



# Reduction of chromium-VI by chromium-resistant *Escherichia coli* FACU: a prospective bacterium for bioremediation

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## Abstract

The release of hexavalent chromium [Cr (VI)] into environments has resulted in many undesirable interactions with biological systems for its toxic potential and mutagenicity. Chromate reduction via chromium reductase (ChrR) is a key strategy for detoxifying Cr (VI) to trivalent species of no toxicity. In this study, ten bacterial isolates were isolated from heavily polluted soils, with a strain assigned as FACU, being the most efficient one able to reduce Cr (VI). FACU was identified as *Escherichia coli* based on morphological and 16S rRNA sequence analyses. Growth parameters and enzymatic actions of FACU were tested under different experimental conditions, in the presence of toxic chromium species. The *E. coli* FACU was able to reduce chromate at 100 µg/mL conceivably by reducing Cr (VI) into the less harmful Cr (III). Two distinctive optical spectroscopic techniques have been employed throughout the study. Laser-induced breakdown spectroscopy (LIBS) was utilized as qualitative analysis to demonstrate the presence of chromium with the distinctive spectral lines for bacteria such as Ca, Fe, and Na. While UV-visible spectroscopy was incorporated to confirm the reduction capabilities of *E. coli* after comparing Cr (III) spectrum to that of bacterial product spectrum and they were found to be identical. The chromate reductase specific activity was 361.33 µmol/L of Cr (VI) per min per mg protein. The FACU (EMCC 2289) 16S rRNA sequence and the *ChrR*-partially isolated gene were submitted to the DDBJ under acc. # numbers LC177419 and LC179020, respectively. The results support that FACU is a promising source of ChrR capable of bioremediation of toxic chromium species.

**Keywords** Bioremediation · Chromate reductase · *E. coli* · Hexavalent chromium · LIBS

## Introduction

Heavy metal contaminants related to industrial and agriculture activities are basic sources of environmental pollution. Metal ions such as Cd, Co, Cr, Cu, Ni, and Zn are implicated in several industrial applications (Rodríguez-Sejjo et al. 2016). Chromium is particularly used in leather tanning, pigments and coatings, stainless steel alloys, glass, and ceramic. The Cr contaminants pose adverse impacts on environment and

biota, which consequently lead to mutations on the molecular and cellular levels (Ortega et al. 2005).

The oxidation states of Cr ranged from 2<sup>+</sup> to 6<sup>+</sup> with two stable states, i.e., 3<sup>+</sup> and 6<sup>+</sup>. Cr (VI) is highly toxic, mobile, mutagenic, and carcinogenic whereas, Cr (III) is less toxic and immobile. Hence, Cr (VI) is solely detoxified by transformation to Cr (III) (O'Brien et al. 2005; Zhitkovich 2011). Cr (VI) is listed as a class A hazardous mutagenic chemical according to the United States Environmental Protection Agency (US-EPA 1998).

Removal of highly soluble metal ions from sewage and leachates using traditional methods is facing several obstacles such as a time/cost consuming process (Sanderson et al. 2018), disposal of residual metal waste, and the ineffectiveness at low concentration of Cr (VI) below 2 mmol/L (Caravaglia et al. 2010). Biotechnological applications offer an alternative “green” approach to detoxify chromate through microorganisms as they have powerful adsorption properties (Congeevaram et al. 2007) and potential enzymatic system

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mechanisms to reduce metal ions (Viti et al. 2003). Therefore, microorganisms not only accumulate heavy metals by adsorption but also convert the highly toxic forms of metals to less toxic ones by their oxido-reduction enzymatic processes (Bhattacharya et al. 2019). Several microorganisms were used to reduce Cr toxicity such as fungi, *Gloeophyllum sepiarium* (Achal et al. 2011); algae, *Spirogyra* sp. and *Spirulina* sp. (Mane and Bhosle 2012); Gram-negative bacteria, *Pseudomonas aeruginosa* (Aguilera et al. 2004) and *E. coli* (Robins et al. 2013) as well as Gram-positive bacteria, *Bacillus* species (Camargo et al. 2003a; Mala et al. 2015). Among these, bacteria are the best candidates for their less nutritional requirements and growth rate viability (Chrysochoou et al. 2013).

The reduction of Cr (VI) to Cr (III) via cytosolic and membrane-bound chromium reductases in aerobic and anaerobic bacteria, respectively, is a viable cellular process (Camargo et al. 2003a). The ability of bacteria to detoxify Cr (VI) has been reported to depend on several environmental parameters such as pH and temperature that affect the transformation and bioavailability of heavy metal (Igiri et al. 2018). In literature survey, few studies were reported about the detoxification of Cr (VI)-containing waste mixtures and more work in the microbial remediation of Cr (VI) in the environment is required (Bhattacharya et al. 2019). Therefore, in this study, an attempt has been made toward finding potential bacterial chromium reductase candidates, we screened several bacterial isolates for their capability of chromium reduction in addition to amplification and characterization of chromium reductase gene. Moreover, rapid and reliable spectroscopic techniques were applied to characterize some of the basic elements of bacterial strains as well as to confirm the reduction state of Cr (VI) to Cr (III).

