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ORIGINAL ARTICLE

Bioreduction of hexavalent chromium by *Pseudomonas stutzeri* L1 and *Acinetobacter baumannii* L2

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Abstract Bioreduction of the very toxic hexavalent chromium ion [Cr(VI)] to the non-toxic trivalent chromium ion [Cr(III)] is a key remediation process in chromium-contaminated sites. In this study, we investigated the bioreduction of Cr(VI) by *Pseudomonas stutzeri* L1 and *Acinetobacter baumannii* L2. The optimum pH (5–10), temperature (27, 37 and 60 °C) and initial chromium Cr(VI) concentration (100–1000 mg L^{-1}) for Cr(VI) reduction by strains L1 and L2 were determined using the diphenylcarbazide method. In the presence of L1 and L2, the bioreduction rate of Cr(VI) was 40–97 and 84–99%, respectively. The bioreduction of Cr(VI) by L2 was higher, reaching up to 84%—than that by L1. The results showed that strain L2 was able to survive even if exposed to 1000 mg L^{-1} of Cr(VI) and that this tolerance to the effects of Cr(VI) was linked to the activity of soluble enzyme fractions. Overall, *A. baumannii* L2 would appear to be a potent Cr(VI)-tolerant candidate for the bioremediation of chromium (VI)-contaminated wastewater effluent.

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Introduction

Chromium (Cr) is an important heavy metal that is used in various industries, including textile dyes, leather tanning, paint and pigment manufacturing, electroplating and metal processing industries (Thacker et al. 2006; Sayel et al. 2012; Adki et al. 2013). The unprocessed chromium (VI) from wastewater effluent is a source of severe environmental pollution (Soni et al. 2013). The valence state of chromium in nature ranges from Cr(II) to Cr(VI), but this metal is mainly found in the hexavalent [Cr(VI)] and trivalent states (Gupta and Rastogi 2009). Hexavalent chromium is toxic, mutagenic and carcinogenic (Garbisu et al. 1998; McLean and Beveridge 2001; Coata 2003), and research has shown that exposure to hexavalent chromium increases the risk of human lung cancer (Cheung and Gu 2003); in comparison to hexavalent chromium, trivalent chromium is much less toxic (Viti et al. 2003). The removal of hexavalent chromium from the environment by reduction or absorption can reduce the risks for human health and the environment. The removal of hexavalent chromium by the reduction of Cr(VI) to Cr(III) can be done by physico-chemical methods or biological methods. The physico-chemical methods include chemical precipitation, reduction, filtration, ion exchange, reverse osmosis, membrane technologies, electrochemical treatment and evaporation recovery (Ahluwalia and Goyal 2007). Generally, these processes are quite expensive and not always effective (Nourbakhsh et al. 1994). Therefore, there is a need to develop novel, low-cost and eco-friendly tools for chromium (VI) removal from wastewater.

Various biological methods have been reported for toxic heavy metal remediation, including bacteria-, fungi-, algae- and yeast-mediated systems (Naja et al. 2005). Several bacterial species have the ability to resist the toxicity of chromium through different mechanisms (Naja et al. 2005). For example, *Pseudomonas olevorans*, *Bacillus cereus*, *Bacillus circulans*, *Acinetobacter* sp., *Cellulomonas* sp., *Bacillus pumilis*, *Cellulosimicrobium cellulans*, *Exiguobacterium* and *Streptococcus* sp. have been reported to be able to convert the toxic Cr(VI) to the less toxic Cr(III) form, which can be recovered and reused further (Mistry et al. 2009; Sundar et al. 2010; Chaturvedi 2011; Essahale et al. 2012; Field et al. 2013; Rehman and Faisal 2015; Wani et al. 2015). However, these bacteria require a long time to convert Cr(VI) to Cr(III). The actual average concentration of chromium in soil is <500 mg kg^{-1} while the levels in industrial wastewater ranges from 0.5 to 270 mg L^{-1} (MINAS-CPCB 2011). Ferdouse et al. (2016) reported that *Bacillus cereus* was tolerant up to 500 mg L^{-1} of chromium (VI) in solution. Various factors influence the reduction of Cr(VI), including pH, temperature and concentration of Cr(VI) (Dey et al. 2014). Here, we tested the effect of pH (5–10), temperature (27, 37, and 60 °C) and Cr concentration (100–1000 mg L^{-1}) on bacteria-mediated Cr(VI) reduction. There have been reports on the reduction of Cr(VI) by bacteria at neutral pH, and a limited number of such studies have been carried out under alkaline conditions (Ye et al. 2004; Stewart et al. 2007). The pH is a key factor for the efficient bioreduction of Cr(VI) due to the high alkalinity of contaminated soil (Kamaludeen et al. 2003; Van Engelen et al. 2008). It has also been shown that gram-positive bacteria have a lower efficiency to bioreduce Cr(VI) bioreduction than Gram-negative bacteria. In this study, two bacterial species, *Pseudomonas stutzeri* L1 and *Acinetobacter baumannii* L2, were successfully tested for their ability to reduce Cr(VI) in a short period of time. We also evaluated these two chromium (VI)-resistant bacteria, *P. stutzeri* L1 and *A. baumannii* L2, for their ability to reduce Cr(VI) to Cr(III) under different pH and temperature conditions.

