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Isolation and Characterization of Cesium-Accumulating Bacteria

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Cesium-accumulating bacteria, strains CS98 and CS402, were isolated from soil by a radioactive autoradiographic method using ^{137}Cs. These strains displayed the rod-coccus growth cycle and contained *meso*-diaminopimelic acid, mycolic acids, and tuberculostearic acids. The major menaquinone of CS98 was MK-8(H_2). On the basis of these characteristics, strain CS98 was identified as *Rhodococcus erythropolis* and strain CS402 was classified in the genus *Rhodococcus*. The maximum values of cesium removal efficiencies in the liquid culture containing 10 μmol of cesium per liter for strains CS98 and CS402 were 90 and 47%, respectively. The maximum cesium contents in strains CS98 and CS402 were 52.0 and 18.8 μmol/g (dry weight) of cells, respectively. Maximum values of cesium concentration factors for strains CS98 and CS402 were 3.5×10^4 and 3.6×10^3, respectively.

Radioactive cesium has been detected in the environment near a weapons test area (23) and in wastewater from an energy-producing plant (10). Released radioactive cesium is often incorporated into aquatic ecosystems. Studies of cesium accumulation by algae have been conducted due to the importance of algae in aquatic ecosystems. Williams (25) reported that the values of the concentration factors of ^{137}Cs for *Euglena intermedia* and *Chlorella pyrenoidosa* were 7.1×10^2 and 1.1×10^2, respectively. Harvey and Patrick (8) examined the ^{137}Cs concentration factors for nine species of freshwater algae and indicated that the average value of the concentration factor of ^{137}Cs was 4.6×10^2.

Recently, radioactive cesium has been released after the nuclear accident at Chernobyl (6). The release of ^{137}Cs at Chernobyl has been estimated to be 10^{17} Bq by Marshall (15). After the Chernobyl accident, the bioaccumulation of ^{137}Cs in various organisms, such as a cyanobacterium (1), fungi (9), mushrooms (13), and mosses (7), was studied as an indicator of radioactive contamination. However, only a few studies of cesium accumulation by bacteria have been conducted.

Studies of the bioaccumulation process could be useful for the removal and monitoring of radioactive compounds, such as uranium (19, 21), neptunium (20), and cesium (16, 25), in the wastewater of nuclear facilities and aquatic environments. An important factor in the design of a bioaccumulation process study is the isolation of the appropriate microorganisms.

The purpose of this study was to isolate the cesium-accumulating bacteria and to determine the characteristics of the isolates. Radionuclides were used for the isolation of zinc- (26) and phosphate-accumulating (18) microorganisms. We used an autoradiographic method for the isolation of cesium-accumulating bacteria.

MATERIALS AND METHODS

Isolation of cesium-accumulating bacteria. The composition of the medium for the isolation of the cesium-accumulating bacteria (BS medium) was as follows (in milligrams per liter): CH_3COONH_4, 2,000; $MgSO_4 \cdot 7H_2O$, 200; Na_2HPO_4, 100; $FeSO_4 \cdot 7H_2O$, 10; $CaCl_2 \cdot 2H_2O$, 10; $MnSO_4 \cdot 4$ or $5H_2O$, 0.6; $Co(NO_3)_2 \cdot 6H_2O$, 0.6; $ZnSO_4 \cdot 7H_2O$, 0.1; $CuSO_4 \cdot 5H_2O$, 0.06; $NiSO_4 \cdot 7H_2O$, 0.06; H_3BO_3, 0.05; H_2SeO_4, 0.04; $Na_2MoO_4 \cdot 2H_2O$, 0.01; and yeast extract, 50. The pH was 7.0. For the solid medium, 1.5% agar was added.

