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NOTE

Enzymatic saccharification of spent wood-meal media made of 5 different tree species after cultivation of edible mushroom *Auricularia polytricha*

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Abstract The chemical characteristics and the suitability for enzymatic saccharification in the spent culture media (SCM) of *Auricularia polytricha* were examined in order to investigate the utilization of the SCM as a biomass resource for alternative energy production. Wood meals from 3 hardwood species (*Quercus serrata*, *Betula platyphylla* var. *japonica*, and *Alnus japonica*) and 2 softwood species (*Pinus densiflora* and *Cryptomeria japonica*) were used as basal culture media. Dry weight of fruiting bodies were higher in the cultural media made of *B. platphylla* var. *japonica* and *A. japonica*. Amount of weight loss in media showed almost the same value among the cultural media made of 5 species, except for media made of *C. japonica.* The amounts of the main chemical components (Klason lignin, holocellulose, and α-cellulose) in SCM showed lower values than those in wood meals (WM) and fresh media (FM). After saccharification of the media by Meiselase for 48 h, the hydrolysis weight decrease dramatically increased in SCM. The amount of glucose in SCM ranged from 10.9 to 19.2 g/100 g dry biomass. The highest amount of glucose was obtained in the SCM from *B. platyphylla* var. *japonica*. These results indicate that SCM of *A. polytricha* is a suitable biomass material to produce fermentable sugars for bio-ethanol production.

Keywords Mushroom cultivation · *Auricularia polytricha* · Enzymatic saccharification · Bio-ethanol

Introduction

Many species of mushroom are cultivated primarily using sawdust bed media. After a certain period of cultivation, the media cannot be used for mushroom production because the productivity of fruiting body declines even under the optimal conditions of cultivation environment. Related with current energy issue, the potential of mushroom cultural wastes is increasing to utilize as a material for bio-ethanol production [1, 2]. In fact, the spent culture media (SCM) can be converted into sugars, and then they were fermented into ethanol.

Enzymatic saccharification is one of the methods to convert mushroom cultural wastes into sugars. It has some merits, such as substrate specificity, lower energy consumption, and environmental safety. However, enzymatic saccharification of lignocellulosics, such as wood meal, is ineffective because lignin surrounding cellulose prevents the enzymes from accessing cellulose.

SCM has advantages for enzymatic saccharification because fungal culture and fruiting body formation resulted in degradation of structural components, such as lignin, cellulose, and hemicellulose. The removal of lignin and/or hemicelluloses during the cultivation can improve the accessibility of enzymes to the cellulose and then increase the hydrolysis reactions [3, 4]. In fact, several studies have performed the enzymatic saccharification and ethanol fermentation of SCM of *Lentinus edodes* [4–6] and

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Pholiota nameko [5]. However, there are no reports on enzymatic saccharification of SCM from *Auricularia polytricha*.

A. polytricha is one of edible mushrooms known as black jelly, and is a member of the class Basidiomycetes, subclass Phragmobasidiomycetidae, order Auriculariales, and family Auriculariaceae. *A. polytricha* is included in the major six mushrooms of the total world production [7]. This mushroom has been cultivated mainly in the tropical and subtropical regions.

The aim of this study is to develop the utilization method of *A. polytricha* cultural wastes. In addition, the feasibility to convert the cultural wastes into ethanol was examined by the chemical analysis and enzymatic saccharification of them.

Materials and methods

Wood meals (WM, 9–80 mesh) from 3 hardwood species (*Quercus serrata*, *Betula platyphylla* var. *japonica*, and *Alnus japonica*) and 2 softwood species (*Pinus densiflora* and *Cryptomeria japonica*) were used as basal culture media. Fresh medium (FM) is a mixture containing wood meal, rice bran, and $CaCO_3$. Wood meal (moisture content (MC) = 8.5–11.0%) and commercial rice bran (Satoh Rice, 9–80 mesh size, MC = 11.0%) were mixed in the weight ratio of 8:1. $CaCO_3$ was also added at the concentration of 6% (w/w) to adjust the pH of culture medium to 6–7. MC of medium was adjusted to 65% by adding tap water to them. FM (200 g) was packed in a polypropylene bag (25 × 8 × 4.5 cm) equipped with a porous sterile filter (MilliSeal, 1 cm diameter pore, Millipore) and then autoclaved at 121°C for 20 min. After inoculation of *A. polytricha* (Aragekikurage 89, Mori & Company, Ltd.), the media were cultured for 40 days in a culture room at 20–23°C with relative humidity (RH) of 70–80% in the dark. Flushing treatment was conducted by diagonally cutting one side of the plastic surface, and the culture media were further cultured in a culture room at 25°C with RH of 80–90% under illumination of fluorescent tubes (24 h/day, 3.5 μmol/m^2 s) for 77 days. Every day, the culture media were watered (about 30 l for all samples in a culture room) in the morning and evening. Fruiting bodies of about 5 cm in diameter were collected, and the fresh and dry weight of fruiting bodies were measured. During the mushroom cultivation, WM, FM, and spent culture media (SCM) which contained remaining mycelia, were used as the experimental materials for chemical analysis and enzymatic saccharification. WM, FM, and SCM were used for chemical analysis. Amounts of extractives with organic solvents, lignin, holocellulose, and α-cellulose were determined. Before chemical analysis, the samples were grinded by a rotary speed mill (P-14, Fritsch) and then sieved to collect samples in 40–80 mesh size. After that, the samples were oven-dried at 105 ± 2°C. In order to determine the amount of the extractives, 5 g oven-dried sample was extracted with 120 ml mixture of ethanol and toluene (1/2, v/v) by a Soxhlet extractor for 6 h. Amounts of Klason lignin, holocellulose, and α-cellulose were determined by ordinary methods [8, 9]. Although the FM and SCM contained rice bran, $CaCO_3$, and mycelia, ordinary methods of chemical analysis for WM were also applied for determining the amounts of chemical components in FM and SCM.

