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Production of Polysaccharide by the Edible Mushroom, *Grifola frondosa*

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The production of polysaccharide according to various developmental stages (mycelium growth, primordium appearance, and fruiting-body formation) in the edible mushroom *Grifola frondosa* was studied. The cap of the mature mushroom showed the highest amount of polysacchride. Mycelial growth and polysaccharide synthesis were optimal at pH 5 and 20°C. Polysaccharide synthesis was maximal after 12 days of cultivation, whereas maximum mycelial growth was shown after 18 days. Mannose, cellobiose and starch increased the level of polysaccharide as well as growth in submerged culture. Glucose and sucrose appeared to be good substrates for fruiting of *Grifola frondosa*.

KEYWORDS: Fruit body, *Grifola frondosa*, Mushroom, Mycelial culture, Polysaccharide

Grifola frondosa is a white rot, wood decay fungus belonging to the Basidiomycetes, Aphyllophorales, polyporaceae, which naturally inhabits many hardwood species in Asia, North America and Europe. Its large size and amazing health benefits are why it has come to be called "the king of mushrooms". It has been used for centuries in China and Japan both as a health food and as a medicine (Mizuno and Zhang, 1995) and nowadays it is increasingly being recognized as a potent source of polysaccharide compounds with dramatic health-promoting potential (Mayell, 2001). Fruiting body of *G. frondosa* has been sold in the market as an edible mushroom and considered to have diuretic, gonorrhea-therapeutic, and antipyretic activities (Mizuno *et al.*, 1995). It has been shown to have anti-tumor, anti-viral properties, antidiabetic activities, and immune reaction (Kubo *et al.*, 1994). The dried powder of the fruiting body of *G. frondosa* has demonstrated a reduction of blood pressure in the spontaneously hypersensitive rat (Kabir, 1989). The fruiting body of *G. frondosa* has also been recommended as a remedy for palsy, neuralgia, and aitis (Mizuno *et al.*, 1995).

Many bioactive compounds have been reported from the fruiting body of *G. frondosa*. The most important materials among these were the polysaccharide fractions obtained mainly from the fruiting body and also partly from the mycelium of *G. frondosa* (Ohno *et al.*, 1984). Considering the increasing popularity of this mushroom, knowledge of the polysaccharide production by *G. frondosa* during cultivation is still very limited. Presently, the methods used to grow the mushroom are mostly adapted from other specialty mushroom cultivation techniques, such as *Lentinula edodes*. No published research on the optimum growth condition of *G. frondosa* for the efficient polysaccharide synthesis is available, partly because of the short cultivation history of this mushroom.

We have been interested in the factors responsible for the polysaccharide production and its regulation in *G. frondosa*. The purpose of this study was to examine the effects of various condition of *G. frondosa* on the growth and the polysaccharide production when cultured under different conditions in order to achieve a better understanding of efficient production of polysaccharides by *G. frondosa*.

Materials and Methods

Microrganism and maintenance. *G. frondosa* (KACC 51146) was obtained from National Institute of Agricultural Science and Technology, Suwon, Korea and maintained on potato dextrose agar (PDA) slant at 4°C and subcultured every 2 months. The mycelia of *G. frondosa* were grown in YMG liquid media prepared by dissolving 2 g of yeast extract, 5 g of malt extract, and 2 g of dextrose in 500 *ml* distilled water. Mycelial plugs of *G. frondosa* grown in YMG media for 20 days at 25°C were seeded to liquid media under aseptic conditions. The flasks were placed on a rotatory shaker (120 rpm, 25°C). After 20 days of incubation, mycelia were collected by filtering through Whatman filter paper NO. 42 and washed 3 times with the same volume of distilled water. Collected mycelia were freeze-dried and weighed.

