Characterization of multi-metal-resistant *Serratia* sp. GP01 for treatment of effluent from fertilizer industries

Hrudananda Sahoo1 · Sushama Kumari1 · Umesh Chandra Naik1,2

Received: 17 December 2020 / Revised: 26 July 2021 / Accepted: 4 August 2021 / Published online: 17 August 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

The effluent generated from fertilizer plants in Paradeep in the coast of the Bay of Bengal is the major pollutant causing health hazard in the vicinity of the area with respect to plants, animals and microbes. Samples of effluent were found to contain heavy metals (mg L⁻¹): Cr (100), Ni (36.975), Mn (68.673), Pb (20.133), Cu (74.44), Zn (176.716), Hg (5.358) and As (24.287) as analyzed by XRF. Indigenous bacterial strains were screened for chromate and multi-metal resistance to remediate the toxic pollutants. The isolated strain G1 was identified as *Serratia* sp. through 16S-rDNA sequence homology. The potent strain *Serratia* sp. GP01 treated with 100 mg L⁻¹ of K₂Cr₂O₇ has shown the efficacy of reducing 69.05 mg L⁻¹ of Cr over 48 h of incubation. Further, presence of chromate reductase gene (*ChR*) in *Serratia* sp. confirmed the enzymatic reduction of Cr(VI). SEM–EDX and SEM mapping analysis revealed substantial biosorption of Cr and other heavy metals present in effluent by *Serratia* sp. GP01. Antioxidant enzymes such as catalase (72.15 U mL⁻¹), SOD (57.14 U mL⁻¹) and peroxidase (62.49 U mL⁻¹) were found to be higher as compared to the control condition. FTIR study also revealed the role of N–H, O–H, C = C, C–H, C–O, C–N, and C = O functional groups of the cell surface of *Serratia* sp. treated with K₂Cr₂O₇ and effluent from the fertilizer industry. Isolated strain *Serratia* sp. could be used for the detoxification of Cr(VI) and other heavy metals in fertilizer plant effluent.

Keywords Fertilizer industry · Heavy metals · Bacteria · Chromate reductase · Antioxidants · Detoxification

Introduction

The fertilizer industry is one of the most polluting industries across the world. The fertilizer industry, mainly phosphate fertilizer plants are known to be a source of heavy metals and radionuclides (Khater 2012). Inorganic fertilizers such as calcium superphosphate, urea, iron sulphate and copper sulphate contain a high concentration of Zn, Co, Cu, Cd, Pb, Ni, Fe, Mn, which lead to deterioration of biological diversity of agricultural soil (Gimeno-Garcia et al. 1996). Heavy metals like Cd, Pb, and As have been found in phosphate fertilizers causing health hazard to plants, animals and microorganisms in surrounding ecosystems (Morgan 2013). Metal-induced oxidative stress is created in living organisms due to the generation of reactive oxygen species like O₂⁻, OH·, and H₂O₂ leading to the damage of lipid, protein and genomic content (Choudhary et al. 2007). Chromium is one of the most potent mutagenic and carcinogenic elements released by fertilizer industries. The toxic effect of hexavalent chromium [Cr(VI)] is attributed to the high solubility property of Cr(VI) as it can easily seep into the groundwater. Cr(VI) can easily pass through the semi-permeable biological membrane and interact with nucleic acid and cellular proteins of living organisms (Sultan and Hasnain 2005). Due to extreme toxic nature of soluble Cr(VI), it not only causes a mutational effect, but is also carcinogenic in nature due to its strong oxidizing nature (Mclean et al. 2000; Camargo et al. 2003). High-concentration Cr(VI) causes harm to biological systems by damaging the cell membrane, disrupting the cellular function, alteration in the specificity of enzymes and damaging the DNA structure (Bagchi et al. 2007).
2002). Cr(VI) causes various diseases like dermatitis, diarrhea, ulcers, eye, bronchial carcinomas and skin irritation, kidney dysfunction and probably lung carcinoma (Baruthio 1992). Exposure to Cr, Cd and Zn concentrations also results in cancer, gastrointestinal and respiratory damage, damages to the heart, brain, and kidney.

