

## Cesium Accumulation and Growth Characteristics of *Rhodococcus erythropolis* CS98 and *Rhodococcus* sp. Strain CS402

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**Growth and cesium accumulation characteristics of two cesium-accumulating bacteria isolated from soils were investigated. *Rhodococcus erythropolis* CS98 and *Rhodococcus* sp. strain CS402 accumulated high levels of cesium (approximately 690 and 380  $\mu\text{mol/g}$  [dry weight] of cells or 92 and 52 mg/g [dry weight] of cells, respectively) after 24 h of incubation in the presence of 0.5 mM cesium. The optimum pH for cesium uptake by both *Rhodococcus* strains was 8.5. Rubidium and cesium assumed part of the role of potassium in the growth of both *Rhodococcus* strains. Potassium and rubidium inhibited cesium accumulation by these *Rhodococcus* strains. It is likely that both *Rhodococcus* strains accumulated cesium through a potassium transport system.**

Bioaccumulation by microbial cells offers a potential alternative to existing methods for decontamination or recovery of radioactive compounds from both the wastewater of nuclear facilities and aquatic environments (7, 9, 15, 17, 18). A large amount of radioactive cesium was released after the nuclear reactor accident at Chernobyl (6). Haselwandter and Berreck reported that after the Chernobyl accident the mean  $^{137}\text{Cs}$  content of basidiocarps was 3.0 to 4.8 times higher than the  $^{137}\text{Cs}$  content before the accident (8). Therefore, much attention has been focused on the fate and removal of radioactive cesium.

We isolated two cesium-accumulating bacteria from soil and identified them as *Rhodococcus erythropolis* CS98 and *Rhodococcus* sp. strain CS402 (19). Important factors in the design of a bioaccumulation process include determining the optimal growth conditions and accumulation characteristics of appropriate microorganisms. However, the optimum conditions for growth and the characteristics of the cesium uptake system of *Rhodococcus* species have not been studied previously.

There have been several reports concerning the effects of cesium on the growth of microorganisms (2, 10, 12, 16). Jasper observed that cesium and rubidium at concentrations ranging from 0.1 to 1 mM stimulated the growth of *Rhodospirillum rubrum* (10). On the other hand, Avery et al. (1) reported that the doubling time of *Synechocystis* sp. strain PCC 6803 increased by 64% and the final cell yield decreased by 70% during growth in the presence of 1 mM CsCl compared with growth in the absence of cesium. These authors also reported that the presence of 1 mM CsCl resulted in a 34% increase in doubling time and an 83% reduction in the final cell yield of *Chlorella emersonii* under photoautotrophic growth conditions compared with controls grown without cesium. Cesium did not affect the growth of either alkaliphilic *Bacillus* sp. strain ASSC-2 at a concentration of 50 mM (12) or *Rhodospirillum rubrum* at a concentration of 0.4 mM (16).

Studies on cesium accumulation by microorganisms were first performed in the 1950s. Williams (20) described the

uptake of  $^{137}\text{Cs}$  by *Euglena* and *Chlorella* species. Avery et al. observed that cesium accumulation by *Synechocystis* sp. strain PCC 6803 (1) was markedly dependent on the external pH and was inhibited by the presence of potassium and rubidium. Plato and Denovan (13) reported that the accumulation multiples for  $^{137}\text{Cs}$  with *Chlorella pyrenoidosa* decreased when the concentration of potassium ions increased. There have been several reports concerning the transport of other cations by potassium transport systems (3-5, 11). In particular, Bossemeyer et al. (5) reported that *Escherichia coli* containing a potassium transport system encoded by the *trkD* gene exhibited an ability to accumulate cesium. However, there have been few studies of cesium accumulation by microorganisms.

In this study, we analyzed the effects of cesium and other monovalent cations, as well as pH and temperature, on cell growth and cesium accumulation by two *Rhodococcus* species.

