Radiocesium Concentrations in Wild Mushrooms and Characteristics of Cesium Accumulation by the Edible Mushroom (*Pleurotus ostreatus*)

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Mushrooms collected from a sub-alpine forest of Mt. Fuji and some other locations in Japan in 1996 were analyzed for radiocesium. The ^{137}Cs concentrations in 37 mushrooms varied widely from 1.6 to 783 Bq kg^{-1} fresh wt. The characteristics of Cs accumulation were analyzed by culturing fruiting bodies of the edible mushroom *Pleurotus ostreatus* (Fr.) Kummer Y-1 (*P. ostreatus* Y-1). The ^{137}Cs and stable Cs accumulation expressed as the concentration ratio (CR, ^{137}Cs or Cs concentration in the dried fruiting body /^{137}Cs or Cs concentration in the fresh medium) were in good agreement, indicating similar migration. The CR of Cs grown on medium containing both 0.1% Cs and 0.1% K, 10.2, showed a decrease of about 30 percent as compared with that containing 0.1% Cs only. These CR values suggested that Cs accumulation by the fruiting bodies of *P. ostreatus* Y-1 is affected by the presence of K similarly to previous observations in the mycelia. The ^{133}Cs-NMR spectra from the fruiting bodies of *P. ostreatus* Y-1 showed two resonance signals, whereas those from the media after harvesting of fruiting bodies showed only one signal. Just before growth of the fruiting bodies, bunches consisting of many mycelia were observed by scanning electron microscopy (SEM). No significant differences in the elemental distribution (Cs, K, P and C) were detected in the mycelium surface by SEM equipped with an energy dispersive X-ray microanalyzer.

Key words —— cesium, accumulation, mushroom, *Pleurotus ostreatus*, gamma-ray spectrometry, scanning electron microscope-energy dispersive X-ray microanalyzer

INTRODUCTION

High-level accumulation of radiocesium by mushrooms has been widely reported after the Chernobyl accident in 1986, and there have also been a few reports of contamination of mushrooms in some European countries by ^{137}Cs derived from fallout from atmospheric nuclear weapon tests.[1,2] Since the Chernobyl accident, in various European countries (Austria, Germany, Sweden, Italy and others) ^{137}Cs concentrations of mushrooms have been found to be markedly increased.[3–8] In Japan, it was also found that concentrations of ^{137}Cs in wild mushrooms were higher than those in agricultural products.[9–11] The concentrations of ^{137}Cs in mushrooms collected in Japan were LTD (less than detectable)–1070 Bq kg^{-1} fresh wt., < 0.4–1260 Bq kg^{-1} fresh wt. and ND (not detectable)–570 Bq kg^{-1} fresh wt.[12–14] However, the characteristics and mechanisms of the high accumulation of radiocesium have not been clarified.

In this study, we collected the mushrooms from a sub-alpine forest of Mt. Fuji and from some other locations in Japan, and investigated their radiocesium levels ten years after the Chernobyl accident. We studied the characteristics of uptake and accumulation of Cs by tracer culture experiments of the saprophytic mushroom *Pleurotus ostreatus* (Fr.) Kummer (*P. ostreatus*) Y-1 fruiting bodies, one of edible mushrooms to Japanese taste. Saprophytic mushrooms are advantageous for analysis of Cs uptake because of their relative ease of cultivation in the laboratory. Cs in *P. ostreatus* was examined using a ^{133}Cs-NMR spectrometer and a scanning electron microscope (SEM)-energy dispersive X-ray microanalyzer (EDX).

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MATERIALS AND METHODS

Samples and Analysis of Wild Mushrooms —— Thirty-seven mushrooms classified into 10 species were collected from the sub-alpine forest of Mt. Fuji (1400–2300 m above sea level) and 4 different locations in Yamanashi Prefecture, Japan, in October 1996. Attached soils and litters were carefully removed, and the samples were kept at 4°C. Samples of 20–80 g were compressed into plastic bottles (50 mm in diameter) and radiocesium levels (gamma-ray peaks; 662 keV for ^{137}Cs and 605 and 795 keV for ^{134}Cs) were determined with a high purity Ge-detector (Tennelec Co., Ltd.) coupled to a multichannel analyzer (Nucleus Co., Ltd.) by counting for 2000–60000 s. IAEA marine alga AG-B-1 was used to validate the analytical quality.

