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Promotive effect of the hot water-soluble fraction from corn fiber on vegetative mycelial growth in edible mushrooms

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Abstract Corn fiber (CNF) is an abundant by-product of the wet corn milling process used to produce corn starch. In light of the need to recycle organic wastes, the effects of adding a hot water-soluble fraction (HWSF) from CNF to a medium on the vegetative mycelial growth of nine edible mushrooms such as *Lentinula edodes* and *Pholiota nameko* were investigated. The results showed that the mycelial growth of these fungi was markedly increased (1.4–9.5 times that of the control) by adding 5%–20% CNF-HWSF to the medium. These promotive effects were also apparent on mycorrhizal mushrooms, such as *Tricholoma matsutake* (3.3-fold) and *Lyophyllium shimeji* (3.7-fold). The promotive effects on mycelial growth were shown in the low-molecular-weight fractions (molecular weight <500 daltons) prepared from CNF-HWSF. The promotive actions were more effective on slow-growing mushrooms (*L. edodes* and *P. nameko*) than on rapidly growing mushrooms (*Pleurotus ostreatus* and *Flammulina velutipes*). The results obtained in this experiment suggest that CNF-HWSF can be used as a promotive substance for cultivating edible mushrooms.

Key words Corn fiber · Edible mushroom · Mycelial growth · Industrial waste · Mushroom production

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Introduction

The indoor cultivation of many kinds of mushrooms using sawdust beds has an advantage in that mushrooms can be produced steadily throughout the year. In addition to the decrease in the amount of wood cut, rapid expansion of mushroom production resulted in a shortage of wood sawdust, which is now a serious problem in mushroom cultivation. Hence, the use of agricultural and industrial wastes as cultivation substrates of mushrooms has been increasing in recent years. For example, cultivation of *Lentinula edodes* uses the residue of bamboo grass leaves[1]; *Armillaria ostoyae* can be cultivated with carrot juice residue[2]; and utilized sake lees,[3] olive oil mill wastes,[4] and coffee pulp[5] are used for culturing *Pleurotus ostreatus*.

Corn fiber (CNF) is a by-product of the wet corn milling process used to produce corn starch in factories. By-products such as corncob meal, corn steep liquor (CSL), and corn gluten meal are produced during this process. Recently, the utilization of these by-products has been examined. Corncob meal has been put to good use in the cultivation of mushrooms on a commercial scale. Also, CSL has been utilized as a media component for the microbiological industry.[6,7] In addition, CSL was found to be the most suitable substrate for cellulose production by *Acetobacter xylinum* due to the presence of lactic acid and methionine in the CSL component.[8] The amount of CNF as a by-product is about 20000 tons per year in Japan, and 4 million tons worldwide.[9] Most is used to make livestock feed after combining corn germ with corn steep liquor except for that utilized as functional food material, such as dietary fiber.[10–12] The composition of CNF is about 60% hemicellulose, about 25% cellulose, and about 6% each of starch and protein.[11] Xylan is the major component of hemicellulose. Mushrooms have many strains that have high xylanase activity during the growth of fruit bodies.[13] Therefore, CNF is a possible alternative mushroom cultivation substrate. In fact, Terashita et al.[14] have reported that the yield of fruit bodies on *Pholiota nameko* and *Hypsizygus marmoreus* increased with CNF as a growth substrate

during sawdust-based cultivation. The suggested that mycelial growth-promoting components may be present in the hot water-soluble fraction (HWSF) of CNF.

This paper describes the promotive effects of CNF-HWSF on vegetative mycelial growth using various edible mushrooms containing mycorrhizal mushrooms. In addition, we investigated its fractionation by organic solvent and ultrafiltration and by a chemical analysis that revealed the promotive substances of CNF-HWSF. The promotive mechanisms of vegetative mycelial growth of these edible mushroom cultivations are also discussed.

