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Cs Accumulation Behavior by *Pseudomonas fluorescens*

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Cs accumulation by *Pseudomonas fluorescens* was examined under conditions of growth and resting to elucidate the interaction between Cs and bacteria. In the growth condition, *P. fluorescens* was cultured in citric acid media containing 50 μM CsCl and 0, 0.1, 1.0, and 10 mM K for 64 h at 30 ˚C. Adsorption and desorption behaviors of Cs were examined by the contact of the cells with 50 μM CsCl solution under the resting condition. Cs-accumulated *P. fluorescens* was exposed to a 1 M CH_3COONH_4 solution to examine the reversibility of Cs accumulation. In the growth condition, *P. fluorescens* did not accumulate Cs irrespective of the presence of K. In the resting condition, the cells quickly adsorbed approximately 5 μmol $Cs/g_{cell\ dry\text{-}weight}$ and subsequently released approximately 90% of the adsorbed Cs with 1 M CH_3COONH_4. The amount of Cs adsorption by cells of *P. fluorescens* varied with changing pH and ionic strength of the solution. These results indicate that Cs accumulation by *P. fluorescens* occurs mainly by reversible adsorption on the cell walls, but not by intracellular accumulation under nutrient conditions.

1. Introduction

Radiocesium (^{137}Cs) released by nuclear weapons tests and nuclear power plant accidents tends to remain in surface soils for a long period.[1, 2] Cesium-137 in surface soils may be transferred to plants grown in the contaminated soil.[3, 4] Thus, the migration behavior of ^{137}Cs within soils should be elucidated for predicting ^{137}Cs contamination in plants.

Bacteria may have an important role in regulating the transfer of ^{137}Cs from soil to plants, because they have the ability to accumulate metal cations on their cell surfaces and inside their cells.[5, 6] It has been reported that K concentrations in solution affect the accumulation of Cs by bacteria, and that K transport systems largely determine the Cs-uptake ability of bacteria.[7, 8] Some bacteria, such as *Rhodococcus erythropolis* and *Escherichia coli*, are known to have a specific K transport system (Kup) through which Cs is transported into cells. *R. erythropolis* has been shown to accumulate less Cs from solutions with higher K concentrations.[8] *Pseudomonas fluorescens*, ubiquitous in the terrestrial environment, accumulates negligible amounts of Cs compared to that by these bacteria.[7] However, the effect of K on Cs accumulation by *P. fluorescens* is still unknown.

Bacterial cell surfaces have functional groups, including carboxylic, phosphate, amine, and hydroxyl groups. These surface functional groups are deprotonated with increasing pH and thus provide the bacteria with a net negative surface charge.[10] Bacterial cell walls have a high affinity for various metal cations through coordination with these functional groups.[5] *P. fluorescens* is known to have these functional groups on its cell walls. However, little is known about the interaction between Cs and its cell walls.

The purpose of this study was to investigate the effect of K on Cs accumulation by *P. fluorescens*, and to elucidate the association of Cs with *P. fluorescens*. The association of Cs with growing cells was investigated in media with 0–10 mM K. Adsorption and desorption experiments were conducted using resting cells at pHs 3.0–9.0 and 1.0–100 mM NaCl at pH 6.0, and the time course of Cs adsorption and desorption was measured.

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2. Materials and Methods

Associations of Cs with growing cells. We used *P. fluorescens* (ATCC 55241) that was isolated from a low-level radionuclides waste disposal site at West Valley, New York.[11] *P. fluorescens* was pre-cultured at 30 ˚C for 24 h in 50 mL of culture media containing 1 mM KCl, 10 mM citric acid, 0.1 M NaCl, 6.7 mM $(NH_4)_2SO_4$, 10 mM Na_2HPO_4, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). The pH of the medium was adjusted to 6.0 before autoclaving at 120 ˚C for 20 min.

