Improving nutritive value of underutilized feed resources for ruminants by culturing with white-rot fungi – Review of my research conducted at The University of Shiga Prefecture

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Abstract

Worldwide, a high proportion of lignocellulose materials are not fully utilized. If high quality roughage for animals is insufficient at regional or country-wide level, lignocellulose materials could provide an appropriate alternative feed source. However, some lignocellulose materials originating from wood and grass contain lignin at high levels. Therefore, it is very difficult to feed them as a diet for animals directly because the cellulose and hemicellulose in lignocellulose materials are strongly tied into the lignin structure. Many studies have investigated the removal of lignin from lignocellulose materials. Chemical treatment, steam-explosion treatment and white-rot treatment are potential methods for removal of lignin from lignocellulose. The white-rot treatment is an environmentally-friendly method because this method does not use dangerous chemical reagents or require high energy input compared with the steam-explosion treatment. Further studies are required to optimize the method of lignin removal by the action of white-rot fungi. The appropriate choice of white-rot fungus, its culturing period and culturing temperature are important to improve the success of utilization of lignocellulose materials. Information on the relationship between *in vitro* digestion and *in vivo* digestion of substrates cultured with white-rot fungi should also be collected. Scale-up of the process for mass culturing lignocellulose material with white-rot fungus must be established to ensure practical use of the process. In the present review, I describe aspects of my research that address the issues as mentioned above.

Keywords: digestibility; feed; lignin;, white-rot fingi

1. Introduction

Many scientific reports have been published concerning increasing the nutritive value and removing the lignin in lignocellulose materials by culturing them with white-rot fungi. The use of white-rot fungi is a method to remove lignin from lignocellulose materials and is an environmentally-friendly method because it does not use dangerous chemical reagents such as sulfuric acid or sodium hydroxide in the treatment process. Kirk and Moore [1] found that the digestibility of aspen and birch wood increased by

Table 1. Effect of culturing temperatue on the digestibilty and lignin content in lignocellulose materias cultured with white-rot fungi

Culture temperature and length		IVOMD (%)	NDFomD(%)	IVGP (ml/g OM)	Lignin content (%)
Fungus: *Lentinula edodes* a)					
Substrate: *Sugarcane bagasse and rice bran (19:1)*					
26 ℃ for 4wks +	25 ℃ for 4weeks	79.1	67.4	208	5.1
26 ℃ for 4wks +	30 ℃ for 4weeks	83.7	75.5	227	4.3
28 ℃ for 8 wks		73.3	60.3	204	5.9
28 ℃ for 4wks +	32 ℃ for 4wks	72.6	60.8	206	5.8
Fungus: *Ceriporiopsis subvermispora* b)					
Substrate: *Cedar wood and rice bran (19:1)*					
28 ℃ for 20 wks		64.7	50.2	138	9.6
32 ℃ for 20wks		68.8	55.3	152	8.9

Source: a) Okano *et al*.[14], b) Okano *et al*. (unpublished data).

culturing it with *Polyporus frondosus, Polyporus berkeleyi, Polyporus resinosus, Polyporus giganteus* and *Cryptoderma yamanoi*. Reid [2] reported the increased digestibility of aspen wood when cultured with *Phlebia tremellosa*. Several authors ([3], [4], [5], [6], [7], [8], [9], [10] and [11]) reported that the digestibility of crop straws was improved by culturing with basidiomycetes such as *Pleurotus* sp., *Coprinus cinereus, Phanerochaete chrysosporium, Polyporus ciliatus, Ceriporiopsis subvermispora* and *Cyathus stercoreus*.

In the reports mentioned above, the evaluation of digestibility of cultured substrate was conducted using the *in vitro* rumen fermentation method, while few studies conducted evaluations using the *in vivo* digestion test. Calzanda *et al.* [6] reported that the digestibility of wheat straw cultured with *Pleurotus sajor-caju* increased slightly from 52% to 55%. Suzuki [12] found that the digestibility of sugarcane bagasse substrate and konara wood cultured with *Pleurotus abalones* and *Lentinus edodes*, respectively, increased when evaluated with the *in vivo* digestion test using sheep. From 2000 onwards, the effect of improving the nutritive value of lignocellulose materials by culturing with white-rot fungi has been examined in more detail than the past reports mentioned above.

