Effect of minerals on accumulation of Cs by fungus *Saccaromyces cerevisiae*

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Abstract

The accumulation of Cs by unicellular fungus of *Saccharomyces cerevisiae* in the presence of minerals has been studied to elucidate the role of microorganisms in the migration of radioactive Cs in the environment. Two different types of experiments were employed: experiments using stable Cs to examine the effect of a carbon source on the accumulation of Cs, and accumulation experiments of radioactive Cs from agar medium containing ^{137}Cs and zeolite, vermiculite, phlogopite, smectite, mica, or illite as mineral supplements. In the former type of experiments, the Cs-accumulated cells were analyzed by scanning electron microscopy equipped with energy dispersive X-ray analysis (SEM-EDS). In the latter type, the radioactivity in the yeast cells was measured by an autoradiography technique.

When a carbon source was present, higher amounts of Cs accumulated in the cells than in the resting condition without a carbon source. Analyses with SEM-EDS showed that no mineral formed on the cell surface. These results indicate that the yeast cells accumulate Cs by adsorption on the cell surface and intracellular accumulation. In the presence of minerals in the agar medium, the radioactivity in the yeast cells was in the order of mica > smectite, illite >> vermiculite, phlogopite, zeolite. This order is inversely correlated to the ratio of the concentration of radioactive Cs between the minerals and the medium solution. These results strongly suggest that the yeast accumulates radioactive Cs competitively with minerals.

Keywords: Radioactive Cs; fungi; migration; mineral, accumulation.

1. Introduction

The nuclear accident at the Fukushima Daiichi Nuclear Power Plant (FDNPP) occurred as a consequence of the massive earthquake and associated tsunami that struck the Tohoku and north Kanto regions of Japan on 11 March 2011. A series of hydrogen explosions occurred from 13 March to 15 March at units 1, 2, and 3 of FDNPP. The release rate of ^{137}Cs on 15 March was estimated between 10^{12} and 10^{15} Bq h⁻¹ (Chino et al., 2011; Kobayashi et al., 2013). This radioactive Cs fallout was dispersed from FDNPP to the ocean (Chino et al., 2011; Hirao et al., 2013) and neighboring land region (Kobayashi et al., 2013). Much of the released radioactive Cs was deposited in the area northwest of FDNPP (Tanaka et al., 2012; Saito et al., 2014). Spatial concentration distributions and depth profiles of radioactive Cs were measured (Tanaka et al., 2013; Saito et al., 2014; Tanaka, Iwatani et al., 2014; Tanaka Shimada, et al., 2014) to estimate the dose rate and the fate of the radioactive Cs in the terrestrial environment.

During migration in the environment, radioactive Cs would have been adsorbed onto both inorganic and organic materials. It is well known that radioactive Cs is irreversibly sorbed by micaceous minerals such as illite and vermiculite (Sawney, 1970; Tamura and Jacobs, 1989; Ohnuki, 1994). Recently minerals other than micaceous minerals have been shown to sorb irreversibly radioactive Cs when the concentration of radioactive Cs is extremely low (Kozai et al., 2012; Ohnuki and Kozai, 2013). These results indicate that many kinds of minerals, including micaceous minerals, affect the migration of radioactive Cs. The sorption of radioactive Cs by organic materials including microorganisms also retards the migration of radioactive Cs. After the Chernobyl accident, the effect of microorganisms on the migration of radioactive Cs,

especially in long-term migration, has been studied (Olsen et al., 1990; Fawaris and Johanson, 1995). Some specific microorganisms were reported to accumulate highly radioactive Cs (Tomioka et al., 1994; Kuwahara et al., 2011). Stemmer et al. (2005) reported that approximately 1.2–2.7% of the total radioactive Cs soil inventory was accumulated in fungal microbes. Many kinds of fruiting bodies of fungi accumulate radioactive Cs (Yoshida et al., 1994). The presence of soil microorganisms significantly enhances the retention of Cs in organic systems, and 70% of the radioactive Cs accumulated in microorganisms at 20°C at pH 7.1 was not extracted for 24 h with deionized water, 0.015 mole L⁻¹ CaCl₂, or 0.01 mole L⁻¹ CaCl₂ + 0.001 mole L⁻¹ CsCl (Parekh et al., 2008). These results indicate that fungal microorganisms affect the migration of radioactive Cs in the environment. However, the accumulation of radioactive Cs by fungal microorganisms in the presence of minerals and role of microorganisms in this migration is not fully understood.