Materials and methods

Sample collection

Crude oil samples were collected directly from the well head of the oil reservoir at Karaikal, Tamil Nadu, India (latitude 10.7694°N, longitude 79.6155°E). The samples were collected in sterile screw capped vials, placed into an icebox and transported from the collection site to the laboratory. Samples were stored at 4 °C until analysis. For bioreactor assays, tannery effluent was collected from the Common Effluent Treatment Plant located at the SIPCOT Industrial Complex, Ranipet, Vellore (latitude 12.9320°N, longitude 79.3334°E). The tannery effluent contains 96 mg L^{-1} Cr(VI).

Isolation and identification of bacteria by 16S rDNA sequencing

To isolate bacteria from the crude oil samples we used a pour plate technique and nutrient agar plates (Hi Media Laboratories Pvt. Ltd., Mumbai, India). The plates were incubated at 37 °C for 24 h. Colonies were streaked and restreaked frequently until individual cultures were obtained. The bacterial strains were identified by 16S rDNA gene sequencing. The genomic DNA analysis of the isolated bacterial strains was conducted using The Genomic DNA Extraction Mini-kit (Hi Media) according to the manufacturer's instructions. The eluted genomic DNA was subjected to PCR amplification by 16S rRNA gene sequencing using universal primers specific for the 16S rRNA gene: forward primer B27F (5′-AGAG TTTGATCCTGGCTCAG-3′) and reverse primer (U1492R 5′-GGTTACCTTGTTACGACTT-3′). The amplifications were performed in a thermal cycler (Eppendorf, Hamburg, Germany) programmed for an initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 2 min, with a final extension step at 72 °C for 10 min. Sequencing was performed using the Big Dye Terminator Cycle Sequencing kit (Applied BioSystems, Thermo Fisher Scientific Corp. Waltham, MA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system.

The sequences of bacterial isolates after sequencing were analysed by using the online NCBI BLAST tool program http://www.ncbi.nib.gov/blast. Phylogenetic analysis was used for comparative genomics to show evolutionary relationships. The analysis was carried out using CLUSTAL W version 2.0 and, after alignment, the phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA v 5.05) software. The evolutionary history/phylogenetic analysis or relationship was inferred using the neighbor-joining method (Saitou and Nei 1987).

Bioreduction optimization of Cr(VI) to Cr(III)

The bioreduction of Cr(VI) to Cr(III) by isolates L1 and L2 was optimized testing the reduction reaction the effect of various pH levels, temperatures and chromium (VI) concentrations. In the pH studies, a set of six sterile nutrient broth flasks were prepared with 100 mg L^{-1} chromium ($K_2Cr_2O_7$), and the pH in each flask was adjusted to pH 5.0, 6.0, 7.0, 8.0, 9.0 or 10. The flasks were then inoculated with 23×10^4 CFU/ml individual bacterial cultures of L1 and L2 and them incubated aerobically at 37 °C in an orbital shaker at 150 rpm for 24 h. The culture was sampled every 4 h for the measurement of

Cr(VI) reduction. The effects of various temperatures on Cr(VI) reduction were studied using the same experimental set-up described for the pH studies, only at pH 7 and incubation at different temperatures (i.e. 27, 37 and 60 °C). The medium was withdrawn after 24 h of incubation and Cr(VI) reduction measured. The toxicity effect of Cr concentration on the ability of L1 and L2 to reduce Cr(VI) was determined using the same set-up described for the pH studies, only at pH 7 and 37 °C and at chromium (VI) concentrations of 100, 200, 300, 400, 500 and 1000 mg L^{-1}. The culture was sampled every 4 h for the measurement of Cr(VI) reduction.

