J Wood Sci (2015) 61:420–430
DOI 10.1007/s10086-015-1483-x

ORIGINAL ARTICLE

Reducing radioactive cesium transfer from sawdust media to *Pleurotus ostreatus* fruiting bodies

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Received: 22 October 2014 / Accepted: 24 April 2015 / Published online: 17 May 2015

Abstract The Fukushima Daiichi nuclear power plant was damaged, and radionuclides such as ^{137}Cs were spread into the environment. Three years have passed since the accident, and ingestion of foods contaminated with radioactive material through the food chain is a health hazard of concern. Mushrooms are known for absorbing radionuclides, and methods for reducing fruiting body radioactivity are needed. We investigated radiocesium transfer activities and methods of reducing radiocesium transfer from sawdust media into fruiting bodies using *Pleurotus ostreatus*. Fruiting body radiocesium activity increased linearly with increasing media radioactivity. The radiocesium activities of fruiting bodies cultivated in different sawdust media were 0.2–0.9-fold (on wet-weight basis) and 1.3–3.7-fold (on dry-weight basis) higher than those of sawdust media, showing that transfer rates vary with cultivation conditions. From the reducing fruiting body radiocesium activity tests by medium additives, Prussian blue was effective and nanoparticle insoluble Prussian blue was more efficient than soluble Prussian blue. However, nanoparticle insoluble Prussian blue is more expensive than soluble Prussian blue. If mushroom growers use Prussian blue, it will be necessary to determine the type and the additional amounts that correspond to used strains, consumer needs, commercial value and cost.

Keywords Radiocesium · *Pleurotus ostreatus* · Reducing transfer · Prussian blue

Introduction

Parts of the Fukushima Daiichi nuclear power plant were damaged by a tsunami caused by the Great East Japan Earthquake on 11 March 2011. Artificial radionuclides, for example ^{131}I and ^{137}Cs, spread into the environment, and much radiation was detected from plants and mushrooms in fields by the fallout. Three years have now passed since the accident. Detectable radionuclides are almost entirely ^{134}Cs and ^{137}Cs, because radionuclides with short half-lives such as ^{131}I have been lost, and the object of concern has changed from fallout to absorption through the food chain. To suppress the additional internal exposure of 1 year within 1 mSv from food and so on, the maximum radioactive cesium activities in general foods were set at 100 Bq/kg by the Ministry of Health, Labor, and Welfare of Japan [1]. The ratios of farm products to soil radioactivities have been reported as about tenth to hundredth [2]. In contrast, ratios of mushroom to substrate radioactivities have been reported as about several times to tenth [3, 4]. Indeed, increased radioactive cesium activities in wild mushrooms in Europe have been seen since the Chernobyl nuclear power plant accident in 1986 [5]. Fruiting body radiocesium activities may easily exceed 100 Bq/kg when contaminated substrates are used for cultivation.

Parts of this study were presented at the 62nd and 64th Annual Meetings of the Japan Wood Research Society, Sapporo, March 2012, and Matsuyama, March 2014, respectively.

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Wild mushroom is sold in the market, but most of the mushrooms that Japanese eat are cultivated products. Management of the medium and of bed log for cultivation is important to avoid mushroom contamination by radioactive cesium. Therefore, the provisional reference index for the mushroom sawdust medium was set at 200 Bq/kg (dry basis) by Forest Agency, the Ministry of Agriculture, Forestry and Fisheries of Japan [6]. However, more than 40 % of consumers want foods of low radioactivity, even if the activities were within the limits set for food in February 2014 [7]. To obtain consumer confidence, methods of reducing fruiting body radioactivity in cultivated mushrooms are needed.

The methods have not been developed, but the abilities of K, zeolite, and vermiculite to reduce radioactive cesium absorption from soil by plants have been reported [8]. The term "zeolite" denotes naturally or artificially produced aluminosilicate minerals with miscellaneous constituent elements, and it was reported that mushrooms absorbed heavy metals [9]. When using zeolite, the heavy metal amounts in the fruiting bodies might be cause for concern. It has been proposed that ^{137}Cs uptake of mushrooms could be prevented by providing additional Rb or K at contaminated sites, given that fungi showed a greater preference for Rb and K than for Cs [10, 11]. Feed containing bentonite, zeolite, and Prussian blue (PB) reduced radiocesium activities in milk and meat [12–14]. PB was effective and safe for use against radioactive intoxication in humans [15]. PB is thought to be an effective additive, but there are two concerns. One is that hypokalemia had been concurrently reported [15]. It means that K in the medium is adsorbed by PB. Another is that PB is a stable compound, but was degraded by a bacterium under alkaline conditions [16]. As a result of its degradation, cyanide compounds might be produced. Iron ion is also produced from PB as a by-product and it has been reported that the content of the *Pleurotus ostreatus* fruiting body was increased with media iron content [17]; therefore the fruiting body iron content would be an index for PB degradation. The present study investigated methods for reducing radiocesium transfer from sawdust media into *P. ostreatus* fruiting bodies and verified a few safety measures when using the additives.

Materials and methods

Strains

Two strains of *P. ostreatus* (Jacq.:Fr.) Kummer coded Forestry Mycology Code (FMC) 239 and 245 were derived from stock cultures at the Mushroom Science Laboratory, Forestry and Forest Products Research Institute, Japan.

Culture media

Four kinds of sawdust denoted by MJ, TS, FH, and FS were used for mushroom cultivation and the characters are listed in Table 1. Rice bran and wheat bran were used as nutrients and the fresh ones were used for all cultivations. KCl, two kinds of zeolites, and two kinds of PB were used for reducing radiocesium transfer. PB is classified as soluble and insoluble PB. A kind of soluble PB (sPB), $Fe^{III}NH_4$-$Fe^{II}(CN)_6$, was provided by Dainichiseika Color & Chemicals Mfg. Co., Ltd. Insoluble PB (iPB), $Fe_4^{III}[Fe^{II}(CN)_6]_3$, which was developed for radiocesium adsorbent (http://www.kanto.co.jp/new_products/pb.html), was purchased from Kanto Chemical Co., Inc.