The pre-cultured cell suspensions were inoculated into 50 mL culture media containing 0, 0.1, 1.0 or 10 mM KCl, 50μM CsCl, 10 mM citric acid, 0.1 M NaCl, 6.7 mM $(NH_4)_2SO_4$, 10 mM Na_2HPO_4, and 10 mM HEPES; then, these cultures were allowed to stand at 30 ˚C. Five milliliters of the cell suspension was withdrawn at 18, 30, 42, and 64 h after inoculation. Cell growth was determined on the basis of the optical density of the culture medium measured at 600 nm (OD). The concentration of citric acid was measured by high-pressure liquid chromatography-mass spectroscopy (HPLC-MS) (Alliance 2695, Waters Corporation, Milford, MA, USA). The Cs concentration was measured by a model AA6200 atomic absorption spectrophotometer (AAS) (Shimadzu Co., Kyoto, Japan).

Cs adsorption on and desorption from resting cells. *Pseudomonas fluorescens* cells grown in a beef-extract solution (3 g L^{-1} beef extract, 5 g L^{-1} polypepton, and 5 g L^{-1} NaCl) were used for the adsorption and desorption experiment. Cells were harvested by centrifugation at 4000 rpm for 10 min. The cell pellet was washed three times with a 10 mM NaCl solution, and cells were resuspended in a 10 mM NaCl solution to obtain a 20 mg mL^{-1} cell suspension on a dry weight basis.

In adsorption experiments, 20 mg of the cell pellet was combined with 10 mL of a 50 μM CsCl solution containing 10 mM NaCl. In order to examine the time course of the Cs adsorption, cells were maintained with Cs at pH 7.0 for 0.5, 3,

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12, and 24 h. In order to examine the effect of pH and ionic strength on the Cs adsorption by *P. fluorescens*, cells were also maintained with Cs at pHs 3.0, 7.0, and 9.0 and in 1, 10, and 100 mM NaCl for 12 h. After the controlled contact, supernatants were separated from the cells by centrifugation at 4000 rpm for 10 min. The Cs concentration in the supernatant was measured by AAS.

The cells maintained with the Cs solution containing 10 mM NaCl at pH 7.0 for 12 h were used for desorption experiments after washing the cells once with distilled water at pH 6.8 and collecting them by centrifugation at 4000 rpm for 10 min. The collected cells were resuspended in a 1 M CH_3COONH_4 solution for 3, 12, 24, and 36 h. The suspensions were centrifuged at 4000 rpm for 10 min. The Cs concentrations in the supernatants were measured by AAS.

3. Results and Discussions

Growth of *P. fluorescens* and its association with Cs. Figures 1a and 1b show the time courses of the OD and the citric acid concentration, respectively, in the media containing different Cs concentrations after the inoculation of *P. fluorescens*. In the absence of K, the OD did not increase and the concentration of citric acid slightly decreased from 20 mM to 17 mM over 64 h. These results indicate that *P. fluorescens* cells did not grow in the absence of K.

In the medium containing 0.1 mM KCl, the OD increased gradually to 0.28 and the citric acid concentration decreased to 0.23 mM for 64 h. In the media containing 1.0 and 10 mM KCl, the OD increased to 0.58 and 0.60, respectively, for 42 h, when the citric acid concentration decreased to below the detection limit, and then gradually decreased through 64 h. These results indicate that the cell growth was influenced by K in the medium. The growth of *Rhodopseudomonas capsulata* cells is enhanced by 0.1 and 1.0 mM Cs even in the absence of K. *R. capsulata* has a specific K transporter (i.e. Kup), which shows low selectivity between K and Cs.[12] However, in this study, the presence of Cs without K did not result in the growth of *P. fluorescens* cells.