## Materials and methods

### Isolation and purification of bacteria from polluted soil

Soil samples from different polluted industrial areas, harboring heavy industries such as chemicals, detergents, dyes, petroleum refining, and tanneries, in addition to the uncontrolled disposal of these wastes (Alexandria: 31°08'46.8"N 29°50'21.3"E; Gharbia: 30°49'33.6"N 30°48'30.8"E; and Giza: 29°53'57.3"N 30°54'13.6"E, Egypt), were collected with a clean scoop and transferred to a sterilized plastic pack, transported to the laboratory within 12 h and analyzed in the same day using standard microbiological technique. Briefly, from each soil sample, 5 g (dry mass) was suspended in 500 mL sterile saline and were kept on a rotatory shaker for 90 min at 37 °C. The isolation was initiated by making tenfold dilutions of these suspensions in sterile saline solution up to

10<sup>-7</sup> dilution. A 100-μL aliquot of the serially-diluted suspensions was spread-plated onto Luria Bertani (LB) agar medium in triplicate and incubated at 37 °C for 24 h. Ten isolates with distinctive morphological appearances designated FACU-FACU10 were picked and re-streaked on the same media to get purified single colonies (Congeevaram et al. 2007).

### Screening for chromate-resistant bacteria

For selection of chromate-resistant bacteria, a LB media supplemented with 100 μg/mL K<sub>2</sub>CrO<sub>4</sub> were inoculated with the pure bacterial isolates (FACU-FACU10) and then incubated at 37 °C on a shaker at 150 rpm. The bacterial growth of the different isolates was determined after 24 h of incubation by the absorbance at 600 nm (A<sub>600</sub>). The bacterial isolate that showed the highest growth was selected for further studies.

### Effect of Cr on bacterial growth

To evaluate chromate resistance, 100 μL of exponential-phase culture was inoculated in 10 mL fresh LB medium supplemented with 100 μg/mL K<sub>2</sub>CrO<sub>4</sub> or without chromium as a control. The cultures were incubated at 37 °C on a shaker at 200 rpm for 72 h then the cell density was determined spectrophotometrically at A<sub>600</sub>. An aliquot of culture was taken out in a sterilized tube, at regular intervals of 0 and 72 h. Negative control, LB medium supplemented with a 100 μg/mL K<sub>2</sub>CrO<sub>4</sub> and without bacterial cells was used to monitor abiotic chromate reduction. The growth represented as cell density was graphically plotted against time. The experiment was repeated three times.

### Identification of the isolated Cr-resistant bacteria using 16S rRNA gene-specific primers

The selected bacterial isolate for Cr resistance was initially identified based on colony morphology, Gram staining, motility, and laboratory biochemical tests including tests of catalase, citrate utilization, indole ornithine, methyl red, oxidase, urease, Voges Proskauer, and lactose fermentation ability (Sneath et al. 1986). The identification was then confirmed using molecular biology technique including amplification of 16S rRNA by PCR based method using two universal primers; 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-TACGGYTACCTTGTTACGACTT-3'. The primers were used to amplify a gene fragment of ca. 1444 bp using 10 ng genomic DNA isolated from the selected bacterial isolate. The ethidium bromide stained-PCR amplicons on 1% agarose gel were visualized under ultraviolet (UV) light.

## Primer design

According to the DNA sequence of *E. coli* K-12 (GenBank accession no. NC\_000913), the primers of chromate reductase gene responsible for heavy metal detoxification were designed according to data in GenBank Database ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) by using the OligoPerfect™ Designer <https://tools.thermofisher.com/content.cfm?pageid=9716>). These primers were named and designed as follows *reduc-f* (5'-ATGTCTGAAAATTGCAGGTGG-3') and *reduc-r* (5'-TTAGATCTTAACTCGCTGAATAAACTC-3').

## Polymerase chain reaction (PCR)

The PCR technique was performed in a total volume of 50 µL, comprised of 1× PCR buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl, 20 mmol/L MgCl<sub>2</sub> pH 8.3, 0.01% (w/v) gelatine), a mix of 0.2 mmol/L dNTPs/each, 2.5 U Taq DNA polymerases, 0.5 mmol/L from each primer, and 50 ng/µL bacterial DNA template. Conditions of PCR: initial denaturation at 94 °C for 3 min and 35 cycles; denaturation at 94 °C for 1 min; annealing at 48 °C for 2 min, extension at 72 °C, followed by final extension at 72 °C for 7 min. The PCR product purification was done with EXOSAP-IT (Ambion, CA), followed by forward and reverse sequencing at HVD Company, Germany using PCR primers.

## Laser-induced breakdown spectroscopy (LIBS)

LIBS technique is now an established optical spectroscopic technique due its superior advantages over other conventional techniques. LIBS is robust, fast, and does not need any sample preparation besides its high sensitivity. In the present study, we used the same setup that has been described in our previous work (El-Hussein et al. 2015) where two drops were taken from the bacteria filtrate and left them on ash-less filter paper to be spread for 10 min. Nd:YAG laser at its fundamental wavelength ( $\lambda = 1064$  nm) was used. The laser energy was 55 mJ at a frequency of 10 Hz. The pulse width was 5 ns. The laser beam was focused by a plano-convex lens whose focal length is 10 cm into the sample where LIBS plasma was obtained. This emitted plasma was fed into the entrance slit of the echelle spectrometer coupled to an ICCD camera via a fiber optic which has a diameter of 6 cm and was at 2 cm above the plasma at 30° with respect to the target surface. The represented LIBS spectra in the results section are the average of taking ten spectra at three fresh location at *y*-axis of our sample by adjusting a movable micrometer stage. Another movable stage on the *x*-axis was employed to make sure the ash-less paper is still intact and was not damaged by the laser pulses. Finally, LIBS++ software with its relevant information has been used for further processing of the obtained spectra.

