The Role of Different Metal and Heavy Metal Ions on Chromium Reduction by *Pseudomonas mendocina* DS0601-FX-P22

Pseudomonas mendocina DS0601-FX-P22'nın Krom İndirgemesi Üzerine Farklı Metal ve Ağır Metallerin Rolü

Research Article

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ABSTRACT

In this study, the effects of different metal ions such as Mn^{2+}, Cu^{2+}, Fe^{2+}, Ba^{2+}, Al^{3+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} on the bacterial chromium reduction by *Pseudomonas mendocina* DS0601-FX-P22 were investigated. Two different initial chromium concentrations (15 mg/L and 25 mg/L Cr(VI)) were studied. The Cr(VI) reduction ability of the bacterium increased in the presence of metal ions like Cu^{2+} and Fe^{2+} and was significantly inhibited in the presence of metal ions like Ba^{2+} and Ni^{2+}. Also, Cu^{2+} was the most efficient metal ion at Cr(VI) reduction for both Cr(VI) concentration (15 and 25 mg/L) in *P. mendocina* DS0601-FX-P22 bacterium.

Keywords

Bioremediation, Chromate reduction, Heavy metal, *Pseudomonas*.

ÖZET

Bu çalışmada, Mn^{2+}, Cu^{2+}, Fe^{2+}, Ba^{2+}, Al^{3+}, N^{i2+}, Co^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} gibi farklı metal iyonlarının *Pseudomonas mendocina* DS0601-FX-P22 tarafından bakteriyel krom indirgeme üzerine etkisi araştırılmıştır. İki farklı krom derişiminde çalışılmıştır (15 mg/L ve 25 mg/L Cr(VI)). Cu^{2+} ve Fe^{2+} gibi metal iyonlarının varlığında bakterinin Cr(VI) indirgeme yeteneği artmış, Ba^{2+} and Ni^{2+} gibi metal iyonlarının varlığında önemli derecede inhibe olmuştur. Ayrıca, *P. mendocina* DS0601-FX-P22 bakterisinin her iki Cr(VI) derişimi (15 ve 25 mg/L) için Cr(VI) indirgemesinde en etkili metal iyonu Cu^{2+} olarak bulunmuştur.

Anahtar Kelimeler

Biyoremediasyon, Krom indirgeme, Ağır metal, *Pseudomonas*.

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INTRODUCTION

Chromium is one of the most frequently used metal contaminants released by activities such as mining, metal plating, wood preservation, ink manufacture, dyes, pigments, glass and ceramics, tanning and textile industries, and corrosion inhibitors in cooling water, induce pollution and may cause major health hazards [1]. Contact with Cr(VI)-compounds over a prolonged period of time is a risk factor for developing lung cancer [2], and inhalation of Cr(VI) can cause severe damage and irritation to the nose, throat, and lungs. The toxicity, solubility and bioavailability of Chromium, depend primarily on its chemical form [3]. Chromium generally exists in two stable oxidation states: trivalent chromium and hexavalent chromium. Cr(VI) compounds tend to be highly soluble, strongly oxidizing, and potentially mutagenic [4]. In contrast, Cr(III) compounds are generally less soluble, with low mobility and toxicity [5]. Thus, the reduction of Cr(VI) to Cr(III) can provide a useful method for Cr(VI) detoxification [6]. Also, removal of Cr(VI), either by reduction or by biosorption, can significantly reduce the risks to human health [7]. Conventional technologies for remediation of chromium-contaminated wastewater, including ion exchange, precipitation, and adsorption on alum or kaolinite, cannot be applied on a large scale due to high cost and subsequent secondary environmental pollution [8]. Additionally, the conventional chemical reduction methods are more suitable for disposing high concentration of Cr(VI)-containing wastes [9]. Alternatively, bioremediation, the use of multifarious microorganisms capable of reducing toxic Cr(VI) to Cr(III), offers efficiency, affordability, and environmentally friendly advantages to traditional chemical methods, especially when dealing with Cr(VI)-containing wastes at low-to-mid concentrations (10~200 mg/L) [8-10].