Yeasts are found in soils worldwide (Botha, 2011). Yeast *Saccharomyces cerevisiae* is a unicellular fungi and its physiology is well known (Walker, 1998). In the present study, accumulation of Cs by *Saccharomyces cerevisiae* was studied to elucidate the role of fungal microbes in the migration of radioactive Cs in the environment.

2. Experimental

2.1 Strain and culture conditions

The yeast strain *S. cerevisiae* X-2180-1B was pre-grown in YPD-broth medium containing 1 g yeast extract, 2 g peptone, and 2 g dextrose in 100 mL of 0.1-M NaCl solution for 48 h at 30°C. The pre-grown yeast cells were inoculated in liquid media or agar media after rinsing twice with 0.1-mol L⁻¹ NaCl solution. In the liquid media experiments, pre-cultured yeast cells at

95 1.0 g L⁻¹ were inoculated in a 1.0×10⁻³-mol L⁻¹ NaCl solution containing CsCl at 1.0×10⁻⁴ mol L⁻¹
96 ¹. The solution contained 1.0×10⁻³ mol L⁻¹ D(+) glucose as a carbon source and 2.5×10⁻² mol L⁻¹
97 HEPES as a pH buffer. The solution pH was adjusted to 7.0 by adding 0.10 mol L⁻¹ HCl or
98 NaOH solution. After exposure of the yeast cells to Cs solution containing glucose at 30°C, the
99 supernatants were sampled at 0, 0.5, 1, 2, 3, 6, 12, 18, 24, 36, and 48 h. The same accumulation
100 experiment was performed in 1.0×10⁻³ mol L⁻¹ NaCl and 1.0×10⁻⁴ mol L⁻¹ Cs solution without
101 glucose to test the effect of glucose activation on the accumulation of Cs by yeast cells.

102 The effect of Cs concentration on accumulation by yeast cells at 1.3 g L⁻¹ under glucose
103 activation was examined for 5.0×10⁻⁵, 1.0×10⁻⁴, 5.0×10⁻⁴, and 1.0×10⁻³ mol L⁻¹ Cs solutions. A
104 2.5×10⁻² mol L⁻¹ HEPES was added as a pH buffer, and the pH was adjusted to 7.0 by adding
105 0.10 mol L⁻¹ HCl or NaOH solution. The supernatants of the solution were collected at 24 h after
106 exposure. The supernatants were filtered through a membrane filter with a pore size of 0.45 µm,
107 and the Cs concentration was determined by ICP-MS. The Cs-accumulating yeast cells in the
108 1.0×10⁻² mol L⁻¹ CsCl solution containing glucose were separated from the solution by filtering
109 through the 0.45-µm-pore membrane filter, and were dried on carbon tape and analyzed by field
110 emission scanning electron microscopy equipped with energy dispersive spectroscopy (FE-SEM-
111 EDS) to investigate the morphology and elemental components.

112 In the agar media experiments, pre-cultured yeast cells were inoculated on a membrane filter
113 with a pore size of 0.2 µm placed on the 10 g YPD-broth agar media containing either 44 Bq g⁻¹
114 of ¹³⁷CsCl or no ¹³⁷CsCl as a control. A schematic figure of the agar media used for the agar
115 media experiments is shown in Supplementary data 1. Since ¹³⁷CsCl was a carrier-free chemical,
116 the chemical concentration of Cs was lower than 10⁻⁹ mol L⁻¹. The yeast cells were exposed to

agar medium containing ^{137}Cs for 48 h at 30°C. Distribution of ^{137}Cs in the yeast cells was determined by autoradiography (AR) analysis using an imaging plate.