Various conventional methods including precipitation, ion exchange and adsorption on coal and activated carbon are employed for the removal of toxic chromium from environment. These methods require high energy and could not address the detoxification of toxic chromium (Avudainayagam et al. 2003). There are reports on various bacterial species as Cr(VI) reducing bacteria such as Bacillus sp. (Elangovan et al. 2006; Das et al. 2014) Microbacterium (Pattanapipitpaisal et al. 2001), Ochrobactrum intermedium (Faisal and Hasnain 2004), Pseudomonas sp. (Rajkumar et al. 2005), Shewanella oneidensis MR-1 (Thompson et al. 2007), Arthrobacter sp., Microbacterium sp. (Gutierrez et al. 2010). The detoxification strategies include biosorption, bioaccumulation and bioreduction by enzymatic reduction (Ramirez-Diaz et al. 2008). Microbial detoxification of Cr(VI) has been identified since the 1970s, which seems to be cost-effective and eco-friendly approach over conventional methods (Camargo et al. 2003). A wide group of Cr(VI)-resistant bacterial species such as Pseudomonas sp. (Rajkumar et al. 2005), Shewanella oneidensis MR-1 (Thompson et al. 2007), Arthrobacter sp., Microbacterium sp. (Gutierrez et al. 2010), Bacillus sp. (Das et al. 2014) have strong chromium metabolizing pathway. Extracellular, intracellular and membrane-bound reductases are involved in the reduction of Cr(VI) into Cr(III) (Joute et al., 2015). Reductases such as aldehyde oxidase, cytochrome p450, DT-diaphorase (Patra, et al. 2010), nitroreductase (Kwak et al., 2003) are involved in resistance and detoxification of Cr(VI). Reports on different chromate resistant genes (chrR, chrB, chrA, chrC, chrF, ruvB and chr2) from various bacteria like Pseudomonas aeruginosa, Ochrobactrum tritici, Ralstonia metallidurans, responsible for resistance to chromate ion and other toxic metalloids by minimizing the oxidative stress in the bacterial system along with the efflux system have been published (Juhnke et al. 2002; Aguilar-Barajas et al. 2008; Gadd 2010; Morais et al. 2011).

The effluent from the fertilizer industry is discharged into water bodies channeled to Mahanadi River near the confluence point in the Bay of Bengal. As a result, the aquatic system of river Mahanadi near Paradeep is polluted which in turn affects the nearby ecosystems causing health effects on humans, animals and plants as well. The untreated acidic effluent from the fertilizer plants is mainly responsible for increasing ammonia, nitrogen and phosphorous concentration. The effluent of fertilizer industries containing toxic heavy metals including carcinogenic chromium aggravates the pollution menace in surrounding regions of the port town of Paradeep, off the coast of the Bay of Bengal. Given this pollution problem, the present study attempts to address the removal and detoxification of Cr(VI) present in the effluent. We have investigated the potency of an indigenous chromate-resistant bacterium (strain-GP01) which could be used for successful bioremediation of Cr(VI) and other heavy metals in effluent released from fertilizer industries.

Materials and methods

Sampling site

Effluent sample was collected from the site (20.262646, 86.630084) in the Paradeep region, Odisha (Fig. 1). Sterile bottles were used for sampling and collected samples were brought to the laboratory in ice packs. pH, redox potential and temperature parameters were recorded during sampling by pocket pH meter (Hanna) and mercury thermometer, respectively.

Elemental analysis by ED-XRF

The elemental composition of effluent was analyzed by the X-ray fluorescence (ED-XRF) spectrometry method (PANalytical Epsilon 5, Bruker S4 PIONEER, Europe) at Advanced Instrumentation Research Facility (AIRF), JNU, New Delhi.

Isolation of bacterial strains

About 1 mL of sample was taken and diluted up to 10 mL with double-distilled water in a test tube as stock solution. A dilution series was prepared with different dilution factors ranging from 10⁻¹ and 10⁻⁶ for isolation of bacterial strains from the samples. Diluted samples (100 µL) were spread onto the nutrient agar petri-dishes (agar–agar 1.8, peptone 1.0, yeast extract 1.0, sodium chloride 1.0 g L⁻¹) and incubated in a microbiological incubator (SDS1-2S, Smita Scientific) at 37 °C for 24–48 h for the growth of bacteria. Based on the morphological features such as size, color, texture, the bacterial strains were isolated into different morphological communities.

