Removal of chromate from industrial effluent by a new isolate of *Staphylococcus cohnii*

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Abstract We have isolated and identified of a new isolate of *Staphylococcus cohnii* from a tannery effluent and have studied the capacity of the organism to bioaccumulate chromium. The *Staph. cohnii* isolate is resistant to Cr, growing well in standard medium supplemented with 1000ppm Cr(VI). Over a treatment period of 96 hrs, the isolate removed about 90% Cr(VI) from medium containing 100ppm Cr(VI) and from contaminated tannery wastewater. Bioaccumulation of Cr from the wastewater was confirmed by atomic absorption. Results further indicate that the organism reduces Cr(VI) to Cr(III).

Keywords Bioaccumulation; chromium; heavy metals; industrial effluent; *Staphylococcus cohnii*

Introduction

Many industries including metal works, leather and electroplating industries, discharge effluent containing relatively high levels of chromium. Chromium is an important environmental and health hazard (Codina, *et al.*, 1995; Hartwig, 1995) and even the presence of relative low concentration of Cr(VI) in effluent can significantly reduce the efficacy of biological sewage treatment (Vankova *et al.*, 1999). On the other hand, reduction of Cr(VI) to Cr(III) is known to significantly lower the toxic effects of chromium, possibly as a result of a decrease in solubility and bioavailability of the trivalent metal (Cooke *et al.*, 1995).

Several physical and chemical methods are available for removing metal ions from industrial effluent. Some disadvantages of these methods include high operating costs, the requirement for preliminary treatment steps and the difficulty of treating the solid waste subsequently generated. Biological methods using microorganisms to remove metal ions from effluent have also been developed. Studies show that microorganisms accumulate metals by a number of different processes such as uptake by transport, bioabsorption to cell walls, entrapment in extracellular capsules, precipitation, and oxidation-reduction reactions (Gadd, 1990; Chen and Hao, 1998).

Chromium is a major contaminant of tannery effluent and its discharge into the environment poses an environmental hazard (Turick *et al.*, 1996). While a number of chromium resistant microorganisms have already been described it is not yet clear what determines bacterial resistance to chromium and how this property is related to the reduction of chromate by bacteria (Silver and Phung, 1996). Furthermore, only limited information is available on the use of microorganisms for bioremediation purposes (Turick *et al.*, 1996; Chen and Hao, 1998).

We report here the isolation of a new strain of *Staphylococcus cohnii*, which demonstrates significant resistance to and bioaccumulates Cr(VI), resulting in more than 90% removal of this heavy metal from tannery effluent. Results indicate that *Staph. cohnii* reduces Cr(VI) to Cr(III).

Materials and method

Microorganism and growth conditions

Industrial effluent was kindly provided by OR Barkan Company, a leather processing industry (Barkan Industrial Estate, Barkan, Israel). The Cr content of the effluent was
determined by standard methods (Standard Methods, 1995). The total Cr content was 9 ppm (8 ppm of Cr(VI) and 1 ppm of Cr(III)). These levels are much higher than the permissible limits of 0.1 ppm for Cr(VI) and 0.25 ppm for Cr(III) (see below). The chemical oxygen demand (COD) of the effluent was determined by the open reflux method and biological oxygen demand (BOD) by the 5-day BOD test with seeding the dilution water with domestic wastewater (Standard Methods, 1995). COD of the effluent was 120 mg/l, which is much lower than the permissible limit for pretreated industrial discharges (2000 mg/l). BOD was 30 mg/l, which is close to the standard for treated domestic wastewater (20 mg/l). All the limits quoted are those published by the Israel Ministry of Environment (1991,1995) or the Israel Ministry of Health (1992).

From this effluent several bacterial colonies were isolated on nutrient agar supplemented with 100 ppm of Cr(VI). Cells were thereafter maintained on nutrient agar or grown in Luria broth (LB) liquid medium with Cr(VI) added as CrO$_4^{2-}$. One of the resulting colonies was further trained by successive culture on LB containing increasing concentration of Cr(VI) ranging from 100 to 2000 ppm. The isolate was identified as *Staphylococcus cohnii* using the MicroScan System (Walkaway 96 model, Dade-Behring, Sacramento, USA).