## UV-visible spectroscopy

UV-vis spectrophotometry (T80-PGT80+PG instruments) was used to investigate the absorption spectrum of normal Cr (III) and to compare that spectrum to that of the bacterial filtrate.

## Chromate reductase enzymatic assay

Chromate reductase enzymatic activity was assayed colorimetrically according to Sau et al. (2010) by determination of Cr (VI) by measuring the absorbance at 540 nm. The assay mixture comprised of 200 µL of 0.2 mmol/L K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution; 400 µL of the bacterial culture cell-free extract as enzyme source; 200 µL of 0.2 mmol/L NADH; incubated at 37 °C and 200 µL of 200 mmol/L phosphate buffer, pH 7.2. After 30 min, 500 µL of 20% trichloroacetic acid was added to stop the reaction. Finally, pink color was developed by adding 2 mL of 1,5-diphenylcarbazide/ethanol (0.5% w/v). Negative controls constitute no bacterial cell-free extract. Initially, Cr level was compensated by 0.2 mL of 0.2 mol/L phosphate buffer, pH 7.2. The remaining Cr (VI) concentration was estimated based on standardization with serial concentrations of 10–100 µmol/L K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>. The unit of chromate reductase (U) was defined as the amount of enzyme which decreased 1 µmol/L of chromate per min at 37 °C. Total protein was determined with the Bradford protein assay (Bradford 1976), using bovine serum albumin as standard. Specific activity of chromate reductase was represented as U/mg.

## Effect of temperature and pH on chromate reductase activity

Different enzyme assay mixtures of the same concentration were prepared and incubated at 25, 30, 37, 45, 50, and 60 °C, activity was assayed and expressed as U/mg. For estimating the effect of different pH on enzyme activity, different buffering systems were prepared as follow: 200 mmol/L glycine-HCl for pH 3.0 and 4.0; 200 mmol/L phosphate buffer for pH 5.0–8.0; and finally, 200 mmol/L glycine-NaOH buffer for pH 9.0 and 10.0 were used. Enzymatic reactions were performed in triplicates and assayed as described above. The bacterial strain was deposited and available in Microbiological Resources Center (Cairo MIRCEN - Egypt), under number EMCC 2289.

## Results

### Bacterial isolation

Ten bacterial isolates (FACU to FACU10) have been isolated and purified from polluted soils. Bacterial isolates' capability

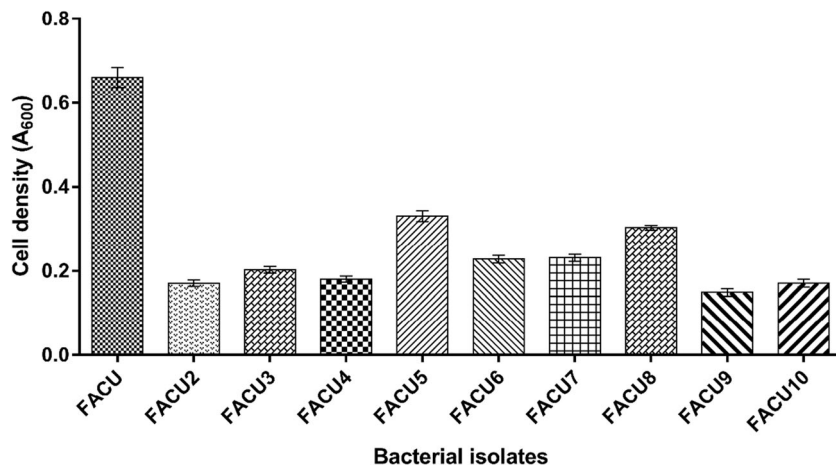
to reduce Cr (VI) was tested by adding  $K_2Cr_2O_7$  (100  $\mu\text{g}/\text{mL}$ ) in the incubation medium. Only one isolate (FACU) was able to tolerate Cr (VI) at 100  $\mu\text{g}/\text{mL}$  concentration up to 24 h (Fig. 1). These results suggested that FACU isolate is capable of reducing Cr (VI).

### Morphological and molecular identification

The results of screening showed that the chromium-resistant property was only detected in one isolate, FACU. This isolate has been characterized by manual identification as follows: Gram-negative bacteria, motile, rod-shaped, and non-spore forming with optimum growth temperature at 37 °C. FACU colonies were non-pigmented. The FACU isolate displayed positive activities for catalase, methyl red, indole ornithine, and lactose fermentation. Whereas, this strain was negative in citrate utilization, oxidase, urease, and Voges Proskauer tests. This isolate was identified as *E. coli* with Bergey's Manual of Systematic Bacteriology (Sneath et al. 1986).