A commercial enzyme, Meiselase (Meiji Seika), was used for the saccharification of each sample. Two hundred mg of oven-dried samples (40–80 mesh size) was put in an L-shaped test tube, and then 50 mg of enzyme, 19 Filter Paper Units (FPU), dissolved in 10 ml of 0.1 M acetate buffer (pH 5.0) was added to it. The L-shaped test tube was reciprocally agitated at 60 strokes/min with a water-bath shaker (NTS-120, EYELA) at 40°C for 48 h. After saccharification, the reaction mixtures were centrifuged at 4,000 rpm for 15 min. The supernatant was freeze-dried, and the residue obtained was dried at 105 ± 2°C. The hydrolysis weight decrease was calculated by the following formula:

$$\text{Hydrolysis weight decrease } (\%) = \frac{W_0 - W_1}{W_0} \times 100,$$

where W_0 (g) is the oven-dried weight of the sample before saccharification, and W_1 (g) is the oven-dried weight of the residue after saccharification.

Monosaccharides were quantified using high-performance anion-exchange chromatography (DX 500, Dionex) equipped with guard column CarboPac™ PA1 (4 × 50 mm, Dionex), analysis column CarboPac™ PA1 (4 × 250 mm, Dionex), and pulsed amperometric detector. The eluent was A (ultrapure water) and B (100 mM NaOH) with A/B (84/16, v/v) at flow rate of 1 ml/min. The column oven (Model 502, EYELA) was maintained at 30°C. Glucose (Kanto Chemical Co.), galactose (Wako Pure Chemical Industries, Ltd.), and xylose (Wako Pure Chemical Industries, Ltd.) were used as standard monosaccharides.

Results and discussion

Table 1 shows the yield of fruiting body in oven-dried weight and weight loss in media after cultivation of *A. polytricha*. The cultural media made of *B. platphylla* var. *japonica* (9.3 ± 1.9 g) and *A. japonica* (9.0 ± 0.9 g) gave higher dry weight of fruiting bodies. Amount of weight loss in media showed almost the same value among the cultural media made of 5 species, except for media

made of *C. japonica.* During fruiting bodies production, *A. polytricha* seemed to degrade wood components to satisfy its carbohydrate requirement [10].

The amount of lignin in WM was higher in softwood species (*P. densiflora* and *C. japonica*) compared to hardwood species (*Q. serrata*, *B. platyphylla* var. *japonica*, and *A. japonica*) (Table 2). In all types of sample, the amount of lignin was low in SCM (8.2–22.9 g/100 g dry biomass in a bag). Amount of lignin was decreased from FM to SCM, indicating that the lignin in FM was degraded partially by *A. polytricha* during its culture. In contrast, the amount of holocellulose in WM of hardwood species was higher than in softwood species, whereas amount of α-cellulose showed similar values in 5 wood species. Amount of holocellulose and α-cellulose in SCM were lower compared to FM. Thus, it is considered that *A. polytricha* also degraded hemicellulose and cellulose as well as lignin during the cultivation.

Table 3 shows hydrolysis weight decrease of the WM, FM, and SCM after enzymatic saccharification. The hydrolysis weight decrease of WM was similar to that of FM, whereas that of SCM dramatically increased. The high level of hydrolysis weight decrease of SCM is considered to be caused by the low lignin content. In the utilization of lignocellulosic materials to produce sugars for ethanol fermentation by enzymatic saccharification, lignin interferes with hydrolysis by blocking access of cellulases to cellulose and by irreversible binding to hydrolytic enzymes [11]. Hence, the removal of lignin from the materials can dramatically increase the hydrolysis rate [4, 5, 11].