Screening for the cesium-accumulating microorganisms was conducted as follows. One hundred fourteen soil samples collected from various kinds of vegetable and crop fields and from urban and rural river areas were used as sources of cesium-accumulating bacteria. The soil suspensions were spread on a BS medium plate and incubated at 30°C for 2 days. Colonies grown on the BS medium plate were transferred to a BSRI medium plate (BS medium plate containing 250 kBq of ^{137}Cs per liter) by the replicate method and incubated at 30°C for 1 day. The specific activity of ^{137}Cs in the BSRI medium plate was 3.1×10^{12} Bq/mol of cesium. Colonies grown on the BSRI medium plate were transferred to sheets of paper (Type L; Fuji Xerox Co., Tokyo, Japan). These sheets were placed on X-ray films (HRH; Fuji Film Co., Tokyo, Japan) with a screen (GRENEX HR-12; Fuji Film) and then exposed for 20 h at −70°C in the dark. Then the films were developed. Colonies on the BS medium plate appearing as dark black spots on the developed X-ray film were picked and purified by repeated streaking on a BS medium plate. These strains were cultivated in CS medium (BS medium with 10 μmol of CsCl per liter) for 2 days at 30°C. The growth and the cesium accumulation ability were determined.

Identification of microorganisms. Cesium-accumulating bacteria were identified by using *Bergey's Manual of Systematic Bacteriology, volume 2* (14). The peptidoglycan diamino acid in the cell wall was analyzed by the method of Rhuland et al. (17). Mycolic acid was examined by the method of Toriyama et al. (24). Fatty acid analyses were conducted by the method of Katayama and Kuraishi (12). Menaquinones were analyzed by mass spectrometry by the method of Collins et al. (3–5).

Determination of cesium accumulation ability. The cesium accumulation ability was determined on the basis of the cesium content in the cells or the decrease in the cesium concentration in the medium. One loopful of agar slant cultures was inoculated into test tubes containing 10 ml of BS medium and incubated at 30°C with shaking for 2 days.

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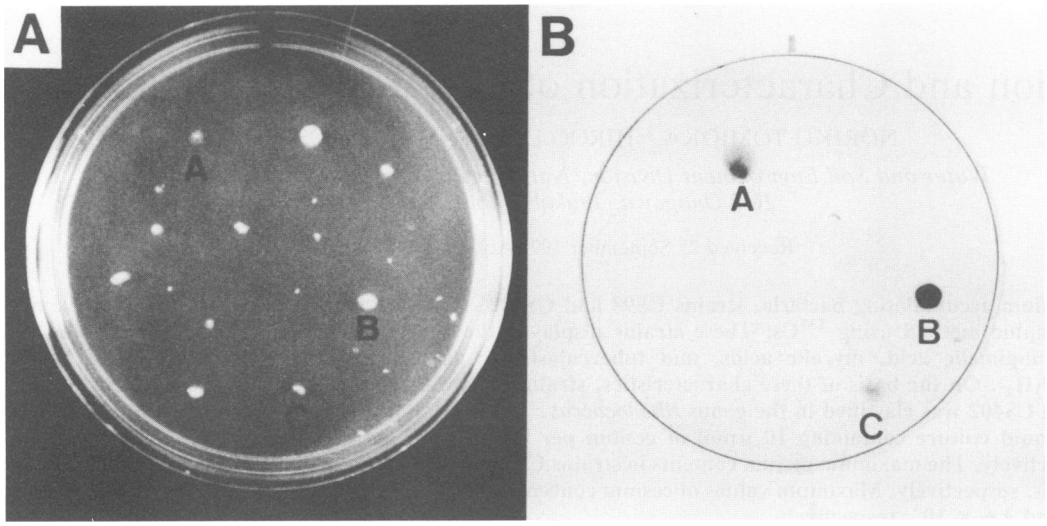

FIG. 1. Autoradiography for the screening of cesium-accumulating microorganisms. (A) Colonies grown on a BS medium plate. (B) Autoradiogram of colonies grown on a BSRI medium plate.

Then 1-ml portions of these cultures (optical density at 550 nm, 1.0) were inoculated into 200-ml Erlenmeyer flasks containing 100 ml of CS medium and incubated at 30°C with shaking. Cells and supernatants were separated by centrifugation. The cells were washed twice with an 0.85% NaCl solution. Washed cells were subjected to acid digestion (HNO_3 and H_2SO_4 at 180°C for 10 h) and neutralized with NaOH. The cesium concentration was measured with a Shimadzu AA640-12 atomic absorption spectrophotometer (Shimadzu Co., Kyoto, Japan). The cell growth was determined on the basis of the optical density at 550 nm.