Molecular techniques were employed to confirm identification of the bacterial isolate at the genetic level. The universal 16S rRNA bacterial primers 27 and 1492R primers were used in molecular identification. The gene of 16S rRNA was amplified and sequenced. A nucleotide sequence of 1444 bp corresponding to the 16S rRNA gene from the *E. coli* FACU isolate was compared with other 16S rRNA sequences on GenBank using the BLAST searches. The isolated FACU exhibited a high degree of sequence homology of 98% with 16S rRNA gene from *E. coli* strain NBRC 102203. The obtained sequence was deposited to the DNA Data Bank of Japan (DDBJ) (<http://www.ddbj.nig.ac.jp>) under accession number LC177419 as *E. coli* FACU strain. The BLAST results for nucleotide sequence of 16S rRNA were performed by Geneious Prime and the data used to build the phylogenetic tree using the MEGAX software (Fig. 2).

**Fig. 1** The growth of different bacterial isolates (FACU-FACU10) in LB media supplemented with 100  $\mu\text{g}/\text{mL}$  of  $K_2CrO_4$ . The data are represented as the mean of triplicate samples  $\pm$  standard error



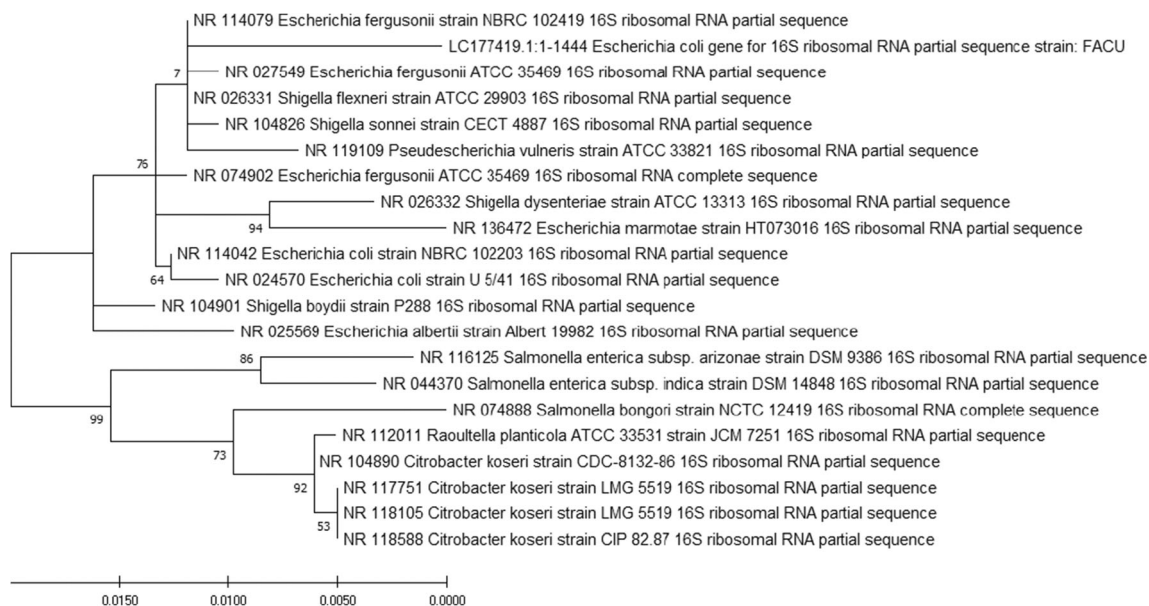
### Ideal growth conditions of *E. coli* FACU strain

The growth of *E. coli* FACU strain supplemented with chromate was monitored spectrophotometrically (Fig. 3). The growth curve demonstrated a rapid growth rate in chromate containing medium. In the first 12 h of incubation, the FACU strain growth rate was rapid and reached the log phase then the growth was relatively decreased compared with control indicating efficient chromate reduction ability under aerobic conditions suggesting that genes conferring chromate resistance could be present in this strain.

The results revealed that *E. coli* FACU strains showed a maximum growth in concentrations of 100  $\mu\text{g}/\text{mL}$  of  $K_2CrO_4$  and maximum enzyme activity at pH 7.0 and any increase in enzyme activity in acidic or alkaline pH lead to a decrease in reduction activities (Fig. 4a), similar to many intracellular enzymes with optimum pH where optimal growth occurs. The optimum temperature the chromate reductase activity was 37 °C (Fig. 4b).