### MATERIALS AND METHODS

**Organisms and growth media.** All of the experiments were performed with *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402, both of which were isolated as cesium-accumulating bacteria (19). These strains were cultured on BSK0, BSK10, and BSK100 media. BSK0 medium contained (per liter) 2,000 mg of  $\text{CH}_3\text{COONH}_4$ , 200 mg of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 100 mg of  $\text{Na}_2\text{HPO}_4$ , 10 mg of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 10 mg of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.6 mg of  $\text{MnSO}_4 \cdot 4$  or  $5\text{H}_2\text{O}$ , 0.6 mg of  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , 0.1 mg of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.06 mg of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.06 mg of  $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.05 mg of  $\text{H}_3\text{BO}_3$ , 0.04 mg of  $\text{H}_2\text{SeO}_4$ , 0.01 mg of  $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ , and 0.2 mg of thiamine. BSK10 and BSK100 media were prepared by adding 10 and 100  $\mu\text{M}$  KCl, respectively, to BSK0 medium. BSK0 medium contained 2 to 4  $\mu\text{M}$  potassium derived from impurities in the other salts.

**Growth experiment.** Cells equivalent to 2 to 3 mg (dry weight) were inoculated into 100 ml of BSK0 medium containing potassium, rubidium, and cesium (added as chlorides) and incubated at 30°C with shaking to determine the effects of cation concentrations on growth. Cells were inoculated into BSK100 medium prepared with various buffers to examine the effects of pH on growth. The following buffers were used: 25 mM  $\text{Na}^+$ -phosphate, pH 6.6 to 7.7; 25 mM  $\text{Na}^+$ -TAPS [*N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid],

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pH 7.8 to 8.2; and 25 mM Na<sup>+</sup>-Bicine, pH 8.2 to 8.6. *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402 cells were inoculated into BSK10 and BSK0 media, respectively, to examine the effects of temperature. Cell growth was measured by measuring increases in optical density at 550 nm.

**Cesium accumulation experiment.** The effects of growth medium cation concentrations on cesium accumulation were investigated by inoculating the equivalent of 2 to 3 mg (dry weight) of cells into 100 ml of either BSK10 (for *R. erythropolis* CS98) or BSK0 (for *Rhodococcus* sp. strain CS402). The media contained various concentrations of cesium, potassium, and rubidium and were incubated for 24 h at 30°C with shaking. After incubation, the cells were separated from the culture medium by centrifugation and were analyzed for their cesium contents.

Cells were cultured in 100 ml of BSK10 or BSK0 medium at 30°C with shaking to study the effects of pH and temperature on cesium accumulation. Cells were collected during exponential growth by centrifugation at 6,000 × *g* for 10 min and were suspended in fresh BSK0 medium prepared with various buffers containing 0.01 mM CsCl to a cell density of 300 to 500 mg (dry weight) of cells per liter. The cells were incubated with shaking under various pH and temperature conditions. The cells were separated from the culture medium by centrifugation after 2.5 h. The following buffers were used for the pH experiment: 25 mM Na<sup>+</sup>-phosphate, pH 6.5 to 8.0; 25 mM Na<sup>+</sup>-TAPS, pH 8.0 to 8.5; and 25 mM Na<sup>+</sup>-Bicine, pH 8.5 to 9.5. The experiments to test the effects of temperature were also performed with Na<sup>+</sup>-TAPS buffer (pH 8.5).

Following incubation the cells were separated from the culture medium by centrifugation at 16,000 × *g* for 4 min, washed twice with a 0.85% NaCl solution, and subjected to acid digestion with HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> at 180°C for 1 h. The cesium concentration was measured with a model AA640-12 atomic absorption spectrophotometer (Shimadzu Co., Kyoto, Japan).

All experiments were performed in duplicate, but only the mean values were plotted. Duplicate data usually differed from the means by less than 5%.

## RESULTS

**Effects of monovalent cations on growth.** Potassium, rubidium, and cesium concentrations affected both the cell yields (Fig. 1A and B) and the specific growth rates (Fig. 1C and D) in BSK0 medium.

*R. erythropolis* CS98 did not grow at all on BSK0 medium to which no cations were added (Fig. 1C). The cell yields and specific growth rates of this strain were significantly higher when the potassium, rubidium, or cesium concentration was 0.02 mM. Almost maximal growth was observed after addition of between 0.1 and 10 mM potassium. The maximum cell yield was 1,180 mg (dry weight) of cells per liter at a potassium concentration of 0.15 mM. The cell yields were essentially constant when the organism was grown in the presence of rubidium and cesium at concentrations between 0.02 and 1.0 mM. The specific growth rate of *R. erythropolis* CS98 increased as the rubidium concentration was increased from 0.02 to 1.0 mM, but was essentially constant at cesium concentrations between 0.02 and 1.0 mM. The cell yield and the specific growth rate were lower in the presence of 10 mM rubidium and 10 mM cesium (Fig. 1A and C).

