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EXPERIMENTAL ARTICLES

Efficient Uptake of Cesium Ions by *Rhodococcus* Cells

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Abstract—Bacteria of the genus *Rhodococcus* were found to be able to accumulate cesium by means of active transport and nonspecific sorption on the cell surface structures. The maximum removal (up to 97%) of cesium from a medium supplemented with ammonium acetate was observed at 28°C, pH 7.8–8.6, and an equimolar content (0.2 mM) of potassium and cesium ions in the medium. The most active cesium-accumulating rhodococcal strains may be useful in biological treatment of industrial wastewaters contaminated with radionuclides.

Key words: Rhodococcus, bioaccumulation, cesium.

Nuclear power plants are the main source of contamination of the environment with radioactive cesium isotopes, some of which are hazardous to animals because of their long half-life (up to 30 years in the case of cesium-137), the high solubility of cesium salts in water, and the similarity to cesium to potassium, a metabolically important chemical element. This poses the problem of developing technologies, including those which employ microorganisms, for the efficient removal of cesium from contaminated areas [1, 2].

The data available in the literature indicate that cesium is accumulated by algae [3, 4], fungi [5, 6], yeasts [7, 8], bacteria [9], cyanobacteria [10, 11], and rhodococci [12, 13]. The mechanism of cesium bioaccumulation is best studied for eukaryotes. Most of the relevant studies on microorganisms are focused on the investigation of their cesium-sorptive ability. Among microorganisms, bacteria of the genus *Rhodococcus* sensu stricto are of great interest because of their diversity, high adaptability, and domination in extreme habitats [14]. Some authors consider rhodococci as the most biotechnologically important microorganisms [15].

The aim of the present work was to evaluate the ability of various *Rhodococcus* species to accumulate cesium, to investigate the effect of growth conditions on this process, and to study the mechanism of cesium bioaccumulation.

MATERIALS AND METHODS

The 43 strains of 7 *Rhodococcus* species (1 *R. coprophilus* strain, 9 *R. erythropolis* strains, 5 *R. fascians* strains, 5 *R. "longus"* strains, 5 *R. opacus* strains, 4 *R. rhodochrous* strains, and 14 *R. ruber* strains) used in this work were obtained from the Regional Collection of Alkanotrophic Microorganisms, Institute of Ecology and Genetics of Microorganisms (IEGM, http://www.ecology.psu.ru/iegmcol/). The strains and the habitats from which they were isolated are described in Table 1.

The strains were grown at 28°C at pH 7.0 on a shaker (130 rpm) in a mineral medium containing (g/l) $MgSO_4 \cdot 7H_2O$, 0.2; Na_2HPO_4, 0.1; $FeSO_4 \cdot 7H_2O$, 0.01; and $CaCl_2 \cdot 2H_2O$, 0.01 [13]. The medium was supplemented with thiamine (0.002 mg/ml), Pfennig's trace element solution (1.0 ml/l), and ammonium acetate (2.0 g/l) as the source of carbon and energy.

The cultivation medium was inoculated with exponential-phase rhodococci grown in the mineral medium supplemented with 1 mM potassium. Cells were collected by centrifugation at 3000 *g* for 15 min, washed thrice with saline (NaCl) solution, resuspended to a density of 3.0×10^9 cells/ml, and added to the cultivation medium in an amount of 1 vol %. The medium was supplemented with potassium and cesium ions at concentrations of 0.01, 0.1, 0.2, 0.5, 1.0, and 5.0 mM in different combinations. Potassium and cesium chlorides were dissolved in distilled water. The minimal inhibitory concentration of cesium was determined by the serial dilution method.

The cesium-accumulating ability of rhodococci was evaluated at 4, 14, 25, 35, and 60°C at pH 3.8 to 6.0 (20 mM NaOH–succinate buffer), 5.8 to 8.0 (20 mM sodium phosphate buffer), 8.6 to 10.6 (NaOH–glycine buffer), and 9.3 to 10.7 (20 mM borate buffer). Samples were taken at 6- to 12-h intervals for 72 h. The mecha-

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Table 1. *Rhodococcus* strains and habitats from which they were isolated

