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Full Length Research Paper

Hexavalent chromium reduction by novel chromate resistant alkaliphilic *Bacillus* sp. strain KSUCr9a

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Alkaliphilic bacterial strain termed KSUCr9a was isolated from soil and water samples collected from various soda lakes located in northern Egypt. KSUCr9a was tolerance up to 75 mM Cr (VI), with minimum inhibition concentration (MIC) value of 80 mM, in alkaline medium (pH 10.5) containing 10% NaCl. Analysis of 16S rDNA of strain KSUCr9a identified this bacterial strain as *Bacillus* sp., with sequence similarity of 99%, and was referred to as *Bacillus* sp. strain KSUCr9a. In addition to its tolerance to Cr(VI), *Bacillus* sp. KSUCr9a showed high resistance to other heavy metals including Cd^{2+} (50 mM), Mo^{2+} (75 mM), Mn^{2+} (100 mM), Cu^{2+} (2 mM), Ni^{2+} (100 mM), Pb^{2+} (75 mM), Co^{2+} (5 mM) and Zn^{2+} (2 mM). *Bacillus* sp. KSUCr9a demonstrated good chromate bio-reduction ability, as it could rapidly reduce up to 100 µM within 24 h. In addition, at initial Cr(VI) concentration of 200 µM, complete chromate reduction was achieved within 48 h. Furthermore, at initial Cr(VI) concentration of 300, 400 and 500 µM, 92.8, 75.5 and 39.8% of chromate reduction was achieved within 72 h. *Bacillus* sp. KSUCr9a was able to reduce Cr(VI) in a wide range of NaCl (0 to 20%), indicating the halotolerance nature of this alkaliphilic bacterial strain. Addition of glucose as an electron donor to the culture medium led to significant increase of both growth and chromate reduction by *Bacillus* sp. KSUCr9a. Maximum Cr(VI) reduction was exhibited in alkaline medium (pH 9) containing 0.8% glucose at incubation temperature of 35°C and under static culture condition. Under optimum Cr (VI) bioreduction conditions, 169.2 µM of Cr(VI) was completely reduced within 24 h, indicating a good ability of *Bacillus* sp. KSUCr9a of Cr(VI) detoxification under alkaline condition. Furthermore, Cr(VI)-reduction by *Bacillus* sp. KSUCr9a was slightly induced in the presence of other heavy metals, such as Mn^{2+}, Co^{2+}, Mo^{2+} and Cu^{2+} at concentration of 50 mg/L along with Cr(VI) in the culture medium. Moreover, *Bacillus* sp. KSUCr9a showed the ability of repeated bioreduction of chromate without any addition of exogenous nutrients, indicating its possible application in chromate detoxification.

Key words: Chromate reduction, bioremediation, heavy metals, *Bacillus* sp., soda lakes.

INTRODUCTION

Hexavalent chromium [Cr(VI)] is acutely toxic and is a known potent carcinogen. Cr(VI) has become a common environmental metal contaminant through the widespread application of Cr(VI) in various industries including electroplating, leather tanning, dye and pigment manufacturing, wood processing, textile dyeing and the steel and alloy industries, and photographic sensitizer manufacturing (Cheung and Gu, 2007). According to the United States Environmental Protection Agency (USEPA), industries in the United States of America (USA) use more than 50,000 tonnes of Cr(VI) every year and release 5000 kg/day into the environment (Poopal and Laxman, 2009). Disposal of these high levels of toxic chromates-containing industrial wastes without specific treatment, results in anthropogenic contamination of environments (Sarangi and Krishnan, 2008). Chromium is an essential micronutrient required for the growth of many organisms. However, at high concentrations, Cr(VI) is toxic, mutagenic, carcinogenic and teratogenic (Costa

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et al., 2006; Desai et al., 2008). Chromate can readily diffuse through cell membranes and their intracellular reduction results in chromate induced toxicity (Desai et al., 2008). Within the cell, Cr (VI) is partially reduced to highly unstable Cr(V) radical, leading to the formation of reactive oxygen species which are considered to be major causes of Cr(VI) toxicity and carcinogenesis (Sarangi and Krishnan, 2009; Opperman et al., 2008). The allowable concentration of Cr(VI) in drinking water is 0.05 mg L^{-1} (WHO, 1993). Thus, it is essential to reduce Cr(VI) concentrations from water/wastewater to the acceptable levels (WHO, 1993; Ozturk et al., 2009).

Cr(VI) undergoes reduction to less toxic Cr(III) in the presence of reducing agents and precipitates as hydroxides. Physicochemical processes for Cr(VI) removal from industrial effluent and contaminated environments have several limitations including high-energy inputs, different chemical treatments and generation of unnecessary sludge, reactive chemical species as secondary wastes, in addition to their high cost (Ahluwalia and Goyal, 2007; Zahoor and Rehman, 2009). Alternatively, bioremediation of toxic metals containing waste by bacteria is getting increased attention due to its efficient, affordable and environmentally friendly advantages (Ozturk et al., 2009; He et al., 2011). Biological reduction of chromate is based on the concept that Cr(III) has negligible toxicity and minimal solubility compared with Cr(VI). Chromate reducing ability has been demonstrated in several bacterial species including *Achromobacter* sp., *Shewanella* sp., *Pseudomonas* sp. and others (Guh et al., 2001; Pal et al., 2004; Puzon et al., 2005; Jimenez-Mejia et al., 2006; Thacker et al., 2006; Sarangi and Krishnan, 2008, 2007; Sultan and Hasnain, 2007). However, there are few reports about Cr (VI) bioreduction under alkaline conditions (Ye et al., 2004). Cr(VI) detoxification under alkaline conditions is important for many bioremediation processes as several Cr(VI)-containing industrial are alkaline effluents (Ye et al., 2004; Stewart et al., 2007). Therefore, alkaliphilic microorganisms are suitable candidates for bio-remediation processes under alkaline conditions, since their optimum growth are under high pH values (pH 9 to 11), in addition to its tolerance to high salt concentrations (Horikoshi, 1999, 2011). In this study, we report the isolation of chromate reducing alkaliphilic bacterium from Egyptian soda lakes and investigation of the effects of influences of various factors on the chromate bioremoval.

