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Uptake of Radiocesium by Hypha of Basidiomycetes –Radiotracer Experiments–

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Uptake of Cs and its effects on mushroom growth were studied using a simulated medium in which K^+ and NH_4^+ concentrations were lowered as much as possible. The hyphae of *H. vinosophyllum* did not grow in the simulated medium with 2 mmol CsCl. The growth increased with the addition of K, indicating that K lowers the toxicity of Cs to mushrooms in the simulated medium. The inhibition of mushroom growth by Cs was greater than that by Rb. The uptake of Cs was reduced by the addition of the monovalent cations of K^+, Rb^+, and NH_4^+.

1. Introduction

Many papers[1–4] have reported high concentrations of radiocesium in mushrooms collected in Ukrainian and European forests after the Chernobyl accident. The concentrations of ^{137}Cs in forest mushrooms were markedly higher than those in autotrophic plants.[5] Mushrooms sometimes affect the behavior of Cs in the environment.[6] Lindner et al.[7] surveyed roe deer contamination with radiocesium isotopes in the rural prealpine area of Oberschwaben in southwestern Germany beginning in the autumn of 1986. They identified characteristic regional and seasonal patterns of contamination that resulted from its transfer from the soil to the grazing plants of these animals. They suggested that the periodic maximum observed in roe deer in the autumn was correlated with the mushroom season in the grazing forests.

Since edible mushrooms for humans also have higher ^{137}Cs concentrations than other foodstuffs, they are important for an estimation of internal radiation doses from ^{137}Cs through foods. Skuterud et al.[8] reported dietary surveys and whole body monitoring at two sites in the Bryansk Region of the Russian Federation in order to estimate the transfer of Chernobyl radiocesium to humans. They found mushroom consumption was the predominant factor in a 60–70% mean increase in radiocesium activity concentrations in humans in the autumn. The contribution from a high concentration of ^{137}Cs in mushrooms was found worldwide because of atmospheric nuclear tests. In our most recent study,[9] the contribution of mushrooms to the total intake of ^{137}Cs in Japanese was estimated to be 28% (on average) of the total intake of ^{137}Cs, while that of ^{40}K was estimated to be only 1.9%. Gaso et al.[10] calculated the intake of ^{137}Cs through 30 local mushroom species collected from 1993 to 1999 in a semi-natural temperate forest in Mexico and found that the mushrooms contributed 37% of ^{137}Cs and 4% of ^{40}K to the total dietary intake of these contaminants. We suggest that it is unreliable to neglect ^{137}Cs in mushrooms when estimating internal doses of ^{137}Cs. Therefore, the accumulation behavior of radiocesium in mushrooms should be elucidated for understanding radiation protection and radioecology.

Accumulation of radiocesium in fruit bodies has been observed by laboratory experiments. We carried out cultivation experiments for four basidiomycetes (*Hebeloma vinosophyllum*, *Flammulina velutipes*, *Coprinus phlyctidosporus*, and *Agrocybe cylindracea*) in a solid growth medium including ^{137}Cs as a radiotracer.[11, 12] Our results showed that fruit bodies of the four basidiomycetes accumulated Cs from the medium, although there were large differences in Cs concentration among mushroom species.[11] The effects of Cs in the medium on the accumulations by the fruit body of *H. vinosophyllum* were investigated, and it was found that the concentration ratios of ^{137}Cs were not highly influenced by coexisting Cs in the medium.[12]

On the other hand, some references have reported that Cs is toxic to organisms. For example, Nishita et al.[13] cultivated bean plants in nutrient solution at various Cs concentrations and a constant K concentration of 20 ppm. Visibly reduced plant growth occurred when the substrate Cs concentration was 5 ppm. At a Cs concentration of 20 ppm, the fruits were dwarfed and deformed. No fruit was produced at a Cs concentration of 30 ppm. Matsudaira et al.[14] cultivated the cellular slime mold, *Dictyostelium discodeum* A-3, in the usual medium with 1 mM CsCl added. They found that the cell doubling time in this medium was 1.1 times as much as that when cultivated in the medium without Cs. The Merck index[15] reports the LD_{50} (medium lethal dose, the quantity of a chemical that is estimated to be fatal to 50% of the organisms tested) of CsCl to be 1.5 g/kg, or about 8.9 mmol/kg, intraperitoneally in rats, but values for RbCl and KCl are not given there.