Strain	Habitat
	Rhodococcus coprophilus
IEGM 600^T	Bottom sediments, United Kingdom
	Rhodococcus erythropolis
IEGM 200, IEGM 201, IEGM 502	Water, oil field, Perm oblast, Russia
IEGM 20, IEGM 253	Oil-contaminated soil, Ukraine
IEGM 270	Soil, oil field, Perm oblast, Russia
IEGM 678, IEGM 690	Soil, United Kingdom
IEGM 198	Bottom sediments, Pim River, Tyumen oblast, Russia
	Rhodococcus fascians
IEGM 35, IEGM 173	Water, Kama Reservoir, Perm oblast, Russia
IEGM 43^T	Oil-contaminated soil, Poltava oblast, Ukraine
IEGM 288	Gazha, Perm oblast, Russia
IEGM 170	Snow, oil field, Perm oblast, Russia
	Rhodococcus "longus"
IEGM 31, IEGM 32, IEGM 33	Oil-contaminated soil, Ukraine
IEGM 68, IEGM 69	Soil, oil field, Perm oblast, Russia
	Rhodococcus opacus
IEGM 56^T, IEGM 57, IEGM 60	Oil-contaminated soil, Ukraine
IEGM 249	Soil contaminated with polyester by-products, Belarus
IEGM 716^T	United Kingdom
	Rhodococcus rhodochrous
IEGM 646, IEGM 647	Water, oil field, Perm oblast, Russia
IEGM 63	Ukraine
IEGM 653	United Kingdom
	Rhodococcus ruber
IEGM 73, IEGM 333	Water, oil field, Perm oblast, Russia
IEGM 85, IEGM 86	Water, Taymyr Peninsula, Krasnoyarsk region, Russia
IEGM 220	Water, Pechora River, Russia
IEGM 223, IEGM 326	Soil, oil field, Perm oblast, Russia
IEGM 241	Chalk rock, Gomel oblast, Belarus
IEGM 78, IEGM 79, IEGM 81, IEGM 243, IEGM 468	Sand rock, Gomel oblast, Belarus
IEGM 72	Core, Gomel oblast, Belarus
	Rhodococcus sp.
IEGM 218	Soil, oil field, Ukraine

Note: The superscript T denotes "type strain." R. "*longus*" is an invalid species name.

nism of cesium uptake was studied using the ionophore valinomycin at a concentration of 0.16 μM and rhodococcal cells, either living or dead (the latter were obtained by autoclaving living cells at 0.5 atm for 20 min).

The amount of accumulated cesium was determined from the measurements of its content in the medium and cells using an AAS 30 atomic absorption spectrophotometer (Carl Zeiss, Germany). The degree of cesium removal from the medium was calculated by the formula: $[(C_0 - C_t)/C_0] \times 100\%$, where C_0 is the initial and C_t is the final concentration of cesium in the cultivation medium of rhodococci.

All experiments were carried out in triplicate. Arithmetic mean, standard deviation, and confidence level were calculated in terms of Student's *t*-test statistics using Excel 2000 and Statistica for Windows software.

RESULTS AND DISCUSSION

All strains of rhodococci were found to be able to accumulate cesium from the medium, the most active being the pigmented *R. rhodochrous* and *R. ruber* species, which removed up to 97% cesium present in the medium (Table 2). Various strains of the same species possessed different cesium-accumulating capabilities (Fig. 1 exemplifies this fact with reference to *R. erythropolis*).

Some researchers distinguish two types of microbial interaction with metal ions—biosorption, which is typical of both living and dead cells [16, 17], and bioaccumulation, which is an active process of metal ion uptake by living cells [18, 19]. The latter process is most common for monovalent cations.

Figure 2 shows that the degree of cesium removal from the medium by living cells drastically increased during the first hour of incubation and then tended to decrease. At the same time, the degree of cesium removal from the medium by dead cells remained at a constant level (about 10%) throughout the experiment.

Experiments with valinomycin, which suppresses energy processes in bacterial cells [21], showed that its addition to cesium-loaded cells led to an intense release of cesium ions into the medium (Fig. 3). In this case, about 10% of the cesium remained bound to the cells, which is in agreement with the data obtained with dead cells (Fig. 2). This indicates that cesium ions are mostly accumulated by rhodococci through the mechanism of active transport.

Experiments with *R. ruber* IEGM 326 showed (Fig. 4) that exponential-phase cells actively accumulated cesium ions, while stationary-phase cells gradually lost them to the value typical of dead and valinomycin-treated (Fig. 3) cells. The maximal uptake of cesium from the medium by this strain (about 85%) was observed at 28°C (Fig. 5), which is the optimum growth temperature for rhodococci. The optimum pH for cesium removal from the medium was 7.8–8.6 (data not shown). It should be noted in this regard that rhodococci grown on ammonium acetate as the sole source of carbon and energy alkalinize the cultivation medium to pH 8.9 ± 0.25.