As standard sawdust media, MJ, rice bran, and wheat bran were mixed in the ratio 75:15:10 (dry-weight basis), and the moisture content was adjusted to 63 % (wet-weight basis). For measuring radioactive cesium transfer from the various sawdust media to the fruiting bodies, the standard sawdust medium and three kinds of media in which the sawdust of the standard sawdust media was replaced with TS, FH, and FS were prepared. For analyzing the relation between the medium and fruiting body radiocesium activities, the MJ contents in the standard sawdust media were changed with FH. The ratios of FH were 0, 25, 50, 75, and 100 % by dry-weight basis. For speculating additive capabilities for reducing radioactive cesium transfer from medium to the fruiting body, standard sawdust media supplemented with the additives were prepared and measured ^{133}Cs contents act as index, because the chemical properties of the radioactive and stable cesium were almost same. The additive amounts in the media were adjusted by their characters. The PB capabilities for reducing radioactive cesium transfer from media to fruiting bodies were analyzed by adding PB to the sawdust media in which the sawdust of the standard sawdust media was replaced with TS. The PB contents in the prepared media ranged from 0.02 to 2 g/kg on dry-weight basis.

Five replicates were prepared for each condition. However, ten replicates were prepared on the occasion of the standard sawdust medium replaced with FS for measuring radioactive cesium transfer from the various sawdust media to the fruiting bodies, because its yield was suspected to be small for measuring accurate radioactivity.

Cultivation conditions

All cultivation conditions were set as follows. The prepared medium (530 g) was packed in 850-mL plastic bottles and autoclaved at 121 °C for 60 min. For sources of inoculum, mycelia were pre-cultured in liquid media containing 1 % sucrose, 1 % malt extract, and 0.4 % yeast extract with

Table 1 Characters of sawdust for cultivating oyster mushroom

Abbreviation	MJ	TS	FH	FS
Place to obtain	Market	Tochigi Prefecture	Grower in Fukushima Prefecture	
Time to obtain	Before the earthquake	20 June 2013	27 July 2011	
Content	*Fagus crenata*	*Quercus acutissima*	Mainly hardwood (unknown species)	Mainly softwood (unknown species)

5 mm ϕ glass beads at 25 °C for approximately 2 weeks in darkness with intermittent shaking. The sterilized sawdust media were inoculated with the 5 mL liquid media containing the pre-cultured mycelia. After incubation at 22 °C in darkness for 20 days, the top surfaces of the media were removed, a little water was sprayed on them, and the media were placed in a developing room at 15 °C and relative humidity at 95 % under fluorescent illumination. All fruiting bodies fruited on one bottle were harvested when caps of more than 2 cm diameter were approximately 80 % open, and approximately 2 cm stipe from the top surface of the sawdust media were cut off for removing sawdust media.

Element analysis

The fruiting bodies and the sawdust media were dried at 105 °C and ground to a powder. The replicates of dried fruiting bodies were combined because the fruiting body amounts from one bottle were too small for measuring their accurate radioactivities. Nonradioactive ^{39}K, ^{56}Fe, and ^{133}Cs contents were measured by inductively coupled plasma mass spectrometry according to the factory instruction manual (Agilent Technologies, Inc.). As internal standards, ^{45}Sc and ^{140}Ce were used. Before the measurement, mushroom and sawdust medium samples (about 0.5 g) were digested with 14 mL concentrated nitric acid (Ultrapur-100, Kanto Chemical Co., Inc.) and 1 mL hydrogen peroxide (Ultrapur, Kanto Chemical Co., Inc.) at 220 °C for 4 h. For measuring nitric acid-soluble iron content in sPB, sPB (about 2 g) was diluted with 5 % nitric acid to 100 mL total, filtered, and centrifuged (10,000$\times g$, 5 min.).

^{134}Cs and ^{137}Cs radioactivities were measured with a coaxial germanium detector equipped with a multichannel analyzer (Canberra Industries, Inc.) according to the manual authorized by the Ministry of Education, Culture, Sports, Science and Technology of Japan [18]. Two-liter Marinelli beakers or U-8 containers were selected, depending on the sample volume; for instance, a 2-L Marinelli beaker was used for culture substrates and a U-8 container for powdered fruiting body. Measurement times were adjusted according to the sample radioactivities. The activities are presented on a dry-weight basis unless otherwise specified.

Statistical calculation

All statistical calculations were performed with R: a Language and Environment for Statistical Computing (http://www.r-project.org).

Results and discussion

Fluctuations of radioactive cesium transfer from media to fruiting bodies

Periods from moving media to developing room until harvesting fruiting bodies (fruiting body developing times), fruiting body wet weights, fruiting body dry weights, transfer factors (the ratios of fruiting body to media radioactive cesium activities) by wet-weight basis, and transfer factors by dry-weight basis are shown in Table 2. The fruiting body wet and dry weights on hardwood media were larger than that of softwood media. The cultivation periods were fixed for 20 days in this study; the fruiting body weights on softwood media would be consequentially smaller than that on hardwood media, because the mycelial growth on softwood media was generally slower than that on hardwood media because of the wood extractives and so on in softwood. There were no differences between the 2 strains with the transfer factors on hardwood media at 5 % risk, because the P values of Student's t test for wet-weight and dry-weight basis were 0.19 and 0.41, respectively. The 2 strains were selected among 36 strains by a selection test based on the fact that larger fruiting body yields were obtained within shorter fruiting body developing times on the standard sawdust media cultivated for 20 days. Then, differences in the strains would not appear because the characters of the 2 strains might be similar. The transfer factors on hardwood media were also higher than those of softwood medium. The small transfer factors on softwood media might also be caused by mycelial growth delay. On the other hand, the fruiting body developing times on the TS media were longer than the other hardwood media, but the transfer factors on the hardwood media were similar. These results showed that the transfer factors might be impervious to the developing time. The transfer factors of *P. ostreatus* cultivated on different culture media, culture periods and so on were reported from 1.3 to 3.4 on wet-