Though it is known that mushrooms concentrate Cs, there are few reports on the role and the toxicity of Cs for mushrooms. It is difficult to evaluate the effects on growth and concentration of Cs by varying the concentrations of monovalent ions in the medium because the usual media for cultivation of fungi have high concentrations of K^+ and NH_4^+. Therefore, we studied uptake of Cs and its effects on mushroom growth using a synthetic medium in which K^+ and NH_4^+ concentrations were reduced as much as possible.

2. Experimental

Hebeloma vinosophyllum NBRC31231 (IFO31231) was used for the cultivation experiment. The medium composition is shown in Table 1. We used a medium proposed by Suzuki[16] with modifications designed to reduce K^+ and NH_4^+ concentrations. A portion of the KH_2PO_4 was replaced with Na_2HPO_4 in order to lower the K content in the medium. Glycine

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TABLE 1: Components of Media

Maltose	40 g
Glycine	1 g
KH_2PO_4	0.14 g
$Na_2HPO_412H_2O$	2.7 g
$MgSO_47H_2O$	0.3 g
$FeSO_4$	0.01 g
$CaCl_2$	1 mg
$ZnSO_47H_2O$	0.3 mg
$CuSO_45H_2O$	0.1 mg
$MnSO_45H_2O$	0.1 mg
$(NH_4)_6Mo_7O_24H_2O$	0.02 mg
Thiamin	0.5 mg
Nicotinamide	0.1 mg
Distilled Water	1 L

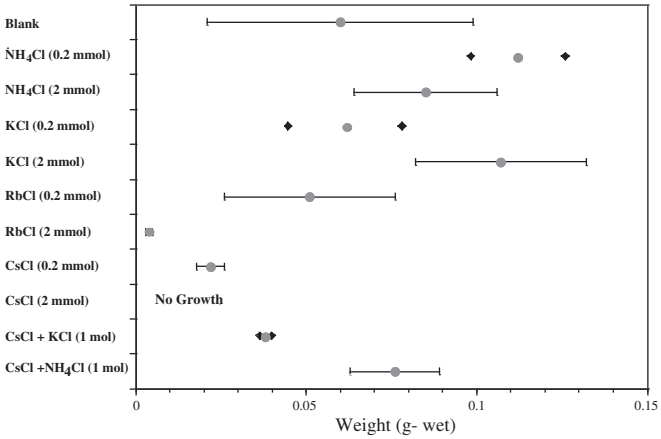

Figure 1. Growth of hyphae of *Hebeloma vinosophyllum* in various media. Arithmetic mean (●) and range of standard deviation (solid lines). Original data are shown by ◆, if only two data are available.

(H_2NCH_2COOH) was added in exchange for NH_4Cl in order to reduce NH_4^+ in the medium. We assumed that cells uptake glycine through different channels from the NH_4^+ channels since glycine is an organic substance. Cells can make NH_4^+ from glycine in the cell by using glycine synthase (see eq 1).[17] Therefore, the amount of glycine was decided by equating the nitrogen content to the NH_4Cl content and assuming that all glycine in the medium was converted into NH_4^+. The amount of Ca was reduced in order to prevent the precipitation of Ca phosphate in the medium.

$$H_3^+NCH_2COO^- + H_4 \text{ folic acid} + NAD^+ \rightarrow CO_2 + NH_4^+ + 5,10\text{- methylene } H_4 \text{ folic acid} + NADH \quad (1)$$

Concentrations of K^+, Rb^+, Cs^+, and NH_4^+ in several medium solutions were controlled by adding additional amounts of KCl, RbCl, CsCl, and NH_4Cl (see Table 2). We refer to the basic medium without the additional chlorides as "blank medium." The amount of K in the blank medium was successfully reduced to 1/25 of that in the medium that we had used previously.[8] Each medium solution was sterilized by filtration through a membrane filter. We poured 25 mL of each medium solution into bottles (50 mL) and added the radiotracer, ^{137}Cs (661.7 keV, half life: 30.07 y), as $^{137}CsCl$. Since the ^{137}Cs used was carrier-free, the amount of elemental Cs added as ^{137}Cs was negligible. Two or three bottles were prepared for each medium solution. The fungus was pre-cultivated on the medium in a petri dish. After pre-cultivation, the fungus with its accompanying medium was taken out using a cork borer and placed on the medium in the bottles. After incubating the bottle contents at 25 ˚C for three weeks, the hyphae were removed using tweezers. The hyphae were washed with radiotracer-free medium by placing them on a membrane filter in a suction funnel.