Materials and methods

Spawn strains

Lentinula edodes (Mori No. 465), *Hypsizygus marmoreus* (Takara No. 1), *Pleurotus ostreatus* (Kitamura), *P. eryngii* (axenic isolate from a commercial mushroom), *Flammulina velutipes* (IFO 7777), *Grifola frondosa* (Mogami), and *Pholiota nameko* (Meiji) were used in these experiments. In addition, two species of fungi, *Lyophyllum shimeji* (MHO 1721) and *Tricholoma matsutake* (IFO 30605), mycorrhizal mushrooms, were also used. These fungi were stored on potato dextrose agar (PDA) medium (Nissui Co.) in a test tube.

Culture media

Potato-dextrose liquid (PDL) medium consists of potato extract (200 g boiled in 500 ml distilled water), 15 g glucose, and 1 mg thiamine hydrochloride per liter of distilled water. PMM liquid medium was prepared according to the method of Terashita et al.[15] This medium consists of 22.7 g glucose, 5 g dried beer yeast (Wako), and 5 g Sunpeal CP (commercially available) per liter of distilled water. The mixture was heated for 30 min, and then 1.0 ml thiamine hydrochloride solution (0.1 g thiamine hydrochloride per 10 ml distilled water) was added after the residue was removed. The initial pH of this medium was adjusted to 5.1 with 1 N HCl.

Preparation of CNF-HWSF

Corn fiber (100 g) was mixed with 1 l of distilled water and extracted for 3 h at 80°C. After the residue was removed by centrifugation at 10000*g* (0°C, 10 min) the supernatant solution was concentrated at 40°C to 100 ml by a rotary evaporator.

Fractionation and isolation of CNF-HWSF

Ethanol (400 ml) was added to 100 ml of CNF-HWSF and left overnight. The sample was separated by centrifugation (0°C, 15 min, 13000*g*) into the supernatant and the precipitate. The supernatant was further separated into molecular weight (MW) substances of <500-dalton and >500-dalton fractions by molecular sieving using ultrafiltration membranes (YC 05; Amicon).

Arabinoxylan from the CNF-HWSF was isolated by the method of Takeuchi.[11] To 3.5 g of freeze-dried CNF-HWSF (this weight is equivalent to 100 ml of CNF-HWSF) was added 500 ml of 2% NaOH. The extraction was performed at room temperature for 18 h. After removing the residues by centrifugation at 13000 g (5°C, 15 min), the supernatant solution was neutralized with 1 N acetic acid, and trichloroacetic acid (TCA) was added to make the final concentration 7% for deproteinization. The solution was dialyzed for 4 days at 4°C and added to 4 volumes of 99.5% ethanol. The precipitate was lyophilized.

The cellulose was prepared as follows: Dried CNF-HWSF 3.5 g was washed with 50 ml of distilled water at 90°–95°C for 3 h and with 50 ml of 2% NaOH at room temperature for 18 h. This manipulation was repeated three times, and the collected precipitate was then lyophilized.

Starch[16] was obtained in a manner similar to the preparation of cellulose: 3.5 g of CNF-HWSF was extracted with 50 ml of distilled water at 90°–95°C for 3 h. The extract solution obtained by filtration was deproteinized with TCA solution and then added to 4 volumes of methanol. The precipitate was dissolved in 20 ml of dimethylsulfoxide (DMSO) and the insoluble component was removed by centrifugation (13000*g*, 25°C, 15 min). The starch was then reprecipitated in 4 volumes of ethanol.

Inoculations and culture conditions

The PDL medium was supplemented with 5%, 10%, and 20% (v/v) CNF-HWSF, soluble starch (Kanto Chemicals), corn starch (Wako), CMC (Wako), avicel (Asahikasei), and xylan (Nakalai Tesque) solution of the same concentration as that of CNF-HWSF and dispensed in 16-ml aliquots in 100-ml Erlenmeyer flasks before autoclaving at 121°C for 10 min. As inoculum, a mycelial block (diameter 5 mm) was cut from a plate culture that had grown on a PDA medium for 14 days at 24°C in a petri dish (diameter 90 mm). The incubation was carried out at 24°C for 15 days. For the mycorrhizal mushrooms that grew on the PDL and PMML media, the incubation was carried out at 24°C for 30 days (*L. shimeji*) or 60 days (*T. matsutake*).

