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SHORT COMMUNICATION

Development of animal feeding additives from mushroom waste media of shochu lees

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Abstract

Purpose Mushroom waste medium can be used as a source of enzymes, but few studies on animal feed improvement using it have been found. In this study, the potential as additives for improving feed efficiency was examined. Extracts from waste media containing shochu lees used for growing three mushrooms cultivation: monkey head mushroom (MHM) *Hericium erinaceus*, oyster mushroom *Pleurotus ostreatus*, and shiitake mushroom *Lentinula edodes* were investigated.

Methods Several polysaccharide hydrolase activities in the extracts were measured and the following feedstuffs were partially digested by the extracts: Italian ryegrass, rice straw, and sweet potato runner. Digestibility of neutral detergent fiber (NDF) in the three feedstuffs was compared by the analysis of sugar composition of residual NDF after hydrolysis with sulfuric acid and trifluoroacetic acid. Antioxidant activity of the extract from waste media was also determined by DPPH radical method.

Results The polysaccharide hydrolase activity differed depending on the kind of mushroom and waste medium composition. The waste media containing sweet potato shochu lees showed higher enzyme activities than the standard media did. The additives reduced NDF content in the feedstuffs, especially in treating sweet potato runner with MHM extract. MHM remarkably decreased xylose in NDF of rice straw, and glucose and xylose in NDF of sweet potato runner.

Conclusions The results suggested that the additives might be effective in improving the efficiency of the feedstuffs for heat-stressed or weakened livestock.

Keywords Feedstuff · Mushroom cultivation waste medium · Neutral detergent fiber · Polysaccharide hydrolase · Shochu lees

Introduction

Shochu is a distilled alcoholic beverage from plant products in Japan. The shochu lees that are generated from shochu production consist of leftover distillation residue which can be recycled. We previously reported that shochu lees generated from sweet potato spirit was a good substrate in culturing mushrooms (Yamauchi et al. 2005, 2007). After that, it was also revealed that these mushroom waste media could be used for animal feed (Yamauchi et al. 2010, 2011). Phan and Vikineswary (2012) summarized the potential uses of spent mushroom media and their enzyme activities for bioremediation, animal feed, and energy feed stock.

Heat stress is known to lower the appetite of an animal and its digestive ability (Jørgensen et al. 1996, Kadzerea et al. 2002). That is, a reduction in feed efficiency is brought about. It has been reported that the addition of hydrolytic enzyme to feed partially degrades the feed and consequently reduces the effect of the stress (Bedford 1995, Ravindran 2013). Although the potential of mushroom waste medium is suggested as a source of such enzymes, it is unclear how much effect it has.

In this study, we revealed higher enzyme activities in the extracts from sweet potato shochu lees waste media of three different mushroom species and examined the digestibility

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of feedstuffs by enzyme preparations of shochu lees media origin. Antioxidant activity was also measured due to its involvement in the deterioration of feedstuff (Decker 1998), which is known to affect heat stress in sheep (Chauhan et al. 2014).

Materials and methods

Culture of mushrooms

Seed cultures of oyster mushroom (OM, *Pleurotus ostreatus* (Jacq.) P. Kumm. Hiratake Tohoku H67) and monkey head mushroom (MHM, *Hericium erinaceus* (Bull.) Pers. KX-Y B04) were purchased from Kinox Co. Sendai, Japan. Seed culture of shiitake mushroom (SM, *Lentinula edodes* (Berk.) Pegler, XR1) was obtained from Mori Co. Kiryu, Japan. Hardwood sawdust made of Japanese beech wood (*Fagus crenata*) and Japanese cedar (*Cryptomeria japonica*) was obtained from Kyozen Co., Nagano, Japan. Sweet potato starch waste, dry sweet potato shochu lees and rice bran were from Kyushukako Co. Kanoya, Southern Green Co. Minamikyushu, and Tsuchimochi Co. Miyakonoji, Japan, respectively.