Chemicals. $^{137}CsCl$ was purchased from Dupont, NEN Research Products (Boston, Mass.). The highest-grade inorganic or organic chemicals were used in this study.

RESULTS

Isolation of cesium-accumulating bacteria. Figure 1A shows an example of colonies grown on a BSRI medium plate replicated from the BS medium plate. Figure 1B shows the presence of black spots on the X-ray film corresponding to the colonies indicated in Figure 1A by autoradiography. The background level on the X-ray film after 1 day of exposure was very low. Colonies A, B, and C gave rise to black spots on the X-ray film. About 1/10 of the colonies grown on a BS medium plate over which soil suspensions were spread showed black spots to some extent on the X-ray film. Fifteen colonies which showed deep, dark spots were picked and purified. After measuring the accumulation ability of these strains, we picked two typical strains which displayed satisfactory growth and accumulation. We designated the two strains CS98 and CS402 and used them for the following studies.

Identification of strains CS98 and CS402. Table 1 shows the characteristics of strains CS98 and CS402. They were gram positive, nonmotile, and strictly aerobic. They displayed a rod-coccus growth cycle, which is typical of *Rhodococcus* spp. The strains contained *meso*-diaminopimelic acid, mycolic acid, and tuberculostearic acid. It was confirmed that CS98 and CS402 belonged to the genus *Rhodococcus* on the basis of these chemotaxonomic analyses.

Strain CS98 could decompose adenine, tyrosine, and urea. The pattern of carbon assimilation of strain CS98 resembled that of *Rhodococcus erythropolis* (except for maltose and benzoate) or *Rhodococcus bronchialis* (except for maltose and *p*-hydroxybenzoic acid). *R. erythropolis* and *R. bronchialis* contained different menaquinone types, MK-8(H_2) and MK-9(H_2), respectively. The molecular ion peak of the menaquinones of CS98 was calculated to be 718 by mass spectrometry, suggesting that the menaquinone type of CS98 was MK-8(H_2). On the basis of these results, it was concluded that strain CS98 belonged to *R. erythropolis*. On the contrary, strain CS402 could assimilate only a few carbon sources. The pattern of carbon assimilation of strain CS402 was similar to those of *Rhodococcus equi* and *Rhodococcus maris*. Strain CS402 differed from *R. equi* in the API-ZYM reactions (data not shown). Moreover, strain CS402 was different from *R. maris* in the oxidation-fermentation test and in glycerol assimilation. It was considered that CS402 was a new strain in the genus *Rhodococcus*.

Cesium accumulation ability. Figure 2 shows the time course of cell growth, pH, and cesium concentration in the medium and cells for strains CS98 and CS402 and for a control strain, *Pseudomonas fluorescens*. All of the strains grew well on the CS medium. The cesium concentration in the medium for strains CS98 and CS402 decreased with cell growth and showed the minimum values at 24 h for CS98 and at 48 h for CS402. Strain CS98 exhibited the maximum removal efficiency of 90% at 24 h of incubation, whereas strain CS402 exhibited the maximum removal efficiency of 47% at 48 h of incubation. In the case of strain CS98, the cesium content in the cells increased rapidly, decreased slowly, and then became stable. The cesium content in CS402 increased over 48 h, decreased, and then became stable. The maximum values of cesium content of CS98 and CS402 were 52.0 μmol/g (dry weight) of cells at 17 h and 18.8 μmol/g (dry weight) of cells at 48 h, respectively. On the other hand, the cesium concentration in the medium for *P. fluorescens* did not decrease throughout the incubation period. The pH increased from 7.0 to 9.1 with the progression of growth for all of the bacteria used.