Cesium uptake by rhodococci was proportional to its concentration in the medium within a range of 0.01 to 1.0 mM, while cesium concentrations higher than 5 mM inhibited bacterial growth and cesium uptake. Some authors believe that cesium accumulation by bacterial cells is closely related to the concentration of potassium ions in the medium and is mediated by potassium uptake systems [4, 9, 20]. For instance, *Escherichia coli* cells accumulate cesium ions through the Kup (TrkD) potassium uptake system [20]. According to our observations, the removal of cesium from the medium by rhodococci was maximal at an equimolar content of cesium and potassium in the medium (0.2 mM). Potassium at a concentration of 1 mM considerably decreased the degree of cesium bioaccumulation. These data strongly suggest that rhodococci absorb cesium ions through the potassium uptake system, whose affinity for cesium ions may be even higher than that for potassium ions.

Table 2. Cesium uptake by *Rhodococcus* sp. cells

Species (number of strains)	Degree of cesium uptake, %	
	minimal	maximal
R. coprophilus (1)	28.4 ± 3.73	
R. erythropolis (9)	27.1 ± 2.81	61.0 ± 2.89
R. fascians (5)	42.1 ± 1.84	58.4 ± 3.56
R. "longus" (5)	36.9 ± 3.78	66.0 ± 1.25
R. opacus (5)	23.5 ± 2.41	47.0 ± 5.26
R. rhodochrous (4)	54.8 ± 3.63	78.8 ± 4.12
R. ruber (14)	46.9 ± 2.15	97.0 ± 2.11

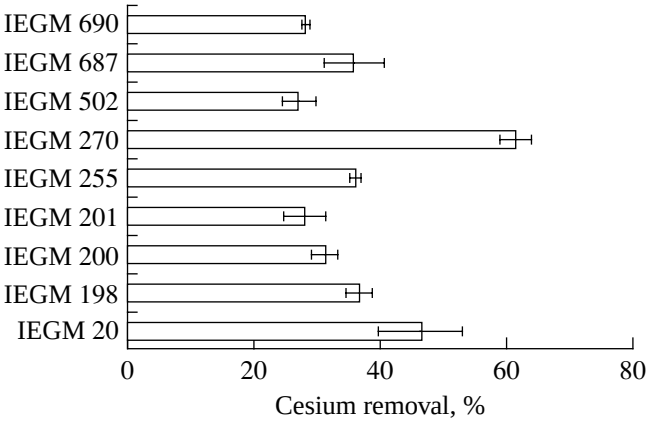

Fig. 1. The degree of cesium removal from the medium by various *R. erythropolis* strains.

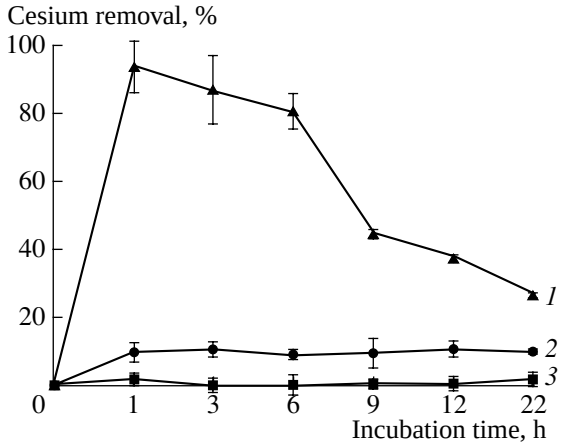

Fig. 2. The degree of cesium removal from the medium by (*1*) living and (*2*) dead *R. ruber* IEGM 326 cells as compared with (*3*) sterile mineral medium.