MATERIALS AND METHODS

Soil and water samples

Samples (soil and water) were collected in sterile tubes from the different locations of Wadi Natrun soda lakes, kept in refrigerator and were transferred to the laboratory (King Saud University, Saudi Arabia) within two weeks. Wadi Natrun valley is located in northern Egypt, extending in a northwest by southeast direction between latitudes 30° 15′ north and longitude 30° 30′ east. The bottom of the Wadi Natrun area is 23 m below sea-level and 38 m below the water-level of Rosetta branch of the Nile. The lowest part of the depression, encircled by contour zero, covers an area of about 272 km^2 (Taher, 1999).

Isolation of Cr (VI) resistant alkaliphilic bacteria

Isolation of Cr (VI) reducing alkaliphilic bacteria were performed using alkaline agar medium containing various concentrations of Cr (VI). The alkaline agar medium (pH 10.5) contained glucose (10 g/L; Sigma), yeast extract (5 g/L; Difco), casamino acids (5 g/L; Difco), peptone (5 g/L; Difco), NaCl (100 g/L), Na_2CO_3 (15 g/L), agar (15 g/L), 300 µL trace elements solution, and K_2CrO_7 (1 to 20 mM). The trace element solution contained: $CaCl_2.2H_2O$ (1.7 g/L), $FeSO_4.7H_2O$ (1.3 g/L), $MnCl_2.4H_2O$ (15.4 g/L), $ZnSO_4.7H_2O.7H_2O$ (0.25 g/L), H_3BO_3 (2.5 g/L), $CuSO_4.5H_2O$ (0.125 g/L), Na_2MoO_4 (0.125 g/L), $CoNO_3.6H_2O$ (0.23 g/L) and 2.5 ml 95 to 97% H_2SO_4. The collected soil samples were suspended in 50 mM glycine-NaOH buffer (pH 10) containing 10% NaCl, and serially diluted up to 10^{-5}. Aliquots (200 µL) of different soil dilutions and water samples were plated on the alkaline agar medium and incubated at 30°C for 3 to 7 days.

Identification of the isolated strains

The obtained bacterial colonies were sub-cultured several times in fresh agar media until single homogeneous colonies were obtained. For bacterial identification, the selected isolate was grown overnight in alkaline broth medium and total bacterial DNA was purified using DNeasy Blood and Tissue Kits (Qiagen) according to the manufacturer's instructions. Universal bacterial-specific forward primer 16F27 (5′-AGA GTT TGA TCC TGG CTC AG-3′) and reverse primer 16R1525 (5′-AAG GAG GTG ATC CAG CCG CA-3′) were used to amplify 16S rDNA gene (Lane, 1991). PCR amplification of 16S rDNA gene was carried out in a final volume of 25 µL, containing 12.5 µL of GoTaq® Green Master Mix (2X), (Promega), 0.5 µL of upstream primer (10 µM), 0.5 µL of downstream primer (10 µM), 2.5 µL DNA template (100 to 200 ng) and 9 µL of nuclease-free water. PCR reaction was run for 35 cycles in a DNA thermocycler under the following conditions: Initial denaturation at 95°C for 5 min, denaturation at 95°C for 1 min, primers annealing at 52°C for 1 min, extension at 72°C for 1.5 min and a final extension cycle for 10 min at 72°C. Amplified PCR 16S rDNA genes were purified using a QIAquick Gel Extraction Kit (Qiagen). 16S rDNA gene was submitted for sequencing (Research center, King Faisal Hospital, Riyadh, Saudi Arabia). The obtained DNA sequence was aligned with the known bacteria available nucleotide database (http://www.ncbi.nlm.nih.gov).

Tolerance to heavy metal ions

The minimum inhibitory concentrations (MICs), defined as the lowest metal concentrations that completely inhibit the growth of the selected bacterial strain was determined in the above alkaline agar medium. Different metal salts including Cr^{6+}, Pb^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Mo^{2+}, Ni^{2+} and Cd^{2+} were prepared in stock solutions and the cross metal resistance was checked by increasing the concentration of respective metal in a stepwise manner in the alkaline agar medium (pH 10.5). Alkaline agar medium supplemented with various concentrations of the metals was inoculated with 50 µL of overnight bacterial culture and incubated at 30°C for 3 to 5 days.

Optimization of growth and Cr(VI)-reduction conditions

Optimum growth and Cr(VI)-reduction conditions of the selected

strain were determined by investigation of the effects of several nutritional and environmental parameters. These included the effect of initial Cr(VI) concentration (10 to 300 mg/L), incubation temperature (25 to 50°C), pH (5 to 12), Na_2CO_3 concentration (0 to 2%), aeration level (shaking with 0 to 300 rpm), glucose concentration (0 to 2%) and NaCl concentration (0 to 20%). Sterile alkaline medium (100 ml) in culture flasks (250 ml) was supplemented with desired Cr(VI) concentration, inoculated from exponential phase bacterial culture and incubated at the appropriate temperature and shaking. Cell-free controls were also used in each experiment to monitor any abiotic Cr(VI)-reduction. Samples were drawn, under aseptic conditions, at defined times interval, centrifuged at 7000×*g* for 10 min and the supernatant analyzed for residual Cr(VI) using the standard diphenylcarbazide method (Thacker et al., 2007). Furthermore, the effects of other heavy metals including Co^{2+}, Mn^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}, Cu^{2+}, Mo^{2+}, and Ni^{2+} (with final concentration of 50 mg/L) on Cr(VI)- reduction by strain KSU9AC were also investigated. One hundred milliliters of alkaline medium in 250-culture flasks was supplemented with Cr(VI) to a final concentration of 300 µL together with other metals (50 mg/L) and incubated for 24 h at the optimum bioreduction conditions. All experiments were carried out in triplicate and the mean values were reported.