The total amount of cesium in the cells and in the medium ranged from 95 to 106% of the added cesium throughout the

TABLE 1. Characteristics of strains CS98 and CS402

Characteristic	CS98	CS402
Gram stain	+	+
Cell morphology	R-C[a]	R-C
Colony morphology	Buff Dull	Pale pink Glistening
Conidia	−	−
Motility	−	−
Acid fastness	−	−
Strictly aerobic growth	+	+
Catalase	+	+
Oxidase	−	−
Metabolism of glucose	−	−
Peptidoglycan diamino acid	mDAP[b]	mDAP
Mycolic acids	+	+
Fatty acids (tuberculostearic acid)	+	+
Decomposition of:		
Adenine	+	+
Tyrosine	(+)[c]	−
Urea	+	+
Growth on sole carbon sources (%, wt/vol)		
Inositol (1.0)	+	−
Maltose (1.0)	−	−
Mannitol (1.0)	+	−
Rhamnose (1.0)	−	−
Sorbitol (1.0)	+	−
m-Hydroxybenzoic acid (0.1)	−	−
Sodium adipate (0.1)	+	−
Sodium benzoate (0.1)	+	−
Sodium citrate (0.1)	+	−
Sodium lactate (0.1)	+	+
Testosterone (0.1)	+	+
L-Tyrosine (0.1)	−	−
Ethanol (1.0)	+	−
Glycerol (1.0)	+	−
Sucrose (1.0)	+	−
Trehalose (1.0)	+	−
p-Hydroxybenzoic acid (0.1)	−	−
Sodium malate (0.1)	+	+
Sodium pyruvate (0.1)	+	+
Sodium succinate (0.1)	+	+
Lipid characteristics (major menaquinone)	MK-8(H_2)	ND[d]

[a] R-C, rod-coccus growth cycle.
[b] mDAP, *meso*-diaminopimelic acid.
[c] (+), weakly positive.
[d] ND, not determined.

cultivation. Therefore, it was concluded that the amount of cesium accumulated in the cells could be determined by measuring the decrease in the amount of cesium in the medium.

Cesium concentration factors. The values of the cesium concentration factors are indicated in Table 2. The concentration factor in this study is the ratio of the cesium concentration in cells (on a dry weight basis) to that in the medium. The maximum values of the cesium concentration factors for *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402 were 3.5×10^4 and 3.6×10^3 at 24 and 48 h of incubation, respectively, whereas the value of the cesium concentration factor for *P. fluorescens* was zero throughout the cultivation.

DISCUSSION

There are only a few reports on cesium accumulation by bacteria and yeasts. We found high-cesium-accumulating bacteria, strains CS98 and CS402, belonging to the genus *Rhodococcus*. About 1/10 of the colonies grown on a BS medium plate over which soil suspensions were spread showed black spots to some extent on X-ray film, and it was considered that many kinds of bacteria were able to accumulate cesium. Strains CS98 and CS402 belonged to the genus *Rhodococcus*, and the other 13 colonies which were picked as cesium-accumulating bacteria belonged to the genus *Rhodococcus* (data not shown). It appears that the genus *Rhodococcus* is characterized by the ability to accumulate cesium. Members of the genus *Rhodococcus* are widely distributed in nature and have frequently been isolated from soil, freshwater, and marine habitats (14). It is interesting to note that members of the genus *Rhodococcus* display a high level of cesium accumulation.

Strandberg et al. (22) estimated the values of the ^{137}Cs concentration factors to be at 16 and 37, respectively (Table 2), on the basis of the wet weight biomass of *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae*. The values of the concentration factors of CS98 and CS402 were much higher than those of *P. aeruginosa* and *S. cerevisiae*.