Table 2 Transfer factors from various media into fruiting bodies

Sawdust	MJ	TS	FH	FS
Medium radioactivity (Bq/kg)				
^{134}Cs	4.6	61	276	254
^{137}Cs	8.7	144	331	302
Fruiting body developing time (day)				
FMC 239	12.5 (0.5)	21.7 (1.2)	12.8 (0.8)	14 (0)
FMC 245	12.1 (0.8)	21.2 (0.5)	12.4 (0.7)	14 (0)
Fruiting body wet weight (g)				
FMC 239	78.0 (6.5)	81.4 (4.4)	74.3 (10.2)	53.6 (9.2)
FMC 245	84.5 (6.4)	91.9 (4.1)	76.5 (7.6)	67.9 (4.0)
Fruiting body dry weight (g)				
FMC 239	7.2 (0.6)	8.2 (0.3)	6.5 (0.8)	4.0 (0.6)
FMC 245	7.7 (0.8)	9.4 (0.3)	6.4 (0.4)	4.3 (0.3)
Transfer factor (wet-weight basis)				
FMC 239				
^{134}Cs	0.8	0.7	0.6	0.2
^{137}Cs	0.9	0.9	0.7	0.3
FMC 245				
^{134}Cs	0.7	0.6	0.7	0.2
^{137}Cs	0.7	0.6	0.8	0.3
Transfer factor (dry-weight basis)				
FMC 239				
^{134}Cs	3.1	3.6	2.8	1.3
^{137}Cs	3.7	3.2	2.8	1.3
FMC 245				
^{134}Cs	2.9	2.9	2.7	1.4
^{137}Cs	3.7	3.1	2.8	1.4

MJ, TS, FH, and FS are as in Table 1

The values in parenthesis are standard deviations

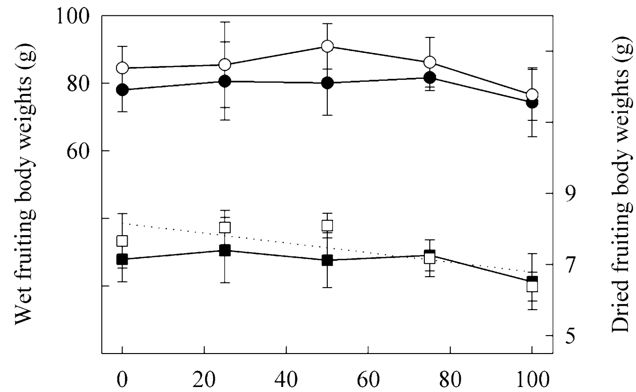

Fig. 1 Fruiting body weight changes on substituting MJ with FH in standard sawdust media. *Circles* and *squares* denote wet and dried fruiting body weights, respectively. *Closed* and *open symbols* denote FMC 239 and 245 fruiting body weights, respectively. The *dotted line* represents the regression line of the dried fruiting body weights of FMC 245, $y^{a} = -1.41x^{b} + 8.17$ ($r^{c} = -0.62$, $P^{d} < 0.01$). FH and MJ are as in Table 1. Standard sawdust media are described in the text. a, Dried fruiting body weights; b, proportions of FH substituted for MJ; c, Pearson's r; d, P value

weight basis [19]. The media water content was 60 %, the fruiting body water contents were assumed to be 90 %, and the calculated transfer factors on a dry-weight basis ranged from 5.2 to 13.7. These showed that the transfer factors easily varied with culture conditions. For verifying radiocesium transfer activity, regression line or curve obtained by evaluating a plurality of data was required than transfer factor obtained by evaluating only one data.

Relationships between fruiting body and sawdust media radiocesium activities

The impacts of the substituted FH contents on the fruiting body cultivation were evaluated first. The effects of the FH content increase on fruiting body developing times were not seen with FMC 239 and 245 at 5 % risk, because the *P* values of Pearson's product–moment correlation coefficient (Pr) were 0.55 and 0.73, respectively. The averages of all the fruiting body developing times for FMC 239 and 245 were 12.5 [standard deviation (σ) = 0.2] and 12.7 (σ = 0.7) day, respectively. As shown in Fig. 1, the effects of the FH content increase on the wet fruiting body weights and the dried fruiting body weights with FMC 239 were not seen at 5 % risk, because Pr values were 0.49 and 0.08, respectively. No clear tendency was seen between the wet fruiting body weights and FH contents with FMC 245 at 5 % risk, because Pr was 0.10. In contrast, there was a significant negative relationship between the dried fruiting body weights and FH contents with FMC 245 at 1 % risk, because the Pearson's product–moment correlation coefficient (r) and Pr were −0.62 and <0.01, respectively. The regression equation was as follows: (dried fruiting body weights) = −1.41 × (FH substituted ratios) + 8.17. The FH content increase caused the FMC 245 dried fruiting body weight decrease, but had no effects on the fruiting body developing times of both strains, the wet fruiting body weights of both strains, and the dried fruiting body weights of FMC 239. These results showed that the impacts of the substituted FH contents on the fruiting body cultivation did not exist for FMC 239 and very less for FMC 245. The FH was substituted for the MJ in the standard media for adjusting medium radiocesium activities, and the effect of substituted sawdust would not be large enough to significantly change the relations between the media and fruiting body radioactivity.

As shown in Fig. 2, there were significant positive correlations and regression lines between fruiting body and media radiocesium activities at 1 % risk, given that all *r*s

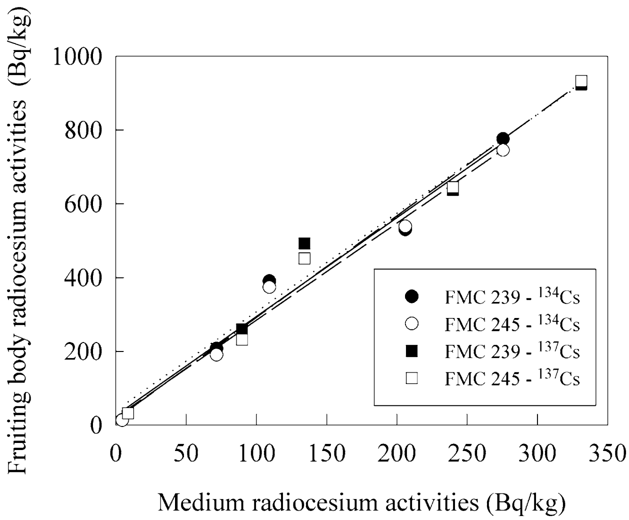

Fig. 2 Relationships between fruiting body and medium radiocesium activities. The *solid line* represents FMC 239–^{134}Cs, $y^a = 2.7x^b + 25.4$. The *dashed line* represents FMC 245–^{134}Cs, $y = 2.6x + 20.2$. The *dotted line* represents FMC 239–^{137}Cs, $y = 2.7x + 39.6$. The *dashed dotted line* represents FMC 245–^{137}Cs, $y = 2.8x + 16.1$. All rs[c] and their Prs[d] of the regression lines were 0.99 and <0.01, respectively. **a** Fruiting body radiocesium activities; **b** media radiocesium activities; **c, d** as in Fig. 1