In order to determine the depth of agar medium at which ^{137}Cs was transported to yeast cells, pre-cultured yeast cells were inoculated for 72 h at 30°C on $^{137}\text{CsCl}$ -containing YPD broth agar medium in a centrifuged tube of 50 mL roughly 8 cm in depth. A cross section of the agar medium in the centrifuged tube was analyzed by AR analysis. The AR image was compared with a control cross-section of agar medium without yeast cells.

For testing the effect of the presence of minerals in the agar media, the following minerals were used: three kinds of zeolite namely, zeolite A (mordenite structure by XRD), clinoptilolite (Kunimine Co., Ltd., Japan), and clinoptilolite Niki (Niki-Cho, Hokkaido, Japan), vermiculite from South Africa (SA) and China (CH), phlogopite from India, four kinds of smectite (Kunipia F, lucentite, Kunipia G, and synthetic saponite), Japanese acid clay, illite, Somasif (synthetic swelling mica), and mica. These minerals were obtained from by TIC Co. Ltd. (Japan). The pre-cultured yeast cells were inoculated on a 0.22- μm -pore membrane filter placed on the 10 g YPD Broth agar medium containing 0.01, 0.05, or 0.1 g of each mineral and ^{137}Cs for 72 h at 30°C. Distribution of ^{137}Cs in the yeast cells was determined by AR analysis using an imaging plate.

For examining the sorption ability of the minerals for ^{137}Cs from the culture medium, the distribution ratio of each mineral for ^{137}Cs was determined by placing the mineral in YPD broth liquid medium containing $^{137}\text{CsCl}$ for 24 h at 30°C. The supernatant of the liquid medium was filtered through a 0.22- μm -pore membrane filter. The radioactivity in the filtrate was measured by liquid scintillation counting. The distribution ratio of minerals for ^{137}Cs in the liquid medium (K_{d_min}) was determined by the following equation:

$$K_{d_min} (\text{ml g}^{-1}) = D(C_0 - C)/C,$$

where D is the liquid-to-solid ratio (ml g^{-1}), C_0 is the initial concentration of Cs, and C is the concentration of Cs after exposure to the liquid medium.

2.2 Analytical methods

The concentrations of Cs were determined using inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer NexIonIII, PerkinElmer). Autoradiography analysis was performed to measure ^{137}Cs using a combined bio-imaging analyzer system (BAS-300, Fuji-film, Japan) with an imaging plate (IP). In the AR analysis, the intensity of IP was measured by a BAS system. Since the relationship between the radioactivity of the standard ^{137}Cs solution and the intensity of the standard ^{137}Cs solution dropped on the membrane filter determined by BAS showed a good linear relationship, the radioactivity of ^{137}Cs in the yeast cells was determined by the intensity measured by the BAS system.

Liquid scintillation analysis was performed by Aloka liquid scintillation system LSC-7200 (Aloka, Japan). The supernatant was mixed with a scintillation cocktail (Ultima Gold, PerkinElmer) for the measurement.

The yeast cells samples in contact with $1.0 \times 10^{-2} \text{ mol L}^{-1}$ CsCl solution were analyzed using field emission scanning electron microscopy (FE-SEM, JEOL JSM-6701F) combined with energy dispersive X-ray spectroscopy (EDS). Topology-sensitive imaging was conducted at an acceleration voltage of 5 kV and EDS analysis was completed at 25 kV. All samples were coated with carbon using a carbon coater (SANYU SC-701C) to ensure conductivity.