Culturing of Fruiting Bodies of the Edible Mushroom *Pleurotus ostreatus* —— The culture medium was prepared as follows; 240 g of sawdust and 60 g of wheatbran were added and then mixed with boiled water and ^{137}Cs standard solution (JAS; 100 kBq g^{-1}, CsCl 0.05 mg g^{-1} in 0.1 N HCl) to a total weight of 800 g (containing 10000 Bq kg^{-1} of ^{137}Cs and 62.5% H_2O) in 1 l bottles. The mixture was sterilized at 120°C for 1 h. This mixed medium was used as a control for the Cs uptake experiment. Three different media groups, with addition of only stable 0.1% Cs (7.5 mM Cs), both stable 0.1% Cs and 0.1% K, and only 0.5% K, were prepared by the same method (containing 10000 Bq kg^{-1} of ^{137}Cs and 62.5% H_2O). Cultivation of fruiting bodies of *Pleurotus ostreatus* (Fr.) Kummer Y-1 (*P. ostreatus* Y-1) was performed as described.[15)]

Measurement of ^{137}Cs and Stable Cs, and K in Cultured Fruiting Bodies of the Mushroom *P. ostreatus* —— The cultured fruiting bodies of *P. ostreatus* Y-1 were separated from the medium with a knife. The fresh fruiting bodies samples were compressed into plastic bottles, and then concentrations of ^{137}Cs were determined with a Ge-detector as described above. For determination of stable Cs by instrumental neutron activation analysis (INAA) and K by flame photometry (FP), the fresh fruiting bodies were freeze-dried and pulverized with a stainless steel blender. Samples of about 100 mg of each dried sample and the standard reference material (NIST, peruvian soil and citrus leaves) were sealed in polyethylene bags and placed into polyethylene capsules. The samples were irradiated for 6 h at a neutron flux of 5×10^{11} n cm^{-2} s^{-1} in the TRIGA II reactor at Rikkyo University. After cooling for 5–7 d, gamma-ray spectrometry was carried out. The activity of ^{134}Cs, produced by ^{133}Cs$(n,\gamma)^{134}$Cs reaction, was measured with HPGe detector for 2000–4000 s. The K content of dried samples and media were determined by FP (Hekisa Co., Ltd. FP-3B) after HNO_3–H_2O_2 wet digestion.

^{133}Cs-NMR Experiments of Cultured Fruiting Bodies of the Mushroom *P. ostreatus* and Substrates —— The cultured fruiting bodies of *P. ostreatus* Y-1 and the media containing 0.1% Cs were added to NMR sample tubes (10 mm in diameter) with the external reference capillary tube containing either 100 mM CsCl and 50 mM Dy $(P_3O_{10})^{7-}$ or 50 mM CsCl and 25 mM Dy $(P_3O_{10})^{7-}$. ^{133}Cs-NMR analyses were carried out with NMR spectrometer (JEOL Co., Ltd. EX 400 FT) operated at 52.3 MHz.

Observation and Elemental Analysis of Cultured Fruiting Bodies of the Mushroom *P. ostreatus* —— The mycelia for cultivation of the fruiting bodies of *P. ostreatus* Y-1 in sawdust media containing 0.2% Cs for about 60 d were observed with SEM (Hitachi Co., Ltd. S-3000N). Simultaneously with SEM observation, elemental analysis was performed using an energy dispersive X-ray microanalyzer (EDX, Horiba Co., Ltd. EMAX-7000).