MHM and OM were cultivated on two types of media: sweet potato shochu lees media (shochu lees media) and hominyfeed or rice bran media (control media) according to the method of Yamauchi et al. (2009, 2011). The former was dry shochu lees: sweet potato starch waste: shell fossil, 60:36:4. The control media were: rice bran: sawdust: shell fossil 60:36:4 for OM, and hominyfeed: sawdust: shell fossil, 31:62:4, respectively. The sawdust used for OM and MHM were Japanese cedar and Japanese beech wood, respectively.

The composition of the shochu lees medium for SM cultivation was shochu lees: sawdust (Fagus): shell fossil at a ratio of 20:76:4, and in the conventional medium, it was rice bran: sawdust (Fagus): shell fossil at a ratio of 20:76:4. The mixture (2.5 kg, 66% water content) was transferred to a culture bag and sterilized by autoclaving for 3 h. Ten g of spawn was inoculated into each culture bag and stored in a culture room, at 22 ± 2 °C, humidity 75 ± 5%, which was lit for approximately 2 h a day. The mycelial growth of SM lasted for 94 days. The bags were then transferred to a developing room maintained at 17 ± 2 °C, humidity 90% to allow the fruit body formation. The room was lit for 9 h/day with 200-lux fluorescent lights. The end of the first harvest was 109 days from inoculation. For the 2nd and 3rd harvests, the cultures were incubated for 2 weeks in the culture room; the watering stimulation and the fruit body formation occurred in the developing room under the same conditions as the first harvest. The ends of the 2nd and 3rd harvests were 137 days and 174 days from inoculation, respectively.

Enzyme preparation

Waste media (wet weight 50 g; dry weight 17 g) were dispersed in 500 ml of 50 mM Na phosphate buffer (pH 7.0). Solutions were then incubated at 4 °C for 2 h, with stirring, before centrifugation at 8000*g* for 20 min. The resulting clear supernatant was sterilized by passing through a cellulose acetate membrane (0.22 µm), and then lyophilized. The lyophilizate obtained from the MHM medium was 7.59 g. The lyophilizate (1 g) was then dissolved in 200 ml of 50 mM Na acetate buffer (pH 5.0), and used as the enzyme preparations.

Enzyme activities

Polysaccharide hydrolase activity was measured using insoluble azurine-cross-linked-(AZCL) conjugates (Megazyme Co. Bray, Ireland) as substrates. α-Amylase, arabinanase, cellulase, galactanase, β1,3-glucanase, β1,4-mannanase, and xylanase were measured using AZCL-amylose, arabinan, HE-cellulose, galactan, curdlan, galactomannan, and arabinoxylan, respectively, as substrates. Hydrolysis was performed using 1 ml of reaction mixture containing 50 mM Na acetate buffer (pH 5.0) and 2 mg (± 0.06 mg) of AZCL-conjugate in a 1.5-ml micro tube, at 30 °C. The reaction was started by the addition of 10–100 ml enzyme preparation, and the micro-tube was continuously shaken (30 rpm) for 20–60 min. The hydrolysis rate was estimated by the absorbance of supernatant at 590 nm. Enzyme activity was expressed as mg/min/g (dry weight of waste medium).

Digestibility of feedstuffs using the enzyme preparations

Lyophilized powders of Italian ryegrass, rice straw and sweet potato runner were incubated with enzyme preparations at 25 °C for 24 h. 0.5 mg of the enzyme preparation was used for 1 g of sample in 5 ml of 50 mM Na acetate buffer (pH 5.0). After incubation, neutral detergent fiber (NDF) was obtained using the method of Van Soest and McQueen (1973). NDF was then hydrolyzed with 2 M trifluoroacetic acid at 120 °C for 1 h. Neutral sugars in the hydrolyzates were derivatized with p-aminobenzene ethyl ester (ABEE) (Kwon and Kim 1993) and ABEE sugars were separated by HPLC using a column of C18 Honenpack column in acetonitrile borate buffer (Yasuno et al. 1997). Detection was performed using a fluorometer at the excitation wavelength 305 nm and emission at 360 nm. The quantity of each sugar was corrected using 2-deoxyglucose as an internal standard. Total glucose was determined using a GluNeo glucose

determination kit (Sinotest Co., Tokyo, Japan) after hydrolysis with 1 M sulfuric acid and the subsequent neutralization.