Scale-up testing was carried out in a 5-L Winpact Air Uplift Bioreactor (AUBR) (Major Science, Saratoga, CA) in a batch administration for 24 h of continuous operation. We added 4.95 L of the tannery effluent with 1 % of nutrient broth to the reactor and 50 ml (1 × 104 cells/ml) of the enriched individual *P. stutzeri* and L2 cultures were inoculated to the reactor. The temperature and pH were maintained at 37 °C and 7, respectively. A constant pH 7 was maintained with the addition of concentrated H_2SO_4, and the oxygen supply was 150 L/h. The sample was withdrawn every 4 h for the measurement of Cr(VI) reduction.

Table 1 Biochemical characterization of *Pseudomonas stutzeri* and *Acinetobacter baumannii* isolated from crude oil

Characteristics	L1	L2
Gram stain	–	–
Colony shape	Circular	Oval
Motility test	+	–
Pigmentation	Yellow	Cream
Cell shape	Rod	Rod
Spore stain	–	–
Indole	–	–
Methyl red test	–	–
Voges-Proskauer	–	–
Citrate	+	+
Urease	–	–
H_2S	–	–
Gelatin hydrolysis	+	+
Oxidase	–	–
Catalase	+	+
Glucose	+	+
Sucrose	–	–
Lactose	–	–
Xylose	+	+
Mannitol	–	–

L1, *Pseudomonas stutzeri* strain, L2, *Acinetobacter baumannii* strain; +, positive response; –, negative response

Estimation of Cr(VI)

The Cr(VI) concentration in the samples was quantified by the diphenylcarbazide method (Thacker et al. 2006). The samples obtained from the culture were centrifuged (6000 *g*, 20 min) to determine the Cr(VI) concentration from the culture supernatant. The reaction mixture contained 200 μl of sample, 800 μl of distilled water to which 330 μl of 6 M sulphuric acid was added and 400 μl of diphenylcarbazide solution; distilled water was added to reach a reaction volume of 10 ml. Diphenylcarbazide reacted with Cr(VI), leading to the formation of a purple color. Absorbance was measured at 540 nm using a UV-spectrophotometer (Double Beam UV–VIS Spectrophotometer SL 210; Elico Ltd, Hyderabad, India).

Permeabilized cell assays

To study the permeabilization of bacterial cells, we first cultured L1 and L2 bacterial cells overnight in 200 ml nutrient broth. The cells were then collected by centrifugation (6000 *g*, 20 min, 4 °C), washed by 10 mM Tris–HCl buffer (pH 7.2) and suspended in the same buffer. Toluene (0.1 ml) and Triton X-100 (0.2 %, v/v) were added to 9.9 ml of cell suspension and the suspension vortexed to obtain permeabilized cells. Chromium (VI) was added at 10 mg L^{-1} and the cells incubated at 37 °C with shaking for 6 h. The control consisted of permeabilized bacterial cells that were heated at 100 °C for 30 min. Following the incubation period, the cells were centrifuged (6000 *g*, 20 min, 4 °C) and the Cr(VI) reduction was quantified from the culture supernatant. All assays were carried out in triplicate.

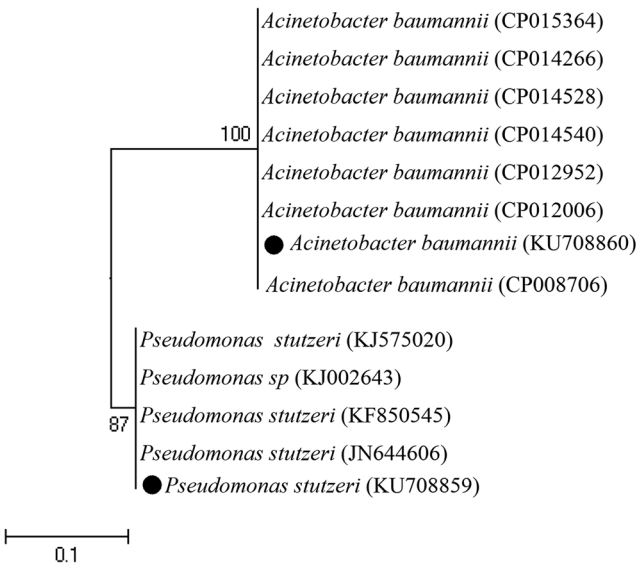

Fig. 1 Phylogenic tree of 16S rDNA gene sequence of *Pseudomonas stutzeri* L1 and *Acinetobacter baumannii* L2