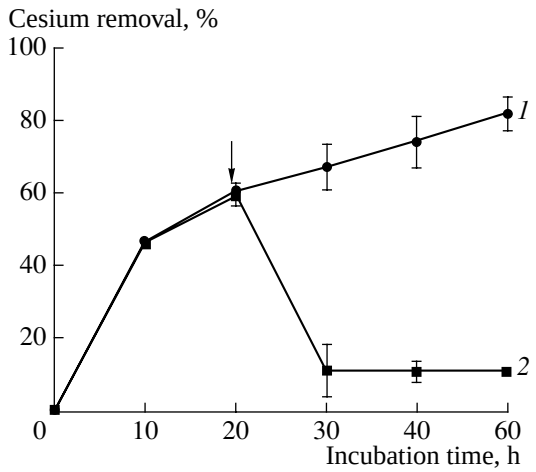

Fig. 3. The degree of cesium removal from the medium by *R. ruber* IEGM 326 cells in the (*1*) absence and (*2*) presence of 0.16 μM valinomycin. The arrow indicates the instant of valinomycin addition.

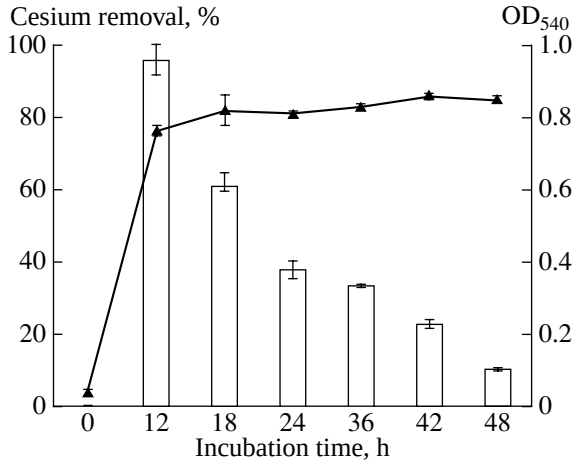

Fig. 4. The dynamics of cesium removal from the medium (bars) by *R. ruber* IEGM 326 cells grown in the mineral medium with ammonium acetate. The curve shows culture growth evaluated in optical density units at 540 nm.

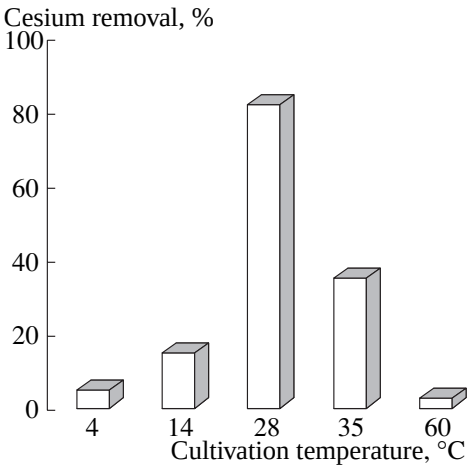

Fig. 5. The effect of the cultivation temperature on the degree of cesium removal from the medium by *R. ruber* IEGM 326 cells.

Thus, bacteria of the genus *Rhodococcus* are able to accumulate cesium from the medium by means of active transport and nonspecific sorption by the cell surface structures. Cesium is most actively accumulated at 28°C, pH 7.8–8.6, and the equimolar content (0.2 mM) of cesium and potassium ions in the medium. The most active cesium-accumulating strains are *R. erythropolis* IEGM 270, *R. fascians* IEGM 173, *R. "longus"* IEGM 31 and 69, *R. rhodochrous* IEGM 63 and 653, and *R. ruber* IEGM 79, 85, 86, 241, 326, and 333, which accumulate from 60 to 97% of the cesium ions present in the medium. These strains can be used to develop biotechnology for the treatment of cesium-contaminated wastewaters.