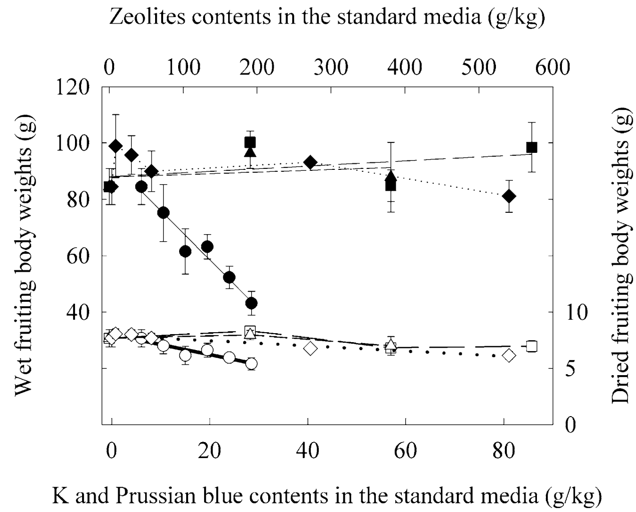

Fig. 3 Fruiting body weight changes with changes in additives content. *Closed* and *open symbols* denote wet and dried fruiting body weights cultivated on standard sawdust media supplemented with additives, respectively. The *circles*, *squares*, *triangles,* and *diamonds* represent the fruiting body weights cultivated on the media supplemented with K, zeolite-1, zeolite-2, and sPB, respectively. The *fine* and *thick solid lines* represent wet and dried fruiting body weights cultivated on standard sawdust media supplemented with K and the regression lines were as follows: $y^a = -1.49x^b + 87.69$ ($r^c = -0.89$, $P^d < 0.01$) and $y = -0.07x + 7.55$ ($r = -0.68$, $P < 0.01$), respectively. The *fine long dashed line* represents the wet fruiting body weights for zeolite-1, $y = 0.03x + 81.54$ ($r = 0.56$, $P < 0.01$). The *fine short dashed line* represents the same parameters for zeolite-2, $y = 0.03x + 80.72$ ($r = 0.46$, $P = 0.047$). The *large dotted line* represents the dry fruiting body weights for sPB, $y = -0.02x + 7.65$ ($r = -0.63$, $P < 0.01$). Standard sawdust media, zeolite-1, zeolite-2, and sPB are described in the text. **a** Wet or dried fruiting body weights; **b** additive contents; **c**, **d** as in Fig. 1

and their Prs were 0.99 and <0.01, respectively. The regression equations were as follows: (FMC 239 fruiting body ^{134}Cs radioactivities) = 2.7 × (medium ^{134}Cs radioactivities) + 25.4, (FMC 239 fruiting body ^{137}Cs radioactivities) = 2.7 × (medium ^{137}Cs radioactivities) + 39.6, (FMC 245 fruiting body ^{134}Cs radioactivities) = 2.6 × (medium ^{134}Cs radioactivities) + 20.2 and (FMC 245 fruiting body ^{137}Cs radioactivities) = 2.8 × (medium ^{137}Cs radioactivities) + 16.1. These regression equations were almost the same. These findings showed that fruiting body radiocesium activities increased linearly with media radiocesium activities and the transferred radiocesium activities from media to fruiting bodies could be represented by the total radiocesium activities. Linear increases of fruiting body ^{137}Cs activities have also been reported using media containing a maximum of 10,000 Bq/kg ^{137}Cs on a wet basis (60 % water content) [19]. Fruiting body radiocesium activities may easily exceed 100 Bq/kg during cultivation on contaminated media by radioactive cesium.

Additives for reducing ^{133}Cs transfer from media into fruiting bodies

The provisional reference index for the mushroom sawdust medium was set at 200 Bq/kg (dry basis) [6]. The ^{137}Cs content in the 200 Bq/kg medium was 6.2×10^{-20} g/kg, as calculated from the ^{137}Cs half-life [20]. In contrast, ^{133}Cs content in the standard sawdust medium was 3.9×10^{-7} g/kg. These concentrations indicate that additives for reducing radioactive cesium transfer from sawdust media to fruiting bodies are needed for the extremely large quantity of ^{133}Cs, because the chemical properties of radioactive and stable cesium were almost same. For speculating the capabilities of K, zeolite, and PB for reducing radioactive cesium transfer from media to fruiting bodies, ^{133}Cs contents were measured as an index using FMC 239 and standard sawdust media. The fruiting body weights and ^{133}Cs contents are summarized in Figs. 3 and 4, respectively.

The calculated K content from the medium substrate in the standard medium was 5.0 g/kg; therefore, K contents in the tested media were set from 5.0 to 25.0 g/kg. However, the actual K content ranged from 6.1 to 28.5 g/kg. The effect of K content increase on the fruiting body developing time was not seen, because Pr was 0.25. The average of all fruiting body developing times was 11.2 ($\sigma = 0.8$) days. In contrast, clear decreasing tendencies were seen on the wet fruiting body weights and the dried fruiting body weights with increasing medium K contents at 1 % risk, and the regression equations were as follows: (wet fruiting