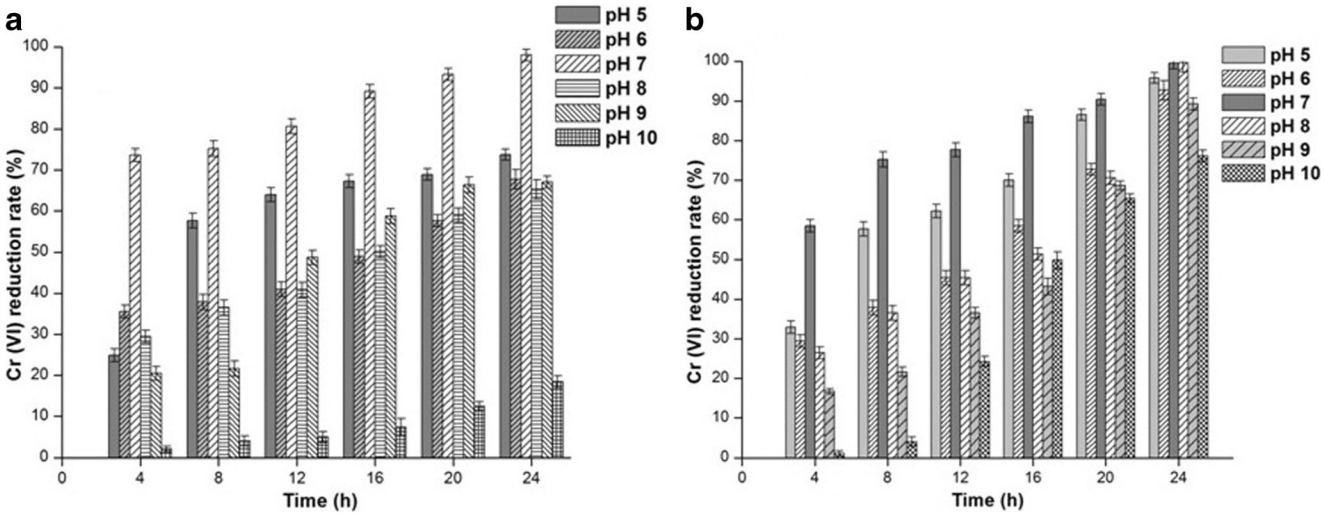

Fig. 2 Effect of pH on reduction of the hexavalent chromium ion [*Cr(VI)*] to the less toxic trivalent chromium ion [Cr(III)] by *Pseudomonas stutzeri* L1 (**a**) and *Acinetobacter baumannii* L2 (**b**) Error bars indicate the standard deviations of 3 measurements

Cell-free assay

To prepare the crude extract, the L1 and L2 bacterial cultures were first grown in 200 ml nutrient broth containing 100 mg L^{-1} chromium (VI) at 37 °C for 24 h. Bacterial cells were collected by centrifugation (6000 *g*, 15 min), washed with 10 mM Tris HCl buffer (pH 7.2) and then suspended in 3 ml of the same buffer. Bacterial cells were disrupted by sonication for 5 min. The bacterial suspension was centrifuged (8000 rpm, 30 min, 4 °C) and the supernatant collected. The culture supernatant was used as a crude extract to test Cr(VI) reduction. The supernatant with a chromium (VI) concentration of 100 mg L^{-1} for 12 h was heated at 100 °C for 30 min crude extracts used as control. After incubation, Cr(VI) reduction was determined using the diphenylcarbazide method.

Results

Isolation and identification of bacteria by 16S rDNA sequencing

Bacteria were isolated from crude oil samples. The total bacterial count was 41× 10^4 CFU/ml, and two different bacterial colonies, namely, *P. stutzeri* L1 and *A. baumannii* L2 were observed. lsolates L1 and L2 were subsequently investigated for their ability to reduce Cr(VI). The morphological and biochemical characteristics of these two strains are presented in Table 1. The bacterial isolates were amplified and sequenced by 16S rDNA gene sequencing. The amplified DNA sequences were analysed using NCBI database, and isolates L1 and L2 were identified as *P. stutzeri* and *A. baumannii*, respectively. The sequence similarity and phylogenetic tree were constructed and showed in Fig. 1. The nucleotide sequences have been deposited in GenBank under sequence accession numbers KU708859 and KU708860.