Screening and growth pattern of isolates strains under K₂Cr₂O₇ stress

The isolated bacterial strains were inoculated in Minimal Salt Media (MSM) amended with 50 mg L⁻¹ to 500 mg L⁻¹ (v/v) of potassium dichromate (K₂Cr₂O₇) and incubated at 35 °C for 72 h at 100 rpm. The growth pattern of bacterial isolate in liquid media was calculated by measuring OD at 600 nm (Naik et al. 2012).
Molecular identification of potent chromium-resistant bacteria

Genomic DNA was isolated from the potent chromium-resistant bacteria strain (G1) by QIAamp DNA Mini Kit from Qiagen. The 16S rDNA was amplified using primer sets of forward primer 27F (5′-AGAGTTT GATCMTGG CTCAG-3′) and the reverse primer 1492R (5′-CGTATTACCT TGTACGACTT-3′). The PCR reaction mixture of 25.0 µL contains 0.5 µM of each primer, Taq buffer (1 x), 1.5 mM of MgCl2, 500 µM of DNTP’s mix, 1 U of Taq polymerase and 50 ng of template DNA. The DNA profiling was carried out by using a thermal cycler (Veriti-96, Applied Biosystems, USA) with the following programs: pre-heating at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 50 °C for 30 s, elongation at 72 °C for 90 s and final extension step at 72 °C for 5 min. Amplified PCR products were purified by using the HiPurA PCR product purification kit from Himedia. The purified PCR product was used for sequencing with Genetic Analyser-3500 (Applied Biosystems, USA), using BDT v3.1 ready reaction mixture. An identified nucleotide sequence was deposited in the National Center for Biotechnology Information (NCBI) and EzBioCloud database (Kim et al. 2012). Corresponding neighbor sequences (Based upon maximum similarity and zero E-value) were downloaded from the NCBI database and valid type strains from the EZBioCloud database. The phylogenetic tree was constructed by aligning the sequences and bootstrapped neighbor-joining relationships (1000 replicates) with MEGA version 7.0 software (Stackebrandt and Goebel 1994; Tamura et al. 2013; Kumar et al. 2016).

Identification of chromate reductase in Serratia sp. GP01

Bacterial chromate reductase genes (ChR and ChrT) were amplified with the genomic DNA of Serratia sp. GP01. PCR amplification of ChR and ChrT genes was carried out in the thermal cycler (Veriti-96, Applied Biosystems, USA), by using the primer sets as given in Table 1 (Patra et al. 2010; Zhou et al. 2017). A total volume of 25.0 µL PCR reaction mixture containing 0.5 µM of each primer, 11.0 µL of 2X-PCR TaqMixture (Himedia), 50 ng of template DNA. Final volume was brought with molecular grade water. PCR workflow was set as following programs: pre-heating at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 53 °C (for ChR) and 56 °C (for ChrT) for 30 s, elongation at 72 °C for 60 s and final extension step at 72 °C for 10 min. Amplified PCR products were separated on 2% (w/v) agarose gel and purified by using the HiPurA-PCR product purification kit (Himedia). The purified PCR product was used for sequencing with Genetic Analyser-3500 (Applied Biosystems, USA), using BDT v3.1 ready reaction mixture. An identified nucleotide sequence

---

Fig. 1 Location map and sampling site of industrial effluent at Paradeep region in India
of the amplified chromate reductase gene was translated and appropriate open reading frame was selected by employing ExPASy tools (http://www.expasy.org/tools/dna.html). Translated chromate reductase was searched in ExPASy-blast search (https://web.expasy.org/blast/) and chromate reductase sequences of different Serratia sp. were retrieved from NCBI. Based on homology similarity, a phylogenetic tree was prepared by aligning the sequences in Clustal W and tree was constructed by using MEGA 7.0 with neighbor-joining method (1000 bootstrap replicates at 50%) (Tamura et al. 2007). Significant alignment of the query sequence of Serratia sp. GP01 with another group of bacteria was carried out by NCBI blastp (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and similarities of sequences were analyzed.

