Resistance and Reduction Potential of *Serratia marcescens* in Hexavalent Chromium

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ABSTRACT

Heavy metal pollution due to legacy waste from the Cold War nuclear proliferation remains a huge problem at federal and industrial sites. Microbial bioremediation is a cost effective method for removing heavy metals from soil and groundwater. This pilot research project looks at the reduction of chromium present at DOE's Savannah River Site and the ability of bacteria population to aid in the reduction of heavy metals. The purpose of this study was to characterize the growth pattern of Serratia marcescens strain 93-1399-1 in the presence of heavy metal hexavalent chromium (Cr [VI]) and its ability to convert Cr (VI) to the less toxic Cr (III). When S. marcescens was grown on LB agar with increasing concentrations of Cr (VI) as potassium dichromate, the bacteria survived at all concentrations tested up to 200 ppm. Growth curve of S. marcescens in Cr (VI) showed that the bacteria exposed to 25 pm grew in similarly to bacteria without Cr (VI), had reduced exponential growth at 50 and 100 ppm and the poor growth in 200 pp after 24 hours. Plate count assays showed that the 65% of the bacteria survived in 25 ppm of Cr (VI) but only 5% survived at 50 ppm. In preliminary chromium reduction assays, S. marcescens was grown for 24 hours in 25 ppm of Cr (VI). S. marcescens reduced 60% of the Cr (VI) to Cr (III) as compared to 4% reduction in broth alone. These data suggest that the S. marcescens strain may be useful in bioremediating Cr (VI) at contaminated sites. Future studies will focus on identifying the genes responsible for Cr (VI) reduction.

INTRODUCTION

Extensive use of metals in various industrial applications has caused substantial environmental pollution. Some organisms are susceptible to heavy metals while others are resistant and this resistance can be used for the bioremediation of metals (Lloyd 2003, Noghabi *et al*., 2007). One of the heavy metals that have contaminated abut 1300 sites across US and is on EPA's high priority list of hazardous substances is Chromium. Cr (VI) and Cr (III) are the principal forms of chromium found in the environment. Cr (VI) is a highly soluble and toxic agent that is also carcinogenic and mutagenic, whereas Cr (III) is less toxic and tends to form insoluble hydroxides (Richards et al., 1992).

The ongoing efforts to develop a more cost effective strategy in the bioremediation of heavy metals are reflected in the works of chromium-reducing bacteria (Lloyd, 2003). Chromium-resistant *Serratia marcescens*, has been shown to present chromium-reduction activity and can grow in the presence of a wide range of industrial contaminants. The reduction of chromium is a potentially useful process for the remediation of chromium-contaminated waters, soils and sites.

The aim of this study is (1) assess the resistance of bacteria to high level concentrations of chromium, (2) generate a growth curve to determine the growth and absorbance of the bacteria in the presence of different concentration of chromium over time, (3) determine the survival rate of *S. marcescens* to chromium, and (4) conduct a reduction assay to reduce Cr (VI) to Cr (III) with the aid of the indicator S-diphenylcarbazide (DPC).

RESULTS

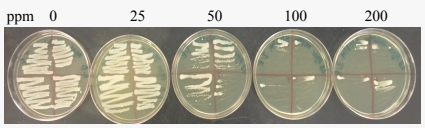


Figure 1: The growth of *Serratia marcescens* on LB agar plates with 0, 25, 50, 100 and 200 ppm of $K_2Cr_2O_7$.

MATERIALS AND METHODS

PLATE ASSAY

To get a qualitative assessment of bacterial resistance to chromium, a single colony of *S. marcescens* was streaked on LB agar plates of 0, 25, 50, 100 and 200 ppm and incubated for 2 days at 25°C.

GROWTH CURVE

A culture of *S. marcescens* in LB broth was prepared and incubated for 24 hours. Following incubation, 15 ml of LB broth and 15 µl of *S. marcescens* were placed into tubes labeled 0, 25, 50, 100 and 200 ppm. $K_2Cr_2O_7$ was added to give the final concentrations of 25, 50, 100, and 200 ppm. The absorbance at 600 nm of 1 ml from each tube was recorded at time intervals 0, 1.5, 3, 6, 12, 24, and 48 hours.