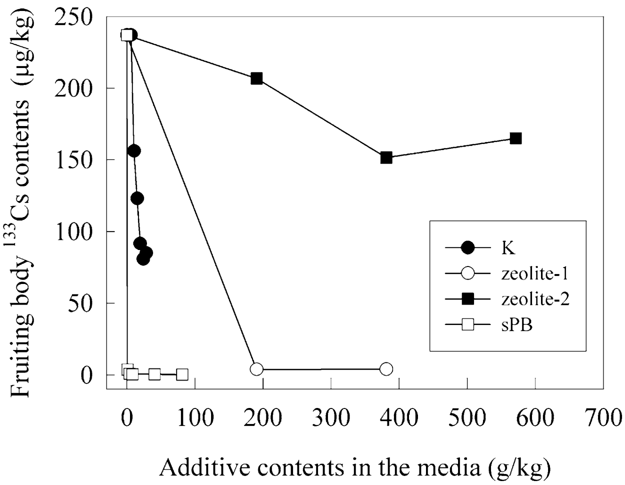

Fig. 4 Fruiting body ^{133}Cs content changes with additive content changes. Zeolite-1, zeolite-2, and sPB are described in the text

body weights) = $-1.49 \times$ (K contents) + 87.69 ($r = -0.89$, $P < 0.01$); (dried fruiting body weights) = $-0.07 \times$ (K contents) + 7.55 ($r = -0.68$; $P < 0.01$). Increasing K contents in sawdust media have been reported to reduce *Pleurotus cornucopiae* var. *citrinopileatus* fruiting body yields [21], and it was thought that nutritional imbalance would reduce the fruiting body yields.

The term "zeolite" denotes naturally or artificially produced aluminosilicate minerals with miscellaneous constituent elements. Then, two zeolites collected in different places, zeolite-1 and zeolite-2, were used. The zeolites were added to the media at rates varying from 190.5 to 571.3 g/kg. With both zeolites, negative correlations were detected between the fruiting body developing times and media zeolite contents at 1 % risk, and the equations were as follows: (fruiting body developing times of zeolite-1 added media) = $-0.004 \times$ (zeolite-1 contents) + 12.064 ($r = -0.66$, $P < 0.01$); (fruiting body developing times of zeolite-2 added media) = $-0.01 \times$ (zeolite-2 contents) + 12.54 ($r = -0.98$, $P < 0.01$). Positive correlations were detected between the wet fruiting body weights and media zeolite contents at 5 % risk, and the equations were as follows: (wet fruiting body weights of zeolite-1 added media) = $0.03 \times$ (zeolite-1 contents) + 81.54 ($r = 0.56$, $P < 0.01$) and (wet fruiting body weights of zeolite-2 added media) = $0.03 \times$ (zeolite-2 contents) + 80.72 ($r = 0.46$, $P = 0.047$). In contrast, the effects of zeolite content increase on the dried fruiting body weights were not seen, because Prs for zeolite-1 and zeolite-2 were 0.32 and 1.00, respectively. The averages of all the dried fruiting body weights of zeolite-1 and zeolite-2 added media were 7.4 ($\sigma = 0.8$) and 7.5 ($\sigma = 0.7$) g, respectively. These results showed that zeolite addition led to the production of fragile and watery fruiting bodies. We inferred that the abilities of zeolites to reduce cesium absorption were low. The zeolite addition amounts were greater than those of the other additives, but the zeolites absorbed little or no water, so that the media with adjusted water content became watery. It has been reported that excessive media water content led to watery fruiting bodies [22]. Such fruiting body production in this study would be caused by excessive media water content.

The sPB was added to the media at rates ranging from 0.8 to 81.1 g/kg. The effects of sPB content on the fruiting body developing times were not seen at 5 % risk, because the Pr was 0.24. The average of all fruiting body developing times was 10.2 ($\sigma = 1.6$) days. The effects of sPB content on the wet fruiting body weights were also not seen at 5 % risk, because the Pr was 0.67. The averages of all the wet fruiting body weights were 90.5 ($\sigma = 6.8$). In contrast, clear decreasing tendency was seen on the dried fruiting body weights with increasing medium sPB contents at 1 % risk, and the regression equations were as follows: (dried fruiting body weights) = $-0.02 \times$ (sPB contents) + 7.65 ($r = -0.63$, $P < 0.01$). There was a significant decreasing tendency when the sPB added up to 81.1 g/kg, but no significant decreasing tendency when the sPB added up to 40.5 g/kg because the Pr was 0.21. These results showed that excessive sPB addition affected the *P. ostreatus* cultivation, for example up to 81.1 g/kg, but the addition of sPB to 40.5 g/kg would not affect the *P. ostreatus* cultivation.

There was no significant correlation between dried fruiting body K contents and media K contents, because its Pr was 0.24. The dried fruiting body K content was constant. The average of all dried fruiting body K contents were 26.4 ($\sigma = 1.6$) g/kg. No linear correlation was seen between dried fruiting body ^{133}Cs contents and media K contents, but a decreasing trend was detected at 5 % risk, because Spearman's rank order correlation coefficient (ρ) and its P value ($P\rho$) were -0.90 and 0.01, respectively. The ^{133}Cs contents were reduced from 237 to 81 μg/kg (34 %) at the maximum by 24 g/kg K addition. With plants, K addition to the media showed a competitive effect on the uptake of radiocesium [23]. A radiocesium transfer-reducing tendency has been reported for *P. ostreatus* mycelia by K addition to the medium [10]. It was thought that fruiting body radiocesium activities would decrease competitively following the K addition to media. In addition, fruiting body K contents have been reported to be relatively large and almost constant when *P. ostreatus* were cultivated on various media [24]. It was thought that cesium absorption would increase to hold fruit body K content constant when media K content was low. K addition to media would reduce fruiting body radiocesium activity, but would also reduce the yields. If K is used for reducing radiocesium activity, it is necessary to determine the K addition amounts considering the balance between the fruiting body radiocesium activity decrease and yield loss.

A linearly decreasing relationship was not seen between the fruiting body ^{133}Cs content and media zeolite content, because Prs for zeolite-1 and zeolite-2 were 0.11 and 0.33, respectively. However, the fruiting body ^{133}Cs contents were reduced from 237 to 152 µg/kg (64 %) by the addition of 381 g/kg zeolite-1 and to 3.8 µg/kg (1.6 %) by the addition of 190 g/kg zeolite-2. A study of zeolite as a ^{137}Cs remover has focused on its ion exchange and adsorption activity [25]. These actions would be expected to differ with zeolite composition, and it was natural that the zeolites collected in different places showed different behavior. Moreover, it was reported that mushrooms absorbed heavy metals [9]. For applying zeolite to reduce radioactive cesium transfer, sufficient pretesting is needed such as capability for reducing radioactive transfer of targeted zeolite, commercial value of fruiting body, and heavy metal contents in fruiting bodies.