RESULTS AND DISCUSSION

Concentrations of Radiocesium in Wild Mushrooms in Japan

The concentrations of ^{137}Cs and ^{134}Cs in wild mushrooms collected from the forest at the base of Mt. Fuji and from some other locations in 1996 are shown in Table 1 with the each fungi type. The mean and the median of ^{137}Cs concentration in the mushrooms at each sampling location are also listed. Thirty-seven mushrooms were classified into 10 species most of which were mycorrhizal fungi. The concentrations of ^{137}Cs in wild mushrooms ranged from 1.6 to 783 Bq kg^{-1} fresh wt. The ^{137}Cs median values for the mushrooms were 254 Bq kg^{-1} fresh wt. (69.5–511 Bq kg^{-1} fresh wt.) at fifth station of Mt. Fuji (2300 m above sea level), 81 Bq kg^{-1} fresh wt. (21.2–455 Bq kg^{-1} fresh wt.) at third station (1800 m above sea level) and 51 Bq kg^{-1} fresh wt. (1.6–310 Bq kg^{-1} fresh wt.) at first station (1400 m above sea level). The mushrooms collected from location A near the sub-alpine forest of Mt. Fuji showed relatively high ^{137}Cs concentration, with a median value of 240 ^{137}Cs Bq kg^{-1} fresh wt. (54.1–397 Bq kg^{-1} fresh wt.). However, ^{134}Cs derived from the

Table 1. Concentrations of ^{137}Cs and ^{134}Cs in Wild Mushrooms in a Sub-Alpine Forest around Mt. Fuji and Other Locations in Japan

Sampling location	Species[a)]	Type[b)]	^{137}Cs (Bq kg^{-1} fresh wt.)	^{134}Cs (Bq kg^{-1} fresh wt.)
Mt. Fuji 5th station	*Camarophyllus virgineus*	M	511	< 70
(2300 m above sea level)	*Hygrophorus camarophyllus*	M	493	< 13
	Rozites caperata	M	489	< 10
	Tricholoma virgatum	M	345	< 15
	Cortinarius hemitrichus	M	163	< 11
	Cortinarius collinitus	M	126	< 8.8
	Cystoderma amianthinum	M	100	< 19
	Boletinus asiaticus	M	69.5	< 11
	Mean		287	
	S.D.		193	
	Median		254	
Mt. Fuji 3rd station	*Camarophyllus pratensis*	M	455	< 23
(1800 m above sea level)	*Hydnum repandum*	M	332	< 23
	Lactarius flavidulus	M	91.9	< 10
	Trichoroma portentosum	M	82.8	< 13
	Clitocybe clavipes	M	78.7	< 5.5
	Boletopsis leucomelas	M	57.0	< 12
	Cystoderma amianthinum	S	42.0	< 9.7
	Tricholoma sejunctum	M	21.2	< 5.6
	Mean		145	
	S.D.		158	
	Median		81	
Mt. Fuji 1st station	*Cortinarius bovinus*	M	310	< 9.0
(1400 m above sea level)	*Lactarius laeticolorus*	M	108	< 19
	Lactarius flavidulus	M	59.6	< 8.7
	Tricholoma vaccinum	M	56.4	< 8.9
	Cystoderma amianthinum	S	44.7	< 9.3
	Pholiota lubrica	S	40.9	< 8.0
	Russula delica	M	18.3	< 5.0
	Clitocybe clavipes	M	1.6	< 1.7
	Mean		80	
	S.D.		98	
	Median		51	
Yamanashi prefecture	*Trichoroma sejunctum*	M	54.1	< 7.9
Location A	*Lactarius chrysorrheus*	M	240	< 5.1
	Tricholoma flavovirens	M	333	< 3.9
	Cortinarius elatior	M	126	< 4.6
	Rozites caperata	M	397	< 10
	Mean		230	
	S.D.		142	
	Median		240	
Location B	*Suillus grevillei*	M	90.5	< 7.0
	Tricholoma portentosum	M	4.4	< 2.4
	Tricholoma flavovirens	M	783	< 10
	Hydnum repandum var. *album*	M	2.6	< 6.3
	Naematoloma sublateritium	S	13.6	< 2.1
	Mean		129	
	S.D.		290	
	Median		7	
Location C	*Suillus grevillei*	M	7.0	< 1.1
	Pleurotus ostreatus	S	1.6	< 1.3
Location D	*Hygrophorus camarophyllus*	M	175	< 13

a) Mushrooms were collected in 1996. *b*) Type of fungi: M, mycorrihizal fungi; S, saprophytic fungi.