TABLE 2: Extra Salt in Medium (medium name in paper)

1) nothing	(Blank medium)
2) 0.2 mmol NH_4Cl	(0.2 mmol NH_4Cl medium)
3) 2.0 mmol NH_4Cl	(2.0 mmol NH_4Cl medium)
4) 0.2 mmol KCl	(0.2 mmol KCl medium)
5) 2.0 mmol KCl	(2.0 mmol KCl medium)
6) 0.2 mmol RbCl	(0.2 mmol RbCl medium)
7) 2.0 mmol RbCl	(2.0 mmol RbCl medium)
8) 0.2 mmol CsCl	(0.2 mmol CsCl medium)
9) 2.0 mmol CsCl	(2.0 mmol CsCl medium)
10) 1.0 mmol CsCl + 1.0 mmol KCl	(1 mmol CsCl+KCl medium)
11) 1.0 mmol CsCl + 1.0 mmol NH_4Cl	(1 mmol CsCl+NH_4Cl medium)

The hyphae were placed in polyethylene vials and weighed. The concentrations of ^{137}Cs in hyphae were determined by means of a NaI scintillation counter (Aloka ARS-380). Decay corrections were made at the start of the measurement. The concentration ratio was calculated as "activity of ^{137}Cs in hyphae of mushroom (Bq/g, wet wt.)" divided by "activity of ^{137}Cs in medium (Bq/g, wet wt.)".

3. Results and Discussion

Effect on growth of hyphae. The hyphal growth in various medium solutions is shown in Figure 1. The growth of the hyphae is usually described as an extension or weight increase of the hyphae; we used the wet weight increase in this report. The growth of *H. vinosophyllum* in 0.2 mmol NH_4Cl medium and 2 mmol KCl medium was larger than that in the blank medium. Addition of NH_4^+ and K^+ promoted the growth of hyphae.

No hyphal growth in 2 mmol CsCl medium was observed by visual inspection. The hyphal growth in 0.2 mmol CsCl medium was less than that in the blank medium. The growth was inhibited by increasing the Cs concentration of the medium. Hyphae grew in the media that contained more than 2 mol Cs if there were sufficient amounts of NH_4^+ and K^+ present.[18] Hence, not only the toxicity of Cs but also the low NH_4^+ and K^+ levels contributed to the inhibition of hyphal growth.

The hyphal growth in the 1 mmol CsCl + KCl medium was larger than that in the 0.2 mmol CsCl medium. In terms of low K content, the hyphal growth in 1 mmol CsCl medium should be less than that in 0.2 mmol CsCl medium. Our results indicate that the toxicity of Cs^+ decreased with the addition of K^+. We thought that the obstruction by Cs of enzyme activities was one of the causes for the growth inhibition of mushrooms by Cs. Some enzymes, such as rabbit liver fructose-1,6-bisphosphatase,[19] have been found to lose activity in the presence of Cs rather than K. The influence of Cs increased as the ratio of Cs to K increased.

The hyphal growth of *H. vinosophyllum* in 0.2 mmol CsCl medium was less than that in 0.2 mmol RbCl medium. The hyphae of *H. vinosophyllum* in 2 mmol RbCl medium grew slightly while the hyphae in 2 mmol CsCl medium did not grow at all. The hyphal growth was inhibited more strongly by Cs than by Rb.

The effect of the cultivation medium on the concentration ratio of ^{137}Cs. The concentration ratio is one parameter that shows the uptake of elements from a medium to an organism. The results for the concentration ratio of ^{137}Cs in hyphae cultivated in various medium solutions are shown in Figure 2.