### Isolation of chromate reductase gene from *E. coli* FACU strain

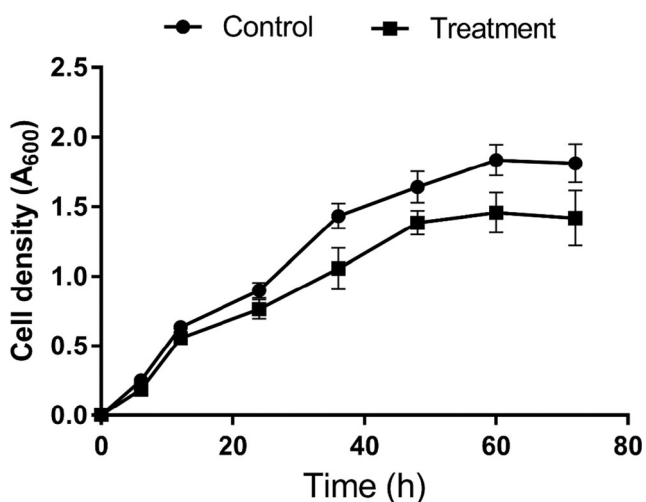
A fragment of ca. 567 bp encompassing the complete coding sequence of the *ChrR* gene was isolated and sequenced (Fig. 5). The sequence analysis of the amplified gene was performed by blasting it with the corresponding sequences in the National Center for Biotechnology Information (NCBI). The nucleotide sequence was deposited in the DDBJ under accession number LC179020. The genetic distance of the gene was shown in Fig. 6. The amino acid sequence of chromium reductase-encoding protein is composed of 188 amino acid residues with an estimated molecular weight of 20.38 kDa. The protein is rich in leucine (10.1%), valine (9.0%) and glutamic acid (9.0%). The deduced isoelectric point is 4.99 implying an acidic nature of protein. The absence of signal peptide indicated an intracellular enzyme localization.



**Fig. 2** The maximum likelihood phylogenetic tree for the 16S rRNA sequences using the MEGAX software. Numbers at nodes represent the percentage values given by 1000 bootstrap analysis

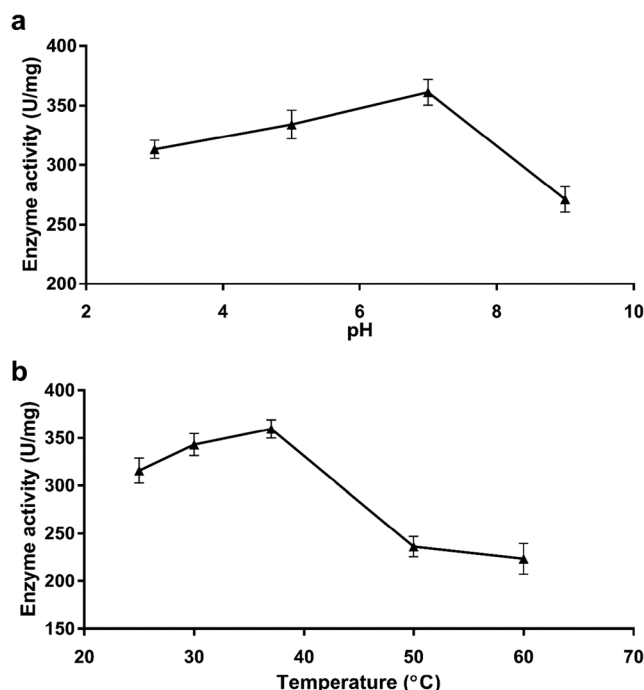
**LIBS and UV-vis spectroscopy**

LIBS spectra in Fig. 7A–E show the characteristic spectral line for Cr and other bacterial spectral lines for Na, Ca, Fe, and Carbon. The results clarify that *E. coli* did not consume Cr, but reduced it into Cr (III). LIBS spectra revealed many Cr spectral lines at 425, 427, 429, 520.5, 520.6, and 520.8 nm. There are many Ca spectral lines as well, but the most prominent lines are those at 395 and 397 nm, whereas Mn spectral line is at 325 nm. Na duplicate spectral line which is a characteristic feature of sodium is found as expected at 590 nm. There are other elements such as C and Mg are seen clearly in the overall spectrum.

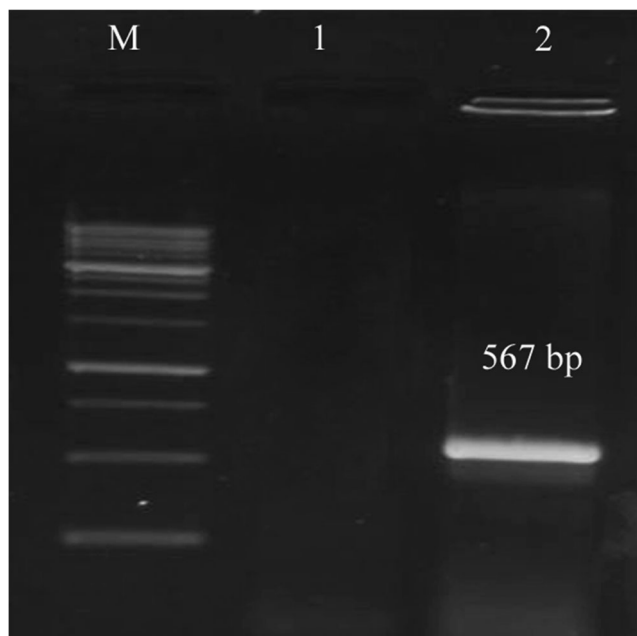


**Fig. 3** Growth curve of FACU strain after 72 h of incubation in LB medium (pH 7.0) as control or supplemented with 100 µg/mL of K<sub>2</sub>CrO<sub>4</sub> (treatment). The data are represented as the mean of triplicate samples ± standard error

To validate this hypothesis, UV-vis spectrophotometry revealed the exact and identical spectral peaks of inorganic Cr (III) to that of the bacterial filtrate as shown in Fig. 8. Where both spectra were identical showing the characteristic absorption peak of Cr (III) at 400 nm.