Antioxidant assay

The scavenging activity of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical (Sharma and Bhat 2009) in mushroom culture media and fruit bodies was measured. They were extracted with 50% ethanol and the extract was incubated with 0.1 mM DPPH (50% ethanol solution). The scavenging activity of the extract was calculated as a Trolox equivalent, from the decrease of absorbance at 517 nm. Super oxide dismutase (SOD) activity was also determined using a SOD-assay kit WST (Dojindo, Kumamoto, Japan), according to the manufacturer's instructions.

Results and discussion

Enzyme activities in waste lyophilizate

Generally, SM grows slowly and takes 2.5–3 times longer to fruit compared with MHM and OM. Fruit bodies of SM were harvested three times during the 174 culturing days. Among the three waste preparations of SM, the last waste showed the highest activities of all the enzymes investigated; the first and second extracts had weaker polysaccharide hydrolase activities.

A comparison of polysaccharide hydrolase activities in shochu lees media waste of MHM, OM and SM (after the third harvest) and conventional media waste of MHM and OM is shown in Table 1. Different enzymes had the different pH optima; in our experiment, pH 5.0 was the most common and was selected for enzyme assays.

Table 1 Polysaccharide hydrolase activities

Enzyme	Waste				
	MHM		OM		SM
	S	C	S	C	S 3rd
	Activity (mg/min/g)				
α-Amylase	0	0	450	520	10.9
Arabinanase	7.3	2.7	117	16.8	9.7
Cellulase	175	15.2	38.7	13.2	14.7
Galactanase	42.4	2.7	55.6	10.3	41.2
β1,3-Glucanase	191	18.1	185	163	14.7
β1,4-Mannanase	272	80.2	2.3	2.1	16.5
Xylanase	102	24.3	19.6	19.6	66.2

S and C, shochu lees medium & conventional medium, respectively

Experimental errors were, at most, 0.6 for values below 20, and less than 3% for values above 20

The enzyme profiles of the three waste extracts differed: MHM preparations displayed the highest activity towards AZCL-substrates such as HE-cellulose, β-galactomannan, and arabinoxylan. In OM preparations, α-amylase and arabinanase showed the highest activity, and β1,3-glucanase activity was comparable with that of the activity measured in MHM preparations. Enzyme activities from MHM and OM in the conventional media containing sawdust and rice bran were lower than those in the shochu lees medium. Schimpf and Schulz (2016) reported that SM medium showed higher cellulase and xylanase activities than MHM. However, Table 1 shows both enzyme activities in the shochu medium of MHM are higher than SM medium. It is evident that mushroom cultivation waste medium of shuchu lees is better as the additive of animal feed.

Sudo et al. (2008) reported glycosidase activities in MHM media with α-galactosidase activity being the highest of all the glycosidases measured. The glycosidase activity profiles differed among the strains used and it is probable that profiles of polysaccharide hydrolyses activities differ among the strains in one mushroom species.

Digestion of feedstuffs by waste lyophilizates

Amylase-digested NDF contains polysaccharides and lignin that are sometimes difficult for domestic animals, such as heat-stressed cows, to digest. A decrease in NDF in feedstuffs after treatment with mushroom waste extracts could increase the nutritional efficiency. The enzyme preparations listed in Table 1 were used in the hydrolysis of NDFs. Table 2 shows the NDF content after enzyme treatment.

MHM and OM waste had comparable effects on NDF. The decrease in NDF was the highest in sweet potato runner. Poaceae plants such as Italian ryegrass and rice contain silica, and sweet potato contains more hemicellulose (Cornelissen and Thompson 1997, Pauly and Keegstra 2008, Vogel 2008). Consequently, it can be inferred that sweet potato runner NDF was most hydrolyzed by MHM extract. The hydrolysis of Italian ryegrass NDF was small (Table 1). Therefore, to confirm the hydrolysis, ten times the amount

Table 2 NDF content in feedstuffs after treatment with enzyme preparation from shochu lees waste media

Waste treatment	NDF content (mg/g)		
	Italian ryegrass	Rice straw	Sweet potato runner
Control	351	493	324
SM	340	477	313
MHM	337	440	285
OM	335	436	304

of extract was used. NDF decreased to 272 and 268 mg/g, by MHM waste and OM waste treatment, respectively. Thus, it was proved that waste enzyme treatment on feedstuffs is effective.