A wide variety of microorganisms such as bacteria, yeast, algae, protozoa, and fungi are found in water, and these microorganisms have developed various mechanisms to protect themselves from heavy metal toxicity, such as adsorption, uptake, methylation, oxidation, and reduction [7]. And, it is known that bacterial chromium reduction is related to multiple factors such as pH, chromium concentration, carbon sources, organic acids, temperature and presence of various metal ions. There are many studies in the literature about the effects of pH, chromium concentration, carbon sources, organic acids and temperature on Cr(VI) reduction [11-13]. Also, we reported the effects of these factors on bacterial Cr(VI) reduction by *Bacillus* and *Pseudomonas* bacteria in our previous study [14-16]. But, there is relatively little information in the literature on the effect of metal ions to Cr(VI) reduction. For this reason, the main objective of this study is, to investigate the effects of various metal and heavy metal ions on bacterial Cr(VI) reduction and to provide a useful reference for further development of effective chromium reduction bioprocesses.

MATERIAL AND METHODS

Chemicals

Unless otherwise stated, all chemicals used in the experiments were reagent grade or better. Water for all experiments was supplied from a Human Power-Pure water system (Zeener Power, Korea). Cr(VI) stock solution was prepared by dissolving 2.829 g $K_2Cr_2O_7$ (294.19 g/mol) (Merck) in 1 L UV-water, which was autoclaved separately and added to the media before experiments. In addition, diphenylcarbazide (Merck) reagent was prepared in acetone. All stock solutions were stored in amber glass bottles in darkness at 4°C.

Culture conditions of microorganism

The wastewater isolates of *P. mendocina* DS0601-FX-P22 obtained from Pamukkale University, Biology Department, Bacteriology Laboratory were used in this study. The bacterial culture was inoculated in growth media tryptic soy broth (TSB) consisting of peptone from casein (17.0 g/L), peptone from soymeal (3.0 g/L), glucose (2.5 g/L), NaCl (5.0 g/L) and dipotassium hydrogen phosphate (2.5 g/L). The culture was inoculated in TSB media at pH 6.0 and aerobically incubated at 37°C with constant shaking at 125 rpm; culture growth was monitored by measuring optical density (OD) at 600 nm. The culture suspension was prepared and adjusted by comparing against 0.5 McFarland turbidity standard tubes (1.5×10^8 cfu/mL) for all tests.

Cr(VI) reduction experiments

The 250-mL flasks containing 100 mL of TSB with a desired Cr(VI) concentration were inoculated with 2 mL of cultures at logarithmic phase. The initial pH of the media was buffered to 6.0 (±0.2) using an appropriate amount of $NaHCO_3$ (0.11 mM). All media were autoclaved at 121ºC for 15 min before use in microbial Cr(VI) reduction experiments. Cultures were then incubated at 37 ºC with constant shaking at 125 rpm. Immediately after inoculation with bacteria, samples were drawn at regular time intervals (every 12 h) and centrifuged at 6000 rpm for 20 min. The concentration of Cr(VI) in the supernatant was determined colorimetrically at 540 nm by UV spectrophotometer using diphenylcarbazide reagent [17]. The growth of cells was also routinely monitored measuring optical density (OD) at 600 nm. The experiments were carried out in duplicate.

Effects of metal ions on bacterial chromium reduction

Effect of various metal cations (Mn^{2+}, Cu^{2+}, Fe^{2+}, Ba^{2+}, Al^{3+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}) supplemented as $MnSO_4.H_2O$ (Merck), $CuSO_4.5H_2O$ (Merck), $FeSO_4.7H_2O$ (Merck), $BaCl_2.2H_2O$ (Merck), $Al(NO_3)_3.9H_2O$ (Merck), $N_2NiO_6.6H_2O$ (Fluka), $CoN_2O_6.6H_2O$ (Fluka), $ZnSO_4.7H_2O$ (Carlo-Erba), $CdN_2O_6.4H_2O$ (Fluka), $Pb(NO_3)_2$ (Merck) on chromate reduction during growth of the bacterium was investigated in TSB medium containing 15 and 25 mg/L Cr(VI). All metals were added in certain amount that can be dissolved in distilled water (52 mg/L for Cu^{2+}, Mn^{2+}, Zn^{2+} and Fe^{2+}, 100 mg/L for Ba^{2+}, Al^{3+}, Ni^{2+} and Pb^{2+} and 20 mg/L for Co^{2+} and Cd^{2+}). The experiments were carried out at pH 6.0 and 37ºC.

