Cesium uptake by edible mushrooms and microorganisms isolated from mushroom substrata

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Abstract. The concentrations of ^{137}Cs and stable Cs were measured in wild mushrooms, mushroom substrata and soils from Mt.Fuji in Japan. We then studied the Cs uptake by the edible mushroom, *Pleurotus ostreatus*, through the cultured experiments using ^{137}Cs and/or a stable Cs tracer. ^{137}Cs concentrations in the fruiting bodies were 2-4 orders of magnitude higher than those in the wild mushrooms collected in Japan. It was demonstrated that ^{137}Cs and Cs were actively uptaken by the mushrooms depending on the ^{137}Cs or Cs concentration in the medium. We subsequently investigated the Cs sensitivity of microorganisms in the mushroom substrata and Cs uptake by actinomycetes, one of the soil microorganisms in mushroom substrata. The number of both bacteria and actinomycetes in the substrata decreased with increasing Cs concentration in the medium. Actinomycetes in the mushroom substrata were more sensitive to Cs than other bacteria. In the presence of 5mM CsCl, Cs uptake by several strains of actinomyces was generally high, and the growth was partially inhibited compared with the Cs resistant strains, *Streptomyces lividans* TK24 and *Streptomyces* sp. TOHO-2.

1. INTRODUCTION

The studies following the Chernobyl accident clarified that the concentration of radiocesium in mushrooms was higher than in other vegetables and types of vegetation, and that mushrooms have a tendency to accumulate radiocesium [1-3]. The number of reports on the concentration and transfer of radiocesium in wild and cultivated mushrooms has been increasing, however a clear explanation of the characteristics and mechanism of ^{137}Cs uptake by mushrooms has not been established. Yoshida et al. noted that the ^{137}Cs concentration in mushrooms reflected the layers in which their mycelia were grown [2]. Additionally, several researchers indicated that microorganisms are involved in a high retention of ^{137}Cs in the surface soil layer [4-6]. We supposed that the process of ^{137}Cs transfer to mushrooms is not only a direct transfer of soluble ^{137}Cs from the substrata, but also an indirect way from the soil microorganisms that accumulate ^{137}Cs, as in a food chain. For example, *Pleurotus ostreatus* (oyster mushroom) and five species of *Pleurotus* were reported to prey on nematodes [7, 8], and soil nematodes prey on soil bacteria with accumulated ^{137}Cs. We supposed that this type of food chain would contribute to a high ^{137}Cs accumulation in wild mushrooms. In this study, we first determined ^{137}Cs and Cs concentrations in mushrooms and mushroom substrata from a sub-alpine forest of Mt. Fuji, and examined the concentration ratios (CRs) of cultured mushrooms, *Pleurotus ostreatus* Y-1 and wild mushrooms. We investigated Cs sensitivity of the microorganisms and Cs uptake by strains of actinomycetes isolated from the mushroom substratum. By scanning electron microscopy (SEM)-energy dispersive X-ray microanalysis (EDX) and ^{133}Cs-NMR spectrometry, the states of Cs in the mycelia were examined.

2. MATERIALS AND METHODS

2.1 Samples and analyses of wild mushrooms

Eleven species of mushroom, surface soil samples (0-10cm) and two soil samples where mushrooms had not grown, were collected from the sub-alpine forest of Mt. Fuji (1600-2200m above sea level) in Yamanashi Prefecture, Japan, in October 1998. Mushroom samples and the substrata were freeze-dried and pulverized. Dried samples were placed in plastic bottles and concentrations of radionuclides were determined with a Ge-detector. Stable Cs was measured by instrumental neutron activation analysis (INAA) after cooling for several months as described by Kuwahara et al. [9].