*Rhodococcus* sp. strain CS402 grew in BSK0 medium containing no cation; the cell yield and specific growth rate were 210 mg (dry weight) of cells per liter and 0.085 h<sup>-1</sup>, respectively (Fig. 1B and D). The cell yields increased to 870, 640,

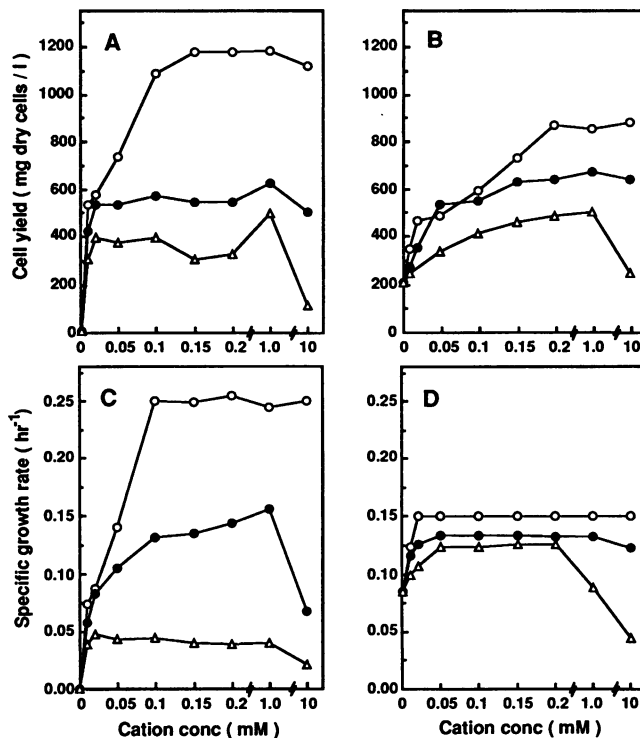


FIG. 1. Effects of cesium, rubidium, and potassium concentrations on the cell yields (A and B) and specific growth rates (C and D) of *R. erythropolis* CS98 (A and C) and *Rhodococcus* sp. strain CS402 (B and D). Symbols: ○, KCl; ●, RbCl; △, CsCl.

and 490 mg (dry weight) of cells per liter when the cation concentrations were 0.2 mM potassium, 0.2 mM rubidium, and 0.2 mM cesium, respectively. However, the cell yield was significantly lower in the presence of 10 mM cesium. The specific growth rate was higher when the potassium concentration was 0.02 mM and also when the rubidium and cesium concentrations were 0.05 mM. At potassium and rubidium concentrations between 0.05 and 10 mM, the specific growth rate was constant, and it was significantly lower at cesium concentrations equal to or greater than 1.0 mM (Fig. 1B and D). Thus, it seems likely that rubidium and cesium could replace part of the potassium required for the growth of *Rhodococcus* species. However, high concentrations of cesium inhibited the growth of both strains.

**Effect of pH and temperature on growth.** The specific growth rate of *R. erythropolis* CS98 was highest at pHs between 6.6 and 8.2 and significantly lower at a pH value of 8.3 or more (Fig. 2). *Rhodococcus* sp. strain CS402, on the other hand, grew at specific growth rates near the maximum rate at pH values ranging from 7.0 to 8.5.

The optimum temperature for growth for both *Rhodococcus* strains was 25°C (Fig. 3). The growth rates at 20 and 30°C were more than 80% of the growth rate observed at 25°C. At temperatures above 35°C, the specific growth rate of *R. erythropolis* CS98 was significantly lower.