Repeated detoxification of Cr(VI)

Bacterial culture at the exponential phase (12 to 16 h old) in 100 ml sterile alkaline broth was supplemented with Cr(VI) to a final concentration of 50 mg/L and incubated at 35°C under gyratory shaking of 150 rpm. Two milliliters culture suspensions were withdrawn every 12 h of the incubation to measure Cr(VI) remaining as described below, and the culture flasks were repeatedly amended with increments of 100 µM Cr(VI) until saturation in Cr(VI) reduction was observed.

Chromium and bacterial growth analysis

For the determination of the bacterial growth, samples (1 ml) were drawn and centrifuged at 7000×*g* for 10 min at 8°C. The obtained cells pellets were resuspended in 1 ml of distilled water and the absorbance was measured at 600 nm against distilled water as blank and was reported as growth of the bacterium. The obtained culture supernatant after centrifugation was used for the measurement of the residual chromium concentration. The residual Cr (VI) was measured calorimetrically as the decrease in chromium concentration with time using hexavalent chromium specific colorimetric reagent (Thacker et al., 2007), 0.25% (w/v) 1,5-diphenylcarbazide (DPC) prepared in acetone (AR) to minimize deterioration. Supernatant (100 µL) was added to 10 ml of glass-distilled water in a test tube, followed by the addition of 1 ml of 1,5-diphenylcarbazide solution and 1 drop of H_3PO_4. The mixture was kept at room temperature for 10 min for color development and then optical density was measured at 540 nm. Calibration curve was made using of $K_2Cr_2O_7$ at concentration ranging from 10 to 300 µg/ml. All experiments were done in triplicate and the mean values were reported.

RESULTS AND DISCUSSION

Isolation of Cr (VI) resistant alkaliphilic bacteria

Cr(VI) resistant alkaliphilic bacteria were isolated from lakes (Wadi Natrun) located in Wadi Natrun area, northern Egypt, using rich alkaline medium supplemented with Cr(VI). Some alkaliphilic bacterial strains that can tolerate up to 20 mM of Cr(VI) were isolated. However, at higher Cr(VI) concentrations, only few strains could survive up to 75 mM. One of those strains termed, KSUCr9a, was selected for further investigation. KSUCr9a was able to tolerate up to 75 mM and with MIC value of 80 mM in alkaline medium (pH 10.5) containing 10% NaCl. The strain was identified using 16S rRNA gene sequence analysis as *Bacillus* sp. with 99% similarity and was referred to as *Bacillus* sp. strain KSUCr9a. It has been reported that the presence of Na and K in chemical structure of this oxyanion seems to be one of the reasons for such a high tolerance to oxyanions. Sodium and potassium are essential elements for ionic pumps and the enzymes activity in alkaliphiles and halophiles and thereby enhance bacterial tolerance to toxic metals (Amoozegar et al., 2007; Horikoshi, 2011). Moreover, *Bacillus* sp. strain KSUCr9a demonstrated high resistance to several other heavy metals including Mo^{2+} (50 mM), Ni^{2+} (50 mM), Pb (50 mM), Mn^{2+} (50 mM), Cd^{2+} (50 mM), Cu^{2+} (1 mM), Zn^{2+} (1 mM) and Co^{2+} (1 mM). Most industrial waste effluent contains mixture of heavy metals. Hence, single microorganism with enhanced abilities for detoxification of several pollutants is highly favorable (Ackerley et al., 2006).

Influence of some parameters on growth and Cr(VI)-bioreduction

Influence of initial Cr(VI) concentration

Bioreduction of Cr(VI) by *Bacillus* sp. strain KSUCr9a was investigated at different initial chromium concentrations ranging from 50 to 500 µM Cr(VI), as potassium dichromate ($K_2Cr_2O_4$). *Bacillus* sp. strain KSUCr9a was able to reduce up to 100 µM within 24 h. In addition, at initial Cr(VI) concentration of 200 µM, complete chromate reduction was achieved within 48 h. Furthermore, at initial Cr(VI) concentration of 300, 400 and 500 µM, 92.8, 75.5 and 39.8% of chromate reduction was achieved within 72 h (Figure 1). These results revealed the high efficiency of *Bacillus* sp. strain KSUCr9a in chromate detoxification in comparison to previously isolated bacteria. *B. sphaericus* AND303 failed to completely reduce 10 mg/L of Cr(VI) (Pal and Paul, 2004). A previously isolated *Brucella* sp. was able to completely reduce 50 mg/L Cr(VI) only within 54 h (Thacker et al., 2007). Mangaiyarkarasi et al. (2011) has reported Cr (VI) reduction by an akalipihilic *Bacillus subtilis* and showed that 50 mg/L of Cr(VI) was completely reduced in 65 h. However, a recently isolated strain of *Lysinibacillus fusiformis* was able to reduce 1 mM Cr(VI) within 12 h (He et al., 2011).