Measurement of vegetative mycelial growth

The vegetative mycelia after incubation were separated from the medium by filtration and washed thoroughly with distilled water, after which they were dried at 80°C for 24 h. Their dry weight was measured after cooling in a desiccator.

Chemical analysis of CNF-HWSF

The concentration of reducing sugars in the CNF-HWSF was analyzed by the Somogyi-Nelson method[17] using a calibration curve obtained with glucose as a standard. Free amino acid was estimated using the ninhydrin reaction with

leucine as standard.[18] The crude protein was determined in freeze-dried CNF-HWSF by the micro-Kjeldahl method.[19] The reducing sugars were analyzed and characterized using thin-layer (TLC) and high-performance liquid (HPLC) chromatography. The TLC analysis was carried out with a Silicagel G chromatography plate (Analtech) at room temperature. Chloroform and methanol (6:4) were the developing solvents. The plate after development was sprayed with a dyeing reagent of 20% sulfuric acid in the methanol solution and kept at 110°C for 10–15 min. HPLC was performed using a Shimadzu type LC-10AS and a RID-10 differential refractive index detector. The separation by HPLC was performed in an Ultron PS-80P column (Shinwa Chemical Industries) (300 × 8 mm) using MilliQ water as a development eluent at a flow rate of 1.0 ml/min and 80°C. Arabinose, xylose, galactose, and glucose were used as internal standards. The qualitative analysis of amino acids was performed with an amino acid automatic analyzer (Hitachi 8500 type).

Results

Effect of CNF-HWSF on mycelial growth of edible mushrooms

The vegetative mycelial growth of *L. edodes* on PDL medium with added CNF-HWSF is shown in Fig. 1. Mycelial growth was enhanced by adding 5%–20% CNF-HWSF to the medium; but at 30% CNF-HWSF the results were almost identical to the control (without CNF-HWSF). In particular, addition of 20% CNF-HWSF to the medium increased the mycelial dry weight to 98.6 mg/flask. This weight was about 9.5 times more than that of the control (10.4 mg/flask).

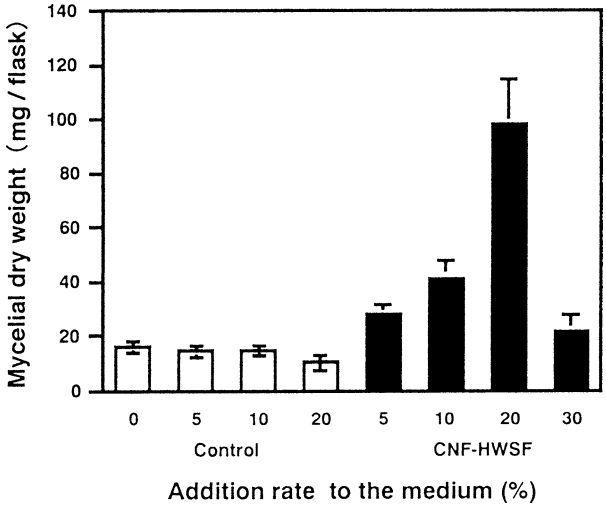

Fig. 1. Promotive effect of corn fiber – hot water-soluble fraction (*CNF-HWSF*) on the vegetative mycelial growth of *Lentinula edodes*. The vegetative mycelia were cultured for 15 days at 24°C. Values are means ± SD ($n = 8$). *Open bars*, potato-dextrose liquid (PDL) medium with added distilled water (control); *filled bars*, potato-dextrose liquid (PDL) medium with added CNF-HWSF

The promotive effect of CNF-HWSF on vegetative mycelial growth of several species of mushrooms is shown in Table 1. When these fungi were cultured on PDL medium with CNF-HWSF added, the mycelial dry weight was greater than that of the control (without CNF-HWSF). The optimal experimentally derived concentrations for mycelial growth were 10% for *H. marmoreus* (2.56 times) and *P. eryngii* (2.23 times) and 20% for *L. edodes* (9.46 times), *P. nameko* (6.94 times), *G. frondosa* (3.68 times), *P. ostreatus* (2.82 times), and *F. velutipes* (2.23 times).

