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Short contribution

Effect of culture medium ions on chromate reduction by resting cells of *Agrobacterium radiobacter*

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Abstract. The influence of some ions in pre-growth culture medium on chromate reduction by resting cells of *Agrobacterium radiobacter* strain EPS-916 was investigated. The reduction was dependent on the Fe^{2+} content of the culture medium: the higher the iron content, the lower the reduction rate. The cells showed maximum chromate reduction when pre-grown in the presence of 0.243 μM Mg^{2+}, 20 μM Ca^{2+} and 3.6 μM Mn^{2+}. Chromate reduction was not affected by the addition of $MgCl_2$, $CdCl_2$, $ZnCl_2$, $MnCl_2$, Na_2SO_4 (1000 μM), and Na_2MoO_4 (100 μM) to the activity assays. However, activity was inhibited by the presence of Na_2SO_4 (10 mM), Na_2MoO_4 (200 μM) and ferric citrate.

Introduction

Heavy-metal pollution of waste-water is a problem for which biodetoxification by microorganisms may be a natural solution (Silver and Walderhaug 1992). Hexavalent chromium is toxic and mutagenic for most organisms (Petrilli and Flora 1977). Reduction of more toxic hexavalent chromate to less toxic trivalent chromium represents a potentially useful detoxification process (Komori et al. 1989). Resting cells of chromate-sensitive bacteria have been reported to be able to reduce chromate to the trivalent form (Bopp and Ehrlich 1988).

Resting cell biomass directs the energy obtained from the endogenous constituents toward a range of activities (Kjelleberg et al. 1987). The activities of non-growing cells is of fundamental importance in the area of biotechnology, since industrial processes might envisage the use of pre-grown, immobilized organisms as a filter through which metal-containing effluents could be passed. With non-proliferative cells, the envelope is the crucial structure that mediates the interaction with their environment (Brown and Williams 1985). The influence of culture conditions on the envelope and other properties of bacteria has long been recognized. Thus, any study on the influence of several significant factors of the culture medium on the chromate reduction by bacterial cells is desirable.

In this paper we report the influence of several ions in pre-growth culture medium on chromate reduction by resting cells of *Agrobacterium radiobacter* from these media.

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Materials and methods

Organism. The bacterium used throughout the study was isolated from soil. This strain was designated EPS-916. This strain was identified as belonging to the species *A. radiobacter* on the basis of its morphological and biochemical properties (Kesters and De Ley 1984). The organism was maintained on Trypticase Soy Agar (TSA) with weekly transfer to a fresh medium.

Growth media and culture conditions. Bacterium was grown in glucose-mineral salts medium (GMS) containing: glucose, 55 mM; NH_4Cl, 50 mM; KH_2PO_4, 40 mM; $MgSO_4 \cdot 7H_2O$, 2.43 μM; $MnSO_4$, 2.31 μM; and $CaCl_2 \cdot 2H_2O$, 4.1 μM. The pH was adjusted to 7.0 with 0.1 M NaOH. Liquid medium did not contain iron and was prepared by using distilled, deionized water from a Milli-Q water purification system (Millipore) with glucose autoclaved separately. When required, $FeSO_4$, $MgSO_4$, $MnSO_4$ and $CaCl_2$ were added at different concentrations. To minimize iron contamination, all glassware was washed before use with 4 M HCl and 50 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.0). Liquid cultures were incubated for 48 h at 30° C on a shaker.

Chromate reduction assays. For resting-cell chromate reduction assays, the organisms were precultured for 48 h in GMS medium without chromium under aerobic conditions. Cells were harvested by centrifugation, washed three times with phosphate buffer (0.05 M, pH 7.0), and resuspended in the same phosphate buffer. The number of viable cells was determined by plating 100 μl of appropriately diluted suspension on TSA and incubating the plates at 30° C for 24 h. Ten millilitres of this suspension was added to 40 ml of a solution of potassium chromate to a final concentration of 0.5 mM in the same phosphate buffer. If indicated, ferric citrate, $MgCl_2$, $CdCl_2$, $ZnCl_2$, $MnCl_2$, Na_2SO_4, and Na_2MoO_4 were added at different concentrations. They were then incubated at 30° C in a incubator shaker. At intervals, samples were centrifuged and chromate determined in the supernatant. To ensure that all chromium was accounted for, chromium was chemically reoxid-

ized in aliquots of the supernatants. Total chromium levels were then compared to those of uninoculated controls. More that 95% of the chromium initially added was always present in the supernatants.

Analytical methods. The chromium content in the supernatants was measured spectrophotometrically using the diphenylcarbazide method (Urone 1955). Total chromium was determined by atomic absorption spectrophotometry (model 460 spectrophotometer, Perkin-Elmer, Norwalk, Conn., USA). All chemicals used were of analytical grade when available.

Results and discussion

Resting cells of *A. radiobacter* strain EPS916 reduced chromate completely when the initial chromate concentration was 0.5 mM, but it appeared to differ from other strains previously described (Bopp and Ehrlich 1988) in that it can reduce chromate without electron donors added. In the absence of cells, no measurable reduction of chromate occurred over a period of several days.