There was no significant linear relation between the fruiting body ^{133}Cs contents and addition of sPB contents because its Pr was 0.50, but there was a significant decreasing trend between them at 1 % risk because its ρ and $P\rho$ were -1.00 and below 0.01, respectively. The fruiting body ^{133}Cs contents were reduced from 236.97 to 0.19 µg/kg (0.08 %) by the addition of 81.1 g/kg sPB. The small amounts of sPB addition to the media that did not affect the fruiting body yields showed notable reduction in ^{133}Cs transfer efficiencies from the sawdust media to the fruiting bodies, because the fruiting body ^{133}Cs contents following 0.81 and 4.05 g/kg sPB addition were 3.69 (1.56 %) and 0.78 (0.33 %) µg/kg. Based on *P. ostreatus* cultivation and additive sPB contents, PB was the most effective among the tested additives.

Radiocesium transfer-reducing effect from the medium into the fruiting body by adding Prussian blue

The average radiocesium activities in the prepared media were 220 ($\sigma = 17$) Bq/kg and fruiting body radiocesium activities increased linearly with media radiocesium activities. Therefore, fruiting body radiocesium activities and sPB contents in the actual media were converted to values equivalent to those in the 220 Bq/kg media.

The effects of sPB contents on the fruiting body developing times with FMC 239 and 245 were not seen at 5 % risk, because the Prs were 0.38 and 0.11, respectively. The averages of all fruiting body developing times for FMC 239 and 245 were 28.0 ($\sigma = 3.4$) and 27.4 ($\sigma = 2.6$) day, respectively. The wet and dried fruiting body weights are summarized in Fig. 5. There were no significant correlations between the wet fruiting body weights and media PB contents at the 5 % level with both strains, because both Prs were 0.06. The averages of all wet fruiting body weights for FMC 239 and 245 were 70.1 ($\sigma = 10.1$) and 77.3 ($\sigma = 6.2$) g, respectively. There were also no significant correlations between the dried fruiting body weights and media PB contents at the 5 % level with FMC 239 and 245, because the Prs were 0.29 and 0.21, respectively. The averages of all dried fruiting body weights for FMC 239 and 245 were 7.6 ($\sigma = 0.8$) and 8.3 ($\sigma = 0.6$) g, respectively. These results showed that sPB addition did not have a negative impact at least within the tested range. PB is a kind of insoluble compound in water; therefore, impacts on fruiting body cultivation will be caused by impurities and so on in the PB suspension.

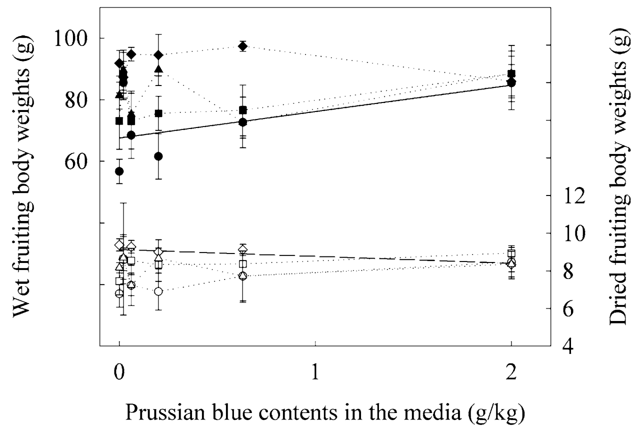

Fig. 5 Fruiting body weight changes with changes in Prussian blue content. *Closed* and *open symbols* denote wet and dried fruiting body weights cultivated on standard sawdust media replaced with TS, respectively. The *circles* and *squares* represent the fruiting body weights of FMC 239 and 245 cultivated on media supplemented with sPB, respectively. The *triangles* and *diamonds* represent the fruiting body weights of FMC 239 and 245 supplemented with iPB, respectively. The *solid line* represents the regression line between the sPB contents and the FMC 239 wet fruiting body weight. The regression line was as follows, $y^a = 8.9x^b + 67.1$ ($r^c = 0.56$, $P^d < 0.01$). The *dashed line* represents the regression line between the iPB contents and the FMC 245 dried fruiting body weight. The regression line was as follows: $y = -0.38x + 9.09$ ($r = -0.42$, $P = 0.02$). Standard sawdust media, TS, sPB, and iPB are described in the text. **a–d** as in Fig. 3

For both strains, there were no significant linear correlations between fruiting body radiocesium activities and sPB contents (both Prs were 0.16); although decreasing trends were observed (ρs and $P\rho$s were -1 and <0.01, respectively, Fig. 6). These results showed that there was a nonlinear relationship between the two parameters. For clarifying the relation between the fruiting body radiocesium activities and the PB contents in the media, several different equations were fitted to the data without success. It was thought that the fruiting body radiocesium activities

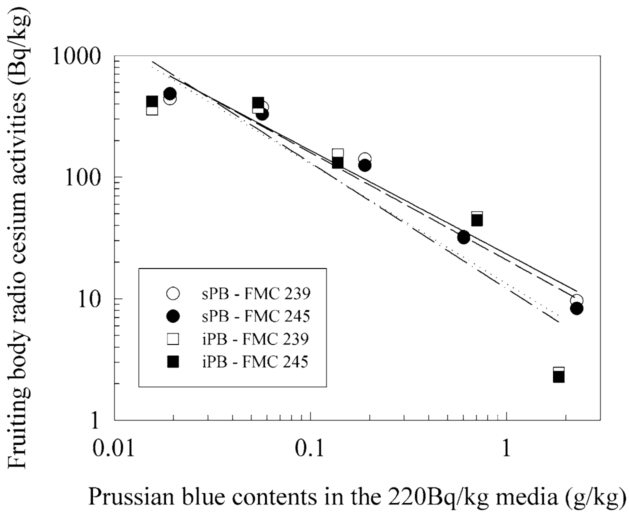

Fig. 6 Fruiting body radiocesium activity changes with Prussian blue content changes converted to 220 Bq/kg sawdust media. The regression curves of FMC 239 and 245 fruiting body radiocesium activities cultivated with sPB were $y^a = 23.3x^{b\ -0.85}$ ($r^c = 0.96$, $P^d < 0.01$) and $y = 20.8x^{-0.88}$ ($r = 0.97$, $P < 0.01$), respectively. The regression curves of FMC 239 and 245 with iPB were $y = 13.3x^{-0.99}$ ($r = 0.82$, $P = 0.03$) and $y = 12.1x^{-1.03}$ ($r = 0.85$, $P = 0.03$), respectively. sPB and iPB are described in the text. **a**–**d** as in Fig. 2