**Cesium accumulation ability.** Cesium accumulation in cells was examined after 24 h of incubation in the presence of various cesium concentrations (Fig. 4). The cellular cesium concentrations of *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402 were saturated when the cesium concentrations in the medium were 0.1 and 0.05 mM, respectively, and further

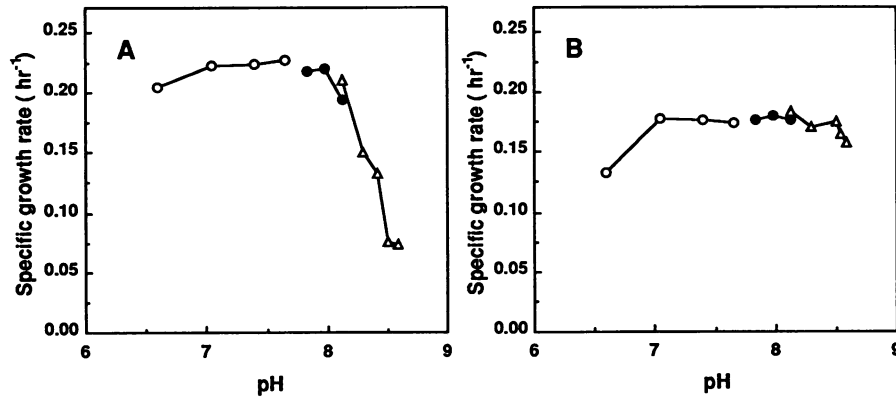


FIG. 2. Effect of pH on the growth of *R. erythropolis* CS98 (A) and *Rhodococcus* sp. strain CS402 (B). Symbols: ○, Na<sup>+</sup>-phosphate; ●, Na<sup>+</sup>-TAPS; △, Na<sup>+</sup>-Bicine.

cesium additions did not result in additional uptake. The maximum cesium content of *R. erythropolis* CS98 was 690 μmol/g (dry weight) of cells (92 mg/g [dry weight] of cells) when the cesium concentration in the medium was 0.5 mM. The maximum cesium concentration in *Rhodococcus* sp. strain CS402 cells was 400 μmol/g (dry weight) of cells (52 mg/g [dry weight] of cells) when the cesium concentration in the medium was 0.1 mM. Thus, *R. erythropolis* CS98 was able to accumulate 1.7 times more cesium than *Rhodococcus* sp. strain CS402.

**Properties of cesium uptake in *Rhodococcus* species.** Cesium accumulation in cells was examined after 24 h of incubation in the presence of various combinations of cesium, potassium, and rubidium (Table 1). Cesium accumulation by *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402 cells decreased with increasing potassium and rubidium concentrations. Cesium accumulation by *Rhodococcus* sp. strain CS402 was more inhibited by potassium and rubidium than cesium accumulation by *R. erythropolis* CS98 was. For *Rhodococcus* sp. strain CS402, potassium inhibition of cesium accumulation was greater than rubidium inhibition. In *R. erythropolis* CS98, the levels of cesium accumulation in the presence of 1 mM potassium were 0.3 and 43% of the level of cesium accumulation in the absence of potassium when the cesium concentrations were 0.01 and 1 mM, respectively. In other words,

potassium inhibition of cesium accumulation at the high cesium concentration was less than potassium inhibition of cesium accumulation at the low cesium concentration.

The effects of pH on the cesium accumulation rate of *Rhodococcus* strains were examined after 2.5 h of incubation (Fig. 5). Cesium accumulation was markedly influenced by pH. The rate of cesium accumulation was maximal at pH 8.5 in both strains. At pH 8.5 the rates of cesium uptake by *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402 were 5.2 and 6.2 μmol/h/g [dry weight] of cells, respectively. The levels of cesium uptake activity exhibited by both *Rhodococcus* strains were dramatically lower when the pH was outside a narrow range of values. In particular, the levels of cesium accumulation by *R. erythropolis* CS98 at pH 7.5 and 9.0 were only 15 and 32%, respectively, of the level of cesium accumulation at pH 8.5.

The effect of temperature on cesium accumulation by *Rhodococcus* strains was examined after 2.5 h of incubation (Fig. 6). The optimum temperatures for cesium uptake were 20 and 25°C for *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402, respectively. More than 64% of the cesium uptake activity observed at the optimum temperature was observed at temperatures of 10 and 35°C.

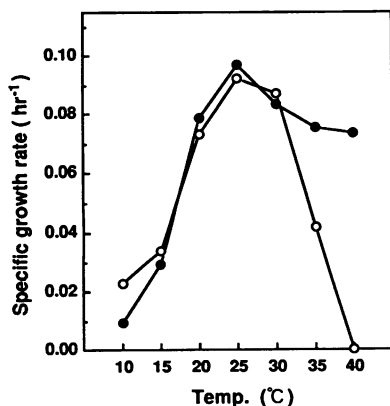


FIG. 3. Effect of temperature on the growth of *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402. Symbols: ○, *R. erythropolis* CS98; ●, *Rhodococcus* sp. strain CS402.

