Short Communication

EFFECTS OF HEAVY METAL CATIONS ON CHROMATE REDUCTION BY *ENTEROBACTER* *CLOACAE* STRAIN HO1

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Wastewaters containing hexavalent chromium (chromate: CrO_4^{2-}) are generated in many industrial processes, including chromium plating, metal cleaning and processing, wood preservation, and alloy preparation (6). Since CrO_4^{2-} is hazardous to fauna and flora in natural ecosystems, these wastewaters must be treated before being discharged into the environment (7, 8, 10, 11).

In our previous studies (2, 4, 5, 9), we demonstrated the potential of a chromate-reducing bacterium, *Enterobacter cloacae* (strain HO1), to remove CrO_4^{2-} from wastewaters. To develop the biological treatment process, however, it must be considered that chromate-bearing wastewaters from industrial processes generally contain heavy metal cations, such as Cu^{2+} , Cd^{2+} , Zn^{2+} , and Ni^{2+} (6). Since the heavy metal cations are known to be toxic for most organisms, they may greatly influence the performance of the biological process for treating CrO_4^{2-} . However, there are presently no data published as to the effects of heavy metal cations on the bacterial reduction of CrO_4^{2-} . In this communication, we demonstrate how heavy metal cations affect CrO_4^{2-} reduction by *E. cloacae* strain HO1.

Growth and reduction experiments were conducted anaerobically in KSC medium, as previously described (6, 13). The pH of the KSC medium was 7.2. The heavy metal cations tested were Ag^{2+} , Cd^{2+} , Co^{2+} , Cu^{2+} , Hg^{2+} , Mn^{2+} , Ni^{2+} , and Zn^{2+} . The metal salts used were of the chloride type, except for ZnSO_4 , and the metal solution was added to the culture at various final concentrations just before the start of incubation. The concentration of viable cells was determined by plating 100 μl of appropriately diluted culture onto nutrient agar and incubating the plates at 37°C for 16-20 h. Chromate was determined spectrophotometrically using

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Fig. 1. Effects of heavy metal cations on the growth (Δ) of and chromate reduction ($\circ$) by *E. cloacae* strain HO1.

diphenylcarbazine (12).

All of the tested metal cations strongly inhibited CrO_4^{2-} reduction by *E. cloacae* strain HO1 (Fig. 1). The experimental conditions were: initial cell concentration, 7×10^6 cells ml^{-1} ; initial concentration of CrO_4^{2-} , 0.5 mM; temperature, 30°C ; incubation period, 24 h. The culture pH was in the range of 7.2 to 7.4 throughout the experiment. The percent inhibition was calculated from the amount of reduced CrO_4^{2-} relative to that of the control culture without added metal cations. The degree of inhibition caused by the tested heavy metal cations was: $\text{Hg}^{2+} > \text{Ag}^+ > \text{Cu}^{2+} > \text{Cd}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Mn}^{2+}$. Hg^{2+} completely inhibited CrO_4^{2-} reduction at concentrations as low as 10^{-6} M. Nearly the same patterns of inhibition were observed with the cell growth, indicating that there is a good correlation between loss of CrO_4^{2-} -reducing activity and cell susceptibility. As far as tested, there was no heavy metal cation that inhibited CrO_4^{2-} reduction without preventing bacteria from multiplying.

We further examined the possible effects of heavy metal cations using a CrO_4^{2-} -bearing industrial effluent. The industrial effluent was taken from a manufacturing process of copper-chromium catalysts, and it contained the heavy metal cations (mM) Cu^{2+} (0.39), Mn^{2+} (0.48), and Zn^{2+} (0.0003). Since the waste

Fig. 2. CrO_4^{2-} reduction by *E. cloacae* strain HO1 in an industrial effluent and KSC medium. ●: untreated effluent, ■: dialyzed effluent, □: KSC medium with 60 mM SO_4^{2-} , △: KSC medium with 60 mM NaCl, ○: KSC medium (control).

solution contained no organic compounds that were available to bacterial cells, appropriate nutrients were provided at the same levels as those of KSC medium. Other experimental conditions were the same as described above. No significant reduction of CrO_4^{2-} was observed with the waste solution to which appropriate nutrients were added (Fig. 2). To remove metal cations, the original waste solution was dialyzed using an anion-exchange membrane (Selemion AMV; Asahi Glass Co., Tokyo, Japan), and then nutrients were added as described above. In the treated waste solution, *E. cloacae* strain HO1 could reduce about 40% of the initial amount of CrO_4^{2-} . This result indicates that the performance of biological treatment of CrO_4^{2-} could be improved by removing heavy metal cations from the industrial effluent. However, complete reduction was not observed with the treated waste solution. The treated waste solution contained a high concentration of SO_4^{2-} (60 mM), and this was likely a reason for the inhibition. In an attempt to identify the factor responsible for the inhibition, we conducted another reduction experiment in KSC medium with and without 60 mM SO_4^{2-} . As can be seen in Fig. 2, CrO_4^{2-} reduction was strongly inhibited by 60 mM SO_4^{2-} . The level of inhibition was essentially the same as that observed with the treated waste solution. No inhibition was observed with 60 mM NaCl, suggesting that the inhibition may not be attributable to the osmotic stress caused by a high ionic concentration. The mechanism for SO_4^{2-} inhibition is presently unknown.

A few papers have reported microbial reduction of toxic CrO_4^{2-} (3). To our knowledge, this is the first report of a study on the inhibitory effects of heavy metal cations on CrO_4^{2-} reduction by bacterial cells. The present study demonstrated that heavy metal cations strongly inhibited the ability of *E. cloacae* strain HO1 to reduce CrO_4^{2-} . The degree of inhibition was also strongly related to that of cell growth. These metal cations are known to generally bind to enzymatically significant proteins and interact with nucleic acids in bacterial cells (1, 14).

However, it is still difficult to describe the specific inhibition mechanism for each metal cation.

CrO_4^{2-} -bearing industrial effluents generally contained heavy metal cations. If the concentrations are low, appropriate dilution may be effective for eliminating the inhibition. As demonstrated in the present study, an ion-exchange membrane is also usable for removing heavy metal cations, though the use of such a membrane may not be conducive to developing a cost-effective treatment process. An alternative means may be to increase the bacterial resistance toward the metal toxicity.

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