2. Appropriate temperature for culturing lignocellulose material with white-rot fungi

Before assessing the effect of white-rot fungi culture on improving the digestibility of lignocellulose materials, the appropriate culturing temperature for mycelial growth of each fungus must be determined. Generally, lignin degradation by white-rot fungi is increased at higher culturing temperatures compared with lower culturing temperatures (Table 1). Incubation at excessive high temperatures can inhibit the growth of mycelium and decrease the degradability of lignin and the digestibility of lignocellulose materials over a constant culturing period. Zadradil *et al.* [15] observed that the *in vitro* dry matter digestibility of wheat straw decreased when wheat straw was cultured with *L. edodes* at 30 ° C compared with 22 ° C and 25 ° C.

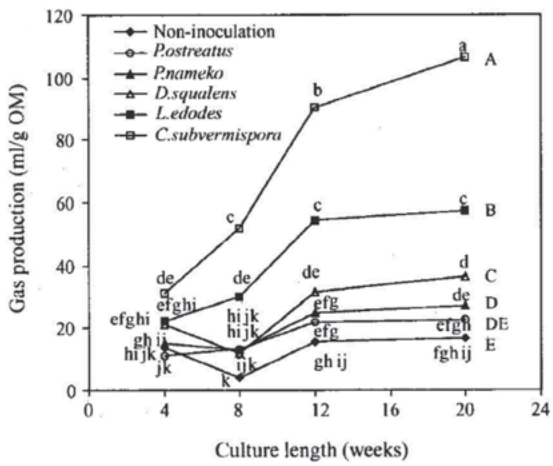

Figure 1. The change in *in vitro* gas production (ml/g OM) of Japanese red cedar wood cultured white-rot fungi for different culturing period [16].

3. Screening fungus for degradation of lignocellulose materials

The combination between the kind of lignocellulose material and fungus is very important (Figure 1). We chose to use *C. subvermispora* for fungal treatment of wheat straw, corncob meal, sugarcane bagasse, reed, bamboo and cedar wood, and checked its effectiveness compared with other fungi such as *L. edodes, Pleurotus eryngii, Pleurotus salmoneostramineus, Pleurotus ostreatus, Pholiota nameko* and *Dichomitus squalens*. Okano *et al.* [13] found that the digestibility of sugarcane bagasse cultured with *L. edodes* or *C. subvermispora* was higher than when cultured with *P. eryngii* or *P. salmoneostramineus*. Meanwhile, Akin *et al.* [11] reported that *C. stercoreus* was more effective in improving the biodegradability of cell walls in alfalfa stems compared with *C. subvermispora*. Culturing rice straw with *C. subvermispora* did not significantly improve its nutritive value (Okano, unpublished data). Yamakawa *et al.* [8] reported that culturing rice straw with *P. ostreatus* improved its digestibility. The use of edible mushrooms, such as *P. ostreatus, P. salmoneostramineus* and *L. edodes*, have an advantage over inedible fungi because the cost of culturing fungi must be recovered by selling their mushrooms.

4. Appropriate culturing period

The appropriate culturing period depends on the kind of lignocellulose materials, the fungal

Table 2. Change in *in vitro* gas production (ml/g OM) at 48 h of substrates mixed materials and supplements or not during culture with *Ceriporiopsis subvermispora*

Materials	Supplements	Culture length (wks)							
		0	2	4	6	8	10	12	15
Reed a)		74	108	178	197	217			
Wheat straw b)		195	125	175	210	230			
Wheat straw b)	Rice bran (19:1)	-	-	-	-	162			
Corncob meal c)	Wheat bran (19: 1)	176	-	223	-	268		274	
Sugarcane bagasse d)	Rice bran (9:1)	143	-	-	-	227		243	
Baboo e)	Rice bran (19:1)	30					151		173
Cedar wood f)	Rice bran (19:1)	21					123		

Source: a) Okano *et al* . [17], b) Kitano *et al* . [18], c) Morita [19], d) Okano *et al* . [20], e) Okano *et al* . [21], f) Okano (unpublished data).

species and any supplements added to the culture medium. Optimizing the culturing temperature accelerates lignin degradation and shortens the culturing period to improve the digestibility of lignocellulose materials as mentioned above.

In Table 2, the changes in *in vitro* gas production during 48 h (IVGP) of reed, wheat straw, corncob meal and sugarcane bagasse digestion are shown. Intact wheat straw contained the most digestible lignocellulose materials (Table 2). Meanwhile, corncob meal was more digestible than wheat straw when they were cultured with *C. subvermispora*. Since the lignin and neutral detergent fiber (NDFom) contents in wheat straw and corncob meal after culturing with *C. subvermispora* were not different but the lignin and NDFom contents in intact corncob meal were higher than those in intact wheat straw, the degradation rate of lignin in corncob meal was higher than that in wheat straw.