The amount of glucose and other monosaccharides based on the dry biomass are shown in Table 4. Glucose was the most abundant among the monosaccharides produced. Amount of glucose and other monosaccharides in WM were similar to those of FM. Furthermore, although amount of holocellulose and α-cellulose in SCM showed

Table 1 Dry weight of fruiting bodies and amounts of weight loss (g) in media after *A. polytricha* cultivation

The same letter shows no significant differences by Tukey–Kramer test at the 5% level

n Number of bags, *SD* standard deviation

Wood species	Fruiting bodies		Weight loss	
	n	Mean ± SD	*n*	Mean ± SD
C. japonica	20	5.0 ± 1.6a	3	14.3 ± 3.7a
P. densiflora	18	6.0 ± 0.8b	3	24.1 ± 1.2b
Q. serrata	19	7.9 ± 0.8c	3	22.7 ± 1.4b
B. platyphylla var. *japonica*	19	9.3 ± 1.9d	3	24.9 ± 2.7b
A. japonica	19	9.0 ± 0.9cd	3	27.9 ± 2.4b

Table 2 Chemical components (g/100 g dry biomass in a bag) of wood meals, fresh media, and spent culture media

Component	Wood meal[a]					Fresh medium[a]					Spent culture medium[b]				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Lignin	32.9	27.4	19.8	13.4	23.2	30.8	25.8	16.2	14.0	19.4	22.9	13.5	11.0	8.2	11.6
Holocellulose	78.2	75.5	83.1	85.5	81.1	73.6	71.8	79.0	78.9	77.4	60.3	59.5	70.9	68.2	62.7
α-Cellulose	51.6	45.2	51.7	43.4	44.3	51.3	47.1	46.5	38.0	39.7	36.5	34.4	33.5	36.2	31.7

Fresh media and spent culture media contained rice bran, and $CaCO_3$, and spent cultura media did *mycelium*

A, *C. japonica*; B, *P. densiflora*; C, *Q. serrata*; D, *B. platyphylla* var. *japonica*; E, *A. japonica*

[a] Results were obtained from 1 replicate

[b] Results were obtained from 3 replicates

Table 3 Hydrolysis weight decrease (%) of wood meals, fresh media, and spent culture media

Respective data were obtained in triplicates. The same letter shows no significant differences by Tukey–Kramer test at the 5% level

Wood species	Wood meal Mean ± SD	Fresh medium Mean ± SD	Spent culture media Mean ± SD
C. japonica	1.5 ± 0.2a	6.6 ± 0.5a	23.6 ± 0.8a
P. densiflora	6.2 ± 0.4c	0.1 ± 1.5cd	45.1 ± 1.2b
Q. serrata	8.6 ± 0.2d	11.9 ± 0.3d	44.5 ± 1.3b
B. platyphylla var. *japonica*	6.5 ± 0.2c	9.8 ± 1.7c	54.6 ± 1.0d
A. japonica	3.4 ± 0.8b	8.7 ± 1.0b	48.9 ± 0.8c

Table 4 Amount of monosaccharides (g/100 g dry biomass) of wood meals, fresh media, and spent culture media

Wood species	Glucose	Galactose	Xylose
Wood meal			
C. japonica	1.8	0.3	0.3
P. densiflora	2.6	0.3	0.5
Q. serrata	3.0	0.3	0.5
B. platyphylla var. *japonica*	1.9	0.2	0.6
A. japonica	2.4	0.3	0.2
Fresh media			
C. japonica	2.5	0.9	0.1
P. densiflora	3.7	1.0	0.3
Q. serrata	4.0	0.9	0.3
B. platyphylla var. *japonica*	3.0	1.0	0.6
A. japonica	3.0	0.7	0.2
Spent culture media			
C. japonica	10.9	2.4	1.2
P. densiflora	12.3	1.1	3.4
Q. serrata	17.1	1.9	5.4
B. platyphylla var. *japonica*	19.2	0.8	7.6
A. japonica	15.7	1.2	5.2

Results were obtained from 1 replicate

the lowest value, the highest amount of glucose and other monosaccharides was obtained in SCM. It is considered that enzyme accessibility toward cellulose is more important than the amount of holocellulose and α-cellulose. Among 5 wood species, the highest amount of glucose was obtained from *B. platyphylla* var. *japonica* SCM. The higher amount of monosaccharides, especially glucose, in SCM is considered to be due to the higher degree of enzymatic saccharification of SCM. In conclusion, this study shows that SCM is a promising biomass material for enzymatic saccharification to produce bio-ethanol.

Conclusion

SCM of *A. polytricha* is a promising biomass material for enzymatic saccharification to obtain large amounts of monosaccharides which are fermented to bio-ethanol. In addition, SCM from hardwood species, especially *B. platyphylla* var. *japonica*, is the most appropriate biomass material for enzymatic saccharification.

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