Effect of pH on Cr(VI) reduction by strains L1 and L2

The reduction of Cr(VI) by L1 and L2 was studied over different pH levels (pH 5–10) in medium initially amended with 100 mg L^{-1} nutrient broth and incubated at 37 °C. The optimum pH for L1 in terms of Cr(VI) reduction was observed to be pH 7, with 97% of the Cr(VI) reduced to Cr(III) after 24 h of incubation. In contrast, Cr(VI) reduction at pH 5, 6, 8, 9 and

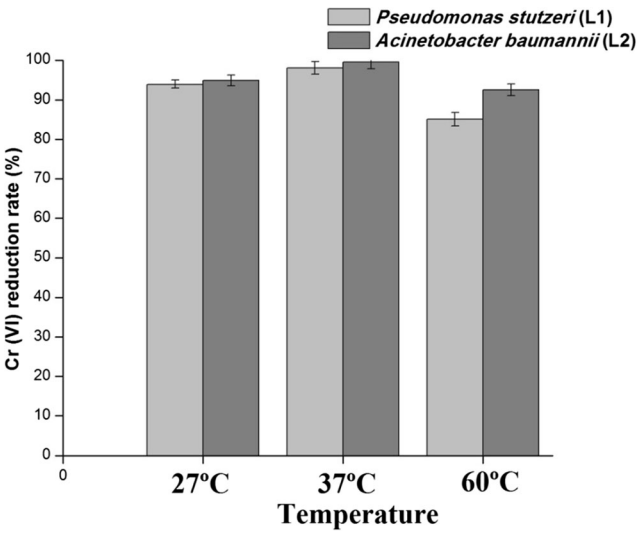

Fig. 3 Effect of temperature on Cr(VI) reduction by *Pseudomonas stutzeri* L1 and *Acinetobacter baumannii* L2, Error bars indicate the standard deviations of 3 measurements

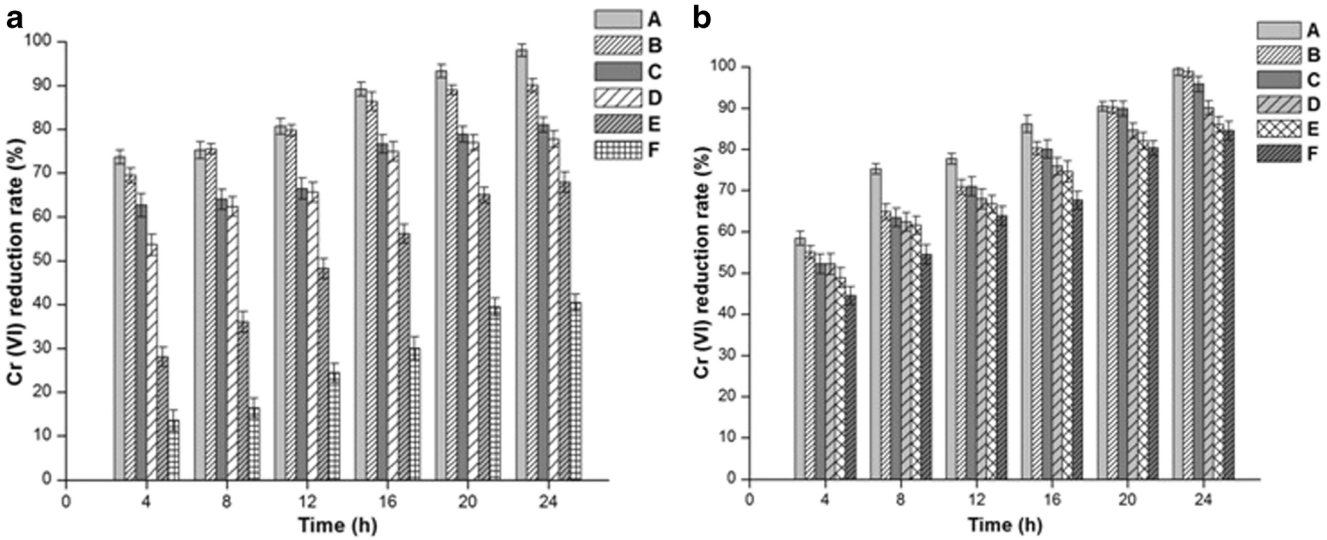

Fig. 4 Effect of chromium concentration on Cr(VI) reduction by *Pseudomonas stutzeri* L1 (**a**) and *Acinetobacter baumannii* L2 (**b**). *A* 100 mg Cr L^{-1}, *B* 200 mg Cr L^{-1}, *C* 300 mg Cr L^{-1}, *D* 400 mg Cr L^{-1}, *E* 500 mg Cr L^{-1}, *F* 1000 mg Cr L^{-1} Error bars indicate the standard deviations of 3 measurements

10 was 73, 67, 65, 67 and 18%, respectively (Fig. 2a). The optimum pH for L2 in terms of Cr(VI) reduction was observed to be pH 7 and 8, with 99.45 and 99.58% of the Cr(VI) reduced to Cr(III), respectively, after 24 h of incubation. In contrast, Cr(VI) reduction at pH 5, 6, 9 and 10 was 95, 92, 89 and 76 %, respectively, (Fig. 2b).