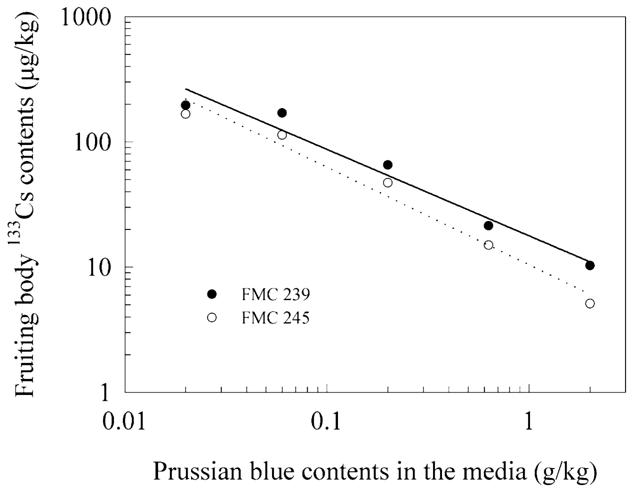

Fig. 7 Fruiting body stable cesium contents changes with Prussian blue content changes. The *solid* and *dashed lines* represent the regression curves of fruiting body stable cesium contents cultivated with PB of FMC 239 $y^a = 17.8x^{b\ -0.69}$ ($r^c = 0.96$, $Pr^d < 0.01$) and FMC 245 $y = 10.4x^{-0.78}$ ($r = 0.98$, $Pr < 0.01$), respectively. The medium ^{133}Cs content was 69 µg/kg. The fruiting body ^{133}Cs contents of FMC 239 and FMC 245 cultivated on no PB-added media were 285 and 224 µg/kg, respectively. **a** Fruiting body stable cesium contents and **b**–**d** as in Fig. 2

ranged widely and the values in the high-activity range might be overestimated by the least-squares method. Therefore, the logarithms of the data were plotted, significant linear correlations were detected, because the *r*s for FMC 239 and 245 were 0.96 and 0.97, respectively, and both Prs were <0.01 (Fig. 6). The regression curves were as follows: (FMC 239 fruiting body radiocesium activities) = 20.8 × (sPB contents)$^{-0.91}$ and (FMC 245 fruiting body radiocesium activities) = 18.4 × (sPB contents)$^{-0.94}$. The required sPB amounts for harvesting 10 and 1 Bq/kg fruiting bodies by wet-weight basis from 220 Bq/kg media by dry-weight basis were calculated based on the regression curves and the average fruiting body water contents (89 %). The sPB amounts for 10 Bq/kg FMC 239 and 245 fruiting bodies were both 0.19 g/kg and that for 1 Bq/kg were 2.9 and 2.5 g/kg, respectively.

The relations between the fruiting body ^{133}Cs contents and the sPB contents in the media were estimated in the same manner as in the case of radiocesium (Fig. 7). There were also significant correlations because the *r*s for FMC 239 and FMC 245, −0.96 ($Pr < 0.01$) and −0.98 ($Pr < 0.01$), respectively. The regression curves were as follows: (FMC 239 fruiting body ^{133}Cs contents) = 17.8 × (sPB contents)$^{-0.69}$ and (FMC 245 fruiting body ^{133}Cs contents) = 10.4 × (sPB contents)$^{-0.78}$. The differences between the strains for reducing cesium transfer efficiency by sPB were not seen with radiocesium, but with ^{133}Cs. It was thought that the cesium absorption amount differences between strains existed, but notable differences did not appear with radioactive cesium because the absolute radioactive cesium amounts were very small. The multiplier factors of the regression curves for stable cesium were lower than that for radiocesium with both strains. The chemical properties of isotopes were generally same, but these results showed that radioactive cesium was adsorbed more than ^{133}Cs by sPB. ^{134}Cs and ^{137}Cs mainly existed on the surface of the medium substrate but with ^{133}Cs inside, while a mixture of ^{133}Cs, ^{134}Cs, and ^{137}Cs in the vicinity of sPB was rapidly adsorbed and ^{133}Cs was supplied from the inside of the medium substrate according to the decrease in mixture.

There are two concerns when using PB. One is that K in the medium is adsorbed by PB. The effects of sPB content on fruiting body K contents with FMC 239 and 245 were not seen at 5 % risk (Prs were 0.32 and 0.81, respectively), and the average contents were 24.7 ($\sigma = 0.8$) and 24.4 ($\sigma = 0.6$) g/kg, respectively. The K content in the fruiting body was conserved and K might be adsorbed by sPB, but these results showed that sPB addition within the tested range did not affect the fruiting body K contents. Also, PB degradation might produce cyanide compounds [16]. Iron was concurrently produced as a by-product and *P. ostreatus* fruiting body iron content increased with media iron content [17]. To evaluate PB degradation, the fruiting body iron contents were analyzed (Fig. 8). No significant correlation was detected between the fruiting body Fe contents

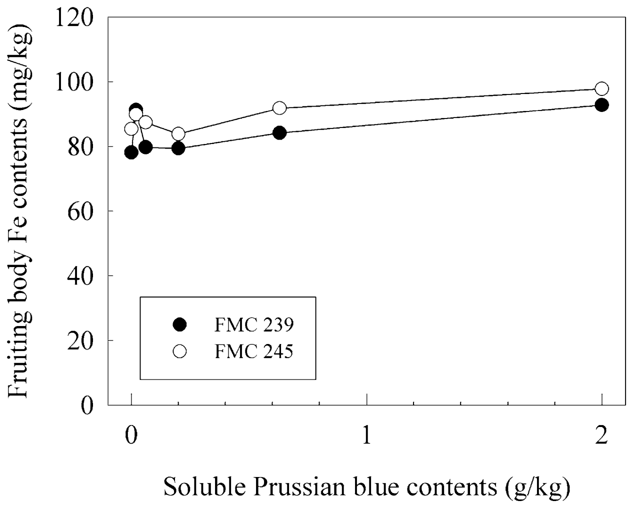

Fig. 8 Fruiting body Fe content changes with soluble Prussian blue content changes

and the media sPB contents with both strains, because the Prs of FMC 239 and FMC 245 were 0.18 and 0.06, respectively. Statistically significant tendencies between them were not detected, but increasing tendencies were observed at first glance. The medium contained 56.9 mg/kg Fe and the sPB also contained 1.44 mg/g nitric acid-soluble Fe. The tendencies might be caused by the soluble Fe in the sPB suspension. These results showed that there was no sPB degradation, so that generation of cyanide compounds would not be of concern. It was reported that the consumption of products from sPB-treated animals would not be expected to have any effects on human health [13]. Using sPB as reducing radiocesium transfer additive for sawdust medium would not be of concern.

