Mechanisms of Hexavalent Chromium Reduction by *Rhodococcus qingshengii* strain SC26

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Abstract
Chromium represents a serious threat for both human health and ecosystems equilibrium. It is mainly present in two stable inorganic forms: trivalent and hexavalent, the latter one being more toxic due to its high solubility and mobility in biological systems. The major pollution sources are electroplating and tannery industries. Among possible remediation strategies, the use of biological systems can be proposed since microorganisms interact with metals by passive adsorption processes and active enzymatic reactions.
In the present study, *Rhodococcus quingshengii* strain SC26 was characterized for its ability to resist to hexavalent chromium (MIC of 300 mg L^{-1}) and to reduce up to 51.14 mg L^{-1} hexavalent to trivalent chromium in growing-cell conditions. The reduction was always paralleled by cell growth. Part of the metal was present on cell pellet (1.9 mg g^{-1}). Not proliferating cells were not able to reduce/adsorb hexavalent chromium, thus excluding passive adsorption processes. Trials conducted with contaminated electroplating wastewaters are ongoing to assess the bioremediation potential of *R. qingshengii* strain SC26 in close-to-real scenarios.

Keywords (maximum 6 in alphabetical order)
Heavy metal removal; hexavalent chromium reduction; *Rhodococcus qingshengii*; wastewater bioremediation.

INTRODUCTION

Nowadays the presence of heavy metals in water bodies represents a serious environmental and human health threat. Chromium (Cr) is known for its widespread utility in various industrial processes such as industrial welding, dyes and pigments manufacturing, electroplating processes, leather tanning and wood preservation (Pushkar, *et al.*, 2021). Therefore, it is one of the most hazardous heavy metals discharged into surface water (Roșca, *et al.*, 2023). Cr is present in the environment mainly in two inorganic stable forms: trivalent (CrIII) and hexavalent (CrVI). Cr(VI) is the most hazardous one, being considered 1,000 times more toxic than Cr(III) (Diaconu, *et al.*, 2020). In some electroplating effluents, Cr(VI) concentration ranges from 6 to 887 mg L^{-1}, being the Cr(VI) and total Cr limits of 0.005 and 0.05 mg L^{-1}, respectively, in surface and ground waters (Directive 2000/60/CE). Therefore, the implementation of strategies to reduce Cr(VI) to Cr(III) and to remove total Cr from industrial wastewater is fundamental to reduce heavy metal pollution (Roșca, *et al.*, 2023). Among these strategies, the use of microorganisms can represent a sustainable alternative to conventional physico-chemical processes (Demarco, *et al.*, 2023). Beside Cr adsorption processes, bacteria can reduce Cr(VI) to the less toxic Cr(III) by intracellular or extracellular reduction mechanisms mediated, respectively, by aspecific ROS-scavenging and Cr(VI)-specific reductase systems (Diaconu, *et al.*, 2020).
The aim of the present study was to characterize Cr(VI) reduction and Cr adsorption mechanisms in *Rhodococcus qingshengii* bacterial strain SC-26, in order to envisage the most promising set-up for the development of a biological system useful to reduce Cr pollution in wastewater.

MATERIALS AND METHODS

Bacterial strains

Nine strains [As3-5a(1), As3-5a(3), As3-5a(4), As3-5a(5), SC5III, SC37, SC3I(2), SC23, and SC26] previously isolated from arsenic- (Cavalca *et al.*, 2010a) and petroleum hydrocarbon-(Cavalca *et al.*, 2010b) contaminated soils, were tested to assess their Cr(VI) resistance and reduction.

Determination of Cr(VI) Minimum inhibitory concentration

Cr(VI) Minimum inhibitory concentration (MIC) was determined on different minimum mineral media: M9 (Kunz and Chapman, 1981), DF (Dworkin and Foster, 1958) and TMM (Mergeay *et al.*, 1985). Na-gluconate (0.6 % *wt/vol*) was added as carbon and energy source. Increasing concentrations of Cr(VI) were tested between 25 and 500 mg L^{-1}, in the form of $K_2Cr_2O_7$ (VWR BDH Chemicals, Radnor, Pennsylvania, US) at pH of 5.67. Bacterial growth was determined by spectrophotometric analysis (OD_{600nm}).