Influence of temperature

Bacterial growth and Cr(VI) reduction by the strain

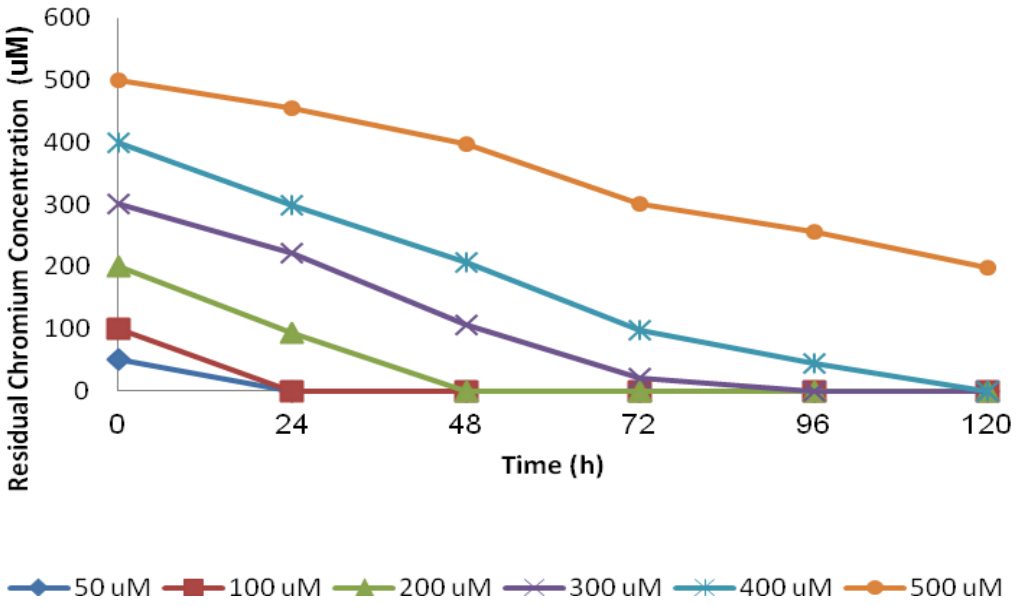

Figure 1. Chromium bio-reduction by *Bacillus* sp. KSUCr9a in alkaline medium with different initial chromium concentration**s** (50-500 µM/L**).** Experiments were performed in triplicates and the mean values were reported. Standard deviation was in range of 2-5 %.

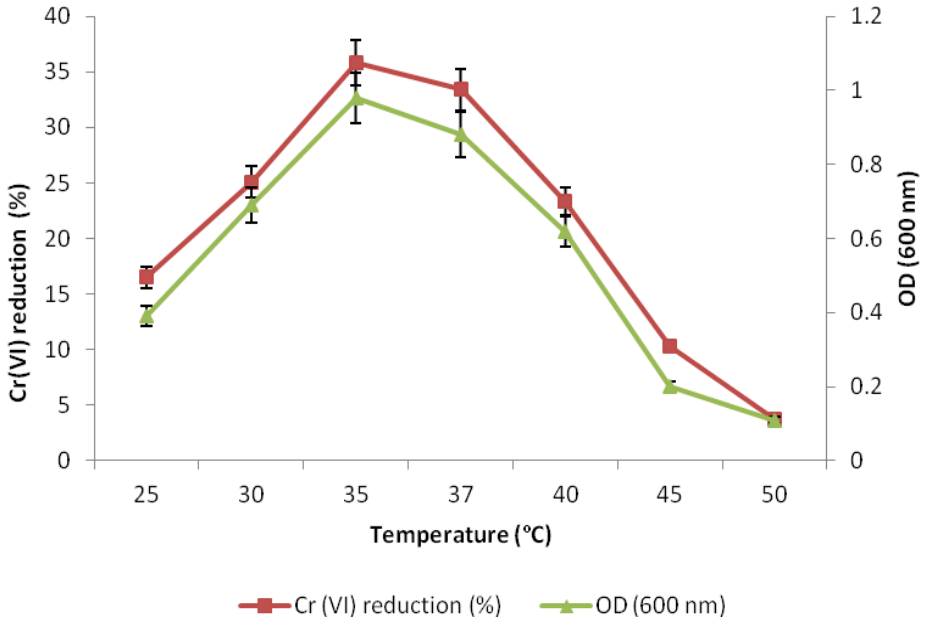

Figure 2. Influence of incubation temperature on growth and Cr(VI) bio-reduction by *Bacillus* sp. strain KSUCr9a. The strain was grown in alkaline medium containing 1% Na_2CO_3, 10% NaCl and 1% glucose and with chromium concentration of 300 µM/L and incubated for 24 h at various temperatures (25 to 50°C) with shaking (100 rpm). Experiments were performed in triplicates, the mean values were reported and deviated bars indicated.

KSUCr9a was studied at various temperatures (25 to 50°C). Growth and chromate bioreduction by KSUCr9a were increased with temperature up to 35°C, which appear to be the optimal temperature for growth of the strain KSUCr9a. At 40°C, chromate reduction was about 65.1% of that at the optimum temperature (35°C). However, above 40°C, both bacterial growth and chromate reduction were severely decreased (Figure 2). Temperature is one of the most important factors affecting microbial Cr(VI)-reduction. It has been reported