Culture condition for the development of fruit body. Mycelia were inoculated into a plant cell culture vessel made from polypropylene containing 20 *ml* of sucrose-asparagine (SA) medium. The mycelia were incubated in the dark at 25°C for 30 days and further incubated in alternate light and dark (12 h-light per day) at 15°C for 30 days. The white light intensity was 1200~1700 Lux. The

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relative humidity was 90%. The SA medium consisted of following constituents per liter of deionized water: 20 g of glucose, 0.88 g of asparagine, 2 g of $NH_4H_2PO_4$, 1 g of L-valine, 0.224 g of $K_2HPO_4 \cdot 3H_2O$, 0.803 g of KH_2PO_4, 0.99 g of $MgSO_4 \cdot 7H_2O$, 0.02 g of $CaCl_2$, 5 *ml* of trace element solution, 5 *ml* of vitamin nucleotide solution. The trace element solution consisted of 0.89 g of $ZnSO_4 \cdot 7H_2O$, 0.765 g of $MnSO_4 \cdot H_2O$, 0.73 g of Fe(III)-citrate, 0.20 g of $CuSO_4 \cdot 5H_2O$ and distilled water to 1000 *ml*. The vitamin-nucleotide solution consisted of 1.60 g of adenosine, 0.02 g of thiamine-HCl and distilled water to 1000 *ml*. To prepare solid agar medium 15 g of agar powder was added to 1 liter of medium solution. In addition to glucose, various sugars were added to examine the effect of carbon sources on the growth, polysaccharide synthesis and fruiting of *G. frondosa*.

Polysaccharide extraction of *Grifola frondosa*. Freeze-dried mycelium was added by distilled water and autoclaved under 1.2 atoms of pressure at 120°C for 1 hr. After cooled off and centrifuged at 8,000 rpm × 20 min, the supernatant was collected. The supernatant was mixed with cold ethanol to final concentration of 70% and left at 4°C for 12 hours. Then the float material was centrifuged at 12,000 rpm for 20 min to collect the material. The material was solved in water and assayed for the amount of polysaccharide. Determination of polysaccharide was done by the anthrone-sulfuric acid using glucose as a reference.

Results and Discussion

Mycelial growth curve and production of polysaccharide. Polysaccharide synthesis in *G. frondosa* was determined with the mycelium, primordium and fruiting body obtained from different stage of development (Table 1). Polysaccharide production was observed to be higher in primordium and fruit body than in mycelium. Polysaccharide production on culture time of *G. frondosa* mycelia was also shown in Fig. 1. The amount of polysaccharide started to increase from 9 to 15 day culture and showed constant production when mycelia were about to grow or actively growing. Polysaccharide production appeared to reach its maximun value after 27 d growth of *G. frondosa* in liquid culture. The mycelia grew at a growth rate of 0.005 per hour with a doubling time 84.7 h and attained maximum value after 18 days of cultivation.

Table 1. Changes of polysaccharide synthesis according to the developmental stages of *Grifola frondosa*

Differentiation	Polysaccharide[a] (mg/mg of protein)
Primordium	7.73
Mycelium	4.83
Fruiting body	7.55

[a]At the end of the growth period, the fungal mat, primordium and fruiting body were separated, freeze-dried, and assayed for the polysacchride amount after extraction. All the values are given as the mean based on results of triplicate experiments.

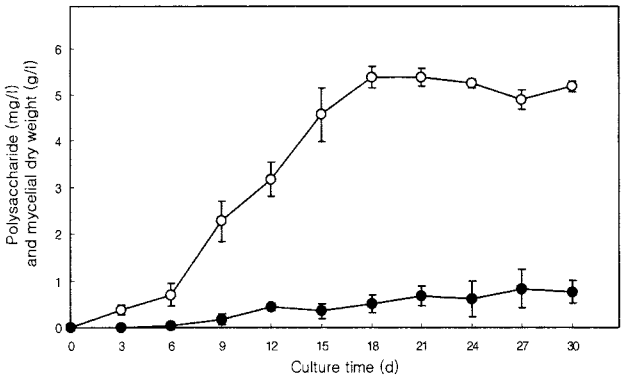

Fig. 1. Mycelial growth and changes in the production of polysaccharide of *Grifola frondosa* grown in 2 liter rich medium using 5 liter culture bottle. Polysaccharide was measured in the water extracts of mycelium cultured in a rich medium: (●), amount of polysaccharide; (○), mycelial dry weight. Data shown represent the mean±S.E. of 4 independent experiments. The absence of error bars indicates that the error was too small to allow its display in those data.