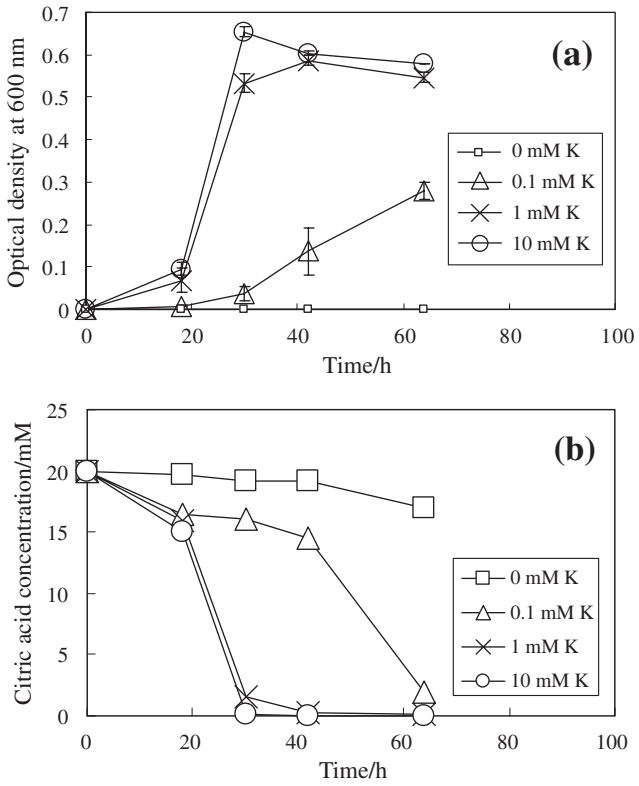

Figure 1. Time-course of the (a) OD and (b) citric acid concentrations (mM) in medium solutions containing Cs of 50 μM and K of 0, 0.1, 1.0, or 10 mM after the inoculation of *P. fluorescens*. The bars show the standard deviation for triplicate measurements of individual K concentrations.

Figure 2 shows the time-course of the accumulation of Cs by *P. fluorescens*, which was calculated from the reduction of Cs concentration in medium solutions. In the media with different K concentration, Cs accumulation was not shown and there was little effect of the difference of K on the Cs accumulation by *P. fluorescens* during the growth period. It has been reported that Cs is transported into bacterial cells in the same way as K to some degree, because of their similar chemical properties.[12, 13] Tomioka et al.[7] showed that Cs was not accumulated by *P. fluorescens* in the medium containing K as a constituent of an yeast extract medium. In the present study, we revealed that Cs was not accumulated by *P. fluorescens* in the culture media containing 0–10 mM K. This suggested that *P. fluorescens* selectively transported K into the cells against Cs and that Cs did not function in place of K for the growth of *P. fluorescens*.

Adsorption and desorption of Cs under resting conditions. The time course of the adsorption of Cs on *P. fluorescens* at resting conditions (Figure 3) showed that the amount adsorbed reached approximately 5.0 μmol $Cs/g_{cell\ dry\text{-}weight}$ at 30 min, and then remained constant up to 24 h. The pHs of the solutions were between 6.6 and 7.0 during the experiment. Note that the amount of cell pellet in contact with Cs solutions was approximately 10 times higher than the maximum amount obtained in the growth experiment. The rapid adsorption of metals on bacterial cells has been widely reported.[14, 15]

The amount of Cs accumulated increased from 1.8 to 4.2 μmol $Cs/g_{cell\ dry\text{-}weight}$ with a rise in the pH of the solution from 3.5 to 7.5 (Figure 4). The final pHs were 3.5, 6.7, and 7.5, changing variously from their starting values of 3.0, 7.0, and 9.0, respectively. The *P. fluorescens* cells have carboxyl and phosphate functional groups on their surfaces.[16] These func-

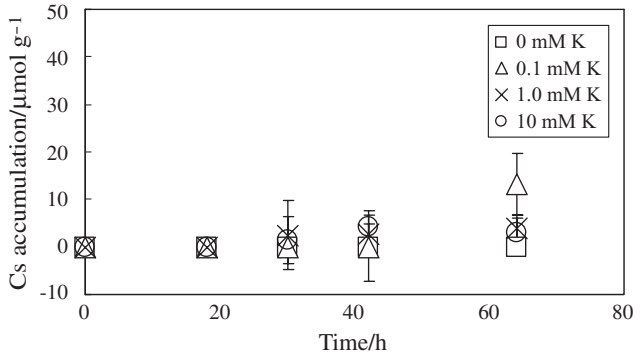

Figure 2. Time-course of the accumulation of Cs by *P. fluorescens* during the growth of the cells, which was calculated from the reduction of Cs concentration in the growth media. The sampling sets were in triplicates.