Effect of CNF-HWSF on mycelial growth of mycorrhizal mushrooms

Table 2 shows the effect of mycelial growth on *L. shimeji* forming mycorrhiza. On the PDL medium for 30 days at 24°C, the dry weight of mycelia increased by 3.72 times when 5% CNF-HWSF was added to the medium. However, the promotive effect of CNF-HWSF in PMML medium was

Table 1. Promotive effect of CNF-HWSF on the vegetative mycelial growth of several edible mushrooms

Mushroom	Promotive effect by supplement concentration		
	5%	10%	20%
Lentinula edodes	1.86	2.78	9.46
Pholiota nameko	–	6.41	6.94
Hypsizygus marmoreus	1.86	2.56	2.19
Grifola frondosa	1.48	3.66	3.68
Pleurotus eryngii	1.71	2.23	1.84
Pleurotus ostreatus	1.40	1.90	2.82
Flammulina velutipes	1.48	1.70	2.23

The growth ratio obtained for the control (distilled water) was set at 1.00
Vegetative mycelia were cultured for 15 days at 24°C in potato-dextrose liquid (PDL) medium
Data represent averages of triplicate experiments, with six Erlenmeyer flasks per experiment
CNF-HWSF, corn fiber – hot water-soluble fraction

Table 2. Promotive effect of CNF-HWSF on vegetative mycelial growth of *Lyophyllum shimeji*

Addition to the medium (%)	Dry weight of mycelium (mg/flask)	
	PDL medium	PMML medium
Distilled water (control)		
5	22.42 ± 3.15	120.68 ± 14.81
10	23.20 ± 7.00	124.20 ± 20.76
20	29.35 ± 7.19	–
CNF-HWSF		
5	83.35 ± 33.86	165.93 ± 15.06
10	45.70 ± 17.06	205.54 ± 30.91
20	0	–

Vegetative mycelia were cultured for 30 days at 24°C in PDL and partly modified matsutake liquid (PMML) medium
Data represent the average of duplicates with six Erlenmeyer flasks (means ± SD)

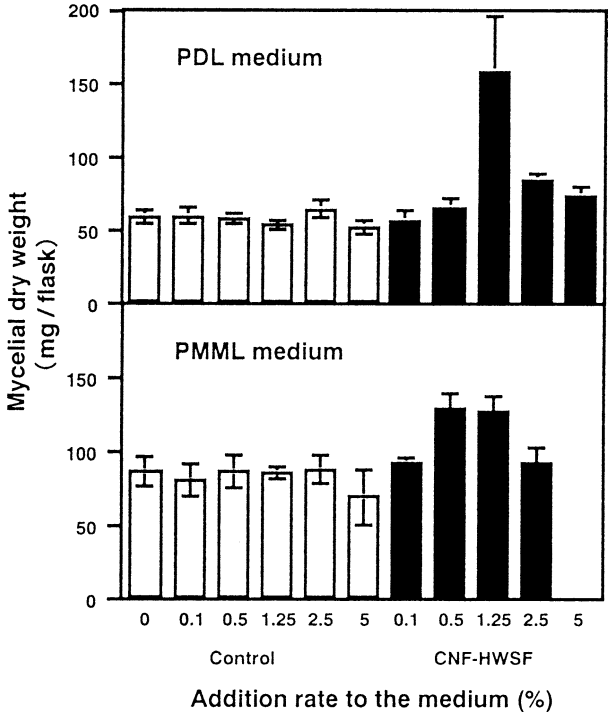

Fig. 2. Promotive effect of CNF-HWSF on vegetative mycelial growth of *Tricholoma matsutake*. The vegetative mycelia were cultured for 60 days at 24°C. Values are means ± SD (n = 6). *PMML*, partly modified matsutake liquid For symbols, refer to Fig. 1