2.2 Measurement of ^{137}Cs, stable Cs and K in cultured fruiting bodies of the edible mushroom *Pleurotus ostreatus*

The culture medium was prepared by the method of Sugiyama et al.[10]. For ^{137}Cs uptake experiments, 10 k Bq kg^{-1} of ^{137}Cs was added to the medium containing 0.1 % stable Cs, both 0.1 % stable Cs and 0.1% K or 0.5 % K. ^{137}Cs concentrations in the fruiting bodies separated from medium were determined with a Ge-detector. The stable Cs concentrations were mesured by INAA, and K content was determined by flame photometry.

2.3 Microorganisms in mushroom substrata and soils

2.3.1 Number of bacteria and actinomycetes from mushroom substrata and soils
Each dried soil sample (1 g) was suspended in 6% yeast extract broth, and aliquots of the suspensions were plated onto YM (yeast extract-malt extract) agar for bacteria and HV (humic acid and vitamin) agar for actinomycetes. After incubation at 27 °C for 7 d, the colonies on the YM and HV plates were counted.

2.3.2 Cs uptake by actinomycetes isolated from a mushroom substratum
Preincubated 3d cultures of the strains of actinomycetes in R2YE medium were used for inoculation. A certain quantity of culture was inoculated into 30ml YM broth containing CsCl and then incubated at 27 °C for 4 d with shaking. Mycelia were harvested by centrifugation, washed twice with distilled water and freeze-dried. The dried mycelia were digested with HNO_3-H_2O_2. The Cs and K contents of mycelia were determined by flame photometry.

2.3.3 SEM observation and elemental analysis of cultured mycelia of actinomycetes
The mycelia of *S. lividans* TK24 and *Streptomyces* sp. TOHO-2 cultured on YM agar containing 10mM Cs for 7d were observed by SEM (Hitachi Co., Ltd. S-3500N). Elemental analysis was carried out as described by Kato et al. [12].

2.3.4 ^{133}Cs-NMR analysis for cultured mycelia of S. lividans TK24
^{133}Cs-NMR analysis of the mycelia of *S. lividans* TK24, cultured in YM broth containing 10mM Cs in the medium, was performed by NMR spectrometry with an external reference capillary tube containing 100mM CsCl and 50mM Dy $(P_3O_{10})^{7-}$, as described by Kuwahara et al. [9].

3. RESULTS AND DISCUSSION

3.1 Concentrations of ^{137}Cs and stable Cs in wild mushrooms, mushroom substrata and soils

Figures 1(a) and (b) show the concentrations of ^{137}Cs and Cs in wild mushrooms, mushroom substrata and soils together with the previous findings of Sugiyama et al. from Mt. Fuji, Japan, respectively [1, 10]. We confirmed that the ^{137}Cs concentrations in the wild mushrooms were 2-4 orders of magnitude higher than those in other vegetables in Japan, although over 10 years have passed since the Chernobyl accident [13]. The ^{137}Cs concentrations in mushroom substrata and soils were several times higher than those of surface soils (0-5cm) collected from various areas in Japan in the same year [13]. The ^{137}Cs concentrations in both mushrooms and substrata were found to have a gradual decrease in comparison with the samples collected in 1989-1990 and 1996 [1, 10]. The wild mushrooms incorporated larger amounts of stable Cs as well as ^{137}Cs than agricultural plants such as potatoes, cabbages and rice collected in Japan [14, 15]. The Cs levels in mushroom

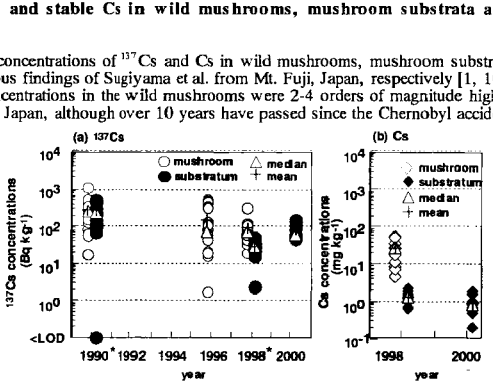

Figure 1: ^{137}Cs and Cs concentrations in mushrooms, substrata and soils. The concentrations in mushrooms were based on fresh weight, and those in substratum were based on dry weight. * Data of Sugiyama et al.[1, 10]

substrata and soils were similar to the other soils from Japan [15].