**Removal of chromium by isolated potent strain Serratia sp. GP01**

Cr(VI) removal potential of Serratia sp. GP01 was measured by inoculating the bacterial isolates in liquid glucose media containing fertilizer industry effluent. Bacteria culture and un-inoculated culture medium (control) were incubated in an orbital shaker incubator at 120 rpm and 30 °C. Reduction of Cr(VI) in the medium was assayed by diphenylcarbazide (DPC) method. About 1 mL of sample was collected after 24, 48 and 72 h followed by centrifugation at 12,000 × g for 10 min at 4 °C. Then about 1 mL of diphenylcarbazide solution (0.25 g diphenylcarbazide dissolved in 100 mL acetone) was added to the collected supernatant in a test tube followed by the addition of one drop of phosphoric acid to lower the pH. Then the solution was allowed to keep at room temperature for the development of color for 5 min and the concentration of Cr(VI) was estimated by spectrophotometer at 540 nm (Zahoor and Rehman 2009). The Cr(VI) reduction was estimated by the following equation:

\[
\text{Cr(VI) reduction} (\%) = \frac{A - B}{A} \times 100,
\]

where \(A\) and \(B\) refer to initial concentration and residual concentration, respectively.

**Antioxidant assay**

Equal volume (10 mL) of effluent-treated and untreated bacteria culture were centrifuged at 10,000g for 10 min at 4 °C. Bacterial pellet were resuspended in 1 mL of lysis buffer [100 mM Tris–HCl (pH 8.0), 2 mM MgCl\(_2\), 0.1 mM EDTA Na\(_2\), 0.2 M NaCl, 1% Triton X-100] followed by sonication (Vibra-m-Cell, Sonic, USA) at 50% amplitude (30 kHz frequency rate) with 5 s on and 5 s off for 15 cycles at 4 °C. The lysate was spun in a pre-chilled centrifuge for 15 min at 20,000g and 4 °C (Zhang et al. 2013). Bacterial crude extracts were estimated by Bradford (1976) assay. Antioxidant enzymes such as superoxide dismutase, catalase and peroxidase were carried out to assess the resistance of Serratia sp. GP01 against toxicity effect of fertilizer industry effluent. Catalase activity was measured in bacterial extract with the methods developed by Aebi (1984). Bacterial extract of 20 μL was mixed with 980 μL of H\(_2\)O\(_2\) buffer and absorption was measured at 240 nm using a UV–vis spectrophotometer for 60 s at interval of 15 s. The activity was calculated by decrease in absorbance of H\(_2\)O\(_2\) at 240 nm concerning time intervals. Peroxidase activity was measured in bacterial crude extract according to the method of Chance and Maehly (1955). The reaction mixture contains 350 μL of 5% (w/v) pyrogallol in 100 mM potassium phosphate buffer at pH 6 and 25 μL of bacteria cell lysate. Blank contains the reaction mixture and 25 μL of un-inoculated media. The reaction was initiated by the addition of 0.5% (v/v) hydrogen peroxide 30% solution and absorption was measured at 420 nm using UV–VIS spectrophotometer per 34 s at 25 °C. SOD activity was also measured in bacterial extract using the protocol as suggested by Ewing and Janero (1995). In this assay, 25 μL of bacterial extract was mixed with 20 μL of reaction buffer [50 mM phosphate buffer, 0.1 mM EDTA, 98 μM NADH, and 62 μM NBT, pH 7.4]. The reaction was initiated by adding 20 μL of an initiating reagent (50 mM phosphate buffer and 33 μM PMS in 0.1 mM EDTA, pH 7.4). The SOD activity was calculated by measuring the absorbance at 560 nm.

**Scanning electron microscope and EDX analysis**

Bacterial cells were fixed in 1% (v/v) of glutaraldehyde and 2% (v/v) paraformaldehyde buffered with 0.1 M of sodium phosphate buffer saline (pH 6.8) for 12–18 h at 4 °C followed by washing with fresh buffer and fixation with osmium tetroxide (1%) in the same buffer at 4 °C for 2 h. Fixed bacterial cells were washed with phosphate buffer followed by dehydration in a series of ethanol–water solution (30%, 50%, 70% and 90% ethanol) for 5 min each and kept.
for drying under a CO₂ atmosphere for 20 min. Bacterial cells were mounted with aluminium stubs and were coated with 90-Å-thick gold palladium (VG Microtech, East Sussex, TN22, England) for 30 min. Coated bacterial cells were viewed at 15 kV with SEM (Model-Zeiss EVO40). Energy-dispersive X-ray spectrometer (EDAX, USA) was run at 20 kV for studying the biosorption of chromium by isolated strain (Naik et al. 2012; Sahoo et al. 2020).