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REFERENCES

1. Khvorychev, M.P., Mareev, I.Yu., and Pomytkin, V.F., The Study of the Ability of Microbial Biomass to Absorb Some Radionuclides, *Mikrobiologiya*, 1994, vol. 63, no. 1, pp. 145–151.
2. Macaskie, L.E., The Application of Biotechnology to the Treatment of Wastes Produced from the Nuclear Fuel Cycle: Biodegradation and Bioaccumulation as a Means of Treating Radionuclide-containing Streams, *CRC Crit. Rev. Biotechnol.*, 1991, vol. 11, pp. 41–112.
3. Avery, S.V., Codd, G.A., and Gadd, G.M., Replacement of Cellular Potassium by Caesium in *Chlorella emersonii*: Differential Sensitivity of Photoautotrophic and Chemoheterotrophic Growth, *J. Gen. Microbiol.*, 1992, vol. 138, pp. 69–76.
4. Avery, S.V., Codd, G.A., and Gadd, G.M., Transport Kinetics, Cation Inhibition, and Intracellular Location of Accumulated Caesium in the Green Microalga *Chlorella salina*, *J. Gen. Microbiol.*, 1993, vol. 139, pp. 827–834.
5. Haselwandter, K., Berreck, M., and Brunner, P., Fungi as Bioindicators of Radiocaesium Contamination: Pre- and Post-Chernobyl Activities, *Trans. Brit. Mycol. Soc.*, 1988, vol. 90, no. 2, pp. 171–174.
6. Bakken, L.R. and Olsen, R.A., Accumulation of Radiocaesium in Fungi, *Can. J. Microbiol.*, 1990, vol. 36, pp. 704–710.
7. Borst-Pauwels, G.W.F.H., Ion Transport in Yeast, *Biochim. Biophys. Acta*, 1981, vol. 650, pp. 88–127.
8. Perkins, J. and Gadd, G.M., Caesium Toxicity, Accumulation, and Intracellular Localization in Yeasts, *Mycol. Res.*, 1993, vol. 97, no. 6, pp. 717–724.
9. Perkins, J. and Gadd, G.M., The Influence of pH and External K^+ Concentration on Caesium Toxicity and Accumulation in *Escherichia coli* and *Bacillus subtilis*, *J. Ind. Microbiol.*, 1995, vol. 14, pp. 218–225.

10. Avery, S.V., Codd, G.A., and Gadd, G.M., Caesium Accumulation and Interactions with Other Monovalent Cations in the Cyanobacterium *Synechocystis* PCC 6803, *J. Gen. Microbiol.*, 1991, vol. 137, pp. 405–413.
11. Singh, S., Negi, S., Bharati, S., and Singh, H.N., Common Nitrogen Control of Caesium Uptake, Caesium Toxicity, and Ammonium (Methylammonium) Uptake in the Cyanobacterium *Nostoc muscorum, FEMS Microbiol. Lett.*, 1994, vol. 117, pp. 243–248.
12. Tomioka, N., Uchiyama, H., and Yagi, O., Isolation and Characterization of Cesium-accumulating Bacteria, *Appl. Environ. Microbiol.*, 1992, vol. 58, no. 3, pp. 1019–1023.
13. Tomioka, N., Uchiyama, H., and Yagi, O., Caesium Accumulation and Growth Characteristics of *Rhodococcus erythropolis* CS98 and *Rhodococcus* sp. Strain CS402, *Appl. Environ. Microbiol.*, 1994, vol. 60, no. 7, pp. 2227–2231.
14. Ivshina, I.B., Bacteria of the Genus *Rhodococcus:* Biodiversity, Detection, and Immunodiagnosis, *Doctoral (Biol.) Dissertation*, Perm, 1997.
15. Warhurst, A.W. and Fewson, C.A., Biotransformations Catalyzed by the Genus *Rhodococcus, Crit. Rev. Biochem.*, 1994, vol. 14, pp. 29–73.
16. Volesky, B., Removal and Recovery of Heavy Metals by Biosorption, *Biosorption of Heavy Metals*, Volesky, B., Ed., Boca Ratton: CRC, 1990, pp. 7–43.
17. Karavaiko, G.I., Kareva, A.S., Avakian, Z.A., Zakharova, V.I., and Korenevsky, A.A., Biosorption of Scandium and Yttrium from Solutions, *Biotechnol. Lett.*, 1996, vol. 18, pp. 1291–1296.
18. Gadd, G.M., Accumulation of Metals by Microorganisms and Algae, *Biotechnology, a Comprehensive Treatise: Special Microbial Processes*, Rehm, H.-J., Ed., Weinheim: VCH Verlagsgesellschaft, 1988, vol. 6b, pp. 401–433.
19. Avery, S.V., Caesium Accumulation by Microorganisms: Uptake Mechanisms, Cation Competition, Compartmentalization, and Toxicity, *J. Ind. Microbiol.*, 1995, vol. 14, pp. 76–84.
20. Akimenko, V.K., *Al'ternativnye oksidazy mikroorganizmov* (The Alternative Oxidases of Microorganisms), Moscow: Nauka, 1989.
21. Bossemeyer, D., Schlosser, A., and Bakker, E.P., Specific Cesium Transport via the *Escherichia coli* Kup (TrkD) K^+ Uptake System, *J. Bacteriol.*, 1989, vol. 171, no. 4, pp. 2219–2221.