**Fig. 4** Effect of different pH (a) and temperature (b) on chromate reductase activity of *E. coli* FACU strain after 72 h of incubation in LB medium supplemented with 100 µg/mL of K<sub>2</sub>CrO<sub>4</sub>. The data are represented as the mean of triplicate measurements ± standard error



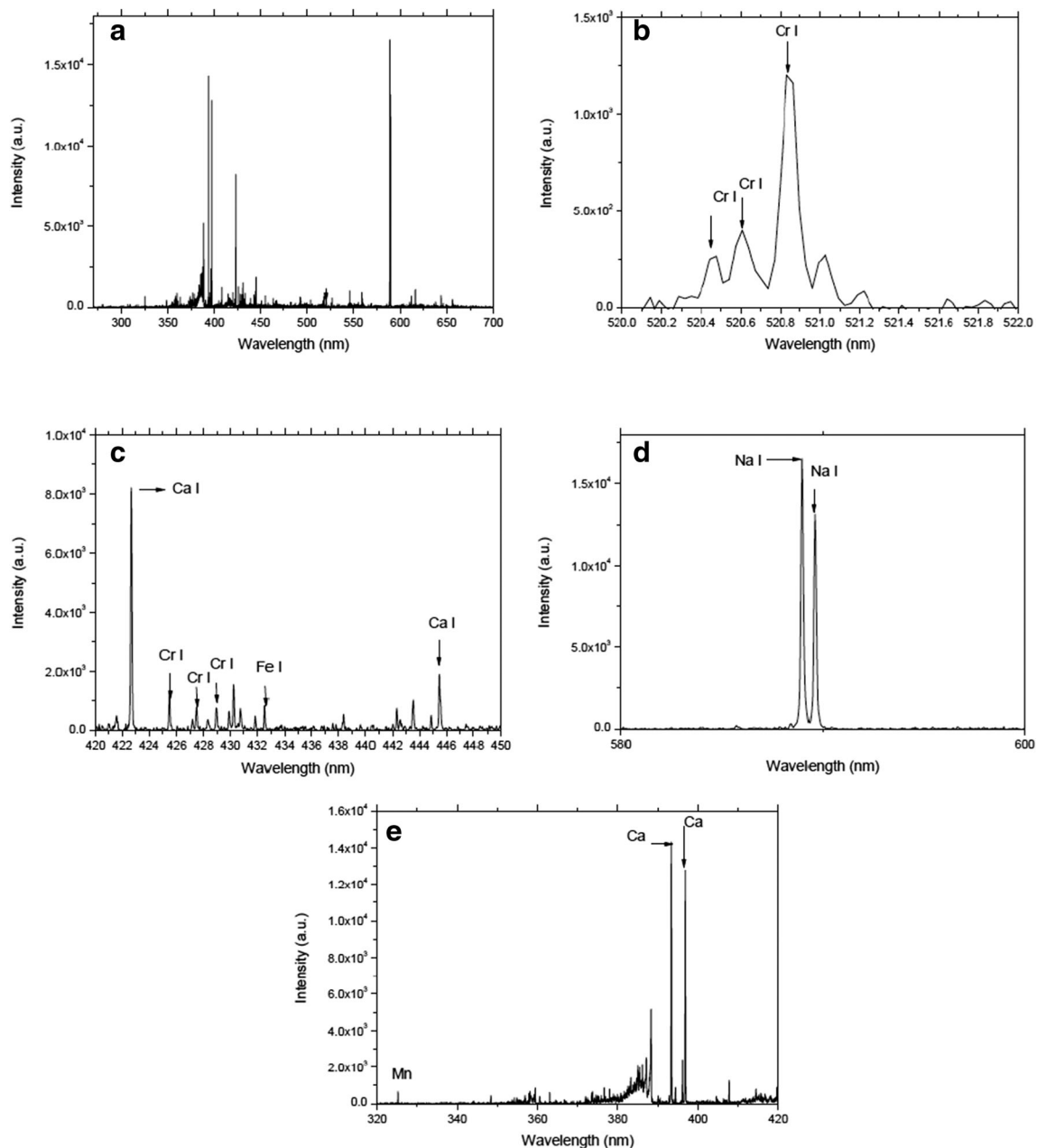
**Fig. 5** The PCR amplification of chromium reductase gene, M is 1 kb ladder markers, 1: negative control and 2: FACU isolate

## Discussion

Several mechanisms for heavy metal removal from aqueous media via microorganisms, emphasizing bacteria, have been reported (Viti et al. 2014). Chromium-reducing bacteria have been isolated from different polluted sources such as leather industrial aqueous wastes (Piñon-Castillo et al. 2010; Chrysochoou et al. 2013) and contaminated soil from chemical industry (Thacker et al. 2006). In this study, a bacterial isolate (FACU) was able to tolerate chromate at 100  $\mu\text{g/mL}$  after 24 h. In this regard, many studies reported the capability of different bacterial genera to withstand chromium toxicity. A dichromate-reducing Gram-positive bacterium was isolated from tanneries effluents (Pattanapitpaisal et al. 2001). This bacterium was able to endure Cr (IV) up to 16  $\mu\text{g/mL}$  under anaerobic conditions only. Other reports demonstrated a very efficient and complete chromate reduction via *Pseudomonas aeruginosa* at concentration of 10  $\mu\text{g/mL}$  at only 2 h (Ganguli and Tripathi 2002). Cell-free extracts of salt-tolerating bacteria *Halomonas aquamarina* indicated a possible role of an extracellular enzyme (Focardi et al. 2012).