Cesium accumulation by algae was studied by several researchers. Harvey and Patrick (8) calculated the ^{137}Cs concentration factors in various algae on the basis of the dry weight of biomass. The highest values of the concentration factors in cyanobacteria, green algae, and diatoms were 9.5×10^2 (*Microcoleus vaginatus*), 1.5×10^3 (*Draparnaldia plumosa*), and 1.6×10^3 (*Navicula seminulum*) (Table 2). The maximum value of the concentration factor in the report of Harvey and Patrick was 1.6×10^3, for *N. seminulum*. The maximum value of the cesium concentration factor for *R. erythropolis* CS98 was 22 times higher than that for *N. seminulum*. Williams (25) reported that the values of the concentration factors of ^{137}Cs on the basis of the wet weight biomass of *E. intermedia* and *C. pyrenoidosa* were 7.1×10^2 and 1.1×10^2, respectively (Table 2). Since the water content of *E. intermedia* was found to account for about 88% of the constituents, the value of the cesium concentration factor for the dry weight biomass of *E. intermedia* was estimated to be 5.9×10^3. The maximum cesium concentration factor for *R. erythropolis* CS98 was about six times higher than that for *E. intermedia*.

Strandberg et al. (22) reported that the values of the cesium concentration factors decreased as the cesium concentration in the medium increased. They used a very low cesium concentration, 0.017 mg/liter, in the medium. In contrast, since we used a very high concentration, 1.33 mg/liter, strains CS98 and CS402 may show a much higher value of cesium concentration factors in the low-cesium-concentration medium.

Strains CS98 and CS402 have a high-level ability to accumulate cesium. However, the cesium accumulated by strains CS98 and CS402 was released after 24 and 48 h of incubation, respectively.

There are a few reports about the cesium accumulation and release mechanisms (1, 2, 11). Avery et al. (1) reported that the cesium accumulation was directly proportional to the extracellular cesium concentration over the range of 0.2 to 2.0 mM cesium, and the accumulated cesium was released after 24 h of incubation by using *Synechocystis* strain PCC 6803. They considered that this release might be a response to an increased internal osmotic pressure.

The cesium accumulation was also markedly influenced by external pH, with increasing cesium accumulation at greater (alkaline) pHs in *Synechocystis* strain PCC 6803 (1). In this study, the pHs were greater than 8 when the accumulated cesium was released from cells of strains CS98 and CS402.