Table 2. Concentrations of ^{137}Cs, Stable Cs and K in Cultured Fruiting Bodies of Mushrooms (*P. ostreatus*) and Concentration Ratio of ^{137}Cs and stable Cs

Group	Medium[a)]	No. of samples	Dry wt./fresh wt. ratio (%)	^{137}Cs (Bq kg^{-1} dry wt.)	Stable Cs (% dry wt.)	K (% dry wt.)
A	Cs blank + K blank	3	21.2±2.3	212000±23000 (21.2)[b)]	0.003±0.003	2.23
B	0.1% Cs + K blank	5	14.4±4.0	141000±14000 (14.1)[b)]	1.37±0.21 (13.7)[b)]	1.95±0.23
C	0.1% Cs + 0.1% K	5	17.3±4.5	99000±12000 (9.9)[b)]	1.02±0.15 (10.2)[b)]	2.25±0.21
D	Cs blank + 0.5% K	5	20.1±5.4	125000±16000 (12.5)[b)]	0.002±0.001	4.72±0.74

a) Medium contained 10000 Bq kg^{-1} of ^{137}Cs activities. *b*) Concentration ratio (CR); Concentration in fruiting body of mushroom (Bq kg^{-1} dry wt. for ^{137}Cs or mg kg^{-1} dry wt. for Cs) /concentration in culture medium (Bq kg-$^{-1}$ fresh wt. for ^{137}Cs or mg kg^{-1} fresh wt. for Cs).

Chernobyl accident was not detected in any of the mushroom samples (< 1.3– < 70 Bq kg^{-1} fresh wt.). These values in the forest of Mt. Fuji were slightly lower than those in the same forest in our previous investigation in 1989–1990, (mean ^{137}Cs value of 259 ± 245 Bq kg^{-1} fresh wt.; LTD–1070 Bq kg^{-1} fresh). However, the ^{137}Cs concentrations in the mushrooms ten years after from the Chernobyl accident were much higher than those in agricultural products.[11)] Sugiyama observed that ^{137}Cs concentration in the forest around Mt. Fuji increased with altitude of the sampling location.[12)] A similar tendency is also observed in this study as described above.

Uptake of ^{137}Cs and Cs, K by Cultured Fruiting Bodies of the Mushroom *P. ostreatus*

Table 2 shows the ^{137}Cs, stable Cs and K concentrations in cultured fruiting bodies of *P. ostreatus* Y-1 and the concentration ratios of ^{137}Cs and stable Cs. The mean value of group A (Table 2) in medium containing 10000 Bq kg^{-1} without addition of Cs and K was 212000 ± 23000 Bq kg^{-1} dry wt. (32300 ± 5900 Bq kg^{-1} fresh wt.). The mean value of ^{137}Cs in this study was similar to the value of 17100 ± 3050 Bq kg^{-1} fresh obtained using sawdust medium in a our previous study.[15)] These ^{137}Cs values in cultured fruiting bodies were 2–3 orders of magnitude higher than those in the wild mushrooms collected from the forest around Mt. Fuji. Concentration ratios are used as parameters to discuss the characteristics of accumulation of elements by plants and mushrooms. The medium-to-mushroom concentration ratio (CR) is defined as follows:

CR = Concentration in mushroom (Bq kg^{-1} dry wt. for ^{137}Cs or mg kg^{-1} dry wt. for Cs)
/Concentration in medium (Bq kg^{-1} fresh wt. for ^{137}Cs or mg kg^{-1} fresh wt. for Cs)

The blank concentrations of ^{137}Cs in medium were LTD by gamma-ray spectrometry, and Cs in medium were not detected by INAA. K concentrations in medium, 1.5%, were calculated from ^{40}K activities analyzed by gamma-ray spectrometry. The means of CR for ^{137}Cs and Cs in this study are shown in Table 2. The CR of group A without addition of stable Cs and K to the medium, 21.2, was similar to the reported CR of the mycelia, about 40–50 calculated from cultivation in YMG medium.[16)] This value was 2–4 orders of magnitude higher than the transfer factors (TF) in agricultural products in Japan.[17)] ^{137}Cs in the medium actively migrated into the mushrooms. Terada suggested that Cs uptake in the mycelia cultivated in YMG medium was competitively affected by K, one of the essential elements for mushrooms and an alkaline element similarly to Cs.[16)] In this study, the CR values of ^{137}Cs for fruiting bodies cultivated in medium containing 0.1% Cs (7.5 mM Cs) and without addition of K (group B), with addition of both 0.1% Cs and 0.1% K (group C) were decreased from 14.1 to 9.9. When there were no addition of Cs in medium, the CR of ^{137}Cs for fruiting bodies in medium with addition of 0.5% K (group D), 12.5, was lower than the CR values of ^{137}Cs in medium without addition of K (group A). Moreover, the CR values of stable Cs in the medium with addition of both 0.1% Cs and 0.1% K (group C), 13.7, were also lower than the CR values in medium with addition of 0.1% Cs only (group B). Migration of ^{137}Cs and Cs into the fruiting bodies of the mushroom was suggested to be based on similar behavior due to the good agreement of CR values between Cs and ^{137}Cs. These results suggested that