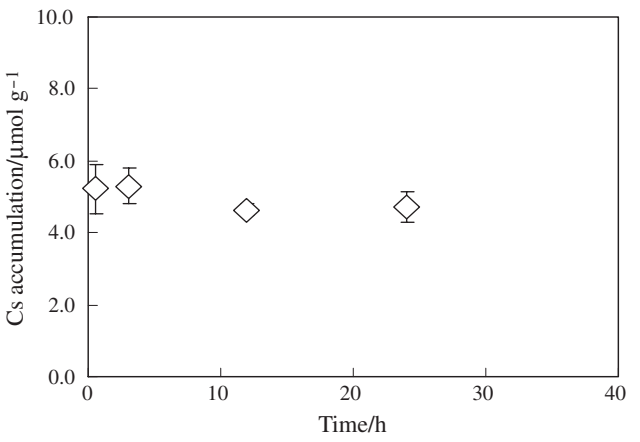

Figure 3. Time-course of Cs concentration adsorbed on the cells of *P. fluorescens* after exposure to a 50 μM CsCl solution of 10 mL.

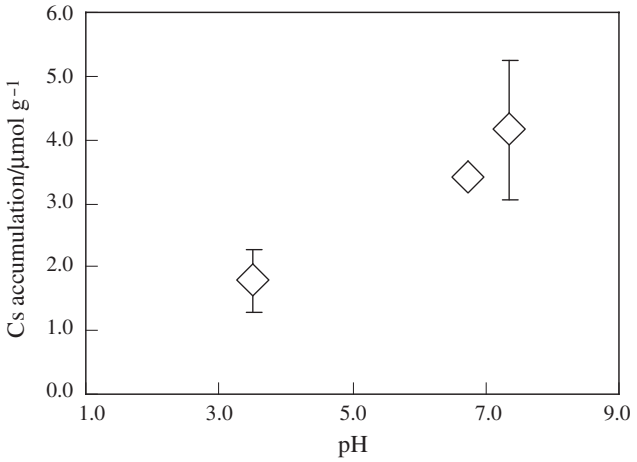

Figure 4. Effect of pH on the accumulation of Cs by *P. fluorescens* cells. The final pHs are shown in the figure.

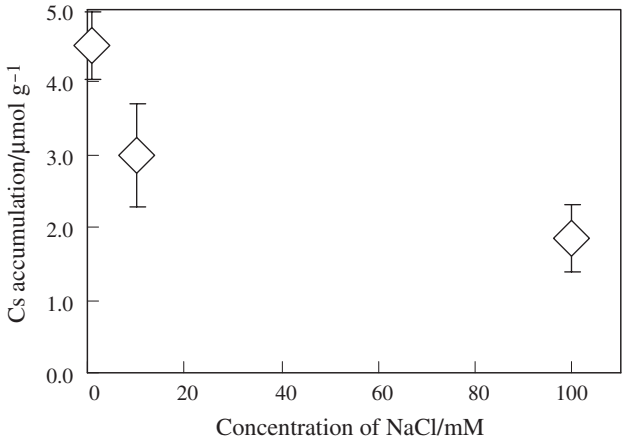

Figure 5. Effect of the NaCl concentration on the accumulation of Cs by *P. fluorescens* cells.

