 5.** Mycelial growth of *Grifola umbellata* at different C/N ratio in the basal medium[a]

| C/N[c] ratio | Colony diameter[b] (mm) | | | |
|---|---|---|---|---|
| | D-Glucose concentration (%) | | | |
| | 1.0 | 2.0 | 3.0 | 4.0 |
| | *G. umbellata* | | | |
| 10:1 | 33.8 | 33.8 | 27.8 | 23.8 |
| 20:1 | 38.0 | 35.4 | 30.0 | 27.6 |
| 30:1 | 39.0 | 37.2 | 33.2 | 35.4 |
| 40:1 | 38.4 | 37.6 | 31.2 | 32.4 |

[a]This was made of $MgSO_4 \cdot 7H_2O$ 0.05 g, $KH_2PO_4$ 0.46 g, $K_2HPO_4$ 1.0 g Thiamine-Hcl 120 μg, agar 20 g, and distilled water 1000 m*l*.
[b]The colony diameter was measured after 15 days of incubation.
[c]The ratios of $NaNO_3$ versus D-glucose were adjusted to the rate of 10:1, 20:1, 30:1 and 40:1, respectively.

**Table 6.** Mycelial growth of *Grifola umbellata* on the basal medium[a] with different organic acids

| Organic acid[e] | Colony diameter[b] (mm) | |
|---|---|---|
| | *G. umbellata* | |
| Succinic acid | 40.2 c[c] | C[d] |
| Gluconic acid | 47.4 b | C |
| Citric acid | 41.8 c | C |
| Fumaric acid | 48.6 ab | C |
| Formic acid | 27.0 e | T |
| Lactic acid | 50.6 a | C |
| Maleic acid | 42.6 c | C |
| Propionic acid | 0 f | - |
| Oxalic acid | 31.8 d | C |
| Control | 48.8 ab | C |

[a]This was composed of $MgSO_4 \cdot 7H_2O$ 0.05 g, $KH_2PO_4$ 0.46 g, $K_2HPO_4$ 1.0 g, Thiamine-Hcl 120 μg, agar 20 g, and distilled water 1000 m*l*.
[b]The colony diameter was measured after 15 days of incubation.
[c]The different letters are significantly different at p=0.01 according to Duncan's new multiple range test.
[d]Mycelial density: C, Compact; SC, Somewhat compact; T, Thin.
[e]Each organic acid was added at the rate of 0.1% (w/v).

considered that our result was similar to that of Chi *et al.* (1996). It was observed that most of 9 different organic acids were unsuitable for a favorable growth of *G. umbellata.*

**Effect of vitamins** The most favorable growth of *G. umbellata* was obtained in the YM liquid media which were supplemented with thiamine-Hcl and recorded dry weight of 12.0 mg after 20 days of the incubation (Table 7). Also, a mycelial growth of *G. umbellata* was favorable in each culture medium which was supplemented with Ca-pantothenic, nicotinic acid and folic acid, respectively. Kim *et al.* (1994) clarified that a favorable growth of *Lentinus lepideus* was observed in the culture media which were treated with Inositol, Ca-pantothenic or thiamine-Hcl. Also, the addition of thiamine-Hcl or Ca-pantothenic seemed to stimulate the mycelial growth of *G. umbellata* (Table 7).

**Effect of mineral nutrients** Inorganic salts necessary to a favorable growth of *G. umbellata* were cosidered as $FeSO_4$ and $MgSO_4$, because the mycelial growths on the media containing $FeSO_4$ or $MgSO_4$ recorded colony diameter of 41.3 mm in $FeSO_4$ and 40.8 mm in $MgSO_4$, respectively as compared with 39.5 mm of the control (Table 8). It has been considered that the optimal concentration of inorganic salts necessary to mycelial growth was different with sorts of mushrooms (Kang *et al.*, 1994; Chi *et al.*, 1996). Kang *et al.* (1994) reported that optimal concentrations of inorganic salts necessary to *Naematoloma sublateritium* were 0.001% in $FeSO_4$ and 0.03% in $MgSO_4$, respectively. However, Chi *et al.* (1996) suggested that *P. linteus* required 0.001% in $FeSO_4$ and 0.02% in $MgSO_4$, respectively. In this study, it was observed that the optimal concentration of inorganic salts necessary to *G. umbellata* was 0.01% (or 0.1 g) in $FeSO_4$ and 0.1% (or 1.0 g) in $MgSO_4$, respectively (Table 8). Shoji (1996) reported that the maximal growth of *Grifola* spp. could be observed in the addition of both $K_2HPO_4$ and $MgSO_4$. However, the addition of $K_2HPO_4$ appeared to be unfavorable to the mycelial growth of *G. umbellata* (Table 8).