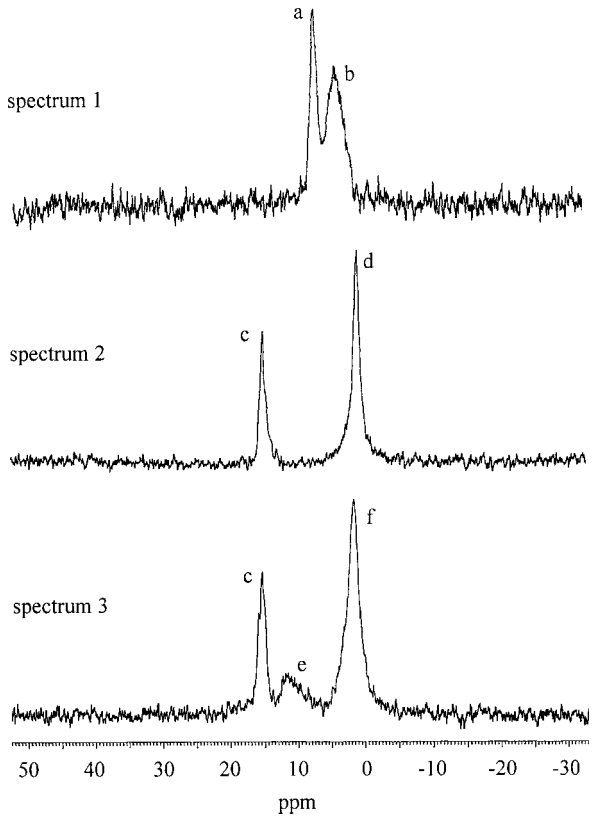

Fig. 1. ^{133}Cs-NMR Spectra (at 52.3 MHz) of the Fruiting Body of *Pleurotus ostreatus* Y-1 and Medium Containing 0.1% Cs

Spectrum 1: medium after sterilization without inoculation. Spectrum 2: medium after growth of fruiting bodies. Spectrum 3: fruiting bodies of *Pleurotus ostreatus*. The signal 'a' at 7.5 ppm in spectrum 1 is derived from 50 mM ^{133}Cs in the external reference and the signal 'c' at 15.5 ppm in spectra 2 and 3 is derived from 100 mM ^{133}Cs in the external reference.

there were also effects of inhibition to Cs or K on ^{137}Cs and Cs uptake by fruiting bodies of *P. ostreatus* Y-1. On the other hand, the concentration of K in the fruiting bodies grown on medium containing 0.5% K as additional concentration and 1.5% K as blank concentration was almost twice as high as those in media addition of either 0.1% K or no K.

State of Cs in Cultured Mushrooms

We applied ^{133}Cs-NMR spectroscopy to estimate the state of Cs in the cultured fruiting bodies of *P. ostreatus* Y-1 in media containing 0.1% Cs. Three ^{133}Cs-NMR spectra, *i.e.*, those of the media before cultivation, the media after harvesting of fruiting bodies and the fruiting bodies, are shown in Fig. 1. One broad peak (signal b) of the media before inoculation of *P. ostreatus* was obtained at a higher magnetic field than the signal of an external reference, 50 mM CsCl 25 mM Dy $(P_3O_{10})^{7-}$, at about 7.5 ppm (signal a). One signal (signal d) in ^{133}Cs-NMR spectrum of the media after harvesting the fruiting bodies was observed at almost the same position as one signal of the fruiting bodies (signal f) with the signal of an external reference of 100 mM CsCl 50 mM Dy $(P_3O_{10})^{7-}$ at about 15.5 ppm. However, two signals besides the signal of an external reference were observed from in the fruiting bodies of *P. ostreatus* cultivated in sawdust containing 7.5 mM Cs (0.1% Cs) (signal e, f). Although Kuwahara reported one signal at about 0 ppm from free ^{133}Cs ion derived from CsCl solution,[18] one signal was shifted to a slightly lower magnetic field than the signal at 0 ppm was observed from each sample, and that the other was observed at a still lower magnetic field from the fruiting bodies. In this experiment, the signals in ^{133}Cs-NMR spectra of three different samples were not in accordance with each other. This suggested that Cs existed in different states in each sample. However, as the results of ^{133}Cs-NMR spectroscopy in living tissue are dependent on a large number of factors, more detailed ^{133}Cs-NMR studies are necessary.