**Fig. 6** The phylogenetic tree of chromium reductase gene



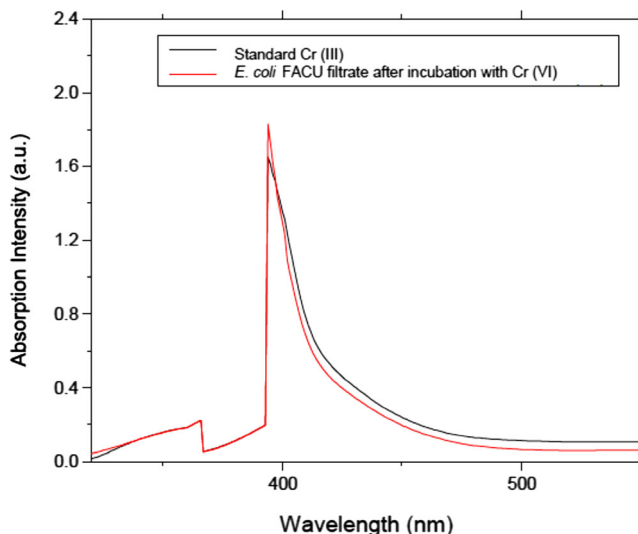


**Fig. 7** LIBS spectra for bacterial filtrate showing: **A** overview spectrum, **B** and **C** Cr spectral lines at 429 nm, 427 nm, 425 nm, 520.5, 520.6, and 520.8 nm. **D** Na lines at 590 nm and **E** Ca lines at 395 and 397 nm and finally Mn line at 325 nm

The reduction of Cr (VI) to Cr (III) is widely present in bacteria and a vital tool in bioremediation (Igiri et al. 2018). A number of chromium-resistant microorganisms were reported, e.g., *E. coli*, *Bacillus cereus*, *Bacillus subtilis*, *P. aeruginosa*, *Pseudohemihyalea ambigua*, *Pseudomonas fluorescens*, *Achromobacter eurydice*, and *Arthrobacter* sp. (Camargo et al. 2003b; Chrysochoou et al. 2013; Robins et al. 2013; Xiao et al. 2017).

These bacteria alleviate the chromium toxicity through activation of the efflux systems, formation of biofilm and/or an array of antioxidant mechanisms (Ackerley et al. 2006; Baldiris et al. 2018).

The growth of *E. coli* FACU strain supplemented with chromate was rapid and reached the log phase then slightly decreased indicating efficient chromate reduction ability under aerobic conditions suggesting that genes conferring chromate resistance are likely present in this strain. The highest chromate reductase specific activity recorded for *E. coli* FACU strain in the presence of 0.2 mmol/L NADH was 361.33  $\mu\text{mol/L}$  of Cr (VI) per min per mg protein. In this context, the Gram-positive bacterium *B. methylotrophicus* reported the production of chromate reductase with specific activity of 356.48  $\mu\text{mol/L}$  of Cr (VI) per min per mg protein in the presence of reduced glutathione as electron donor (Mala



**Fig. 8** UV-Vis spectrum for bacterial filtrate after incubation with Cr (VI) compared to standard Cr (III) showing identical absorption peak at 400 nm and 370 nm shoulder

et al. 2015). However, the fungi *Pichia* sp. recorded lower chromate reductase activity than bacterial strain with only 3.0 nmol/L of Cr (VI) per min per mg protein (Martorell et al. 2012). These results suggest an enhanced enzyme activity produced by *E. coli* FACU that is able to reduce Cr (VI) possibly by converting it into the less toxic Cr (III) form. The bacterial reduction of Cr (VI) can be enhanced by many enzymatic and/or non-enzymatic mechanisms such as reduced glutathione and cysteine by their reducing properties resulting in transfer of electrons directly to Cr (VI) (Thatoi et al. 2014). The three-electron reduction process of Cr (VI) to Cr (III) that occur simultaneously without the formation of the unstable toxic Cr(V) intermediate has been previously identified in *E. coli* (Barak et al. 2006).

The ability to reduce Cr (VI) to Cr (III) in the cell membrane consequently occurs after the adsorption of Cr (VI) on bacterial cell surface (Vendruscolo et al. 2017). The adsorption of Cr (VI) on the bacterial cell is affected by the pH of the media because at low pH the biomaterial surface becomes more positive which enhances the binding of anionic Cr (VI) ion species with the positively-charged groups (Mehta and Vaidya 2010). This is in line with the results obtained in this study that indicated sharp reduced chromate reductase enzymatic activity at high pH value.