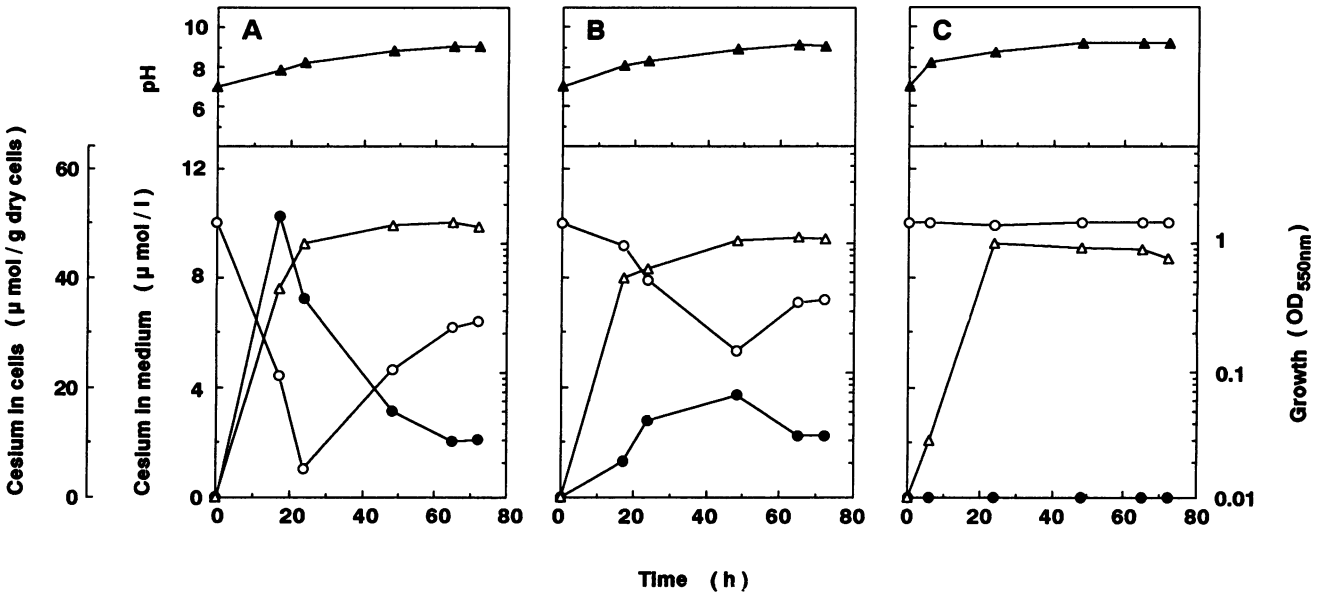

FIG. 2. Accumulation of cesium by strains CS98 (A) and CS402 (B) and by *P. fluorescens* (C). The cultivation was conducted in CS medium (containing 10 μmol of CsCl per liter). Symbols: ○, cesium concentration in medium; ●, cesium concentration in cells; △, optical density at 550 nm; ▲, pH.

Therefore, the pH may not be the reason for the cesium release.

It is said that the cesium accumulation is based on a potassium transport system (1, 2). Bossemeyer et al. (2) reported that *Escherichia coli* containing a potassium uptake system encoded by the *trkD* gene had a high cesium accumulation ability. *Rhodopseudomonas capsulata* required potassium (or rubidium or cesium as an analog of potassium) for growth. These cations were accumulated by the cells by a Michaelis-Menten saturation kinetics. The monovalent cation transport system had K_ms of 0.2 mM potassium, 0.5 mM rubidium, and 2.6 mM cesium (11). Therefore, cesium uptake is closely related with the monovalent cation concentration. Plato and Denovan (16) reported that the cesium accumulation by *C. pyrenoidosa* was inhibited above a potassium concentration of 2 mg/liter. Strains CS98 and CS402 showed cesium accumulation abilities in CS medium which contained 3.9 mg of potassium per liter. Because the concentration of potassium in fresh water is approximately 2 to 3 mg/liter, it was anticipated that strains CS98 and CS402 could be used for the removal and monitoring of radioactive cesium in natural environments.

TABLE 2. Cesium concentration factors of microorganisms

Microorganism	Concentration factor	Reference
R. erythropolis CS98	3.5×10^{4a}	This work
Rhodococcus sp. strain CS402	3.6×10^{3a}	This work
P. fluorescens	0.0^{a}	This work
P. aeruginosa	1.6×10^{b}	22
S. cerevisiae	3.7×10^{b}	22
M. vaginatus	9.5×10^{2c}	8
D. plumosa	1.5×10^{3c}	8
N. seminulum	1.6×10^{3c}	8
E. intermedia	7.1×10^{2b}	25
C. pyrenoidosa	1.1×10^{2b}	25

[a] $\frac{\text{μmol of Cs/g (dry weight) of cells}}{\text{μmol of Cs/g of water}}$, maximum value.
[b] $\frac{\text{cpm of } ^{137}\text{Cs/g (wet weight) of cells}}{\text{cpm of } ^{137}\text{Cs/g of water}}$.
[c] $\frac{\text{cpm of } ^{137}\text{Cs/g (dry weight) of cells}}{\text{cpm of } ^{137}\text{Cs/g of water}}$.

It is generally said that microbial uptake of metallic elements is required for metabolism or adsorption. Strandberg et al. stated that *S. cerevisiae* and *P. aeruginosa* did not require uranium uptake for metabolism (21). Isolates CS98 and CS402 accumulated significant levels of cesium in the logarithmic growth phase and released it in late stationary phase. It was reported that cesium accumulation was energy dependent in *Synechocystis* strain PCC 6803. Incubation in the dark showed an inhibitory effect on cesium accumulation (1). Because monovalent cation uptake is usually energy dependent, cesium accumulation by strains CS98 and CS402 may be energy dependent. Therefore, the mechanism of cesium removal by strain CS98 and CS402 is not simple adsorption.

Further research is necessary to clarify the cesium accumulation mechanism. It may be possible that the removal of radioactive compounds from the wastewater of nuclear facilities can become practical by further study of the bioaccumulation process.

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