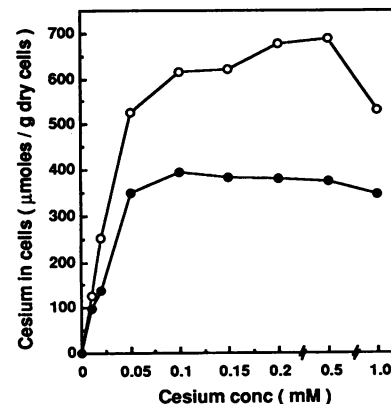


FIG. 4. Effect of cesium concentration in the medium on cesium accumulation by *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402. Symbols: ○, *R. erythropolis* CS98; ●, *Rhodococcus* sp. strain CS402.

TABLE 1. Effects of cation concentrations on cesium accumulation by *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402

Strain	Ion added to medium	Concn (mM)	Cs <sup>+</sup> concn in cells (μmol/g [dry wt] of cells) in the presence of:		
			0.01 mM Cs <sup>+</sup> <sup>a</sup>	0.1 mM Cs <sup>+</sup>	1 mM Cs <sup>+</sup>
<i>R. erythropolis</i> CS98	None		127	615	533
	K <sup>+</sup>	0.1	31.5	68	350
	K <sup>+</sup>	1	0.4	12	229
	Rb <sup>+</sup>	0.1	38.7	ND <sup>b</sup>	ND
<i>Rhodococcus</i> sp. strain CS402	None		98.3	395	348
	K <sup>+</sup>	0.1	0	0	0
	K <sup>+</sup>	1	0	0	0
	Rb <sup>+</sup>	0.1	10.5	ND	ND
	Rb <sup>+</sup>	1	0	ND	ND

<sup>a</sup> Concentration of Cs<sup>+</sup> added to the medium.

<sup>b</sup> ND, not determined.

## DISCUSSION

Various effects of rubidium and cesium on bacterial growth have been reported previously. Jasper noted that rubidium and, less efficiently, cesium could replace potassium during the growth of *Rhodopseudomonas capsulata* (10). Rhoads et al. (14) reported that the potassium requirement of *E. coli* was only partially fulfilled by rubidium. In contrast, cesium did not stimulate the growth of the alkalophilic *Bacillus* sp. strain ASSC-2 (12) and *Rhodopseudomonas sphaeroides* (16). Moreover, Avery et al. (1) reported that the growth rate, the final cell yield, and the cellular potassium content of *Synechocystis* sp. strain PCC 6803 cultured in the presence of 0.46 mM potassium were decreased by the addition of a large amount of cesium (1 mM) and suggested that cesium toxicity in *Synechocystis* sp. strain PCC 6803 arose through replacement of cellular potassium by cesium and the inability of cesium to substitute for potassium in metabolic processes. In this study, the growth of two *Rhodococcus* strains was stimulated by the addition of low concentrations of rubidium and cesium when no potassium was added to the medium (however, the medium did contain 2 to 4 μM potassium derived from impurities in salts in the medium). However, the cell yields, growth rates, and cellular potassium contents (data not shown) of the two *Rhodococcus* strains were lower when high cesium concentra-

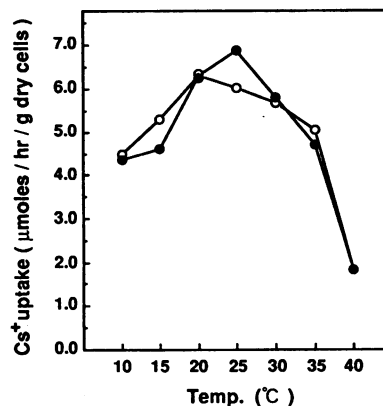


FIG. 6. Effect of temperature on cesium accumulation by *R. erythropolis* CS98 and *Rhodococcus* sp. strain CS402. Symbols: ○, *R. erythropolis* CS98; ●, *Rhodococcus* sp. strain CS402.

tions were present in the medium. These results indicated that rubidium and cesium replaced potassium to some extent for growth of both strains, but could not replace this element completely in metabolic processes.