3. Results

163 3. 1 *Effect of carbon source on accumulation of Cs by yeast cells from liquid media*

164 The time course of Cs concentrations accumulated in the yeast cells from the liquid medium
165 containing $1.0 \times 10^{-4} \text{ mol L}^{-1}$ CsCl in the presence of glucose as a carbon source (Fig. 1) showed
166 that the concentration of Cs in the yeast cells increased abruptly up to approximately $5 \times 10^{-2} \text{ mol}$
167 kg^{-1} with increasing time to 4 h, followed by gradual increase up to $7 \times 10^{-2} \text{ mol kg}^{-1}$. In the
168 absence of glucose, the concentration of Cs in the yeast cells increased gradually to $2 \times 10^{-2} \text{ mol}$
169 kg^{-1} at 6 h, and then the concentration of Cs became almost constant within 48 h. These results
170 indicated that the presence of glucose resulted in higher accumulation of Cs than that in the
171 absence of glucose. Since glucose is a carbon source for metabolites, yeast accumulates higher
172 amounts of Cs in the metabolically active condition than in the resting one without a carbon
173 source.

174 Larger amounts of Cs were accumulated when a higher concentration of Cs was added under
175 glucose activation in the liquid medium (Fig. 2). The concentrations of Cs in the yeast cells and
176 solution showed a linear relationship, with the Cs accumulation in the yeast cells correlating with
177 the concentration in solution. The K_{d_cell} was determined by the following equation:

178 $K_{d_cell} (\text{ml g}^{-1}) = \text{Cs concentration in the yeast cells} / \text{Cs concentration in solution}.$

179 The K_{d_cell} was determined by the relationship between Cs concentration in the yeast cells and Cs
180 concentration in solution to be 100 ml g^{-1} within R^2 of 0.99.

181 SEM images of the yeast cells (Fig. 3) showed that the morphology was not changed after
182 exposure to Cs-containing solution. In addition, no precipitate containing Cs was observed by
183 SEM. The EDS spectrum of the yeast cells showed distinct Cs L_{α} and L_{β} peaks, indicating the
184 presence of Cs on and in the yeast cells. Other elements of O, Na, Mg, S, K originated from the
185 yeast cells.

3. 2 Accumulation of Cs by yeast cells from agar media

Figure 4 shows photographs of the yeast cells cultured in YPD-broth agar medium with and without ^{137}Cs , and AR images of the yeast cells. The photographs show yeast cells grown in agar medium with and without ^{137}Cs . No blackish area was observed in the AR image without ^{137}Cs , indicating that natural ^{40}K in the yeast was below the detection limit of the AR analysis in the present detection time. When ^{137}Cs was contained in the agar medium, the AR images showed that the blackish area coincided with the yeast cells in the photograph. This result indicated that the yeast accumulated ^{137}Cs in the cells from the agar medium.

AR images of thin sections with ca. 5 mm of agar media in the centrifuged tubes with the yeast cells inoculated at the top of the tube and without yeast cells are shown in Fig. 5, along with the depth profiles of the intensity of radioactivity determined by BAS. The depth profile of the intensity with the yeast cells showed that the intensity increased up to around 10 mm from the top, followed by constant intensity to 70 mm, and then decreased. On the other hand, the intensity was almost constant up to 70 mm in the depth profile without yeast cells. The intensity was determined as summation of the whole area of the same depth, indicating that a decrease of the intensity near the bottom with and without yeast cells resulted from the shape of the centrifuge tube. The decreasing trend toward the top of the profile with the yeast cells indicated the removal of ^{137}Cs from the agar medium by the yeast cells. The depletion of ^{137}Cs was revealed as far as 10 mm deep, suggesting that the yeast cells accumulated ^{137}Cs from the top of the agar medium within the first 3 d of exposure.