Effect of temperature on Cr(VI) reduction by strains L1 and L2

Three different temperatures (27, 37 and 60 °C) were studied for their effect on Cr(VI) reduction by L1 and L2. The optimum temperature for Cr(VI) reduction was found to be 37 °C for both L1 and L2. The maximum Cr(VI) reduction by L1 and L2, was 97 and 99 %, respectively, was observed after 24 h of incubation at 37 °C. In the presence of L1 and L2, Cr(VI) reduction was 93 and 94%, respectively, at 27 °C and 85 and 92%, respectively, at 60 °C (Fig. 3).

Effect of chromium concentration on Cr(VI) reduction by strains L1 and L2

The effect of the chromium (VI) concentration on Cr(VI) reduction by L1 and L2 was determined in nutrient broth at a constant pH and temperature of 7 and 37 °C, respectively. Cr(VI) reduction by L1 was 97, 90, 81, 77, 67 and 40% at chromium (VI) concentrations of 100, 200, 300, 500 and 1000 mg L^{-1}, respectively, after 24 h incubation (Fig. 4a). Cr(VI) reduction by L2 was 99, 98, 95, 90, 86 and 84 % at chromium (VI) concentrations of 100, 200, 300, 500 and 1000 mg L^{-1}, respectively (Fig. 4b). Cr(VI) reduction in tannery effluent was 98 and 99% by L1 and L2, respectively, after 24 h incubation (Fig. 5).

Permeabilized cell assay

The Cr(VI) levels were reduced by 27 and 43 % by L1 and L2, respectively, after 6 h of incubation in the permeabilized cell assay (toluene, (0.01 % v/v; Triton X-100 (0.2 % v/v). Bacterial reduction of Cr(VI) to Cr(III) is mediated by cell membrane-bound enzymes or the soluble enzyme fraction. Cr(VI) reduction activity was found in the bacterial culture supernatant. The chromium reductase activity in L1 and L2 was mostly linked to the soluble fraction of the enzyme.

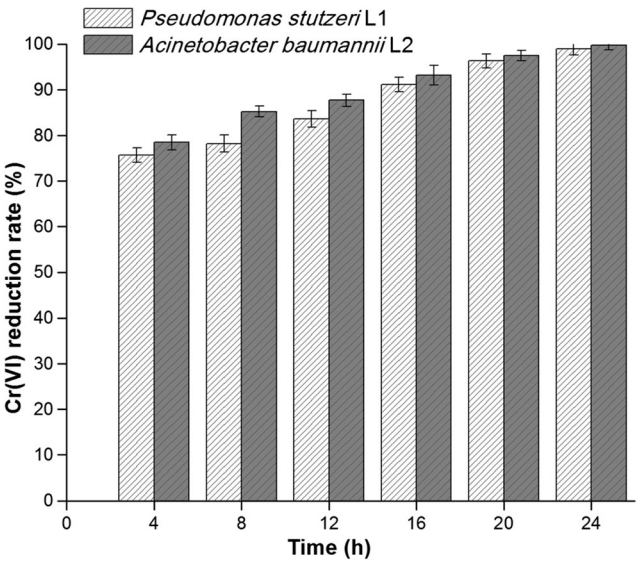

Fig. 5 Cr(VI) reduction in tannery effluent by *Pseudomonas stutzeri* L1 and *Acinetobacter baumannii* L2, Error bars indicate the standard deviations of 3 measurements

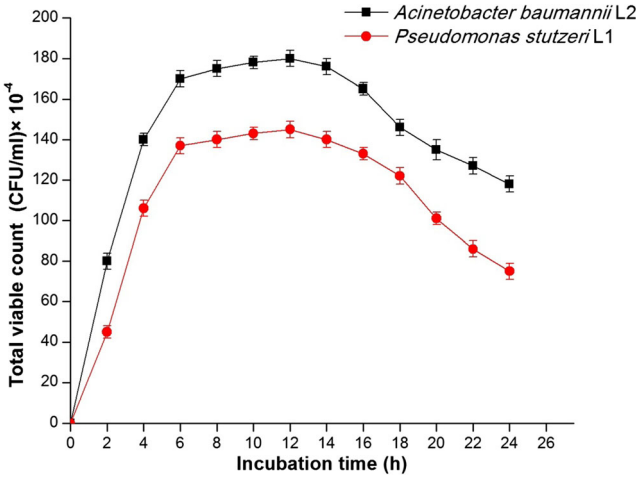

Fig. 6 Colony forming units (*CFU*) in *Pseudomonas stutzeri* L1 and *Acinetobacter baumannii* L2

Cell-free assay

The ability of crude extracts from the L1 and L2 cultures to reduce Cr(VI) to Cr(III) was tested. The cell-free extracts of L1 and L2 were treated with chromium (VI) concentrations of 100 mg L^{-1} and the Cr(VI) reduction rate checked after 12 h of incubation at 37 °C. The cell-free extract of L1 and L2 showed Cr(VI) reduction rates of 51 and 60 %, respectively.