RESULT AND DISCUSSION

Optimization studies on bacterial removal of chromium mainly focuses on the effects of environmental factors such as pH, Cr(VI) concentration, carbon sources, electron donors, and temperature. No doubt that, at the same time very different ecosystem, such as soil or water also contaminated with metal-heavy metals. In these ecosystems, both the relationship of microorganisms with such heavy metals and the interactions of heavy metals with each other impact of the chromium reduction. But, there are not detailed studies about the effect of heavy metals on chromium reduction according to our literature reviews. In this study, the effect of ten different metal ions on bacterial Cr(VI) reduction was evaluated at 15 and 25 mg/L Cr(VI) concentration. The results that performed with 15 mg/L Cr(VI) was given in Figure 1. Some of the metal ions were decrease the bacterial chromium reduction time, some of them were not affect the reduction time and some of them were extend the reduction time according the control (without metal ions). According to Figure 1, while the 15 mg/L Cr(VI) was reduced in 72 h when there is no metal ions in the growth medium, it was 12, 24, 24 and 48 h in the presence of Cu^{2+}, Fe^{2+}, Co^{2+} and Mn^{2+} in the growth medium respectively. We also studied the effect of the metal ions for 25 mg/L Cr(VI) concentration. The results show that (Figure 2), among the metal ions used in the study, Cu^{2+}, Fe^{2+}, Co^{2+}, Mn^{2+}, and Al^{3+} accelerated the bacterial chromium reduction. Although 25 mg/L Cr(VI) was completely reduced at 96 h in the control, in the presence of Cu^{2+}, Fe^{2+}, Co^{2+}, Mn^{2+} and Al^{3+} the reduction time was decreased at 36, 36, 48, 60 and 84 hours respectively. Similar result was observed by Desai et al. 2008. They reported that Cu^{2+} was increased the chromate reductase activity in *Pseudomonas* sp. G1DM21 strain at the rate of 33%. Additionaly, stimulation of chromate reductase activity by Cu^{2+} has been observed in case of *Bacillus* sp. ES 29 [18] and *Pseudomonas* CRB5 [19]. The role of Cu^{2+} in stimulation of Cr(VI) reductases has been attributed to its action as a redox centre shuttling electrons between protein subunits or as a protective agent against oxygen [18]. Besides the Cu^{2+}, Fe^{2+}, Co^{2+} and Mn^{2+} were also increased the chromium reduction rate of *P. mendocina* DS0601-FX-P22 both for 15 mg/L and 25 mg/L Cr(VI) concentrations. There are some literature reports supported to our results about the stimulatory effects of Co^{2+} and Mn^{2+} on Cr(VI) reduction of different bacteria; *Ochrobactrum intermedium* [20] and *Bacillus sphaericus* [21,22].

Another metal ion used in the study was Al^{3+}. Adding of Al^{3+} in growth medium which containing 15 mg/L Cr(VI) was not considerable affect the Cr(VI) reduction. But, interestingly it had positive

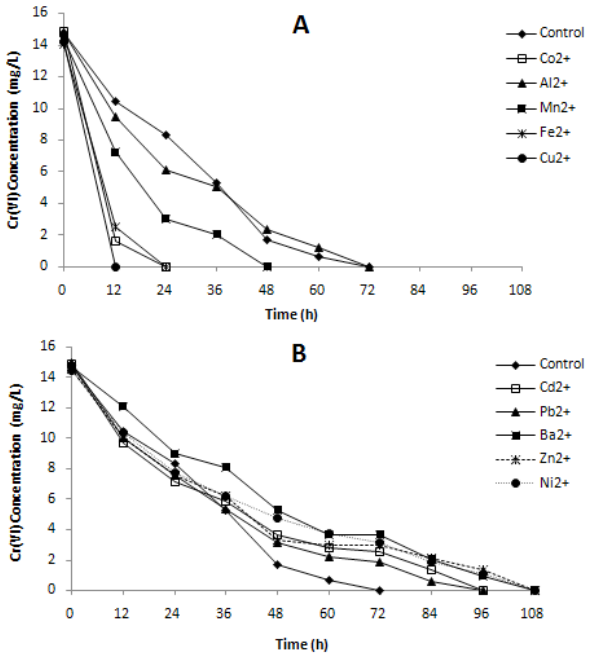

Figure 1. Effect of various metal ions on Cr(VI) reduction by *P. mendocina* DS0601-FX-P22 in 15 mg/L Cr(VI) containing media, A; Effect of Co^{2+}, Al^{3+}, Mn^{2+}, Fe^{2+} and Cu^{2+}, B; Effect of Cd^{2+}, Pb^{2+}, Ba^{2+}, Zn^{2+} and Ni^{2+}.