3.2 Uptake of ^{137}Cs, Cs and K by cultured fruiting bodies of the mushroom *P. ostreatus*

The ^{137}Cs concentrations in the cultured fruiting bodies of *P. ostreatus* Y-1 in medium with 10 kBq kg^{-1} were 2-3 orders of magnitude higher than those in wild mushrooms, as mentioned above. The values decreased dependent on the addition of Cs and/or K to the medium, as shown in Figure 2. The ^{137}Cs uptake by fruiting bodies was inhibited by K and/or Cs. This suggests that the mushroom *P. ostreatus* Y-1 might uptake ^{137}Cs via K transport systems.

Concentration ratios are used as parameters for discussing the characteristics of elements accumulated by plants and mushrooms. The substratum-to-mushroom concentration ratio (CR) is defined follows:

$$CR = \frac{\text{Concentration in mushroom (Bq kg}^{-1}\text{ dry wt. for }^{137}Cs\text{ or mg kg}^{-1}\text{ dry wt. for Cs)}}{\text{Concentration in substratum (Bq kg}^{-1}\text{ fresh wt. for }^{137}Cs\text{ or mg kg}^{-1}\text{ fresh wt. for Cs)}}$$

Figure 3 shows the range, median and mean of the CR for ^{137}Cs and Cs in cultured mushrooms along with the wild mushrooms examined in this study. The CRs of ^{137}Cs and Cs nearly agreed for both wild and cultured mushrooms, and the CRs of cultured mushrooms were markedly higher than those of wild mushrooms. From these findings, it was demonstrated that ^{137}Cs and Cs are actively uptaken by mushrooms depending on the ^{137}Cs or Cs concentrations in the medium.

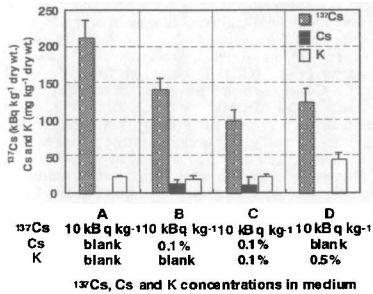

Figure 2: Concentrations of ^{137}Cs, Cs and K in cultured fruiting bodies of mushroom (*P. ostreatus* Y-1) in media at various ^{137}Cs, Cs and/or K concentrations.

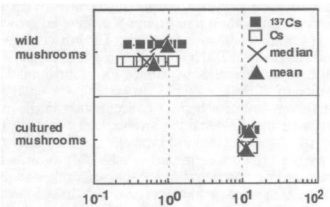

Figure 3: Ranges of CR*s of ^{137}Cs and Cs and median and mean for wild and cultured mushrooms. *CR indicates concentration ratio.

3.3 Experiments on microorganisms in mushroom substrata and soils

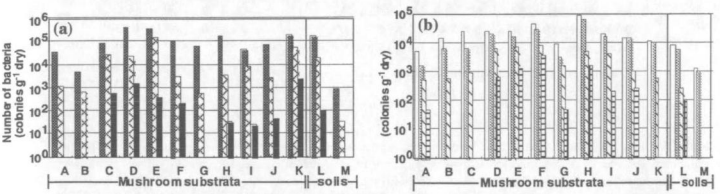

Figure 4: Number of microorganisms in mushroom substratum and soils. (a) bacteria grown on YM agar containing 25 to 100mM Cs and (b) actinomycetes grown on HV agar containing 0 to 25mM Cs.