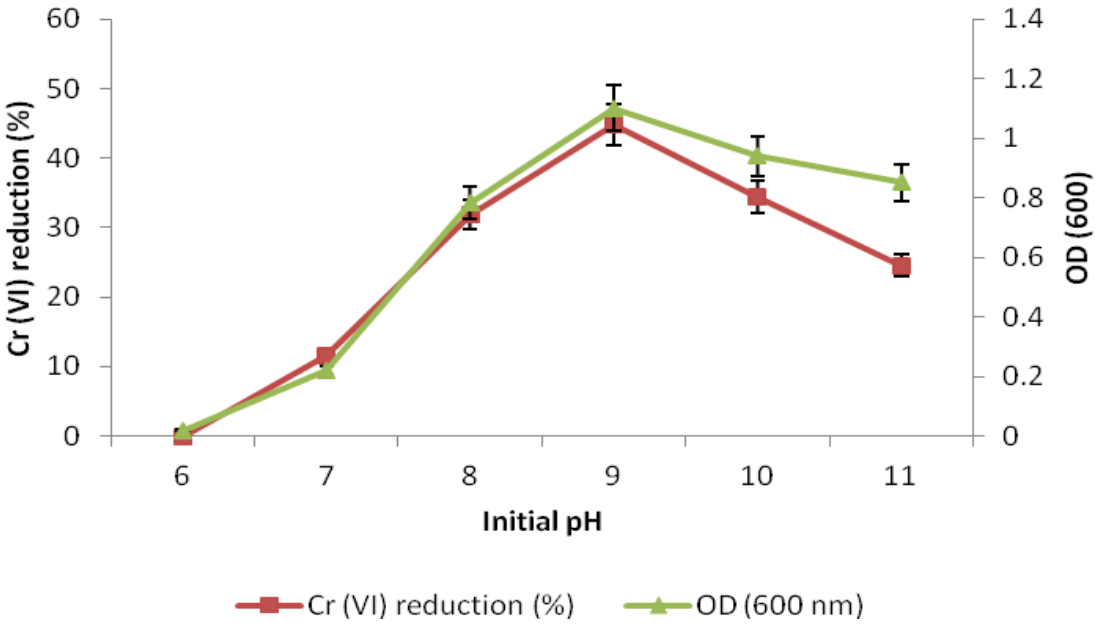

Figure 3. Influence of pH on growth and Cr(VI) bio-reduction by *Bacillus* sp. KSUCr9a. The isolate was grown in media with different initial pH (5-11), containing 10% NaCl, 1% glucose and with chromium concentration of 300 µM/L and incubated for 24 h at 35°C with shaking (100 rpm). Experiments were performed in triplicates, the mean values were reported, and deviated bars indicated.

that the optimal temperature of Cr(VI) reduction could be in the range of 30 to 37°C (McLean et al., 2000; Amoozegar et al., 2007; Cheung and Gu, 2007; He et al., 2009). However, Opperman et al. (2008) has reported chromate reductase from thermophilic *Thermus scotoductus* SA-01 with an optimum temperature of Cr(VI)-reduction at 65°C.

Influence of pH

The influence of pH on growth and chromate bioreduction by *Bacillus* sp. strain KSUCr9a was investigated using culture media with various initial pH, ranging from 6 to 11. The results presented in Figure 3 indicated that KSUCr9a was able to reduce Cr(VI) in a wide pH range (7 to 11) with an optimum growth and reduction yield at pH 9. At pH 10 and 11, the reduction yield was 77 and 54.8% at the optimum pH, respectively. This results is similar to that reported for a Gram-positive bacterium and alkaliphilic *B. subtilis*, showing optimum Cr(VI) bio-reduction at pH 9 (Shakoori et al., 2000; Mangaiyarkaras et al., 2011), and close to that reported for *Ochrobactrum* sp. CSCr-3 (pH 10) by He et al. (2009).

Influence of aeration level

Culture aeration is one of the most important parameters affecting microbial growth and Cr(VI)-reduction. The effect of aeration level on bacterial growth and Cr(VI) reduction by strain *Bacillus* sp. strain KSUCr9a was investigated by incubating the cultures at various shaking level ranging from 0 to 300 rpm. As shown in Figure 4, the bacterial growth was increased with aeration level up to 150 rpm. However, maximum chromate reduction by KSUCr9a was found to be under static conditions (71.5%), indicating that Cr(CVI) reduction by the strain KSUCr9a occurred under low aeration conditions. Generally, bacterial bio-reduction of Cr(VI) is taking place either under aerobic or anaerobic conditions (Michel et al., 2001; Chardin et al., 2002; Pal and Paul, 2004; Poopal et al., 2009).

Influence of glucose

The effect of glucose as electron donors on Cr(VI)-reduction and bacterial growth was studied (Figure 5). Addition of glucose to the culture medium significantly increased both growth and chromate reduction by *Bacillus* sp. strain KSUCr9a. Maximum chromate bioreduction (78.4%) by KSUCr9a was seen at glucose concentration of 0.8%, while optimum bacterial growth was achieved at 1.5%. These results were consistent with other reports indicating requirement of glucose as electron donor for Cr(VI)-reduction. Glucose has been reported to act as an electron donor and demonstrated to significantly increase Cr(VI) reduction by *Bacillus* sp. (Liu et al., 2006; Pal et al., 2005), *Ochrobactrum* sp. CSCr-3