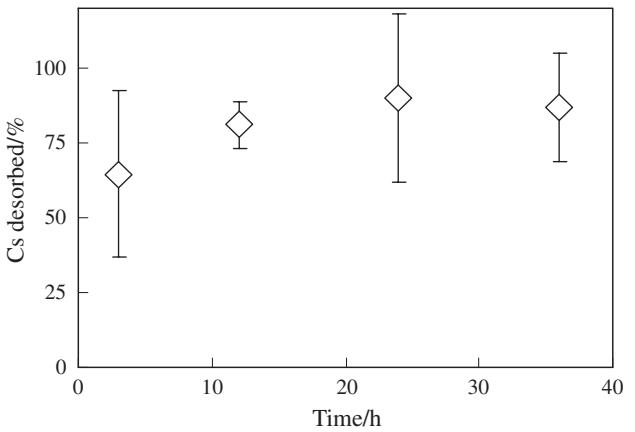

Figure 6. Time-course of the Cs concentration desorbed from cells of *P. fluorescens* by a 1 M CH_3COONH_4 solution. The Cs fraction was defined to be 100% when all the adsorbed Cs was desorbed.

tional groups deprotonate with increasing pH and consequently associate with cations.[10] The pH dependence in the Cs adsorption showed that the adsorption of Cs occurred through the exchange with H^+ on functional groups of the cell surfaces of *P. fluorescens*.

Figure 5 shows Cs accumulation by *P. fluorescens* cells at 1, 10, and 100 mM NaCl at pH 6.9 ± 0.1. The amount of the Cs accumulated was 4.5, 3.0, and 1.9 μmol $Cs/g_{cell\ dry\text{-}weight}$ at 1.0, 10, and 100 mM NaCl, respectively. The decrease in the amount of Cs accumulation with increasing Na concentration results from competition in the adsorption of Cs and Na by the functional groups of the cell surfaces. Intracellular Cs accumulation through Na transport channels is unlikely to occur because no Cs was accumulated under the growing condition (Figure 2).

The time course of the desorption of Cs from the Cs accumulated *P. fluorescens* at pH 7.0 for 12 h (Figure 6) showed that the adsorbed Cs was almost completely desorbed within 24 h by 1 M CH_3COONH_4. A 1 M CH_3COONH_4 solution is typically used as an extract solution to estimate the amount of the reversible cations in soils.[17] Thus, Cs is reversibly adsorbed on the functional groups of the cell surfaces of *P. fluorescens*. The Cs desorption rate was slower than the Cs adsorption rate. Ohnuki and Kozai[18] reported that the desorption and the adsorption rates were almost equivalent in the case of smectite. *P. fluorescens* has peptidoglycan layers on its cell walls, which consist of a cross-linked mesh structure.[19] The results suggest that adsorption of Cs on the cell surfaces of *P. fluorescens* differs from that on the reversible site in smectite.

Oughton et al.[20] reported that approximately 10% of radiocesium was extracted with 1 M CH_3COONH_4 from contaminated soils collected at 3 places in the 30 km exclusion zone surrounding the Chernobyl reactors. Even with a heated and consequently highly reactive acid solution (pH 2.0, 80 ˚C), more than 60% of the radiocesium could not be extracted.[20] The Cs fixation in soils can be explained as an irreversible adsorption of Cs on micaceous clays through the formation of inner-sphere complexes.[21, 22] On the other hand, it was reported that Cs fixation on clay minerals was reduced by adding humic substances because of a decrease in the average affinity of the cation adsorption sites of Cs.[23] It is well known that reversible cations in the adsorption phase are available to plants. Our results suggest that Cs adsorption on the bacterial cell surfaces of *P. fluorescens* plays a less important role in restricting the migration of Cs than micaceous clays but might enhance the plant availability of Cs, because Cs was adsorbed on the bacterial cells reversibly.

No investigation has been conducted on preferential adsorption of Cs between micaceous clays and *P. fluorescens*. *P. fluorescens* releases strong chelate reagents (i.e. siderophore),[24] which may enhance Cs desorption from micaceous clays by dissolving the inner-sphere structure complexes. Even though *P. fluorescens* accumulated only a small amount of Cs, further investigation should be performed to elucidate the effects of bacteria on the migration behavior of Cs in soils.

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