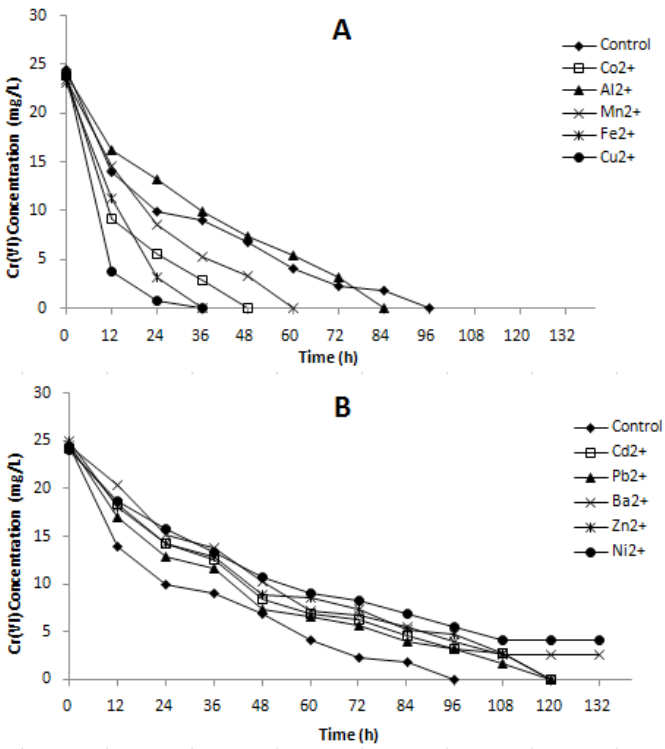

Figure 2. Effect of various metal ions on Cr(VI) reduction by *P. mendocina* DS0601-FX-P22 in 25 mg/L Cr(VI) containing media, A; Effect of Co^{2+}, Al^{3+}, Mn^{2+}, Fe^{2+} and Cu^{2+}, B; Effect of Cd^{2+}, Pb^{2+}, Ba^{2+}, Zn^{2+} and Ni^{2+}.

effect in 25 mg/L Cr(VI) containing medium. In this medium (with 25 mg/L Cr(VI)) Al^{3+} was increased the Cr(VI) reduction rate approximately 14% according to control (without Al^{3+}).

On the other hand, the divalent cations of Pb^{2+}, Ba^{2+}, Ni^{2+}, Zn^{2+} and Cd^{2+} did not exhibit any stimulatory effect on the Cr(VI) reduction of *P. mendocina* DS0601-FX-P22 for both Cr(VI) concentration. In the presence of Pb^{2+}, Zn^{2+} and Cd^{2+} the reduction time of 25 mg/L Cr(VI) was extend from 96 h to 120 h. Especially Ba^{2+} and Ni^{2+} lead to extend the chromium reduction time of *P. mendocina* DS0601-FX-P22. According to our results, inhibitory effects of the metal ions were Ni^{2+}>Ba^{2+}>Cd^{2+}>Zn^{2+}>Pb^{2+} in growth medium containing 25 mg/L Cr(VI).

CONCLUSION

Bacterial detoxification of Cr(VI) under certain environmental conditions has attracted considerable interest, because of microorganisms can tolerate and reduce Cr(VI). The effects of heavy metals on Cr(VI) reduction to understand metal-Cr(VI) relation by the *P. mendocina* DS0601-FX-P22 has been reported in the present study. It has been found that although; Mn^{2+}, Cu^{2+}, Fe^{2+}, Al^{3+} and Co^{2+} stimulated the reduction, Zn^{2+}, Cd^{2+} Ba^{2+}, Ni^{2+} and Pb^{2+} not stimulated the reduction. Moreover, they extent the Cr(VI) reduction time.

AUTHORS' INFORMATION

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