DROP PLATE ASSAY

50 µl of *S. marcescens* culture and 5 ml LB broth were placed into tubes labeled 0, 25, 50, 100 and 200 ppm. $K_2Cr_2O_7$ was added to give a final concentration of 25, 50, 100 and 200 ppm. For drop plating, 25 µl of each sample and 225 µl of 0.9% saline was placed in a 96 well round bottom polystyrene plate and a 10-fold serial dilution of the samples was conducted. 10 µl of the diluted samples were placed onto two agar plates with different chromium concentrations at time 0 and 24 hours and incubated at 25°C.

REDUCTION ASSAY

Bacterial Culture

S. marcescens was streaked on LB agar plate and incubated at 25°C for 2 days and then inoculated into 3 ml of LB broth. This culture was incubated in a shaking incubator at 25°C for 2 days. Two samples of chromium with bacteria in one was prepared and centrifuged at 13,000 rpm for 3 minutes at time 0 and 24 hours.

Assay for Chromium Reduction

A 2-fold serial dilution of $K_2Cr_2O_7$ ranging from 3.125 to 200 ppm was prepared and placed in wells of a 96 flat bottom polystyrene plate. 225 µl of MOPS/DPC solution was added to 25 µl the chromium standards and samples. A microplate reader was used to determine the absorbance of the samples in the wells at 540 nm.

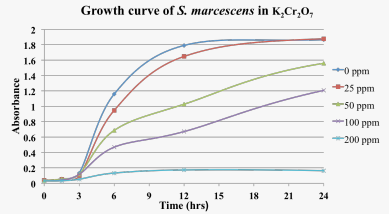
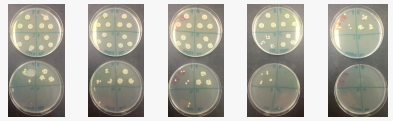
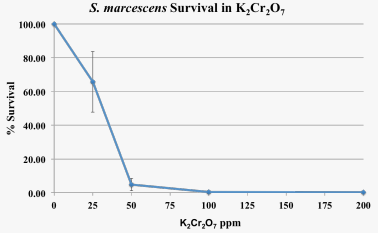


Figure 2. Growth of *S. marcescens* over 24 hours in the presence of 0, 25, 50, 100, and 200 ppm of $K_2Cr_2O_7$. Experiment was repeated at least three times.

Figure 3. Survival of *S. marcescens* in $K_2Cr_2O_7$ by drop plate assay



Bacteria were cultured in 0, 25, 50, 100, or 200 ppm $K_2Cr_2O_7$ diluted and plated in LB agar plates at 0 and 24 hours. 24 hour plating shown.



The percentage survival was calculated after 24 hour exposure to $K_2Cr_2O_7$. The experiment was repeated at least three times.

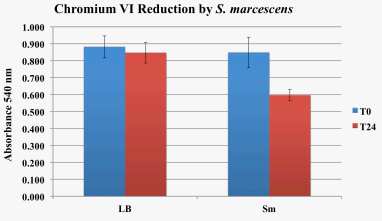


Figure 4. Bacteria were cultured for 24 hours in 50 ppm $K_2Cr_2O_7$ and assayed for Cr VI reduction using DPC. The experiment was repeated at least three times.

CONCLUSION

S. marcescens was capable of growth on solid medium containing 25 and 50 ppm $K_2Cr_2O_7$.

S. marcescens grew in liquid medium containing 25, 50 and 100 ppm of $K_2Cr_2O_7$ although it grew at a slower rate in the presence of 50 and 100 ppm. The bacteria did not grow in 200 ppm of $K_2Cr_2O_7$.

65% of the bacteria survived in 25 ppm $K_2Cr_2O_7$ after 24 hours whereas 4% survived in 50 ppm. Less than 1% of the bacteria survived in 100 and 200 ppm of $K_2Cr_2O_7$.

When Cr^{6+} in $K_2Cr_2O_7$ is reduced to Cr^{3+} by DPC, the DPC is converted into the purple compound diphenylcarbazone (DPCO). When *S. marcescens* was incubated with 50 ppm of $K_2Cr_2O_7$, the absorbance of DPCO decreased by 33% after 24 hours as compared to 4% reduction for LB only. This result indicated that the was less Cr^{6+} in the bacterial sample than in the LB only presumably due to reduction of Cr^{6+} by the bacteria.

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