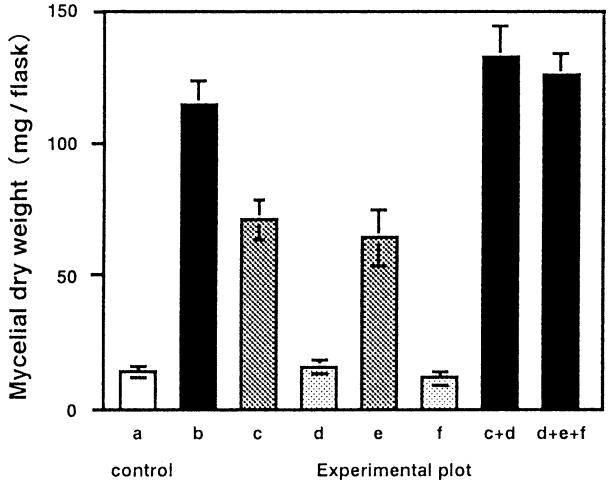

Fig. 3. Influence of CNF-HWSF components on vegetative mycelial growth of *L. edodes*. The vegetative mycelia were cultured for 15 days at 24°C in PDL medium with added CNF-HWSF (20%). Values are means ± SD. *a*, control; *b*, CNF-HWSF; *c*, supernatant after ethanol treatment; *d*, precipitate after ethanol treatment; *e*, 500-L by ultrafiltration; *f*, 500-M by ultrafiltration. MW, molecular weight; 500-L, MW ≤500 fraction; 500-M, MW >500 fraction

only 1.37-fold (5% supplemented) or 1.65-fold (10% supplemented). The effects of CNF-HWSF on mycelial growth of *T. matsutake* are shown in Fig. 2. The preliminary experiments examined the same concentrations (10% and 20%) of additives for this mushroom, but the hyphae did not grow with these CNF-HWSF concentrations. The effect of mycelial growth of *T. matsutake* was then tested again at lower CNF-HWSF concentrations. As a result, the best promotive effect of CNF-HWSF was shown to be at a concentration of 1.25% (3.33 times that of the control) in PDL medium and at concentrations of 0.5% (1.53 times) and 1.25% (1.51 times) in PMML medium.

Effect of each CNF-HWSF fraction on vegetative mycelial growth of *L. edodes*

After CNF-HWSF was separated into components by ethanol treatment and ultrafiltration, the promotive effects of the fractions on mycelial growth were examined. The results are shown in Fig. 3. The promotive effect was indicated in the supernatant solution after ethanol treatment and in low-molecular-weight components after ultrafiltration (500-dalton membrane). There were no effects when starch, cellulose, arabinoxylan fraction, or chemical reagents were added (data not shown).

To examine the promotive components of CNF-HWSF, mixtures of two fractions (c and d) or three fractions (d, e, f) from CNF-HWSF were added again to the PDL medium; and *L. edodes* mycelia were then cultured. The results indicated that the promotive effect on vegetative mycelial growth recovered to the most effective level, corresponding to that of the CNF-HWSF. These results suggested that the enhanced ingredients contained in CNF-HWSF were dispersed by fractionation.

Table 3. Chemical components of CNF-HWSF

Parameter	Content (mg/dl)[a]		
	Protein	Free amino acid	Reducing sugar
CNF-HWSF	559.8	225.9	425.3
Supernatant obtained from ethanol treatment	–	219.7	330.0
Ultrafiltration[b]			
500-L	–	198.0	326.3
500-M	–	10.2	14.1

Protein, free amino acid, and reducing sugar contents were analyzed by the micro-Kjeldahl method, ninhydrin method, and Somogyi-Nelson method, respectively
500-L, MW ≤500 daltons fraction; 500-M MW >500 fraction; MW, molecular weight
[a] The amount was converted to 100 ml (dry weight 3.5 g) CNF-HWSF
[b] Ultrafiltration was done using membrane YC 05 (Amicon Co.) for the supernatant after the ethanol treatment