Discussion

The hexavalent chromium ion [Cr(VI)] is a very toxic and carcinogenic heavy metal that poses risks and hazards to humans and the environment alike (Zayed and Terry 2003). A possible alternative method to the conventional chemical methods for Cr(VI) reduction is to reduce Cr(VI) to Cr(III) by biological systems (Mukherjee et al. 2015). In the study reported here, we focused on the Cr(VI) reduction ability of two bacteria, *P. stutzeri* L1 and *A. baumannii* L2. The optimum pH and temperature for Cr(VI) by these bacteria were determined, as were the effects of chromium (VI) concentration. Our results showed maximum Cr(VI) reduction by L1 and L2 at pH 7 and pH 7 and 8, respectively. L2 can reduce the Cr(VI) level by 95 and 76 % at pH 5 and pH 10, respectively, after 24 h incubation. As control, the pH was measured at the end of each experiment, and no changes in pH were observed in the medium. L2 was found to be able to grow and reduce Cr(VI) under both acidic and alkaline conditions and can thus be used under both conditions to reduce Cr(VI). Umesh et al. (2012) also reported that pH 7 was the optimum pH for Cr(VI) reduction by *Bacillus cereus* IST 105 isolated from electroplating effluent. Also, *Bacillus pumilis*, *Cellulosimicrobium cellulans* and *Staphylococcus capitis* showed maximum Cr(VI) reduction at pH 7 (Zahoor and Rehman 2009; Rehman and Faisal 2015).

Temperature is also an important factor for bacterial growth and Cr(VI) reduction. *Pseudomonas stutzeri* L1 and *A. baumannii* L2 showed the maximum Cr(VI) reduction at 37 °C after 24 h of incubation. *Bacillus* sp. and *Staphylococcus capitis* were found to have maximum growth and maximum chromium (VI) reduction at 37 °C (Zahoor and Rehman 2009), and *Bacillus pumilis* and *Cellulosimicrobium cellulans* showed maximum Cr(VI) reduction at 37 °C (Rehman and Faisal 2015). *Acinetobacter* was found to have maximum growth at 30 and 37 °C, while incubation at 40 °C severely affected its growth and ability to reduce chromium (VI) (Essahale et al. 2012). No chromium (VI) reduction was observed at 60 °C by

Table 2 Optimization of hexavalent chromium ion bioreduction by *Pseudomonas stutzeri* L1 and *Acinetobacter baumannii* L2: analysis of variance

Treatment	*Pseudomonas stutzeri* L1			*Acinetobacter baumannii* L2		
	F value	*df*	*P*	*F* value	*df*	*P*
pH 5	389, 78	5	<0.01	590, 28	5	<0.01
pH 6	142, 40	5	<0.01	499, 78	5	<0.01
pH 7	110, 62	5	<0.01	224, 25	5	<0.01
pH 8	169, 71	5	<0.01	628, 41	5	<0.01
pH 9	441, 71	5	<0.01	1232, 46	5	<0.01
pH 10	58, 91	5	<0.01	1579, 66	5	<0.01
Temperature	60, 87	2	<0.01	16, 65	2	<0.01
Cr(VI) 100 mg L^{-1}	110, 62	5	<0.01	261, 22	5	<0.01
Cr(VI) 200 mg L^{-1}	87, 48	5	<0.01	295, 81	5	<0.01
Cr(VI) 300 mg L^{-1}	542, 01	5	<0.01	176, 03	5	<0.01
Cr(VI) 400 mg L^{-1}	60, 90	5	<0.01	127, 49	5	<0.01
Cr(VI) 500 mg L^{-1}	155, 23	5	<0.01	116, 50	5	<0.01
Cr(VI) 1000 mg L^{-1}	78, 80	5	<0.01	141, 60	5	<0.01