3.3 Effect of presence of minerals in medium

Without minerals in the agar medium, a black-colored region was clearly recognized in the AR image (Fig. 6, No mineral). With clinoptilolite of 0.1 weight% (Fig. 6, clinoptilolite 10 mg), the black color in the AR image of the yeast was paler than that without mineral content. The degree of the black color decreased as the amounts of clinoptilolite in the agar medium increased. A similar tendency for the black color in the AR image was obtained for the yeast grown in the agar medium containing vermiculite of South Africa (Fig. 6). These results indicated that the presence of minerals in the agar medium reduced the amount of ^{137}Cs that accumulated in the yeast cells.

Figure 7 shows the radioactivity in the yeast cells after culturing in the YPD broth agar medium containing $^{137}\text{CsCl}$ and 1.0 weight% of each mineral. Radioactivity levels in the yeast cells in the medium containing zeolite A, clinoptilolite, clinoptilolite (Niki), vermiculite (SA), vermiculite (CH), and phlogopite (IN) were lower than 150 Bq mg^{-1} (Inset of Fig. 7). Somasif and Japanese acid clay were reduced by approximately 500 Bq mg^{-1} , being one-third of the radioactivity in media without minerals. The radioactivity was approximately 1500 Bq mg^{-1} in the presence of the smectite Kunipia F and smectite Kunipia G4, and of illite, being nearly the same as that without minerals. Interestingly, the radioactivity of the yeast cells grown in the agar medium containing mica, lucentite, and synthetic saponite were larger than that without the minerals, indicating that the presence of these minerals enhanced the accumulation of ^{137}Cs in the yeast cells. Radioactivity in the yeast cells with different minerals was present in the order of mica, lucentite, synthetic saponite > Kunipia F, Kunipia G4, illite >> vermiculites, phlogopite, clinoptilolite, zeolites.

The relationship between the radioactivity of ^{137}Cs and the dry weight of the yeast cells grown in YPD broth agar medium containing 1, 5, or 10 g L⁻¹ minerals is shown in Fig. 8. Figure 8 shows that lower radioactivity in the yeast cells was obtained in media containing higher amounts of minerals. For instance, approximately 600 Bq mg⁻¹ ^{137}Cs was accumulated in yeast cells with clinoptilolite of 1 g L⁻¹, the accumulation of ^{137}Cs fell to ca. 150 Bq mg⁻¹ at 5 g L⁻¹ clinoptilolite, and to ca. 50 Bq L⁻¹ at 10 g L⁻¹.

These results indicate that accumulation of radioactive Cs depends not only on the kinds of minerals present, but also on the amount of minerals in the medium.

On the other hand, in the absence of the yeast cells, the distribution ratios ($K_{d\text{ min}}$) of ^{137}Cs in the various mineral supplementations with YPD broth liquid medium are plotted in Fig. 9. Distribution ratios of ^{137}Cs for zeolite A and clinoptilolite were approximately 2000 ml g⁻¹, being higher than those for the vermiculite, mica, illite, and smectite. The distribution ratios were on the order of zeolites > clinoptilolite > phlogopite > vermiculites > smectite, mica, illite. This order was inversely correlated to the radioactivity in yeast cells in the presence of these various minerals.

4. Discussion

The cell surfaces of fungal species of *S. cerevisiae* contain a range of organic acid functional groups that can adsorb metal cations from solution (e.g., Naeem et al., 2006; Wang and Chen, 2006). The pK_a values of the functional groups of the cell surface of *S. cerevisiae* obtained by Naeem et al. (2006) are nearly the same as those of bacteria compiled by Borrok et al. (2005). The metal cations of Cd, Pb, Sr, and Zn are adsorbed by *S. cerevisiae* (Naeem et al., 2006) in a

similar was as bacteria (Yee and Fein, 2001; Ginn and Fein, 2008), indicating that Cs ions are adsorbed by the functional groups of the cell surface of *S. cerevisiae* as is the case with bacteria (Nakao et al., 2005).

Analyses with SEM-EDS of the yeast cells exposed to Cs-containing glucose medium showed the accumulation of Cs on and/or inside cells (Fig. 3). Higher accumulation of Cs in the culture condition than in the resting condition (Fig. 1) indicated that the accumulation of Cs by the yeast cells in the culture condition resulted from adsorption on the functional groups of cells and by a process other than adsorption on the functional groups of the cell surface.