Cells were grown in 250 ml flask containing 100 ml LB (tryptone, 10g/L; yeast extract, 5 g/L; NaCl 10 g/l; pH 7.2) at 37ºC with shaking at 200 rpm. Growth of bacterial cells was followed spectrometrically by absorbence at 600 nm. The cells were harvested during the logarithmic phase (usually after 20 hr) by centrifugation at 4000 g for 10 min at 4ºC and washed twice in distilled water.

**Bioaccumulation of chromium**

Two types of experiments were performed. The first type was designed to provide quantitative data on the bioaccumulation of Cr(VI) by *Staph. cohnii* in standard LB medium to which was added varying concentrations of Cr(VI). In other experiments the ability of the bacteria to accumulate Cr from the tannery effluent was determined.

For experiments with standard medium 250 ml Erlenmeyer flasks were prepared with 100 ml of sterile LB. Various volumes of a Cr(VI) stock solution of 100mg/ml (prepared by dissolving K$_2$CrO$_4$ in 100 ml double distilled sterile water) were added to each flask to give initial Cr(VI) concentration value ranges from 0 to 1000 ppm and 2% v/v of exponential growing culture was then added. All flasks were incubated at 37ºC on an orbital shaker at 200 rpm. Samples were removed at 6, 12, 18, 24, 48, 72 and 96 hr, centrifuged and supernatants analyzed for remaining chromium concentration. Analysis of Cr(VI) and Cr(III) in supernatant of the culture medium were carried out by colorimetric methods (Standard Methods, 1995).

For confirmation of bioaccumulation, intracellular chromium was determined. *Staph. cohnii* biomass was obtained after a 96 hr incubation in either 100 ml LB alone or LB containing 100 ppm of Cr(VI). Cells were isolated by centrifugation at 4000 g for 10 min at 4ºC, washed twice and dried at 105ºC until constant weight. The dried biomass was digested with 5 ml of nitric acid and brought to final volume of 10 ml (Standard Methods, 1992). Total chromium was determined by atomic absorption flame mode (Model 5000, Perkin Elmer, USA) and calculated against standard solutions of chromium (Merck).

For experiments with the tannery wastewater, 90 ml of leather effluent was inoculated with 2% v/v exponential growing culture of *Staph. cohnii* and 10 ml of sterile LB. The LB was added due to the low content of organic material present in the effluent (BOD=30 mg/ml). Samples were removed after 6, 12, 18, 24, 48, 72 and 96 hr incubation and the chromium concentration determined colorimetrically.
Results and discussion

Since the initial report of chromate and dichromate reduction from industrial wastewater by *Pseudomonas dechromaticans* (Romanenko and Korenkov, 1977) the list of microbial strains reported to accumulate, resist and/or reduce hexavalent Cr either aerobically or anaerobically has grown. This list now includes additional strains of *Pseudomonas* (Horitsu et al., 1987; Cervantes et al., 1990; De Leo and Ehrlich, 1994), *Enterobacter* (Wang et al., 1989), *Alcaligenes* (Niels et al., 1989), *Escherichia*, *Micrococcus* and *Bacillus* (Gvozdyak et al., 1986; Fujie et al., 1994; Shen and Wang, 1994) as well as mixed culture populations (Fude et al., 1994; Turick et al., 1996; Bader et al., 1999). A critical comparison of the Cr-related activities of these and other organisms has recently been published (Chen and Hao, 1998). We are not aware of the previous isolation of a *Staph. cohnii* with the ability to both accumulate and reduce Cr(VI) to Cr(III). This organism was isolated from a leather industry effluent contaminated with 8 ppm Cr(VI).

Figure 1 shows a typical growth curve for *Staph. cohnii* and the corresponding decrease in chromium content from an initial Cr(VI) concentration of 1000 ppm. A high initial concentration of Cr was used intentionally in order to stress the resistance of the organism and its ability to remove the heavy metal. A maximum decrease of 75% in Cr(VI) content was achieved after 96 hr growth. Similar experiments using an initial Cr(VI) content of 100 ppm
showed a maximum decrease of total Cr of about 90% after 96 hr growth (Table 1 and Figure 2). The ability of bacteria to tolerate high concentrations of Cr(VI) was also reported by Fude et al. (1994) and Badet et al. (1999), although both studies employed a mixture of organisms making a meaningful comparison with the *Staph. cohnii* isolate difficult.