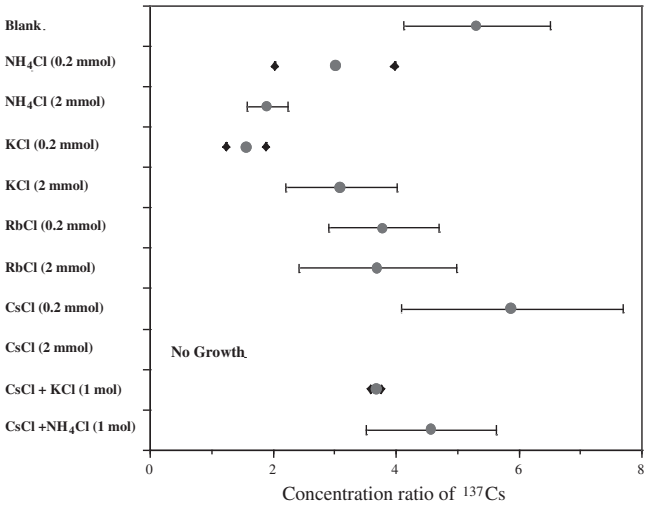

Figure 2. Concentration ratio of ^{137}Cs in hyphae of *Hebeloma vinosophyllum* cultivated in various media. Arithmetic mean (●) and range of standard deviation (solid lines). Original data are shown by ◆, if only two data are available.

The concentration ratio of ^{137}Cs in hyphae cultivated in the blank medium was about 5.3 ± 1.2. The concentration ratio (5.8 ± 1.8) of ^{137}Cs in hyphae cultivated in the 0.2 mmol CsCl medium was just slightly different from that in the blank medium. This indicates that the concentration ratios of ^{137}Cs in the hyphae were not affected by that of Cs^+ in the medium.

The concentration ratios of ^{137}Cs in hyphae cultivated in the medium solutions containing KCl, RbCl, or NH_4Cl were lower than the ratio of ^{137}Cs in hyphae cultivated in the blank medium. This indicates that the concentration ratio of ^{137}Cs was inhibited by monovalent cations other than Cs^+. In the case of the addition of 2 mmol cations to the medium, the concentration ratio of ^{137}Cs was in the order $NH_4^+ > K^+ > Rb^+ > Cs^+$. Ammonia ions inhibited the uptake of ^{137}Cs, though NH_4^+ is not an alkali metal ion. The growths of hyphae in the 1 mmol CsCl + NH_4Cl medium and the 1 mmol CsCl + KCl medium were larger than that in the 0.2 mmol CsCl medium (Figure 1). This was caused by decreasing the absorption of Cs through the addition of NH_4^+ or K^+ in the medium. As a result, the toxicity of Cs in the medium decreased.

Terada et al.[20] investigated the uptake of ^{137}Cs and Cs by a mushroom (*Pleurotus ostreatus* (Fr.) Kummer Y-1) in the presence of alkali metal elements to study the accumulation mechanism of radiocesium. The concentration ratio of ^{137}Cs in hyphae decreased with increasing K or Rb concentrations. Ohnuki et al.[21] also reported micro-PIXE analyses results suggesting a decrease in the accumulation of K, P, and Fe by *Saccharomyces cerevisiae* during Cs uptake. These results agreed with our results. However, Terada et al.[20] reported that the concentration ratio of ^{137}Cs also decreased with increasing Cs concentration in the media. This might be caused by the difference of species and by the K concentration in the medium. The concentration ratio of ^{137}Cs in hyphae cultivated in 2 mmol KCl medium was higher than that cultivated in 0.2 mmol KCl medium. This difference in the concentration ratio was caused by better hyphal growth.

In contrast, the concentration ratio of ^{137}Cs in hyphae cultivated in 2 mmol RbCl medium was as low as that in those cultivated in 0.2 mmol RbCl medium whereas the hyphal growth decreased in the former.

The Cs concentration ratio in the hyphae in this study was lower than the concentration ratio in mushroom fruit bodies in our previous study[11] even taking into account the difference of media. However, Cs might be a physiologically non-essential element for *H. vinosophyllum*. It has been reported that fruit bodies have an ability to change Cs from an ionic form to complexes with pigment; Aumann et al.[22] reported complexation of ^{137}Cs by the cap pigments (norbadione A) of the bay boletus (*Xerocomus badius*).

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