Cesium is accumulated inside cells through the K-channel (Avery, 1995). Glucose acts as an energy source for the biosynthesis of both cytoplasmic and membrane transport proteins that have been repressed during prior growth or starvation (Serrano, 1984). These facts and our results strongly suggest that adsorption on functional groups and intracellular accumulation are the uptake mechanisms of Cs by yeast in culture conditions.

Sugiyama et al., (2008) observed the precipitation of Cs with polyphosphate in vacuoles or other organelles of the mushroom *Pleurotus ostreatus*, when the medium contained 2.5×10^{-2} mol L^{-1} Cs. In the present study, the concentration of Cs was 1.0×10^{-2} mol L^{-1} , lower than that in Sugiyama et. al. (2008). These facts suggest that a higher Cs concentration is required for the precipitation of Cs in yeast cells. The concentration of radioactive Cs in the Fukushima area after the accident is below 10^{-9} mol kg^{-1} in soil, suggesting that radioactive Cs likely did not precipitate with the polyphosphates in fungi.

The ratio of Cs accumulated in yeast cells and the concentrations in glucose medium (K_d) was constant at different Cs concentrations (Fig. 2). In general, K^+ is the main ion which transports across cell membranes, and the concentration of K^+ inside cells is higher than that outside cells

(Phillips, et al., 2009). The concentration of K^+ is higher than that of Cs^+ and the transport process of Cs^+ across the cell membrane resembles that of K^+ , resulting in a constant ratio of Cs inside and outside the yeast cells, even though the concentration of Cs changed.

Yeast cells accumulate the radioactive Cs dissolved in the agar medium (Fig. 4). The AR image of cross section of the agar medium indicated that the yeast cells accumulate Cs^+ present around the cells. When minerals were present in the medium, the amount of radioactive Cs accumulated in the yeast cells was reduced (Fig. 6). These results indicate that minerals inhibit the transport of radioactive Cs from agar medium to yeast cells. The inhibition degree is not the same among the minerals; it is high for clinoptilolite, zeolite, vermiculite, and phlogopite, and low for the smectites Kunipia F and Kunipia G4, illite, mica, lucentite, and synthetic saponite. A high distribution ratio of minerals results in a high inhibition degree (Fig. 9), strongly suggesting that the yeast accumulates radioactive Cs competitively with minerals in the agar medium.

Minerals adsorb radioactive Cs. Recently, the adsorption and desorption of radioactive Cs by 9 kinds of minerals have been studied (Ohnuki and Kozai, 2013). The smectite Kunipia F, Kunipia G4, lucentite, and synthetic saponite have high CEC of approximately 100 (meq 100g⁻¹) (Ohnuki, 1994). The smectite group consists of 2:1 layer structured minerals with a lower charge per formula unit of 0.6–0.25 (Schulze, 1989). In montmorillonite, Mg substitution for Al in the octahedral sheet produces a layer charge (Schulze, 1989). Since cations are loosely adsorbed in the expandable interlayer of smectite, the adsorbed Cs is easily exchanged with other cations, such as Na^+ (Borchardt, 1989). The liquid media contained 0.1 mol L⁻¹ NaCl solution and organic materials for the accumulation experiments (Fig. 9). Smectite may adsorb Na^+ ions in the expandable interlayers, causing low $K_{d\ min}$ for Cs in the liquid medium.

The zeolite structure of mordenite possesses high CEC of 420 (meq 100g⁻¹) (Ming et al., 1989). Approximately 55% of the radioactive Cs adsorbed by mordenite was desorbed by a 1-mol L⁻¹ KCl solution (Ohnuki and Kozai, 2013). The Cs is adsorbed in the non-expandable tetrahedral framework of mordenite (Ming et al., 1989), being unexpandable with cations including Na⁺. This structural difference of zeolite from smectite results in the high K_{d min} of zeolite for radioactive Cs in the liquid medium and in the high inhibition degree of zeolite in the transport of radioactive Cs to the yeast cells in the agar medium.