The supplementation of rice bran or wheat bran was effective to increase the IVGP of lignocellulose materials without wheat straw in this study. Since excessive supplementation of nutrients to the substrate in artificial mushroom production inhibits the degradation of cellulose and lignin, a sufficient readily available carbohydrate to support the growth of the fungal mycelium must be contained in the wheat straw. Amirta *et al*. [22] reported that the supplementation of wheat bran with cedar wood cultured with *C. subvermispora* increased methane production.

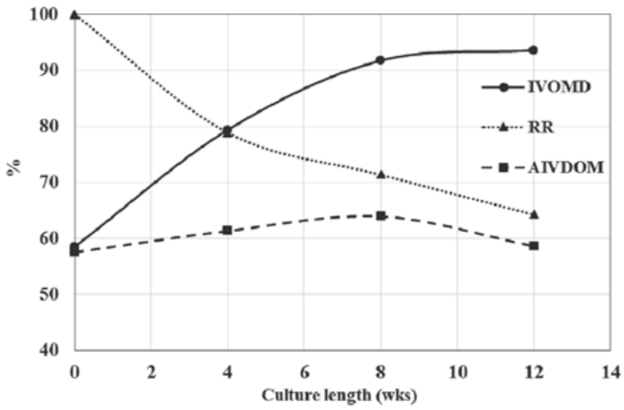

Figure 2. Changes in *in vitro* organic matter digestibility (IVOMD, %), residual ratio (%) of substrate during culture and the amount of *in vitro* digestive organic matter (IVDOMD, %) cultured corncob meal substrate with *Ceriporiopsis subvermispora*.

The changes in *in vitro* organic matter digestibility (IVOMD), residual ratio of substrate during culturing (RR) and the amount of *in vitro* digestive organic matter (IVDOM) of corncob meal substrate cultured with *C. subvermispora* are shown in Figure 2. The IVOMD increased following culturing with *C. subvermispora* for 4, 8 and 12 weeks. In contrast, the value of RR decreased following culturing with *C. subvermispora*. It followed that the amount of IVDOM at 12 weeks was lower than at 4 and 8 weeks. This phenomenon occurs in other lignocellulose materials. Accordingly, we must judge the appropriate culturing period based on whether our aim is to make feed or material for methane production. In the case of feed for ruminants, OM digestibility should be higher than 50-60% .

5. Relationship between *in vitro* and *in vivo* digestion of substrate cultured with white-rot fungi

The *in vitro* digestion method is a very convenient procedure that is able to evaluate feed value within 3 or 4 days, and therefore *in vitro* rumen fermentation methods are used to evaluate the nutritive value of decayed lignocellulose materials in many studies. However, it was unclear whether it was appropriate to apply this method to the evaluation of nutritive value in lignocellulose materials because white-rot fungi metabolize sugars, starch and hemicellulose in preference to cellulose and lignin.

The correlations between IVOMD and *in vivo* OM digestibility, and between IVGP and *in vivo* OM digestibility are shown in Figures 3 and 4, respectively. The coefficient of determination (R^2) for IVGP was higher than for IVOMD. Miki and Okano [24] observed that the IVGP of intact rice straw was higher than that of un-sterilized rice straw cultured with *P. salmoneostramineus*, but the IVOMD of intact rice straw was lower than that of unsterilized rice straw. Menke *et al*. [25] proposed that the metabolizable energy in ruminant feed could be estimated by the values of IVGP and chemical composition. Accordingly, IVGP is a better indicator than IVOMD to evaluate the digestibility of feed in ruminants.

6. Procedure of mass production for culturing lignocellulose material with white-rot fungi

Zadrazil *et al*. [14] proposed the procedure of mass production for culturing wheat straw with white-rot fungi and reported that this system could treat 1.5 ton of wheat straw with *P. sajor-caju*. Okano *et al*. [21] reported that they sterilized 4 kg of substrate/plastic box and could culture 24 boxes simultaneously with *C. subvermispora*. Kumar and Gomes [26] discussed the performance evaluation of reactors for bioconversion of wheat straw to animal feed using white-rot fungi. Further studies are required to establish the optimal procedures for bioconversion with white-rot fungi.