Not-proliferating cell experiment in the presence of Cr(VI)

In biofilm-based system, 4 and 8 mL of bacterial suspensions (OD_{600nm} of 2), prepared in distilled water, were separately deposited onto 0.2 µm cellulose acetate filter (Millipore, Burlington, MA, USA). 50 mg L^{-1} Cr(VI) prepared as $K_2Cr_2O_7$ (VWR BDH Chemicals) solution were vacuum-forced across the biomass-activated filter, with a few seconds of contact. Metal content of the flow through was analyzed by inductively coupled plasma spectrophotometry (ICP-MS).

In planktonic cell system, biomass was resuspended in 12 mL of 50 mg L^{-1} Cr(VI) solution added as $K_2Cr_2O_7$ (VWR BDH Chemicals) and stirred in plastic tubes (100 rpm at 23 °C). The effect of contact time on Cr(VI) reduction and biosorption was determined on cell biomass and supernatant after 0, 4, 72, and 168 h of incubation. Bacterial suspensions were centrifuged (15 min at 10,000 rpm) and total Cr on biomass and Cr(VI) in supernatant were analyzed by ICP-MS and by diphenyl carbazide (DPC) spectrophotometric methods, respectively. Each experiment was conducted in triplicate and the abiotic controls were always analyzed.

Growing cell experiment in the presence of Cr(VI)

R. qingshengii strain SC26 (inoculum 2% *vol/vol*, $OD_{600\ nm}$ of 2) was separately incubated for 7 days in DF mineral medium and in Luria Bertani (LB) rich medium, in the presence of approximately 50 mg L^{-1} Cr(VI) prepared from $K_2Cr_2O_7$ (VWR BDH Chemicals) solution. At subsequent incubation times, bacterial growth was determined by OD_{600nm}. Total Cr present on cell pellet was determined by ICP-MS analysis. Cr(VI) in the supernatant was determined by DPC spectrophotometric method (OD_{540nm}) according to Dean and Beverly (1958). Each experiment was conducted in triplicate and the abiotic controls were always analyzed.

RESULTS AND DISCUSSION

Cr(VI) MIC of isolated strains

Cr(VI) MIC was 25 mg L^{-1} for most of the tested strains, the only exception being *R. qingshengii* strain SC26 with a MIC of 300 mg L^{-1}. Resistance towards Cr is very variable in bacterial isolates. The MIC value of strain SC26 is consistent with Cr tolerance previously reported by Sevak *et al.* (2023) who found that only a few tested bacterial isolates were resistant to 300 mg L^{-1} Cr(VI) and only one isolate could tolerate up to 500 mg L^{-1}. Due to Cr resistance and the ability of the *Rhodococcus* genus to cope with different pollutants (Nazari, *et al.*, 2022), strain SC26 was chosen for further study.

Cr(VI) reduction to Cr(III) by SC26 not-proliferating cells

Not-proliferating cells didn't perform Cr removal or Cr(VI) reduction in biofilm-based and planktonic cell experiments (Table 1), being the amount of Cr present in abiotic solution always close to that of biotic ones. Total Cr biomass content was negligible.

Table 1. Cr (VI) concentrations in biofilm-based and planktonic cell experiments.

	SC26 biofilm		SC26 planktonic			
	OD_{600nm}=2	OD_{600nm}=4	0 h	4 h	72 h	168 h
Cr(VI) (mg L^{-1})	49.55 ± 1.01	49.88 ± 0.64	50.706 ± 3.737	51.065 ± 5.457	51.245 ± 4.609	49.807 ± 2.549

Cr(VI) reduction to Cr(III) by SC26 growing cells

Growing cell experiments performed in the presence of increasing concentrations of Cr(VI) from 32 to 146 mg L^{-1} (Figure 1) showed that Cr(VI) reduction to Cr(III) was inversely related to bacterial growth: increasing metal concentrations exerted cell toxicity thus affecting SC26 ability to reduce Cr(VI). These preliminary experiments evidenced that Cr(VI) transformation was strictly related to the growth of SC26 bacterial cells.