Mica-like structural minerals tightly associate radioactive Cs (Tamura and Jacobs, 1989; Sawhney, 1970; Comans et al., 1991). Vermiculite, mica, phlogopite, Somasif, and illite are belong to mica-like structure minerals. Vermiculite, which is one of the weathered products of mica, has a layer charge per formula unit of 0.9 to 0.6 and contains exchangeable hydrated cations in interlayer (Schulze, 1989). High charge per formula unit of vermiculite causes high CEC and fixation of K⁺ and Cs⁺ in the interlayer (Schulze, 1989). These facts reflect on the high K_{d min} of vermiculite for radioactive Cs in the agar medium, and the high inhibition degree of vermiculite on the transport of radioactive Cs to the yeast cells.

Illite is defined as clay-sized mica, which often has poor crystallinity, lower K content, and higher H₂O content than mica (Schulze, 1989). It is well known that the "frayed edge" sites of mica minerals are energetically more stable for the sorption of Cs than the reversible sites of smectite and kaolinite structures (Goulding, 1986). The capacity of the "frayed edge" sites of illite is sufficient for the concentration of radioactive Cs to fix in the interlayer (Ohnuki and Kozai, 2013). On the other hand, a pure mica structure reversibly adsorbs Cs (Nakao et al., 2008; Fanning et al., 1989). When the concentration of Cs was 10⁻⁴ mol L⁻¹, approximately 80% of the Cs adsorbed by illite and mica was desorbed by a 1-M KCl solution at pH 7 (Ohnuki and Kozai,

2013). These results indicate that fixation of Cs at the "frayed edge" site is limited in illite and mica. In the accumulation experiments (Fig. 9), the liquid media contained 0.1-mol L⁻¹ NaCl solution and organic materials. Sodium cations may be adsorbed by the "frayed edge" as by expandable interlayers, causing the low $K_{d\ min}$ of illite and mica for Cs in the liquid medium. Thus, inhibition of mica and illite was very low in the transport of radioactive Cs from the agar medium to yeast cells. Somasif is an expandable synthetic mica produced by Co-op Chemical, Co. Ltd., Japan, with a higher "frayed edge site" than illite and mica. Radioactive Cs is probably fixed in the interlayer of Somasif, resulting in the inhibition of the transport to the yeast cells from the agar medium. Phlogopite comprises trioctahedral micas and is less resistant to weathering than dioctahedral mica (Schulze, 1989). This fact suggests that the weathered phlogopite contributes to the inhibition of the transport of radioactive Cs from agar medium to the yeast cells, as with Somasif.

Even though "frayed edge sites" are present in minerals, the yeast cells accumulate radioactive Cs from the agar medium. The presence of "frayed edge sites" in illite and mica does not affect the transport of radioactive Cs from agar medium to the yeast cells. The amount of "frayed edge sites" in minerals is a key factor in the accumulation of radioactive Cs by yeast fungi.

The importance of soil microorganisms in the migration of radioactive Cs within organic soil systems has been reported (Parekh et al., 2008). That paper indicated that a higher fraction of radioactive Cs was more tightly associated with organic soils without sterilization than that with sterilization. Similar results have been reported by Sanchez et al (2000). Our results suggest that even in inorganic soil systems fungi have an important role to retard the migration of radioactive Cs in the environments.