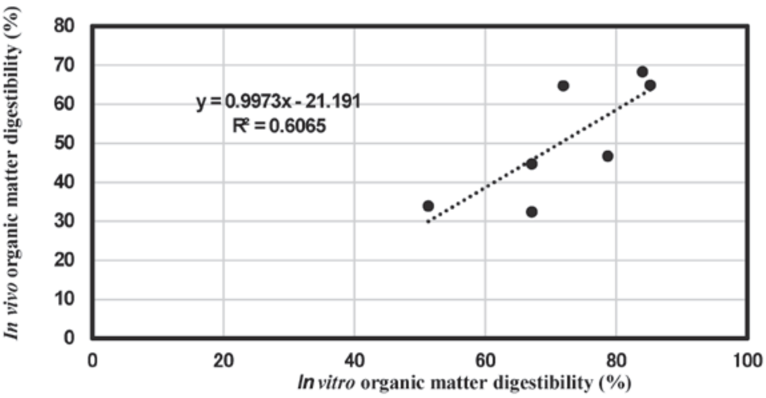

Figure 3. Correlativity of organic matter digestibility between *in vivo* and *in vitro*.

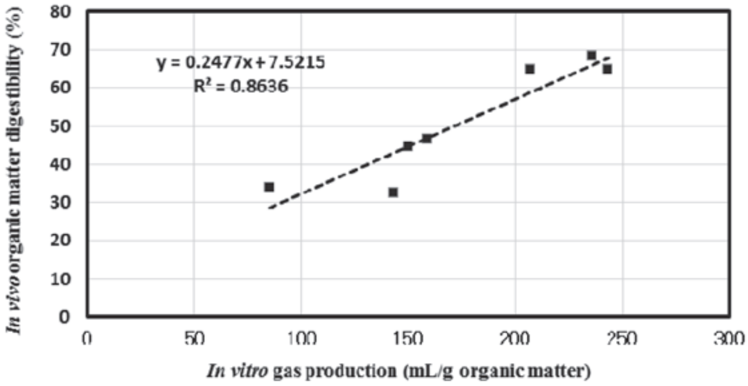

Figure 4. Correlativity of *In vivo* organic matter digestibility and *in vitro* gas production

7. Conclusion

The choice of the appropriate white-rot fungus, temperature and time period of culture is important in improving the nutritive value of lignocellulose material in culture with white-rot fungi. The evaluation of digestibility for lignocellulose materials should be done by determining *in vitro* gas production. The use of edible white-rot fungi is interesting in the treatment of lignocellulose materials because the cost of edible white-rot fungal treatment is lower than that with inedible fungi.

References

[1] Kirk, T.K., Moore, W.E., 1972. Removing lignin from wood with white-rot fungi and digestibility of resulting wood. Wood Fiber 4, 72-79.

[2] Reid, I.D., 1989. Effects of nitrogen supplements on degradation of aspen wood lignin and carbohydrate components by *Phanerochaete chrysosporium*. Appl.

Environ. Microbiol. 45, 830-837.

[3] Zadrazil, F., 1977. The conversion of straw into feed by basidiomycetes. European J. Appl. Microbiol. 4, 273-281.

[4] Burrows, I., Seal, K.J., Eggins, H.O.W., 1979. The biodegradation of barley straw by *Coprinus cinereus* for the production of ruminant feed. In "Straw decay and its effect on diposal and utilization" (ed. by Grossbard, E.), pp.147-154. John Wiley & Sons, Chichester.

[5] Langar, P.N., Sehgal, J.P., Rana, V.K., Singh, M.M., Garcha, H.S., 1980. Chemical change in wheat and paddy straws after fungal cultivation. Indian J. Anim. Sci. 50, 942-946.

[6] Calzada, J.F., de Porres, E., de Leon, R., Rolz, C., Franco, L.P., 1987. Production of food and feed from wheat straw by *Pleurotus sajor-caju*. Mushroom J. Tropics. 7, 45-46.

[7] Jung, H.G., Valdez, F.R., Abad, A.R., Blanchette, R.A., Hatfield, R.D., 1992. Effect of white rot basidiomycetes on chemical composition and *in vitro* digestibility of oat straw and alfalfa stems. J. Anim. Sci. 70, 1928-1935.

[8] Yamakawa, M., Abe, H., Okamoto, M., 1992. Effect of incubation with edible mushroom, *Pleurotus ostreatus*, on in vitro degradability of rice straw. Anim. Sci. Technol. (Jpn.) 63, 180-185 (in Japanese, with English abstract).

[9] Karunanandaa, K., Fales, S.L., Varga, G.A., Royse., D.J., 1992. Chemical composition and biodegradability of crop residues colonized by white-rot fungi. J. Sci. Food Agric. 60, 105-112.