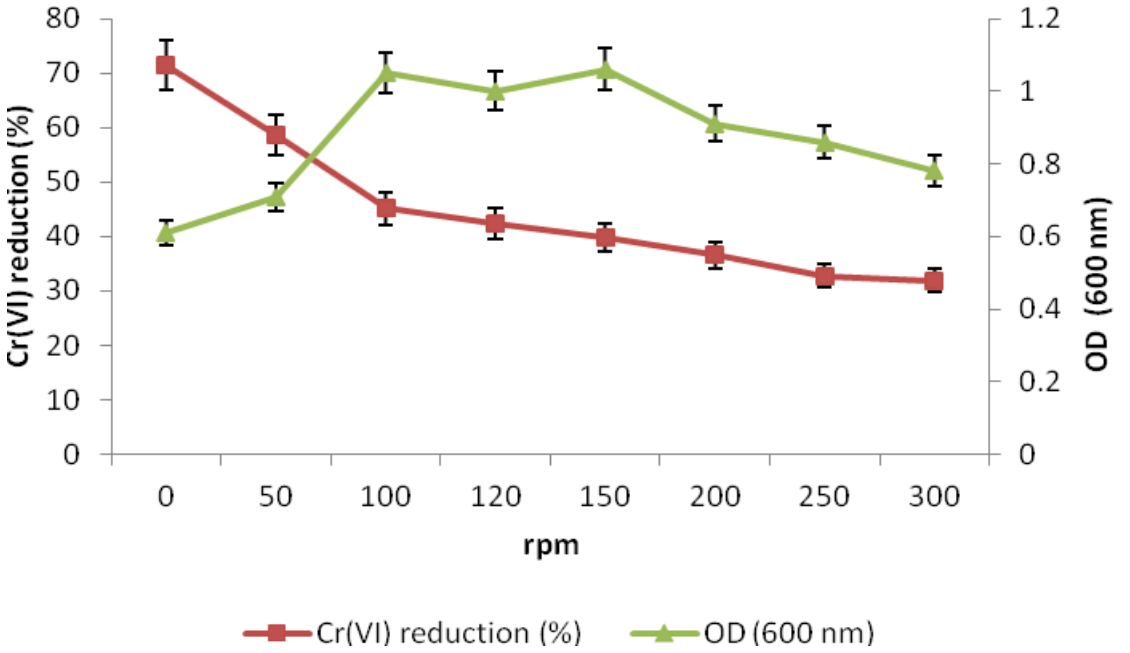

Figure 4. Influence of aeration level on the growth and Cr(VI) bio-reduction by *Bacillus* sp. KSUCr9a. The strain was grown in alkaline medium (pH 9) containing 10% NaCl and with Cr (VI) concentration of 300 µM/L, and incubated for 24 h at 35°C with shaking (0 to 300 rpm). Experiments were performed in triplicates and the mean values were reported and deviated bars indicated.

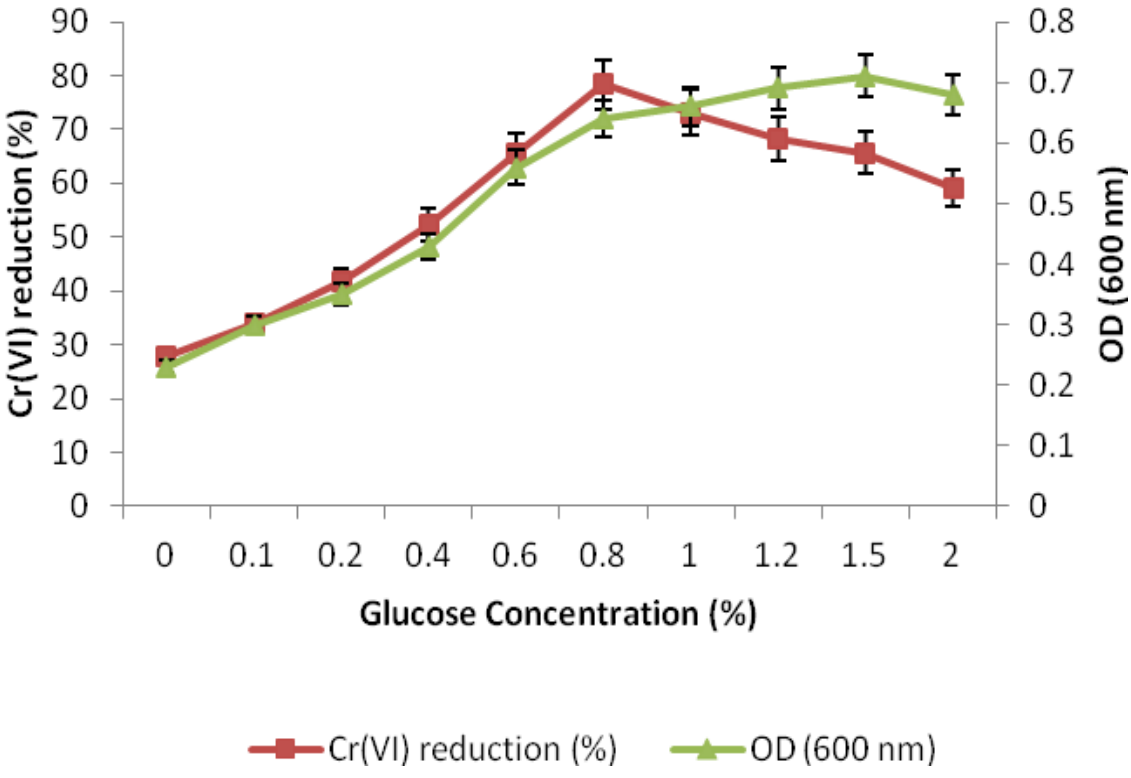

Figure 5. Influence of glucose concentration on growth and Cr(VI) bioreduction by *Bacillus* sp KSUCr9a. The strain was grown in alkaline medium (pH 9) containing 10% NaCl, glucose (0-2%), 10% NaCl and with Cr (VI) concentration of 100 mg/L, and incubated for 24 h at 35°C under static conditions. Experiments were performed in triplicates and the mean values were reported and deviated bars indicated.