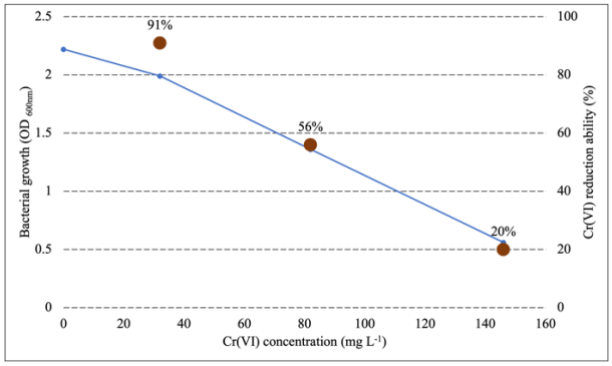

Figure 1. Cr(VI) reduction ability (%) ● of strain SC26 in growing cell experiment —●— at increasing Cr(VI) concentrations (32, 82 and 146 mg L^{-1}).

Analysis performed at increasing incubation times conducted in the presence of 50 mg L^{-1} Cr(VI) (Figure 2) confirmed that the reduction of the metal was parallel by cell growth, either in DF mineral minimal medium and in LB rich medium. After 7 d incubation, Cr(VI) present in the supernatant was 31.59 and 4.45 mg L^{-1} in DF and LB media, respectively, and the specific total Cr content of cell biomass was 7.62 and 1.92 mg g^{-1} cell d.w.. These data permit to determine that the strain was able to reduce 16.46 and 51.14 mg L^{-1} Cr(VI) to Cr(III) when grown in DF and LB medium, respectively. Abiotic controls did not show any Cr(VI) reduction. The ability of reducing Cr(VI) to Cr(III) while increasing in biomass and cell density was also reported by Guatam *et al.*, (2022), who observed the secretion of chromate reductase in the culture medium and intracellular enzyme activity.

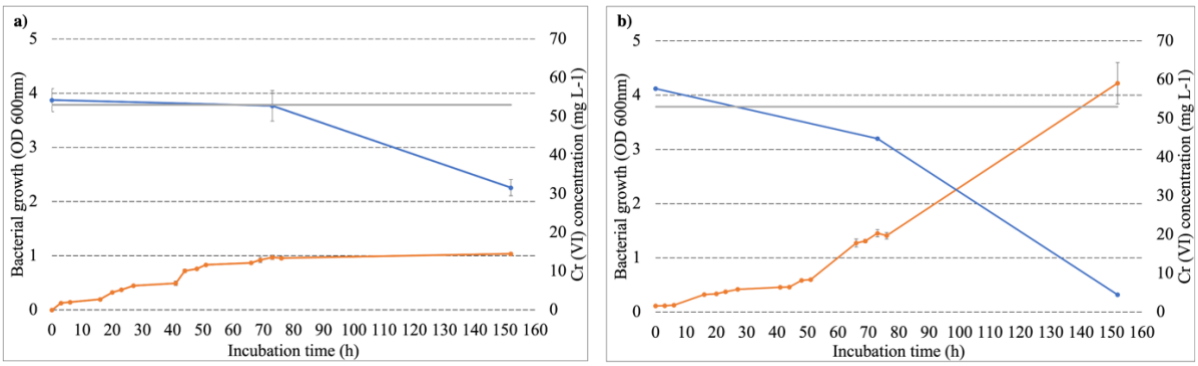

Figure 2. Cr(VI) reduction in *R. qingshengii* strain SC26 growing cell experiment performed in DF (a) and LB (b) media, in the presence of 50 mg L^{-1} Cr(VI) OD_{600nm} —●—, Cr(VI) —●— and total Cr concentrations — over seven days (152 h) of incubation.

The data obtained suggest that *R. qingshengii* strain SC26 is able to lower Cr toxicity either by reducing Cr(VI) to Cr(III) and by adsorbing Cr onto the cell biomass. Further analysis will determine the adsorbed Cr form. Experiments in the presence of real wastewaters are on-going.

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