[10] Jalc, D., Zitnan, R., Nerud, F., 1994. Effect of fungus-treated straw on ruminal fermentation *in vitro*. Anim. Feed Sci. Technol. 46, 131-141.

[11] Akin, D.E., Morrison Ⅲ, W.H., Rigsby, L.L., Gamble, G.R., Sethuraman, A., Eriksson, K.-E.L., 1996. Biological delignification of plant components by the white rot fungi *Ceriporiopsis subvermispora* and *Cyathus stercoreus*. Anim. Feed Sci. Technol. 63, 305-321.

[12] Suzuki, Y., 1995. Study on nutritional upgrading of underutilized feed resources by basidiomycetes. Ph.D. thesis, Kyoto University, Kyoto (in Japanese, with English abstract).

[13] Okano, K., Iida, Y., Samusuri, M., Prasetya, B., Usagawa, T., Watanabe, T., 2006. Comparison of *in vitro* digestibility and chemical composition among sugarcane bagasses treated by four white-rot fungi. Anim. Sci. J. 77, 308-313.

[14] Okano, K., Nishimoto, Y., Usagawa, T., 2008. Effect of shiitake mushroom (*Lentinus edodes*) strains and culture conditions on the digestibility improvement of sugarcane bagasse. Nihon Chikusan Gakkaiho 79, 483-490 (in Japanese, with English abstract).

[15] Zadrazil, F., Kamra, D.N., Ishikhuemhen, O.S., Scuchardt, F., Flachowsky, G., 1996. Bioconversion of lignocellulose into ruminant feed with white rot fungi – Review of work done at the FAL, Braunschweig. J. Appl. Anim. Res. 10, 105-124.

[16] Okano, K., Kitagawa, M., Sasaki, Y., Watanabe, T., 2005. Conversion of Japanese red cedar (*Cryptomeria japonica*) into a feed for ruminants by white-rot basidiomycetes. Anim. Feed Sci. Technol. 120, 235-243.

[17]Okano, K., Minemori, Y., 2014. Improving the nutritive value of reed, *Phragmites australis*, for ruminants by culturing with the white-rot fungus *Ceriporiopsis subvermispora*. The 6th I-SEC, Udonthani, Thailand (in press).

[18] Kitano, E., Petlum, A., Kitagawa, M., Takeuchi, A., Okano, K., 2012. Effect of length of culture period on nutritive value and chemical composition of wheat straw cultured with the white-rot fungus *Ceriporiopsis subvermispora*. The 15th AAAP, Pathum Thani, Thailand.

[19] Morita, M., 2007. Study on upgrading feed value of corncob meal substrate cultured or cultivated with white-rot fungi. MA thesis, Ibaraki University, Ami, Ibaraki, Japan (in Japanese).

[20] Okano, K., Tanaka, T., Usagawa, T., 2011. Improving the nutritive value of sugarcane bagasse for ruminants by culturing with a white-rot fungus *Ceriporiopsis subvermispora*. The 8th International symposium on the nutrition of herbivores. Aberystwyth, Wales, UK.

[21] Okano, K., Ohkoshi, N., Nishiyama, A., Usagawa, T., Kitagawa M., 2009. Improving the nutritive value of madake bamboo, *Phillostachys bambusoides*, for ruminants by culturing with the white-rot fungus *Ceriporiopsis subvermispora*. Anim. Feed Sci. Technol. 152, 278-285.

[22] Amirta, R., Tanabe, T., Watanabe, T., Honda, Y., Kuwahara, M., Watanabe, T., 2006. Methane fermentation of Japanese cedar wood

pretreated with a white rot fungus, *Ceriporiopsis subvermispora*. *J. Biotechnol*. 123, 71-74.

[23] Okano, K., Morita, M., Adachi, Y., Aisaka, I., Inatomi, S, Usagawa, T., 2010. *In vivo* digestibility of spent corncob meal substrate cultured with *Pleurotus eryngii*. Kansai Chikusan Gakkaiho 166, 11-18 (in Japanese, with English abstract).

[24] Miki, S, Okano, K., 2005. *In vitro* digestibility of unsterilized rice and wheat straws cultured with *Pleurotus salmoneostramineus*. Nihon Chikusan Gakkaiho 76, 407-414 (in Japanese, with English abstract).

[25] Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. J. agric. Sci. Camb. 93, 217-222.

[26] Kumar, S., Gomes, J., 2008. Performance evaluation of reactors designed for bioconversion of wheat straw to animal feed. Anim. Feed Sci. 144, 149-166.

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