Changes in sugar composition of NDF

Table 3 summarizes the changes in sugar composition of NDF after waste enzyme treatment. The amount of sugar was higher in the Italian ryegrass treated with SM waste than in the untreated (control) and MHM treated groups (Table 2).

The NDF content of Italian ryegrass decreased after SM waste treatment. If there had been equal degradation of sugar and lignin, the sugar ratio compositions would have been unchanged between the treated and control groups. The amount of sugar in the NDF was increased, suggesting that the degradation of lignin preceded the degradation of polysaccharide during the initial stages of SM waste treatment. Leatham (1985) reported that during SM growth, lignin was degraded faster than polysaccharides; however, lignin degradation activity was still observed during the final stages of growth. After the treatment with MHM waste, there was a notable decrease of xylose in the NDF of rice straw, and large decreases in glucose and xylose were observed in sweet potato runner NDF. These results suggest that the addition of enzyme preparations effectively improves the digestibility of some feedstuffs.

Takabatake et al. (2016) reported that the addition of polysaccharide-degrading enzymes into sawdust-based culture media containing *Hericium erinaceus* and *Pholiota microspora* efficiently increased the fruiting body yield. These results depend on the profiles of enzyme which mushroom secrete being different. We suggest that further improvement on feedstuff digestibility will be possible based on a combination of enzyme preparations from MHM and SM or MHM and OM.

Antioxidant activities

All of the antioxidant activities were lower in the waste media than in the fruit bodies. Figure 1 shows the antioxidant activities of waste media. Scavenging activities in MHM and OM waste media were lower than in the start media (media before spawn inoculation), irrespective of medium composition.

SM antioxidant activity was the highest of the mushrooms examined, especially in the shochu lees waste after the second harvest. Floegel et al. (2011) reported DPPH scavenging activity of many foods were lower than 100 trolox unit/g.

In the similar manner, SOD activity was in the range of 50–400 SOD unit in the media for SM, OM, and MHM, though we found around 1500 SOD activity in fruit bodies of OM. Previously, Niwa and Sasaki (2003) reported that SOD activity was around 200 in leaves of Japanese cedar. These results indicate that SOD activity in the mushroom cultivation waste media is normal.

Table 3 Changes in NDF sugar composition after enzyme treatment from mushroom waste

Sugar Treatment	Italian ryegrass			
	Galactose (mg/g)	Glucose (mg/g)	Arabinose (mg/g)	Xylose (mg/g)
Control	11.4 ± 0.3	373 ± 8.3	1.5 ± 0.2	230 ± 8.1
SM	12.2 ± 0.4	398 ± 8.3	1.6 ± 0.6	241 ± 8.0
MHM	11.8 ± 0.3	384 ± 7.6	1.0 ± 0.3	214 ± 6.3
OM	10.9 ± 0.3	380 ± 7.6	0.6 ± 0.1	242 ± 6.3
Sugar treatment	Rice straw			
	Galactose (mg/g)	Mannose (mg/g)	Glucose (mg/g)	Xylose (mg/g)
Control	16.2 ± 0.5	1.1 ± 0.2	472 ± 9.3	242 ± 7.3
SM	14.2 ± 0.3	1.2 ± 0.3	453 ± 10.3	216 ± 6.1
MHM	12.3 ± 0.3	1.3 ± 0.2	430 ± 9.1	175 ± 5.8
OM	11.8 ± 0.3	1.2 ± 0.3	441 ± 7.6	220 ± 6.3
Sugar treatment	Sweet potato runner			
	Galactose (mg/g)	Mannose (mg/g)	Glucose (mg/g)	Xylose (mg/g)
Control	9.6 ± 0.3	6.5 ± 0.3	493 ± 9.3	114 ± 3.7
SM	12.8 ± 0.5	7.4 ± 0.4	385 ± 12.6	101 ± 6.3
MHM	7.8 ± 0.2	3.8 ± 0.2	272 ± 5.5	87.0 ± 2.6
OM	9.3 ± 0.2	7.1 ± 0.3	372 ± 8.9	104 ± 5.3