3.3.1 Number of bacteria and actinomycetes in mushroom substrata and soils

The number of bacteria and actinomycetes present in mushroom substrata and soils when CsCl was added to YM agar and HV agar are shown in Figure 4. Both of the number decreased with increasing Cs concentration in agar. Colonies of bacteria were observed from all substrata and soil samples up to 50mM Cs in YM agar. In 9 of 13 samples, bacterial colonies were observed even in the presence of 100mM Cs. In contrast, colonies of actinomycetes from all samples were seen only up to 5mM Cs in HV agar. The colonies appeared in 92% of all samples at 10mM Cs, and in 62% at 15mM Cs. In the presence of 25mM Cs, no actinomycetes could be detected any samples. These findings show that actinomycetes in mushroom substrata and soils are more sensitive to Cs than bacteria. There was no distinct difference in the patterns of appearance of bacteria and actinomyces from substrata and soil samples. Additionally, the Cs concentrations in substrata and soils examined in this study did not influence on the number of soil microorganisms.

3.3.2 Cs uptake by actinomycetes isolated from mushroom substratum

We investigated the Cs sensitivity of the strains of actinomycetes isolated from an edible mushroom substratum. Approximately 60% of the actinomycetes isolated from mushroom substratum could not grow in the presence of over 10mM Cs. Figures 5(a), (b) and (c) show the growth and Cs uptake of several strains of actinomycetes, which differ in Cs sensitivity, in YM broth containig 5mM Cs. The most Cs-sensitive strains shown in Figure 5(a) showed growth only up to 5mM Cs in medium, while 5(b) was up to 10mM Cs and 5(c) was 15mM Cs. The growth and Cs uptake of Cs-resistant strains, *S. lividans* TK24 and *Streptomyces* sp. TOHO-2, are shown in Figures 5(d) and (e), respectively. *S. lividans* TK24 showed growth in the presence of 50mM Cs in medium. *Streptomyces* sp. TOHO-2 showed growth even in the presence of 200mM Cs [12]. In general, Cs uptake by the Cs-sensitive strains was higher than that by the Cs-resistant strains when Cs concentration in the medium was low (5 mM). The concentration of K in the mycelia of the Cs-sensitive strains was relatively constant (the range of 10-19 mg g^{-1} dry weight). The range of Cs concentration in mushroom substrata was 0.62-2.2 mg kg^{-1} dry weight (corresponding to 0.2-1.5 mg kg^{-1} fresh weight and 1.5-11 μM) as shown in Figure 1, and the Cs concentrations in nature were extremely low compared to those used in this study. The actinomycetes in mushroom substrata tended to uptake Cs present at low levels in substrata more than the Cs-resistant strains, and to accumulate Cs in mycelia. Therefore, we suppose that the highest ^{137}Cs concentrations were found in the surface layers in most cases, since microorganisms such as actinomycetes in the surface soils will uptake ^{137}Cs and store it in the cells or mycelia.

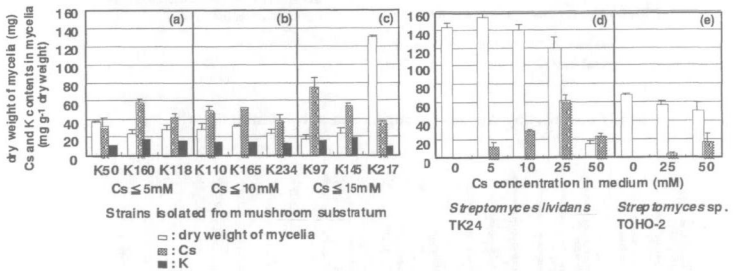

Figure 5: Concentrations of Cs and K incorporated into actinomycetes, and growth. Strains in (a, b and c) were isolated from mushroom substratum. (a), (b) and (c) could grow with up to 5, 10 and 15mM Cs in media, respectively. These strains were cultured in the presence 5mM Cs for 4 d. *S. lividans* TK24 (d) and *Streptomyces* sp. TOHO-2 (e) showed growth in up to 50mM and 200mM Cs and were cultured at various Cs concentrations.

3.3.3 SEM observation and elemental analyses of cultured mycelia of actinomycetes

The mycelia of *S. lividans* TK24 and *Streptomyces* sp. TOHO-2 grown on YM agar containing 10mM and 50mM Cs, respectively, were examined (Figure 6). We observed brilliant spots in the mycelia of both strains, but no such spots were observed in the mycelia grown on Cs-free medium (data not shown).