Effect of pH and temperature for mycelial growth and polysaccharide production. Optimal mycelial growth of *G. frondosa* and polysaccharide production were both attained with an initial culture pH value of 5 (Table 2). Optimum pH values for growth of *G. frondosa* has been reported to be 5.2 (Chung *et al.*, 1991) which is similar to our present finding. Optimal polysaccharide production also was obtained at pH 5, whereas extracellular polysccharide production by *Phellinus linteus* was reported as being 6 to 7 (Chi *et al.*, 1996). Optimum temperature for

Table 2. Effect of initial culture pH and on mycelial growth (dry weight) and polysaccharide production in submerged culture of *G. frondosa*

pH	Mycelia (g/*l*)[a]	Polysaccharide (mg/*l*)[b]
4.0	1.9	0.28
4.5	3.8	0.32
5.0	4.2	0.35
5.5	4.0	0.30
6.0	4.1	0.29
6.5	3.9	0.26
7.0	3.6	0.27
7.5	3.6	0.23
8.0	3.2	0.21

[ab]The submerged culture as performed in 250 *ml* flasks containing 100 *ml* rich medium on a rotary shaker (12 rpm, 26°C, 20 day). All the values are given as the mean based on results of triplicate experiments.

Table 3. Effect of temperature on mycelial growth and polysaccharide production in *G. frondosa*

Temperature (°C)	Mycelia (g/*l*)[a]	Polysaccharide (mg/*l*)[b]
15	3.2	0.19
20	4.5	0.35
25	4.1	0.37
30	2.8	0.25
35	2.1	0.20

[ab]The culture was performed in 250 *ml* flasks containing 100 *ml* rich medium on a rotary shaker (120 rpm, 26°C, 20 day). All the values are given as the mean based on results of triplicate experiments.

growth and polysaccharide production by *G. frondosa* appeared to be 25°C respectively as shown in Table 3. Optimum temperature for growth of *P. linteus* was reported to be between 25~30°C which is little higher than our present study.

Effect of carbon sources on the development and production of polysacchride in *G. frondosa*. Carbon source effect on mycelial growth and polysaccharide production in *Grifola frondosa* was shown in Table 4. The submerged culture was done in SA media containing various kinds of sugar compounds to SA media. Mannose, cellobiose and starch increased the level of polysaccharide as well as growth. However, it should be noted that it is not known whether the increase was inductive yet. *Grifola frondosa* produced primordium and subsequently fruit bodies from vegetative mycelia on a synthetic medium, when incubated in the alternate light and dark for additional 20 days at 15°C after the incubation in the dark for 30 days 25°C. However, the fungus remained in mycelium form if they were continuously incubated in the dark at 15°C. These results are consistent with the previous observations (Mayuzumi and Mizuno, 1997) that light

Table 4. Carbon source effect on mycelial growth and polysaccharide production in *G. frondosa*

Carbon source	Mycelia (mg/*l*)[a]	Polysaccharide (μg/*l*)[b]
Trehalose	62.6	55.0
Xylose	96.2	12.4
Galactose	8.0	12.8
Arabinose	24.5	91.6
Mannose	285.8	119.7
Saccharose	45.7	29.0
Starch	226.1	291.4
Cellobiose	294.4	147.8
Fructose	76.5	12.7
Glucose	16.5	29.7

[ab]The culture was performed in 250 *ml* flasks containing 100 *ml* SA medium on a rotary shaker (120 rpm, 26°C, 30 day). All the values are given as the mean based on results of triplicate experiments.

stimulated development of fruit body from vegetative mycelia. Effect of carbon source on the growth and fruiting body formation of *G. frondosa* was shown in Table 5. Interestingly, we found that glucose and sucrose were good substrates for fruiting eventhough it remains undetermined which compounds in medium play important roles in fruiting of *G. frondosa.*

Although the exact mechanism of polysacchride synthesis and fruiting in *G. frondosa* is not properly understood, the present study suggest that a combination of mechanism might be in polysaccharide production. It also provide basic parameters to solve the mechanism of polysaccharide synthesis, which is physiologically important in fungi.