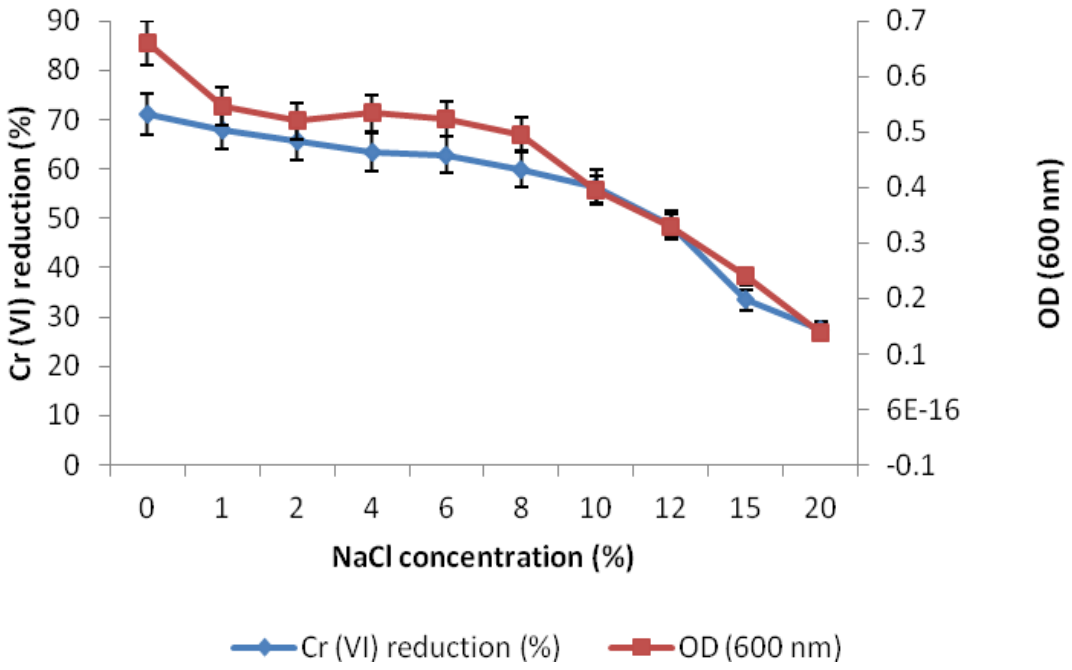

Figure 6. Influence of NaCl concentration on growth and Cr(VI)-reduction by *Bacillus* sp. KSUCr9a. The strain was grown in alkaline medium (pH 9) containing 0.8% glucose, NaCl (0 to 2%), and with Cr(VI) concentration of 300 µM/L and incubated for 24 h at 35°C under static conditions. Experiments were performed in triplicates and the mean values were reported and deviated bars indicated.

(He et al., 2009) and *Streptomyces griseus* (Poopal and Laxman, 2009). However, other electron donors like formate, fructose and carbonate have also been reported to increase Cr(VI) reduction (Myers et al., 2000; Desai et al., 2008b).

Effect of NaCl concentration

The influence of NaCl concentrations on growth and Cr(VI) reduction by *Bacillus* sp. strain KSUCr9a was investigated (Figure 6). Despite the fact that maximum chromate reduction was achieved in the absence of NaCl, KSUCr9a was able to reduce Cr(VI) in the presence of up to 10 to 12% NaCl. Chromate reduction at NaCl of 10 and 15% was about 79.4 and 47% of that in the absence of NaCl, respectively indicating the halo-tolerance nature of *Bacillus* sp. strain KSUCr9a. Complete Cr(VI) reduction by halophilic *Nesterenkonia* sp. strain MF2 was achieved only in the presence of 1 M NaCl (Amoozegar et al. 2007).

Effects of other metals on Cr(VI)-reduction

Contaminated soils and industrial effluents often contain other heavy metals. Thus, the effect of other heavy metals on Cr(VI)-reduction by *Bacillus* sp. strain KSUCr9a was studied in this work. As shown in Figure 7, the presence of 50 mg/L of Mn^{2+}, Co^{2+}, Mo^{2+} and Cu^{2+} together with Cr(VI) in the culture medium slightly increased Cr(VI)-reduction, whereas Zn^{2+}, Ni^{2+}, Cd^{2+} and Pb^{2+} had no effect on Cr(VI)-reduction by strain KSUCr9a. However, Hg^{2+} showed significant inhibition of Cr(VI) reduction by KSUCr9a. Induction of Cr(VI)-reduction in the presence of Cu^{2+} has been also reported in other bacterial strains including *Bacillus* sp. ES 29, *Ochrobactrum intermedium* strain SDCr-5, *Ochrobactrum* sp. strain CSCr-3 (Camargo et al., 2003; Sultan and Hasnain, 2007; He et al., 2009). The stimulatory mechanism of Cr(VI) reduction activity by Cu^{2+} and other metals is not clear. It has been established that Cu^{2+} is acting as a prosthetic group for various bacterial reductase enzymes (Abe et al., 2001; Camargo et al., 2003). In contrast to our results, chromate reduction by *B. sphaericus* was inhibited by the presence of Ni^{2+}, Co^{2+} and Pb^{2+}, even at low concentration (20 mg/L), (Pal and Paul, 2004).

Repeated detoxification of Cr(VI)

The chromate detoxification ability of *Bacillus* sp. KSUCr9a was further investigated by several repeated addition of 100 µM Cr(VI) as $K_2Cr_2O_4$ every 15 h. *Bacillus* sp. KSUCr9a showed complete bioreduction of 100 µupto five consecutive addition (Figure 8). Moreover, *Bacillus* sp. KSUCr9a was able to reduce 55% of the sixth