FTIR analysis on functional groups

Fourier transform infrared spectroscopy (FTIR) was used to identify the changes in functional groups of bacteria under different stress conditions. Both control and experimental (10% effluent and 100 mg L⁻¹ of K₂Cr₂O₇) bacterial biomass were harvested at exponential phase (A₆₀₀ = 0.6). The biomass was dried in a hot air oven at 60 °C for complete dryness and cool in a desiccator. Approximately 5 mg of dried biomass was made to a fine powder along with 50 mg of potassium bromide, using an agate mortar and pestle (Thomas Scientific), (Kowalczuk and Pitucha 2019). The powdered samples were pressed and KBr pellets were prepared for analysis. The infrared spectra (%T) were recorded by using a Spectrum RX I Perkin Elmer, FTIR Spectrometer (Thermo scientific), within the range 400 to 4000 cm⁻¹.

Results and discussion

Elemental analysis of effluent

Elemental analysis of effluent from the fertilizer industry revealed the presence of heavy metals (mg L⁻¹): Cr(100), Ni (36.975), Mn (68.673), Pb (20.133), Cu (74.44), Zn (176.716), Hg (5.358) and As (24.287) as analyzed by XRF analysis (Table 2). The effluent was found to be highly acidic in nature with the pH (1.36) and redox potential (289). Report published by Institute of Minerals and Materials Technology (IMMT) shows the presence of different heavy metals beyond the permissible limits (Dharmendra et al. 2020) at the Paradeep region. Previous studies have also confirmed the existence of heavy metals such as Cd, Co, Cu, Ni, Pb, Zn, Fe and Mn in different inorganic fertilizers like urea, calcium superphosphate, iron sulphate and copper sulphate (Gimeno-Garcia et al. 1996). Further, Mekki and Sayadi (2017) have reported that the heavy metal accumulation increased over time in phosphate fertilizer treated soil samples with average values in the following order: iron (Fe 252.72 mg L⁻¹) > zinc (Zn 152.95 mg L⁻¹) > lead (Pb 128.35 mg L⁻¹) > copper (Cu 116.82 mg L⁻¹) > cadmium (Cd 58.03 mg L⁻¹).

| Compound | Corr. (cps/mA) | Conc | Unit |
|----------|---------------|------|------|
| P        | 10.134        | 1.170 | %    |
| S        | 1.705         | 0.292 | %    |
| Cl       | 0.520         | 377.390 | ppm |
| K        | 1.971         | 404.017 | ppm |
| Ca       | 66.262        | 0.985 | %    |
| V        | 0.576         | 113.599 | ppm |
| Cr       | 0.907         | 100.285 | ppm |
| Mn       | 0.981         | 68.673 | ppm |
| Fe       | 19.567        | 971.749 | ppm |
| Ni       | 0.703         | 36.955 | ppm |
| Cu       | 3.090         | 74.444 | ppm |
| Zn       | 8.055         | 176.716 | ppm |
| As       | 2.230         | 24.287 | ppm |
| Se       | 0.000         | 0.000 | ppm |
| Sr       | 3.959         | 155.732 | ppm |
| Zr       | 0.234         | 6.581 | ppm |
| W        | 13.275        | 925.007 | ppm |
| Hg       | 0.129         | 5.358 | ppm |
| Pb       | 0.732         | 20.133 | ppm |

Growth pattern of isolates strain under K₂Cr₂O₇ stress

The bacterial population was analyzed from the effluent site near the fertilizer industry located at Paradeep, Odisha, India. Samples exhibited different number and pattern of colonies on pour plate culture incubated for 24 h at 37 °C. Based on the morphological features such as size, color, texture, the bacterial strains were isolated into different morphological communities. Bacterial isolates G1 and G2 incubated in MSM amended with 50–500 mg L⁻¹ of K₂Cr₂O₇, have shown contrasting growth pattern over an incubation period of 72 h. Strain G1 has shown a higher growth rate as compared to G2 after 24, 48 and 72 h of incubation period than strain G2. It was observed that G1 strain showed high growth in media enriched with 50 and 100 mg L⁻¹ of K₂Cr₂O₇ and decreased growth was found in culture media enriched with 250, 500 mg L⁻¹ over 72 h (Fig. 2). The growth of the isolated bacterial strain was found to decrease with an increasing K₂Cr₂O₇ concentration. Phospho-gypsum is the major by-product of phosphate fertilizer industry effluent which contains toxic metals like Cd, Cr, Hg, Pb, As, Zn and a few radioactive elements like uranium and thorium (Khater 2012). Apart from that, pH is an important factor as all the chemical reactions of aquatic bodies are controlled by pH. The water sample collected from the effluent site was found to have
a pH of 1.35 at a temperature of 29.6 °C. The toxicity of heavy metals could be further enhanced by lower pH.