Given that PB is a blue pigment, the blue color of the medium increased with PB content and parts of the fruiting body were stained. To avoid reducing the commercial value and so on, added PB amounts must be reduced as much as possible. Recently, a kind of iPB was developed as a radiocesium adsorbent. The abilities of the iPB were studied in the same manner as sPB.

The effects of iPB content on the fruiting body developing times were not seen with FMC 239 at 5 % risk ($Pr = 0.09$), but seen with FMC 245 at 5 % risk ($Pr = 0.04$). The averages of all fruiting body developing times for FMC 239 were 27.0 ($\sigma = 5.9$). The regression equation for FMC 245 was as follows: (fruiting body developing times) $= 2.4 \times$ (iPB contents) $+ 26.2$ ($r = 0.38$, $P = 0.04$). The effects of iPB contents on the wet fruiting body weights with FMC 239 and 245 were seen at 5 % risk, because the Prs were both 0.01. The regression equation was as follows: (FMC 239 wet fruiting body weights) $= 12.0 \times$ (iPB contents) $+ 61.9$ ($r = 0.45$, $P = 0.01$) and (FMC 245 wet fruiting body weights) $= 6.3 \times$ (iPB contents) $+ 74.2$ ($r = 0.45$, $P = 0.01$). There was no significant correlation between the dried fruiting body weights and media iPB contents at 5 % risk with FMC 239 and 245 because the Prs were 0.06 and 0.07, respectively. These results showed that the iPB addition to the sawdust media made the fruiting body developing times long. The iPB addition also made the fruiting bodies statistically watery, but the actual fruiting body water contents of FMC 239 and 245 were 89.2 % ($\sigma = 1.5$ %) and 89.1 % ($\sigma = 1.4$ %), respectively. The iPB suspension included sodium salt as a dispersant; these admixtures might affect the fruiting body developing times, water contents and so on, but differences in appearance of fruiting bodies were not observed. It was thought that the impacts of the iPB addition on fruiting body cultivation were small, except for the fruiting body developing times.

The regressions relating fruiting body radiocesium activities to medium iPB contents were calculated in the same way as for sPB, and there were significant correlations between the data at 5 % risk (both Prs were 0.03, Fig. 6). The required iPB amounts for harvesting 10 and 1 Bq/kg fruiting bodies by wet-weight basis from 220 Bq/kg media by dry-weight basis were calculated in a manner similar to sPB but the average water contents were 90 %. The iPB amounts for 10 Bq/kg FMC 239 and 245 fruiting bodies were both 0.13 g/kg and that for 1 Bq/kg were 1.3 and 1.2 g/kg, respectively. If iPB was used instead of sPB, the additive contents could be reduced by about 32 and 54 % when wet fruiting body radiocesium activities are set to 10 and 1 Bq/kg, respectively.

The reducing radioactive cesium transfer actions by PB from media to fruiting bodies were produced by the cesium adsorption capabilities of PB; therefore the *P. ostreatus* would not be able to absorb the cesium adsorbed by PB. For the reason, the reducing effects were prospective for not only *P. ostreatus*, but also for other cultivated mushrooms. Insoluble PB has been reported to have high capacity for cesium ions in comparison with soluble PB [26, 27]. These results were obtained by using PB suspension solution. The sawdust media were solid, but there would be free water in them and cesium ions would be adsorbed through the water. Organic compound did not contribute to the selective retention of cesium [28, 29], but cesium ion movement was constrained by the medium substrates and only cesium ions in the vicinity of PB were adsorbed. Therefore, the higher adsorption amounts of iPB than sPB was caused by the larger surface areas per unit volume of iPB than sPB.

A kind of prepared iPB is approved as an oral medicine by the US Food and Drug Administration, for instance Radiogardase (HEYL Chemisch-pharmazeutische Fabrik GmbH & Co. KG.). The safety of sPB, which is used as a pigment and for other purposes, has been reported for humans [13]. PB is expected to be safe even if humans ingest it by mistake. However, use of non-pharmaceutical products, with possible contaminants, might pose a problem. In addition, it should be noted that the added PB that absorbed radiocesium did not have to mix into the edible parts.

Rumors about the danger of mushrooms still remain, and more than 40 % of consumers want foods of low radioactivity, even if the activities are within the limits set for food in February 2014 [7]. The transfer factors were different under different culture conditions and were different for strains [3]. The calculated PB addition amounts for the tested strains showed coincidentally similar values, but that might be caused by their similar characters. Insoluble PB was more effective than soluble PB for reducing radiocesium transfer, but iPB was more expensive than sPB. If mushroom growers use PB, it will be necessary to determine the type and the additional PB amounts that correspond to used strains, consumer needs, commercial value and cost.

Acknowledgments A part of this study was supported by research and development projects for application in promoting new policies for agriculture, forestry, and fisheries, supported by the Ministry of Agriculture, Forestry and Fisheries of Japan (Grant No. 23063). We would like to specially thank Mr. Toshio Hattori, a group deputy general manager of Dainichiseika Color & Chemicals Mfg. Co., Ltd, for providing Prussian blue (in 2011); Dr. Atsushi Kumata, forest resources director of Forest Research Center of Fukushima Pref., for introducing sawdust providers (in 2011); Mr. Yoji Ohashi, a senior researcher of Tochigi Prefectural Forestry Center, for providing *Fagus crenata* logs; and Ms. Keiko Hata for her helpful assistance.

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