Elemental analysis of *P. ostreatus* Y-1 mycelia cultivated in sawdust media containing 0.2% Cs was performed using SEM-EDX. The mycelia, forming a white-colored block, were separated from the upper layer of the media just before growth of fruiting bodies. Figure 2 shows the morphology of the mycelia at a minute area. Many mycelia were seen to be grouped together on SEM images, whereas thin mycelial tips (2–3 μm in diameter) were observed on SEM images of the mycelia cultivated in YMG media.[19] Distributions of the four elements of interest in the mycelia are shown in Fig. 3. K is an alkaline element similarly to Cs, and K, P and C are essential elements for mushrooms. The distribution of Cs in the mycelia, as analyzed using SEM-EDX, was not significantly different from those of K and P.

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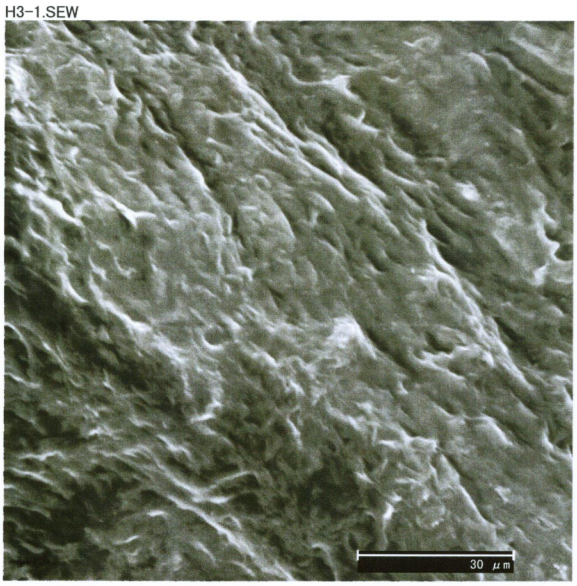

Fig. 2. Morphology of the Mycelia of *Pleurotus ostreatus* Cultured in the Sawdust Medium at Minute Area by SEM Bar indicates 30 μm

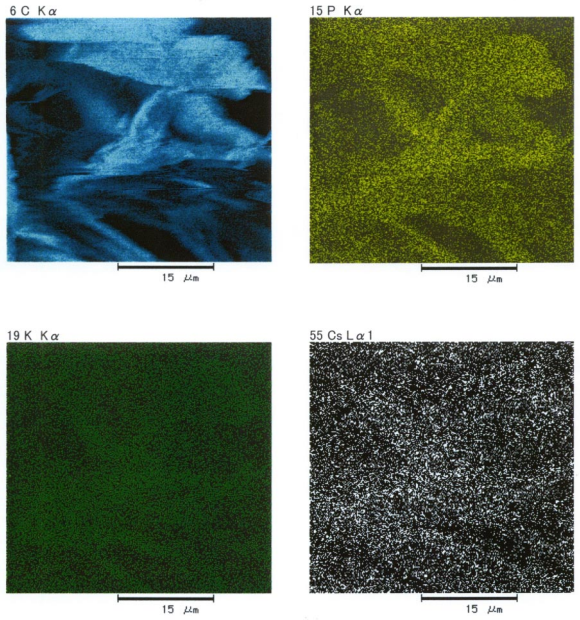

Fig. 3. SEM-EDX Images Showing C, P, K and Cs Distributions in the Mycelia of *Pleurotus ostreatus* Cultured in the Sawdust Medium

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