5. Conclusion

The accumulation of radioactive Cs in yeast cells was studied to elucidate the role of fungal microbes in the migration of radioactive Cs in the environment. The yeast cells accumulate higher amounts of Cs in culture condition than in resting condition. Thus, adsorption on the functional groups of the cell surface and intracellular accumulation are possible accumulation mechanisms in the culture condition. In the presence of minerals in the agar medium, the radioactivity in the yeast cells with different minerals was in the order of mica, lucentite, synthetic saponite > Kunipia F, Kunipia G4, illite >> vermiculite, phlogopite, clinoptilolite, zeolite. This order is inversely correlated to the adsorption ratio of the minerals for radioactive Cs in medium solution. These results strongly suggest that the yeast accumulates radioactive Cs competitively with minerals in the agar medium.

Even though the high CECs of smectite and zeolite and the presence of the “frayed edge sites” of vermiculite, phlogopite, Somasif, mica, and illite suggest the high performance of the inhibition of transport of radioactive Cs from agar medium to yeast cells, the inhibition degree of zeolite, vermiculite, phlogopite, and Somasif was higher than of smectite, illite, and mica. Structural differences of zeolite and smectites, and the amounts of “frayed edge sites” in minerals are key factors in the accumulation of radioactive Cs to yeast fungi.

These results strongly suggest that fungi have an important role to retard the migration of radioactive Cs in the environments, even in the soil where inorganic components are dominant.

Supplementary data

Supplementary data: In the agar medium experiments, a pre-grown culture of the yeast cells was inoculated on a 0.45- μ m pore membrane filter placed on agar medium in a plastic petri dish. The grown cells were recovered with a membrane filter to measure radioactivity by AR.

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Figure captions

Fig. 1 Time course of Cs concentrations accumulated in the yeast cells in the presence and absence of glucose as a carbon source in the liquid medium at $1.0 \times 10^{-3} \text{ mol L}^{-1}$

Fig. 2 Cs accumulated in the yeast after 1-day exposure to glucose medium containing 55.0×10^{-5} , 1.0×10^{-4} , 5.0×10^{-4} , and $1.0 \times 10^{-3} \text{ mol L}^{-1}$ Cs. K_{d_cell} is the ratio of Cs concentration in the cells and solution.

Fig. 3 SEM image of yeast cells grown in the medium containing $1.0 \times 10^{-2} \text{ mol L}^{-1}$ Cs. The dot in SEM photograph with EDS is the point at which the EDS spectrum of yeast is measured. The peak of Cs was distinguished in the spectrum.

Fig. 4 Photographs and autoradiography images of yeast grown in agar medium with and without ^{137}Cs .

Fig. 5 AR images of thin sections of agar medium after yeast was grown for 3 d with yeast cells (left) and the control without yeast cells, and the depth profiles of intensity of radiation detected by AR in thin sections with and without yeast cells.

Fig. 6 Photographs and AR images of yeast grown in agar medium containing ^{137}Cs and the minerals clinoptilolite and vermiculite (SA) at 10 (0.1 weight%), 50 (0.5 weight%), and 100 mg (1 weight%).

Fig. 7 Radioactivity in yeast cells after culturing in the YPD broth agar medium containing $^{137}\text{CsCl}$ and each mineral at 1 weight%. No mineral shows yeast cells grown in agar without minerals. Inset shows enlarged radioactivity for zeolite A, clinoptilolite, clinoptilolite (Niki), vermiculite (SA), vermiculite (CH), and phlogopite (IN).

Fig. 8 Radioactivity of ^{137}Cs and weight of yeast grown in YPD broth agar medium containing 1 g, 5 g, or 10 g L^{-1} mineral.

Fig. 9 Distribution ratios of ^{137}Cs for each mineral in YPD broth medium in the absence of yeast cells.

Vermiculite(SA)

Autoradiography analysis showed that presence of larger amounts of mineral of vermiculite collected in South Africa induced less accumulation of radioactive Cs in yeast cells from the medium.

Figure 1

Fig. 1

Fig. 2

Fig. 3

With ^{137}Cs

Without ^{137}Cs

Fig. 4

Fig. 5

No mineral

Clinoptilolite

Vermiculite(SA)

Fig. 6

Fig. 7

Fig. 8

Fig. 9