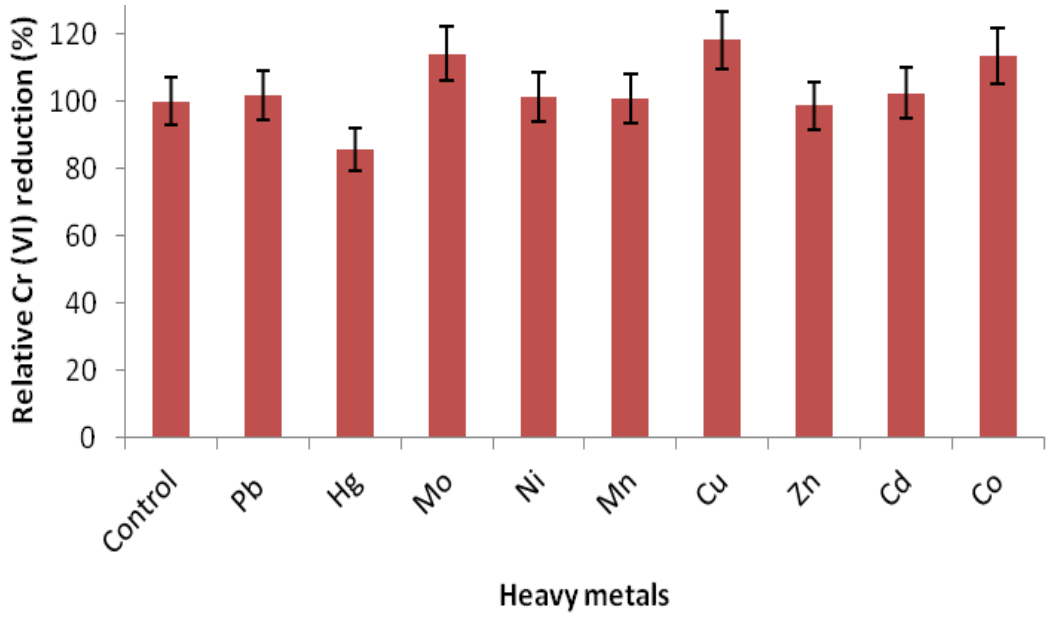

Figure 7. Influence of heavy metals on Cr(VI) bio-reduction yield by *Bacillus* sp. KSUCr9a. The strain was grown in alkaline medium (pH 9) containing 0.8% glucose, 1% NaCl, and with Cr(VI) concentration of 300 µM/L together with 50 mg/L of different metals, and then incubated for 24 h at 35°C under static conditions. Experiments were performed in triplicates and the mean values were reported and deviated bars indicated.

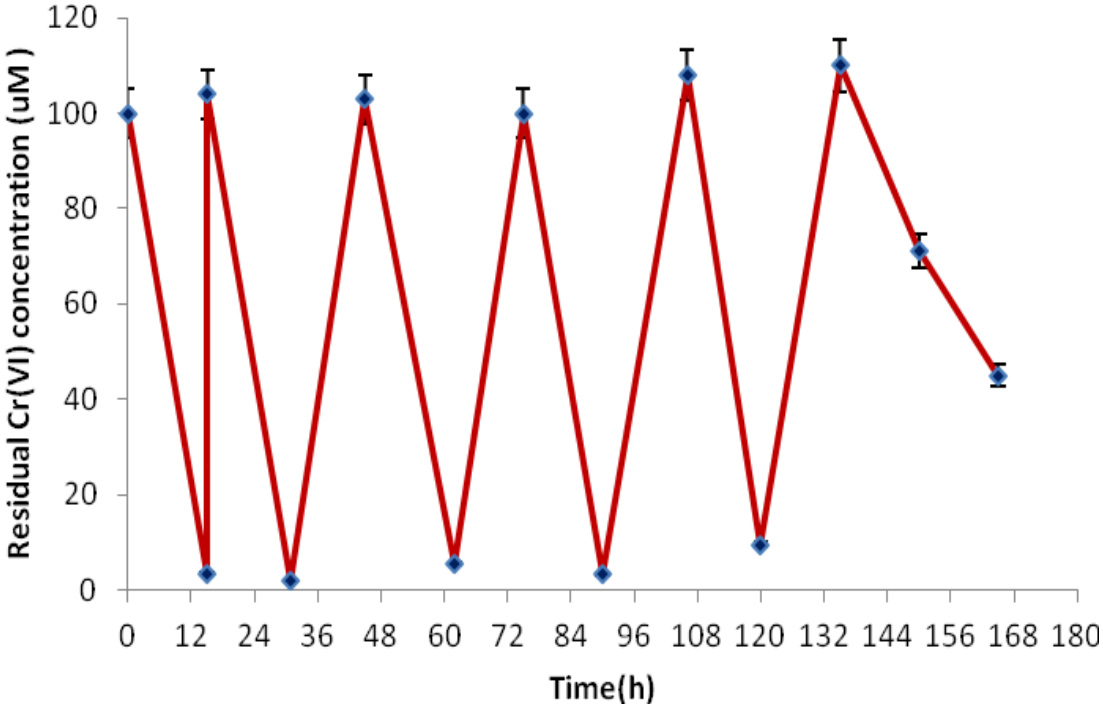

Figure 8. Repeated detoxification of 100 µM *Bacillus* sp. KSUCr9a under the optimum conditions of Cr(VI) bioreduction.

addition within further 30 h. The ability of *Bacillus* sp. KSUCr9a to repeatedly reduce Cr(VI) without any amendment of nutrients, suggests its potential application chromate detoxification.

Conclusion

In this study, we reported the isolation of Cr (VI) reducing alkaliphilic *Bacillus* sp KSUCr9a from Egyptian soda lakes. *Bacillus* sp KSUCr9a can effectively reduce toxic Cr(VI) to non toxic form [Cr(III)] under alkaline condition, high sodium chloride concentration, wide range of temperatures and high Cr(VI) concentrations (50 to 500 µM). Under optimum Cr(VI) bioreduction conditions, complete reduction of Cr(VI) at concentration of 169.2 mg/L was achieved within 24 h, indicating a good ability of *Bacillus* sp KSUCr9a for chromate detoxification. Furthermore, in addition to its tolerance to Cr(VI), it exhibited high tolerance to other heavy metals such as Cd, Mo, Mn, Cu, Ni, Pb, Co and Zn, which are usually present in the industrial effluents. Moreover, *Bacillus* sp. KSUCr9a showed the ability of repeated bioreduction of chromate without any addition of exogenous nutrients, indicating its possible application in chromate detoxification.

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