Avery et al. reported that the level of cesium accumulation by *Synechocystis* sp. strain PCC 6803 was directly proportional to the extracellular cesium concentration over the range from 0.2 to 2.0 mM (1). In both strains used in this study, the level of cesium accumulation was also directly proportional to the extracellular cesium concentration up to 0.05 mM. However, at cesium concentrations greater than 0.05 mM, the cellular cesium content of *Rhodococcus* strains was not proportional to the extracellular concentration. To clarify the relationship between cellular cesium content and extracellular cesium concentration, further studies will be needed.

Plato and Denovan (13) reported that potassium ion concentrations greater than 15 mg/liter (0.4 mM) reduced the accumulation multiples for <sup>137</sup>Cs in *Chlorella pyrenoidosa*. Avery et al. also reported that reductions in the levels of cesium accumulation by *Synechocystis* sp. strain PCC 6803 (1) and *C. emersonii* (2) occurred when the potassium concentration in the medium was increased and suggested that in these organisms potassium transport systems that are not very specific play a role in cesium accumulation. Furthermore, Bossemeyer et al. (5) directly showed that *E. coli* containing the Kup system, the potassium transport system encoded by the *trkD*

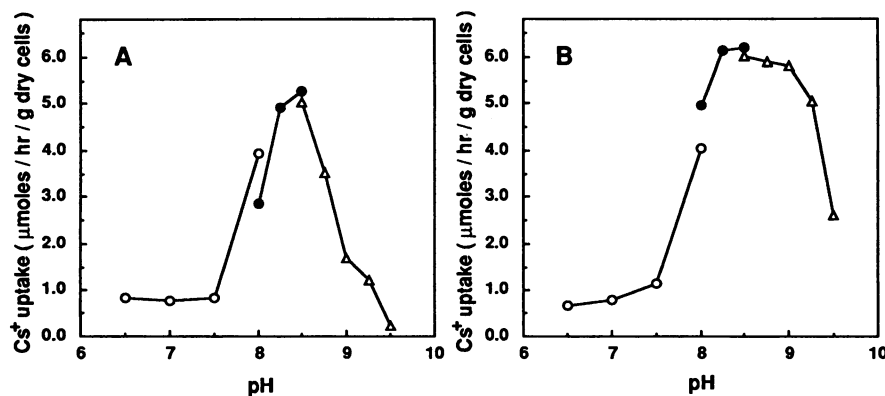


FIG. 5. Effect of pH on cesium accumulation by *R. erythropolis* CS98 (A) and *Rhodococcus* sp. strain CS402 (B). Symbols: ○, Na<sup>+</sup>-phosphate; ●, Na<sup>+</sup>-TAPS; △, Na<sup>+</sup>-Bicine.

gene, was able to accumulate cesium. We found that potassium and rubidium inhibited cesium accumulation in *Rhodococcus* species. Therefore, it is likely that the two *Rhodococcus* strains which we studied accumulated cesium via a potassium transport system that is not very specific, such as the Kup system.

Avery et al. reported that cesium accumulation in *Synechocystis* sp. strain PCC 6803 was greater when the pH of the medium was increased to 10 (1). Both *Rhodococcus* strains which we studied also exhibited maximal levels of cesium accumulation under alkaline conditions (pH 8.5). However, a neutral pH was the optimum pH for growth of *R. erythropolis* CS98, and the pH range for growth of *Rhodococcus* sp. strain CS402 was broad. Therefore, the optimum pH values for cesium accumulation for both *Rhodococcus* strains were not same as the optimum pH values for growth. Further studies will be needed to clarify this phenomenon.

The optimum temperature for cesium uptake was between 20 and 25°C for both *Rhodococcus* strains, and even at 10 and 35°C cesium uptake activity remained high. This characteristic is important for utilization of the  $^{137}\text{Cs}$  accumulation abilities of these species in the environment.

Growth and cesium accumulation characteristics of two cesium-accumulating bacteria were investigated in this study. Rubidium and cesium assumed part of the role of potassium in the growth of both *Rhodococcus* strains. Potassium and rubidium inhibited cesium accumulation by these *Rhodococcus* strains. It is likely that both *Rhodococcus* strains accumulated cesium through a potassium transport system. Our results are the first results describing the characteristics of cesium accumulation in *Rhodococcus* species.

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