Chromate reduction was affected by the presence of Na_2MoO_4 (200 μM), Na_2SO_4 (10 mM), and ferric citrate (89 μM) in the reduction assay, concentrations where cell viability was not significantly affected. No significant inhibition was observed for $MgCl_2$ (500 μM), $CdCl_2$ (500 μM), $ZnCl_2$ (500 μM) and $MnCl_2$ (200 μM).

Metal uptake in resting cells is mediated via the cell activities induced during heavy-metal-free pre-growth (Mackaskie et al. 1992). Consequently, it is important to know the culture parameters that commonly influence the properties of microbial cells. Cells that were grown in the presence of low magnesium concentration (0.024–0.243 μM Mg^{2+}) showed an increase in the chromate-reducing activity. When the magnesium concentration in the pre-growth medium was increased from 0.243 μM to 2.43 μM, the level of residual chromate gradually increased (Fig. 1).

When resting cells of *A. radiobacter* were pre-grown at a low Mg^{2+} concentration (0.243 μM) in the presence of low concentrations of Ca^{2+}, the chromate-reducing activity increased. However, when cells were grown at higher Ca^{2+} concentrations, the ability to reduce chromate gradually decreased (Fig. 1).

Figure 1 shows the results of the influence of the Mn^{2+} concentration in the pre-growth medium on chromate-reducing activity of resting cells of *A. radiobacter*. When the Mn^{2+} concentration increased from 0 μM to 3.6 μM, a decrease in residual chromate was observed. However, when the Mn^{2+} concentration in the culture medium increased from 3.6 μM to 23.1 μM, the chromate-reducing activity gradually decreased.

Examination of chromate reduction by resting cells grown with various concentrations of iron indicated that the activity was significantly affected by Fe^{2+} (Table 1). As the initial iron concentration increased the level of reduction decreased. Furthermore, addition of Fe(III) (89 μM and 357 μM) to the reduction assay inhibited the chromate reduction by 25% and 40%, respectively. This could indicate competition between Fe(III) and Cr(VI) by the uptake system.

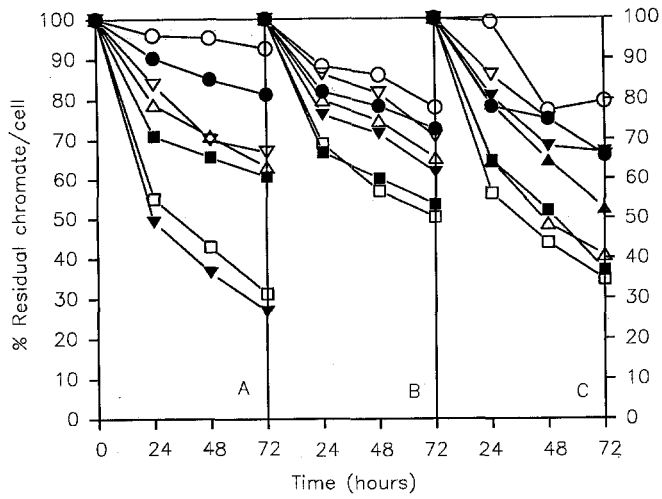

Fig. 1A–C. Effects of ions in the pre-growth medium on chromate reduction by resting cells. **A** $MgSO_4$ concentration (μM): ○, 0.024; ●, 0.048; ▽, 0.122; ▼, 0.243; □, 0.608; ■, 1.220; △, 2.430. **B** $CaCl_2$ concentration (μM): ○, 0.000; ●, 2.040; ▽, 4.080; ▼, 10.020; □, 20.040; ■, 30.060; △, 40.080. **C** $MnSO_4$ concentration (μM): ○, 0.000; ●, 0.500; ▽, 2.300; ▼, 1.100; □, 3.600; ■, 8.870; △, 11.500; ▲, 23.100

Table 1. Chromate reduction by resting cells pre-grown in the presence of several concentrations of Fe^{2+} [a]

$FeSO_4$ (μM)	Residual chromate concentration (mM)				
	Time (h)				
	0	2	6	8	10
86.300	0.500	0.491	0.306	0.243	0.105
43.100	0.500	0.455	0.216	0.141	0.099
21.500	0.500	0.440	0.189	0.102	0.074
8.630	0.500	0.419	0.177	0.080	0.042
4.310	0.500	0.327	0.078	0.000	0.000
2.150	0.500	0.288	0.000	0.000	0.000
0.860	0.500	0.279	0.000	0.000	0.000
0.000	0.500	0.276	0.000	0.000	0.000

[a] Inocula contained 2.5×10^{10} cells·ml^{-1}

Active transport and/or reduction of chromate ion has been shown to be a factor in intracellular accumulation. Chromate is actively transported into bacterial cells mainly via the sulphate transport system, but also by undetermined additional transport systems (Ohtake et al. 1987). Our results suggest that to increase the ability of *A. radiobacter* resting cells to reduce chromate, it appears necessary to control the presence and amount of ions in the pre-culture medium, mainly iron. The ability of *A. radiobacter* to reduce chromate could be related to the induction or repression of the transport systems of some ions. This could provide a practical interest for the use of microorganisms in the resting state in biotechnology and bioremediation.

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