Chemical analysis of CNF-HWSF

Table 3 shows the results of the chemical analysis of the components from CNF-HWSF. The protein, free amino acid, and reducing sugar contents were 0.6%, 0.2%, and 0.4%, respectively. The characterization of polysaccharides, amino acids, and reducing sugars is shown in Table 4. CNF-HWSF contained 0.82 g starch, 0.70 g arabinoxylan, and 0.007 g cellulose. The main components of the reducing

Table 4. Main components of polysaccharides, free amino acids, and reducing sugars in CNF-HWSF

Component	Amount[a]
Polysaccharides (g)[b]	
Starch	0.82
Arabinoxylan	0.70
Cellulose	0.07
Reducing sugars (mg)[c]	
Glucose	126.78
Arabinose	123.74
Xylose	75.48
Free amino acids (mg)[d]	
p-Serine	3.9
Asparagine	4.0
Threonine	3.2
Serine	3.2
Glutamic acid	3.3
Alanine	8.3
Valine	5.7
Isoleucine	3.6
Leucine	15.2
Tyrosine	5.8
Phenylalanine	1.4
Arginine	6.8

[a] Amount converted to 100 ml (dry weight 3.5 g) CNF-HWSF
[b] Polysaccharides are reported as dry weight of component fractionated from CNF-HWSF
[c] Reducing sugars were measured with thin-layer chromatography and high-performance liquid chromatography
[d] Free amino acids were determined by amino acid automatic analyzer

sugars were glucose (126.78 mg) arabinose (123.74 mg), and xylose (75.48 mg). Among the amino acids, the fraction was rich in leucine.

Discussion

The effects of CNF-HWSF on mycelial growth of seven edible mushroom species were examined. The results showed that the mushrooms tested grew well when CNF-HWSF was added. The promotive effect of CNF-HWSF (10%–20%) was more efficient in the slow-growing mushrooms such as *L. edodes* and *P. nameko* than in the rapid-growth species such as *F. velutipes* and *P. ostreatus*. However, mycelial growth was inhibited in 30% of the CNF-HWSF sample of *L. edodes*. This effect was confirmed using mycorrhizal mushrooms (*L. shimeji* and *T. matsutake*). CNF-HWSF showed a stronger promotive effect on mycelial growth of these fungi in PDL medium than in PMML medium. It was thought that PMML medium is richer in nutrition for mycorrhizal species than PDL medium. Hence, these mushrooms did not necessarily utilize the CNF-HWSF (especially the macromolecular substances as nutritional substrate) in PMML medium. The increase in the mycelial dry weight of *T. matsutake* when 1.25% CNF-HWSF was added to the PDL medium can be explained by the promotive factor in the CNF-HWSF supplied.

In previous reports of growth-enhancing agents for mushrooms, lignin sulfonate of MW 1000–2000 daltons, which is fractionationed from sulfite pulp waste,[20,21] and lignin from commercial suppliers[22] have demonstrated positive effects. Moreover, it has been recognized that lignin sulfonate is involved in the enhanced mycelial growth on *T. matsutake*.[23]

Generally, mushrooms use glucose as their primary carbon source; but when the glucose and nitrogen source concentrations are high in the medium, mycelial growth is inhibited.[22,24] The mycelial growth of *L. edodes* is also impeded by the presence of acetic acid, which is generated from beech sawdust sterilized by autoclaving.[25] However, acetic acid enhances hyphal growth on *G. frondosa*, *Wolfiporia cocos*, and *Dendropolyporus umbellatus*.[26]

The effect of CNF-HWSF, as mentioned above, was greater on the slow-growing species. This result is similar to the reports from Terashita et al.,[14] who noted that the increased yield of fruit bodies due to CNF supplement in sawdust-based cultivation was more marked on mushrooms that take a long time to cultivate. Kitamoto et al.[27] reported that rapidly growing species rely on mycelium and medium nutrients, whereas slow-growing species depend almost entirely on nutrients in mycelia for fruit-body development. These differences in nutritional physiology were suggested to reflect mycelial growth.