**Effect of cereal extract media** It is meaningful to investigate if prominent substrates

**Table 7.** Effect of vitamins for the mycelial growth of *Grifola umbellata* on the YM medium[a]

| Vitamin[d] | Mycelial dry weight (mg)[b] |
|---|---|
| | *G. umbellata* |
| Ca-Pantothenic | 6.0 bc[c] |
| Nicotinic acid | 3.0 c |
| Folic acid | 8.0 b |
| Thiamine-Hcl | 12.0 a |
| Inositol | 2.2 c |
| Control | 2.3 c |

[a]This was composed of peptone 5 g, yeast extract 3 g, malt extract 3 g, dextrose 10 g and distilled water 1000 m*l*.
[b]The dry weight was measured after 20 days of incubation.
[c]The different letters are significantly different at p=0.01 according to Duncan's new multiple range test.
[d]Each vitamin was added at the concentration of 0.5 ppm per 50 m*l* of YM liquid medium.

**Table 8.** Concentration of different nutrients necessary to a maximal growth of *Grifola umbellata* on the YM medium[a]

| Nutrients | Range of concentration (g) | Colony diameter (mm)[b] |
|---|---|---|
| | | *G. umbellata* |
| $KH_2PO_4$ | 0.02-0.1 | 31.8 C[d] (0.1)[c] |
| $K_2HPO_4$ | 0.02-0.1 | 29.3 C (0.04) |
| $ZnSO_4$ | 0.02-0.1 | 26.0 C (0.02) |
| $FeSO_4$ | 0.02-0.1 | 41.3 C (0.1) |
| $MgSO_4$ | 0.2 -1.0 | 40.8 C (1.0) |
| Control | | 39.5 C |

[a]This was composed of peptone 5 g, yeast extract 3 g, malt extract 3 g, dextrose 10 g and distilled water 1000 m*l*.
[b]The colony diameter was measured after 15 days of incubation.
[c]These figures were obtained as the optimal concentration (gram) necessary to a maximal growth of *G. umbellata.*
[d]Mycelial density: C, Compact.

**Table 9.** Mycelial growth[a] of *Grifola umbellata* on four cereal extract media[b] mixed with nutrient source

| Nutrient source (%)[d] | *G. umbellata* Colony diameter (mm)[c] | | | |
|---|---|---|---|---|
| | Polished rice | Wheat | Corn meal | Unpolished rice |
| Silkworm Pupae | | | | |
| 10 | 70.0 | 59.0 | 52.0 | 25.5 |
| 20 | 67.5 | 60.0 | 48.0 | 48.0 |
| 30 | 66.0 | 60.5 | 47.0 | 64.5 |
| Milk solution | | | | |
| 10 | 39.0 | 23.5 | 28.0 | 34.7 |
| 20 | 34.5 | 22.0 | 29.0 | 28.5 |
| 30 | 28.0 | 28.7 | 14.0 | 26.0 |
| Rice bran | | | | |
| 10 | 54.5 | 45.0 | 52.0 | 56.5 |
| 20 | 56.5 | 46.5 | 49.0 | 54.0 |
| 30 | 46.5 | 42.0 | 47.0 | 60.0 |
| Control | 66.5 | 53.5 | 24.0 | 56.5 |

[a]The mycelial growth was measured after 20 days of incubation at 25°C.
[b]These media were made by mixing agar 20 g with stock solution 1000 m*l* which was extracted from cereal powder 40 g.
[c]Each treatment is the mean of 3 replications.
[d]Each nutrient source was added to the cereal extract medium in the range of 10~30% (w/v).

for stimulating a favorable growth of *G. umbellata* can be screened from various substrates including plant materials. Among 4 cereal extract media, the mycelial growth of *G. umbellata* which was cultured on the polished rice extract medium was most favorable. On the polished rice extract medium which was mixed with 10 g of silkworm pupae as one of nurient sources, *G. umbellata* recorded colony diameter of 70.0 mm (Table 9). However,the gradual rise of silkworm pupae seemed to suppress a mycelial growth of *G. umbellata* on the polished rice extract medium. Based on the obtained basic data, the next study will be focused on the possibility that *G. umbellata* can produce a lot of its fruiting bodies on the culture media. Sooner or late, it will be possible to develop a new culture medium suitable for mass production of *G. umbellata*.

## 적 요

저령의 균사생장은 20°C의 온도와 pH 4의 조건에서 가장 양호하였으며, Hoppkins 배지에서 생장이 가장 양호하였다. Glucose, Fluctose 및 Manitol과 같은 탄소원이 저령의 양호한 생장을 촉진하였다. 또한,질소원의 하나인 Valine도 저령의 균사생장을 촉진하였다. 배지에 탄소원을 1%로 고정시킨후 C/N비를 30:1로 하였을 때, 저령의 균사의 생장이 가장 양호하였다. 유기산으로서 Lactic acid를 처리 하였을때 저령의 생장이 좋았으며, 비타민으로는 Thiamine-Hcl을 처리하였을때 생장이 양호하였다. $FeSO_4$ 및 $MgSO_4$같은 미량원소의 처리에서 저령의 생장이 가장 양호하였다. 저령의 최적생장을 위한 미량원소의 농도는 $FeSO_4$를 첨가할 경우에는 0.01%였고, $MgSO_4$를 첨가할 경우에는 0.1%였다. 처리된 4종의 식물질 추출배지 중에서, 쌀 추출물과 번데기가루를 혼합 처리한 배지가 저령의 양호한 균사 생장을 나타내었다.

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