Cr(VI), Hexavalent chromium ion

Wang et al. (1990). In our study, *P. stutzeri* L1 and *A. baumannii* L2 displayed maximum Cr(VI) reduction at 37 °C. We noted that *P. stutzeri* L1 and *A. baumannii* L2 can grow and reduce Cr(VI) at 60 °C since both of them were thermotolerant. The optimum temperature for L1 and L2 was 37 °C. The effect of initial chromium (VI) concentration on the Cr(VI) reducing ability of *P. stutzeri* L1 and *A. baumannii* L2 was studied, with the results showing that L1 had maximum growth and maximum Cr(VI) reduction up to 500 mg chromium L^{-1} and that L2 had maximum growth and maximum Cr(VI) reduction up to 1000 mg L^{-1}. In our study, 100 mg L^{-1} of Cr(VI) was reduced to 97 and 99 % by *P. stutzeri* L1 and *A. baumannii* L2, respectively, within 24 h of incubation. In other studies, *Bacillus* sp. reduced the chromium (VI) level by 54% after 24 h of incubation, at an initial chromium (VI) concentration of 100 mg L^{-1} (Masood and Malik 2011). *Bacillus sphaericus* reduced the Cr(VI) concentration by 72% after 24 h (Pal et al. 2005). *Bacillus* sp. and *S. capitis* reduced Cr(VI) at a concentration of 100 μg/ml by 40 and 29%, respectively, after 24 h of incubation (Zahoor and Rehman 2009).

In our assays, the Cr(VI) concentration by L1 and L2 was reduced by 27 and 43%, respectively, after 6 h of incubation at 37 °C while the bacterial cells were permeabilized using toluene (0.01% v/v) and Triton X-100 (0.2% v/v). Bacterial reduction of Cr(VI) to Cr(III) is mediated by cell membrane-bound enzymes or the soluble fraction of enzymes. Megharaj et al. (2003) and Thacker et al. (2006) showed that the bacterial reduction of Cr(VI) to Cr(III) is mediated by soluble proteins from the bacterial cell membrane, with chromium reductase activity mostly due to the soluble fraction of the cell. In our study the bacterial cell crude extracts of L1 and L2 strains reduced the Cr(VI) by 51 and 60%, respectively, at a chromium (VI) concentration of 100 mg L^{-1}. Ganguli and Tripathi (2001) reported that *Pseudomonas aeruginosa* completely reduced 10 μg/ml of chromium (VI) within 2 h. In another study, *Bacillus* sp. and *S. capiitis* were found to reduce 100 μg/ml of chromium (VI) by 30 and 28%, respectively (Zahoor and Rehman 2009). In our study, the crude extracts of *P. stutzeri* L1 and *A. baumannii* L2 had soluble fractions with enzymes that were mainly responsible for Cr(VI) reduction. Gram-positive bacteria are more tolerant to chromium (VI) than Gram-negative bacteria (Ross et al. 1981; Viti and Giovannetti 2001, 2005). However, in our study the Gram-negative *P. stutzeri* L1 and *A. baumannii* L2 also showed a high ability to reduce Cr((VI) to Cr(III). The total viable count (expressed in CFU/ml) of L1 and L2 at optimized conditions is given in Fig. 6. The analysis of variance results are presented in Table 2. Gram-positive bacteria are less resistance to chromium (VI) than Gram-negative bacteria due to the physiology of the individual isolates (Agostinho et al. 2012). Zahoor and Rehman (2009) and Thacker et al. (2007) reported that Gram-negative bacteria *S. capitis* and *Brucella* sp. could efficiently reduce Cr(VI) and confirmed that the Cr(VI) reduction was due to the cell membrane-bound or soluble fraction of enzyme.

Conclusions

In this study, two potent Cr(VI)-reducing bacteria, *P. stutzeri* L1 and *A. baumannii* L2, were isolated from crude oil samples. Biomolecular identification of L1 and L2 by 16S rDNA gene sequencing confirmed their identity as *P. stutzeri* L1 and *A. baumannii* L2, and their accession numbers were KU708859 and KU708860. The optimization of Cr(VI) reduction by L1 and L2 was evaluated using different pH, temperatures and Cr(VI) concentrations. The possible mechanism of Cr(VI) reduction by L1 and L2 was identified as the soluble fractions of the enzymes responsible for the higher efficiency of bioreduction of Cr(VI). Overall, the results confirm that *P. stutzeri* L1 and *A. baumannii* L2 have the ability to reduce the toxic Cr(VI) to the relatively non-toxic Cr(III) under an optimum pH and temperature of 7 and 37 °C, respectively. At optimized conditions the reduction of Cr(VI) by L1 and L2 in tannery effluent in the bioreactor was 98 and 99%, respectively. The application of the bioreduction process is an effective, cheap and eco-friendly technique for achieving Cr(VI) reduction. Hence, our results reveal that *P. stutzeri* L1 and *A. baumannii* L2 can be used for the bioremediation of wastewater effluent containing hexavalent chromium.

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Compliance with ethical standards

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that they have no conflict of interest.

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