Subsequently, we performed elemental analyses of the brilliant spots in the mycelia of *S. lividans* TK24 by SEM-EDX. In areas containing a brilliant spot, distinctive peaks for P and Cs were observed, whereas only weak peaks for P and Cs in other areas were seen [12]. These observations indicate that the Cs incorporated into the mycelia of *S. lividans* TK24 was condensed and accumulated in particular regions where the P concentration was high. This finding may be related to the intracellular detoxification of Cs by organisms.

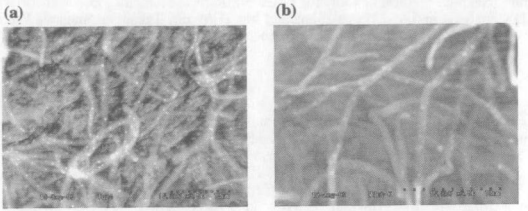

Figure 6: Scanning electron micrographs for *S. lividans* (a) and *Streptomyces* sp. TOHO-2 (b). The mycelia were inoculated onto membrane filters on YM agar containing 10mM (a) or 50mM CsCl (b) for 10d. The mycelia on the membrane filter were subjected to SEM. Bar indicates 10 μm.

3.3.4 ^{133}Cs-NMR analysis for cultured mycelia of S. lividans TK24

We perfomed ^{133}Cs-NMR analyses to estimate the state of Cs in the mycelia of *S. lividans* TK24 cultured in YM broth containing 10mM Cs. Figure 7 shows two representative ^{133}Cs spectra from cultured mycelia in the presence of 10mM Cs (a) and the medium after harvesting the mycelia (b). In the spectra from the mycelia of *S. lividans* TK24, two relatively broad signals at approximately 2 and 5 ppm in the lower field were observed, with the external reference of 100mM CsCl and 50mM Dy $(P_3O_{10})^{7-}$ at 14.5 ppm. In the medium after harvesting, one signal at 0 ppm corresponding to free Cs^+ was seen. We supposed that these lower field signals in the spectra of mycelia were derived from the Cs^+ trapped subcellulary, as in the cytoplasm and vacuoles, as reported by Pfeffer P. E. et al. [16].

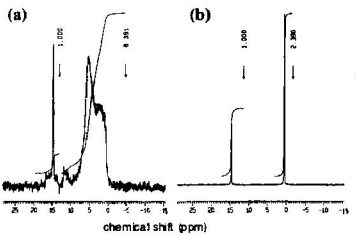

Figure 7: ^{133}Cs-NMR spectra (at 52.3 MHz) of the cultured mycelia of *S. lividans* TK24 (a) and medium containing 10mM Cs (b) . The mycelia of *S. lividans* TK24 cultured in YM broth with 10mM CsCl for 4 d(a) and medium after harvesting the mycelia (b) . The signals at 14.5 ppm in both spectra (a) and (b) are derived from 100mM ^{133}Cs in the external reference.

4. CONCLUSION

In this study, the concentrations of ^{137}Cs and Cs in wild mushrooms and in the substrata, the habitat of the fruiting body mycelia, were determined. We investigated the Cs sensitivity of microorganisms in the substrata, and found that actinomycetes were more sensitive to Cs than other bacteria, and that they accumulated Cs in mycelia. In addition, there are some reports that other bacteria, such as *Rhodococcus* sp. strains CS98 and CS402, *Escherichia coli* and *Bacillus subtilis* incorporated Cs into the cells [17, 18]. ^{137}Cs in mushroom substrata might be concentrated there because these microorganisms inhabit the substrata and accumulate Cs.

The findings of SEM-EDX and ^{133}Cs-NMR analyses suggest that Cs taken up by the mycelia of *S. lividans* TK24 might be concentrated in limited regions, such as vacuoles, for detoxification of intracellular Cs.

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