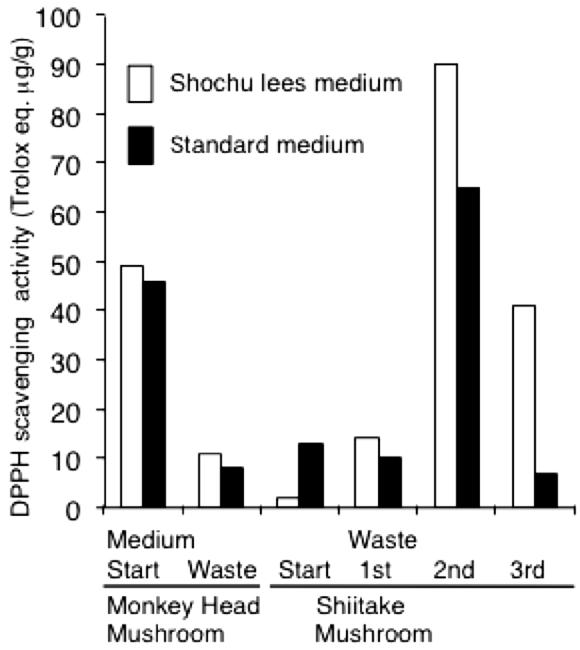

Fig. 1 DPPH scavenging activity of waste media. DPPH scavenging activity of waste and start media of monkey head mushroom and shiitake mushroom were measured. Shiitake mushroom 1st, 2nd, and 3rd are the waste media after 1st, 2nd, and 3rd harvest, respectively

Taken together, antioxidant activity was judged to be not so high in the mushroom waste medium.

Conclusion

Extracts from mushroom cultivation waste media, especially of sweet potato shochu lees contain high enzyme activity and are effective in reducing neutral detergent fiber, suggesting that they could be used as enzyme additives to animal feeding. Total recycling of mushroom cultivation waste medium is idealistic, but keeping the bulky medium for long time is difficult from many view points. In this study, we showed the usefulness of lyophilizate that will be helpful in recycling of mushroom cultivation waste.

References

Bedford MR (1995) Mechanism of action and potential environmental benefits from the use of feed enzymes. Anim Feed Sci Technol 53:145–155

Chauhan SS, Celi P, Lenry BJ, Clarke IJ, Dushea FR (2014) Dietary antioxidants at supranutritional dose improve oxidative status and reduce the negative effects of heat stress in sheep. J Anim Sci 92:3364–3374. https://doi.org/10.2527/jas.2014-7714

Cornnelissen JHC, Thompson K (1997) Functional leaf attributes predict litter decomposition rate in herbaceous plants. New Phytol 135:109–114. https://doi.org/10.1046/j.1469-8137.1997.00628x

Decker EA (1998) Antioxidant mechanisms. In: Akoh CC, Min DB (eds) Food lipids, chemistry, nutrition and biotechnology. CRC Press, Boca Ratom, pp 397–421

Floegel A, Kim D-O, Chung S-J, Koo SI, Chun OK (2011) Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J Food Comp Anal 24:1043–1048. https://doi.org/10.1016/j.jfca.2011.01.008

Jørgensen H, Zhao X-Q, Eggum B (1996) The influence of dietary fibre and environmental temoperature on the development of the gastrointestinal tract, digestibility, degree of fermentation in the hind-gut and energy metabolism in pigs. Brit J Nutr 75:365–378. https://doi.org/10.1079/BJN19960140

Kadzerea CT, Murphya MR, Silanikoveb N, Maltzb E (2002) Heat stress in lactating dairy cows: a review. Livest Prod Sci 77:59–91. https://doi.org/10.1016/S0301-6226(01)00330-X

Kwon H, Kim J (1993) Determination of monosaccharides in glycoproteins by reverse-phase high-performance liquid chromatography. Anal Biochem 215:243–252. https://doi.org/10.1006/abio.1993.1582