Intracellular reduction of Cr (VI) to Cr (III) led to deposition of Cr (III) on cell exterior. This mechanism is usually not plasmid-associated (Viti et al. 2014). In general, bacteria can tolerate the toxicity of Cr (VI) by using indirect chemical way, direct enzymatic pathway or by proton motive force efflux mechanism of Cr (IV). The Cr (IV)-efflux genes such as *P. aeruginosa* *ChrA* were reported on plasmids (Ramirez-Diaz et al. 2008). *E. coli* is reported to possess different genes involved in chromate reduction namely; *chrR* (previously

named; *yieF*) and *nfsA* genes. According to sequence homologies, chromate reducing enzymes were classified into two major classes, Class I or Class II (Ackerley et al. 2004). The direct reduction of Cr (VI) to Cr (III) was reported by the *ChrR* (*YieF*) of the class I family, through the two electron reducers (i.e., simultaneously transfer of four electron), in which three electrons were consumed in reducing Cr (VI) and one generating reactive oxygen species by transferring one electron to molecular oxygen (Park et al. 2002). However, higher reactive oxygen species were generated during the one electron reducers mediated by class I family, *NfsA* forming the Cr(V) intermediate leading to continuous shuttle between Cr (VI) and Cr(V) forms (Barak et al. 2006; Thatoi et al. 2014). Taken together, *E. coli* FACU *ChrR*-encoding gene shares a high homology (approximately 98.6%) with the previously reported *E. coli* *chrR* (Ackerley et al. 2004). Therefore, it is regarded as a promising enzyme for chromate detoxification compared with reductase enzyme from class I family (Viti et al. 2003).

Most Cr (VI) reducing microorganisms are ubiquitous in environment with their ability to simultaneously remove organic contaminants as a natural bioremediation process, e.g., *B. megatarium*, *Halomonas* sp. (Cheung and Gu 2005; Mala et al. 2015). The exact mechanism potentiating extracellular/intracellular chromium reduction remains unclear, but Cr (VI) is transported into the cells where it is reduced intracellularly (Pei et al. 2009). A possible involvement of heme proteins, such as flavoproteins/NADPH in reduction process has been reported (Ackerley et al. 2004). Soluble reductases are constitutively produced; however, it can be induced intracellularly (Cheung and Gu 2007). In this respect, the FACU strain can reduce Cr (VI) to Cr (III) as quantified by measuring chromium reductase activity, this activity was acting optimally under neutral pH conditions and mild temperatures regimes. The main mechanism of Cr (VI) is electron transfer from NADPH, a coenzyme, to Cr (VI) that is reduced to Cr (III) (Ramirez-Diaz et al. 2008) which likely occurred in the present investigation.

LIBS is capable of spectrochemically identifying different samples of variable natures, like bacteria. This approach can be effectively used to evaluate the presence of living organisms in heavily contaminated environments. LIBS with nanosecond and femtosecond pulses can discriminate between different bacterial species as well as between living and dead ones with the privilege of avoiding time consuming and other drawbacks of conventional methods (Sivakumar et al. 2015). *E. coli* is genetically well characterized where its outer membrane is abundant in divalent  $Mg^{2+}$  and  $Ca^{2+}$  ions that can be detected by LIBS and, hence identifying these elements. C, Ca, Mg, and Na are major elements that are used extensively in identifying bacterial species. The distribution of spectral lines of LIBS would be used to discriminate between living and dead bacteria as fingerprint tool and can be used in



reactive oxygen species detection (El-Hussein and Hamblin 2017). In this context, we demonstrated that LIBS can identify the fingerprint elements like Na, Ca, Mg, and C for living bacteria with the abundance of Cr that has been reduced by *E. coli*. UV-Vis spectroscopy is widely used for differentiating and identifying Cr ions with different oxidation number. The absorption spectrum of chromium is much affected by the pH, solvent and the oxidation state. In literature, there are extensive studies interested in the carcinogenic and mutagenic Cr (VI), and less on Cr (III). Our results are matching coherently with the UV-Vis absorption peak of different Cr (III) complexes that were done by Perez-Benito and Martinez-Cereza (2018). There is an important point that one should bear in mind while analyzing the UV-Vis spectrum of Cr (III) as its absorption spectrum is dependent on the present ligand in the solution. Our results confirm that *E. coli* has reduced Cr (VI) to Cr (III) as the spectrum is lacking the characteristic peak of Cr (VI) at 440 nm that are well described in many spectrophotometric studies (Sanchez-Hachair and Hofmann 2018).

In the current study, the *E. coli* FACU strain has a large capability of decreasing Cr (IV) to Cr (III). Hence, it can act as a promising agent in bioremediation of toxic chromium species under aerobic environmental conditions.

**Authors' contributions** AAA and MSMM conceived and designed the study. MSMM and NIE performed experiments, drafted, and edited the manuscript. SA contributed to chemicals and facilities. AE performed laser-based detection of metals. All authors read and approved the final manuscript.

**Availability of data and materials** All datasets supporting the conclusions of the manuscript were included in the article. All sequencing data are available in the NCBI database, under acc. # numbers LC177419 and LC179020. The strain used in the study is available at Microbiological Resources Center (Cairo Egypt), under the number EMCC 2289.

## Compliance with ethical standards

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

**Research involving human participants and/or animals** None. This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed consent** NA

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