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Table 5. Effect of carbon sources on the growth and fruiting of *G. frondosa*[a]

Carbon source	Mycelium colony radius (cm)	Mycelium density	Approx. days to primordium formation after exposure to light	Approx. days to mature fruit body after exposure to light
Glucose	2.2	thin	14	27
Galactose	5	thin	13	N.F.[c]
Melobiose	6	thick	N.P.[b]	N.F.
Saccharose	5.3	thick	N.P.	N.F.
Starch	5.2	thick	N.P.	N.F.
Sucrose	3	thick	11	23
Raffinose	3.5	thin	17	35
Arabinose	5	thin	N.P.	N.F.
Maltose	2.4	very thick	12	26
Xylose	2	thick	14	30
Mannose	3.8	very thick	14	30
Fructose	5.6	very thick	N.P.	N.F.
Lactose	4.3	thin	N.P.	N.F.

[a]All the values are given as the mean based on results of triplicate experiments.
[b]Primordium was not formed in 20 days after exposure to light.
[c]Fruit body was not formed in 30 days after exposure to light.

the biotechnology, The province of Chungbuk, Korea.

References

Chung, K. S., Koo, Y. J., Yoo, J. I., Choi, S. Y. and Shin, D. H. 1991. Mycelial growth of *Ganoderma lucidum* and *Grifola frondosa* in milk whey. *Kor. J. Mycol.* **19**(1): 61-65.

Hwang, H. J., Kim, S. W., Xu, C. P., Choi, J. W. and Yun, J. W. 2003. Production and molecular characteristics of four groups of exopolysaccharides from submerged culture of *Phellinus gilvus*. *J. Appl. Microbiol.* **94**(4): 708-719.

Kim, D.-H., Yang, B.-K., Jeong, S.-C., Park, J.-B., Cho, S.-P., Das, S., Yun, J.-W. and Song, C.-H. 2001. production of a hypoglycemic, extracellular polysaccharide from the submerged culture of the mushroom, *Phillinus linteus*. *Biotech. Lett.* **23**: 513-517.

Kodama, N., Harada, N. and Nanba, H. 2002. Polysaccharide, extract from *Grifola frondosa*, induces Th-1 dominant responses in carcinoma-bearing BALB/c mice. *Jpn. J. Pharmacol.* **90**(4): 357-360.

_____, Komuta, K., Sakai, N. and Nanba, H. 2002. Effects of D-Fraction, a polysaccharide from *Grifola frondosa* on tumor growth involve activation of NK cells. *Biol. Pharm. Bull.* **25**(12) :1647-1650.

Kubo, K., Aoki, H. and Nanba, H. 1994. Anti-diabetic activity present in the fruit body of *Grifola frondosa* (Maitake). *I. Biol. Pharm. Bull.* **17**(8): 1106-1110.

Mayell, M. 2001. Maitake extracts and their therapeutic potential. *Altern. Med. Rev.* **6**(1): 48-60.

Mayuzumi, T. and Mizuno, T. 1997. Cultivation methods of maitake (*Grifola frondosa*). *Food Rev. Int.* **11**: 357-364.

Mizuno, T., Zhuang, C. and Maitake, C. 1995. *Grifola frodosa*: Pharmacological effects. *Food Rev. Int.* **11**: 135-149.

Nakai, R., Masui, H., Horio, H. and Ohtsuru, M. 1999. Effect of maitake (*Grifola frondosa*) water extract on inhibition of adipocyte conversion of C3H10T1/2B2C1 cells. *J. Nutr. Sci. Vitaminol* (Tokyo). **45**(3): 385-389.

Ohno, N., Suzuki, I., Oikawa, S., Sato, K., Miyazaki, T. and Yadomae, T. 1984. Antitumor activity and structural characterization of glucans extracted from cultured fruit bodies of *Grifola frondosa*. *Chem. Pharm. Bull.* **32**: 1142-1151.

Shen, Q. and Royse, D. J. 2001. Effects of nutrient supplements on biological efficiency, quality and crop cycle time of maitake (*Grifola frondosa*). *Appl. Microbiol. Biotechnol.* **57**(1-2): 74-78.

Suzuki, I., Hashimoto, K., Oikawa, S., Sato, K., Osawa, M. and Yadomae, T. 1989. Antitumor and immunomodulating activities of a beta-glucan obtained from liquid-cultured *Grifola frondosa*. *Chem. Pharm. Bull.* **37**(2): 410-413.