Hemicellulose is a major component of CNF (about 50%); and as an arabinoxylan, it is a typical component of graminaceous monocots.[28,29] Arabinoxylan is a complex polymer consisting of β-1,4-linked xylosyl residues, which can be acetylated or have covalently linked arabinosyl and glucuronic acid side groups attached.[30] The sugar composition of the hemicellulose in CNF has been determined by several groups.[31–33] The constitution reported for each sugar was as follows: D-xylose (48%–54%), L-arabinose (33%–35%), galactose (5%–11%), and D-glucuronic acid (3%–6%), although here is considerable variation among research groups. Hemicellulose has a water-insoluble fraction (hemicellulose A) and a water-soluble fraction (hemicellulose B); fraction A has few side chains, whereas fraction B has many.[12]

It is reported that the physiological activity of soluble polysaccharide is higher than that of the insoluble fraction.[30] The hemicellulose of CNF has been linked to intestinal disorders and the regulation of cholesterol; and these effects are stronger in the soluble component than in the insoluble component.[10–12] Ebringerová et al.[30] reported that soluble arabinoxylan has a role in immunological control. They noted that the principal factor is a disaccharide (2-*O*-β-D-Xylp-α-L-Alaf) found in arabinoxylan alone.

Based on our results, CNF-HWSF had a powerful effect on vegetative mycelial growth of the small-molecular-weight fractions of mushrooms. Therefore, it is possible that CNF contains some ingredient that affects nutritional and biochemical metabolism during mushroom growth.

Many studies have explored the relation between mycelial growth and the carbon source of the medium. Azuma and Kitamoto[22] reported that glucose, maltose, and starch were the most suitable carbon sources for *L. edodes*, and fructose and sucrose regulated mycelial growth. They stated that the optimal glucose concentration was 2%–3%. *Coriolus pubescens* and *L. tigrinus* are also reported to grow

well on xylan and cellulose (avicel and CMC), and growth is almost the same as when glucose is added to the medium.[34] Moreover, in the case of *Volvariella volvacea*, when 13 carbon sources (e.g., glucose, starch, and xylan) were added to the medium, only arabinose and sorbose could not be utilized.[35]

The natural material CNF is thought to contain numerous components. The major constituents of CNF-HWSF extracted with hot water were protein, starch, and arabinoxylan, which are sufficient growth substrates for mushrooms. It was shown that the promotive effects on mycelial growth of edible fungi were shown in low-molecular-weight fractions, which contained reducing sugars and free amino acids (MW 500 daltons or less) prepared from CNF-HWSF. On the other hand, the polysaccharide fractions fractionated from CNF-HWSF and the commercially available reagents used in our studies have not been linked to increased mycelial growth. A marked promotive effect of CNF-HWSF probably arises from components other than the carbon sources because the low-molecular-weight carbohydrates accounted for only 0.4% of the fraction. We expected that this promotive effect on the mycelial growth of mushrooms was due to the complex effects caused by the CNF-HWSF ingredients. Based on this idea, the mycelial dry weights obtained from this experiment were approximately equal to that of the CNF-HWSF fraction when all the fractions from CNF-HWSF were pooled, added to PDL medium, and cultured. The promotive effect of the polysaccharide fraction is shown only when this fraction coexists with the low-molecular-weight fraction. We also observed that adding the low-molecular-weight fraction from CNF-HWSF to the medium stimulated several carbohydrase activities in the medium, such as those of amylase, xylanase, and cellulase (these results will be reported in the near future). The CNF-HWSF in our experiments seem to influence significantly the metabolic pathway concerning substrate utilization for vegetative growth in mushrooms.

Conclusions

Based on the results obtained here, it is thought that CNF-HWSF is sufficient for promoting vegetative mycelial growth of edible mushrooms. However, further research is necessary concerning the isolation and characterization of promotive substances to reveal the mechanisms involved. More studies are also needed to examine the utilization CNF and CNF-HWSF in practical culture applications in light of the need to recycle organic waste.

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