Leatham GF (1985) Extracellular enzymes produced by the cultivated mushroom *Lentinus edodes* during degradation of a lignocellulosic medium. Appl Environ Microbiol 50:859–867

Niwa Y, Sasaki Y (2003) Plant self-defense mechanisms against oxidative injury and protection of the forest by planting trees of triploids and tetraploids. Ecotoxicol Environ Saf 55:70–81

Pauly M, Keegstra K (2008) Cell-wall carbohydrates and their modification as a resource for biofuels. Plant J 54:559–568. https://doi.org/10.1111/j.1365-313X.2008.03463.x

Phan CW, Vikineswary S (2012) Potential uses of spent mushroom substrate and its associated lingocellulosic enzymes. Appl Microbiol Biotechnol 96:863–873. https://doi.org/10.1007/s00253-012-4446-9

Ravindran V (2013) Feed enzymes: the science, practice, and metabolic realities. J Appl Poult Res 22:628–636. https://doi.org/10.3382/japr.2013-00739

Schimpf U, Schulz R (2016) Industrial by-products from white-rot fungi production. Part I: generation of enzyme preparations and chemical, protein biochemical and molecular biological characterization. Process Biochem 51:2034–2046. https://doi.org/10.1016/j.procbio.2016.08.032

Sharma OP, Bhat TK (2009) DPPH antioxidant assay revised. Food Chem 113:1202–1205. https://doi.org/10.1016/j.foodchem.2008.08.008

Sudo M, Yoshida M, Fukuda K, Takabatake K, Kaneko S (2008) Biodegradation of sugar beet pulp by edible mushroom *Hericium erinaceus*. Rep Natl Food Res Inst 72:65–81 **(in Japanese)**

Takabatake K, Igarashi K, Samejima M (2016) Effects of polysaccharide-degrading enzyme addition on the sawdust-based cultivation of *Hericium erinaceus* and *Pholiota microspora*. Mushroom Sci Biotechnol 24:121–128 **(in Japanese)**

Van Soest PJ, McQueen KW (1973) The chemistry and estimation of fibre. Proc Nutr Soc 32:123–130. https://doi.org/10.1079/pns19730029

Vogel J (2008) Unique aspects of the grass cell wall. Curr Opin Plant Biol 11:301–307. https://doi.org/10.1016/j.pbi.2008.03.002

Yamauchi M, Imaya R, Masuda S, Yamada M, Kihara M, Yoneyama K, Harada H (2005) Study on the development of mushroom cultivation technology using dried shochu lees from sweet potato. Environ Eng Res 42:545–553. https://doi.org/10.11532/proes1992.42.545 **(in Japanese)**

Yamauchi M, Imaya R, Yamada M, Masuda S, Kihara M, Yoneyama K, Harada H (2007) Practicality of a high value-added edible mushroom (Eringi, *Pleurotus eringii*) using sweet potato shochu lees. Environ Eng Res 44:481–490. https://doi.org/10.11532/proes1992.44.481 **(in Japanese)**

Yamauchi M, Yamada M, Yagi F, Masuda S, Yamaguchi T (2009) Growth and component characteristics of Yamabushitake (*Hericium erinaceum*) using food waste (Shochu lees and starch waste). Environ Eng Res 46:117–127 **(in Japanese)**

Yamauchi M, Komura H, Yamada M, Dairokuno H, Uchida I (2010) Construction of a recycling system for dried shochu lees that is based primarily on mushroom production. Jpn Soc Mater Cycles Waste Manag 21:155–164. https://doi.org/10.3985/jjsmcwm.21.155 **(in Japanese)**

Yamauchi M, Dairokuno H, Yamada M, Yagi F, Harada N, Masuda S, Yamaguchi T (2011) Utilization of food wastes (*Shochu lees and starch waste*) for cultivation of some mushrooms and adaptability of waste culture media to feed. JSCE 67III:449–459 **(in Japanese)**

Yasuno S, Murata T, Kokubo K, Yamaguchi T, Kamei M (1997) Two-mode analysis by high-performance liquid chromatography of p-amino benzoic acid ethyl ester-derivatized monosaccharides. Biosci Biotechnol Biochem 61:1944–1946. https://doi.org/10.1271/bbb.61.1944