The *Staph. cohnii* isolate was then used to treat an industrial effluent containing 9 ppm chromium (8 ppm Cr(VI) and 1 ppm Cr(III)). A 2% (v/v) exponential culture was added to the effluent and samples removed for analysis at different times. The time course of Cr(VI) removal was of similar shape to that depicted in Figure 1. After 96 hrs, about 90% of Cr(VI) had been removed.

A qualitative analysis of Figure 2 allows consideration of Cr(VI) removal kinetics in more detail. A feature of the removal kinetics is the appearance of a reproducible shoulder in the log-phase of growth corresponding to a retardation of the removal process at this time. This is followed by a period of rapid removal and then a final, slower phase. This means that the rate of the Cr(VI) removal has a maximum and a minimum. The retardation is due to an initial, sharp decrease in Cr(VI) concentration resulting from its accumulation in the cells. Such behavior is due to the use of a growing biomass. The use of constant biomass would result in a monotonic rate decrease. The subsequent increase in removal rate is related to an increase in growth rate during the log phase (Figure 1). Thus the rate of Cr(VI) removal is proportional to the product of Cr(VI) concentration and the cell concentration. In principle, the presence of the noted extremes on the rate curve would allow derivation of the rate constant for Cr(VI) removal and its relation to the corresponding constant for cell growth. This will require more detailed measurements of growth kinetics.

The reduction of Cr(VI) to Cr(III) was observed (Table 1). Cr(III) was first measurable in the medium after 24 hr growth. After 96 hr the Cr(III) level had reached about 10% of the initial Cr(VI) content. As Cr(VI) is a known toxin and mutagen (Codina et al., 1995), it is probable that intracellular chromium only accumulates in the form of Cr(III). The appearance of Cr(III) in the culture medium would therefore be due to its release from the cells.

The bioaccumulation of chromium was confirmed by atomic absorption analysis of the biomass. The dry biomass obtained from a 96 hr culture grown in the presence of 100 ppm Cr(VI) contained 3.5 mg Cr/g cells, while a comparable biomass obtained from cells grown in control medium alone contained only 0.015 mg Cr/g cells.

Previous studies in other bacterial and yeast strains suggest an inverse relationship between Cr(VI) resistance and intracellular accumulation (Baldi et al., 1990; Cen and Hao, 1998). This might be due to both accelerated efflux of the hexavalent ion (Cervantes and

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**Table 1** Removal of Cr(VI) over time by *Staphylococcus cohnii* from standard medium contaminated with 100 ppm Cr(VI) and subsequent appearance of Cr(III)

<table>
<thead>
<tr>
<th>Incubation time (hr)</th>
<th>Total Cr (ppm)</th>
<th>Cr (VI) (ppm)</th>
<th>Cr (III) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>82.0</td>
<td>82.0</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>77.8</td>
<td>77.7</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>56.3</td>
<td>56.1</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>47.5</td>
<td>46.3</td>
<td>1.2</td>
</tr>
<tr>
<td>48</td>
<td>23.2</td>
<td>19.6</td>
<td>3.6</td>
</tr>
<tr>
<td>72</td>
<td>14.8</td>
<td>8.8</td>
<td>6.0</td>
</tr>
<tr>
<td>96</td>
<td>11.4</td>
<td>1.6</td>
<td>9.8</td>
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</table>
Silver, 1992) or decreased membrane passage with simultaneous reduction of Cr(VI) to Cr(III) (Horitsu et al., 1983). There is also no necessary link between Cr(VI) resistance and the ability of an organism to reduce this ion to Cr(III) (Silver and Phueng, 1996). Despite the isolation of a membrane-associated protein postulated to be responsible for the outward translocation of Cr(VI) (Cervantes and Silver, 1992) the molecular mechanisms of Cr(VI) accumulation, resistance and reduction are still not clearly understood. Our results, which demonstrate that Staph. cohnii is resistant to high concentrations of Cr(VI), can accumulate Cr intracellularly and may reduce it to Cr(III) suggest that this new isolate might process Cr(VI) by an alternate mechanism.

Conclusions
1. We have isolated a new strain of Staphylococcus cohnii from an industrial effluent which is resistant to high concentrations of Cr(VI) and it able to bioaccumulate Cr.
2. The isolate can remove 75–90% Cr(VI) from contaminated standard medium or tannery effluent, depending on initial Cr(VI) concentration.
3. The isolate reduces Cr(VI) to Cr(III).
4. The kinetics of Cr(VI) removal rate under conditions of growing biomass has characteristic features of maximum and minimum.

References