The strain G1 has a greater population of $21 \times 10^2$ cfu mL$^{-1}$ than strain G2 having $11 \times 10^2$ cfu mL$^{-1}$. The result shows that strain G1 has potential resistance and a high growth rate in comparison to strain G2 against toxicity of heavy metals present in the effluent. Previous report has also suggested that increasing concentration of heavy metals is attributed to decreased cfu mL$^{-1}$ of microbial groups. Alterations in the composition of microbial communities could be taken as the indicator of anthropogenic and industrial effects on soil and water ecosystems (Ashok et al. 2017). The toxic effects of heavy metals result mainly from the interaction of metals with proteins (enzymes) and inhibition of metabolic processes in the bacterial cell. The isolated strains might have a high resistance and detoxifying capacity as they are indigenous to that environmental condition.

Molecular identification of potent bacterial strain

A sequence of amplified genomic DNA of potent bacterial strain G1 was compared with the existing database of the gene bank, National Centre for Biotechnology Information (NCBI). The bootstrap consensus sequence was drawn with the neighbor-joining multiple sequences alignment method using software mega version 7.0 with different species of bacteria. The isolated strain has shown 99% sequence similarity with the 16S rDNA sequence of the corresponding sequences of Serratia sp. and hence the selected strain was identified as Serratia sp. GP01 (Accession number: MH971240) (Fig. 3).

Analysis of chromate reductase genes in Serratia sp. GP01

The result of PCR-amplified products on agarose gel (Supplementary Fig. 1) has shown the presence of chromate reductase gene ($ChR$) in Serratia sp. GP01. Partially amplified chromate reductase ($ChR$) gene product was about 268 bp long. Phylogenetic analysis of partially identified chromate reductase gene ($ChR$) with other groups of bacteria (Fig. 4). Chromate reductase ($ChR$) of strain GP01 (query) has shown close relationship with Class-I_chromate_reductase_flavoprotein, putative NADPH dependent FMN reductase, flavin reductase of E. coli, chromate reductase of Bacillus sp., and other groups of bacteria including, Shigella sonnei, Enterobacteriaceae bacterium, Shigella flexneri, Trichuris trichiura. However, $ChR$ of Serratia sp. GP01 and chromate reductases of other Serratia sp. strains were found to be distantly related. Multiple alignments of different chromate reductase through NCBI blastp (https://blast.ncbi.nlm.nih.gov/Blast.cgi) has shown the amino acid similarity matches with other groups of bacteria (Supplementary Fig. 2). In the present study nucleotide sequences of chromate reductase gene ($ChR$) as analyzed by sequencing followed by ExPASy analysis was submitted to NCBI (GenBank) under the accession number (MZ476040). Aguilar-Barajas et al (2008) have reported the $ChrR$ gene from Pseudomonas aeruginosa which is responsible for chromate resistance. Kalsoom et al. (2021) have also reported the presence of chromate resistance genes ($ChrR$) from Staphylococcus simulans against chromate toxicity. It was evident that in the present study, the identified potent strain GP01 has the chromate reductase gene ($ChR$), which helps in the
reduction of the Cr(VI) and helps the bacteria to withstand the extreme toxic conditions.

**Removal of chromium by Serratia sp. GP01**

The selected bacterial isolate G1 exhibits different Cr(VI) reduction potency at 100 mg L\(^{-1}\) concentration under different incubation times in the liquid media. Growth of the isolated strain-G1 (*Serratia* sp. GP01) was found to be higher in 100 mg L\(^{-1}\) concentration of chromium and it showed maximum reduction capacity of 62.43, 69.05, 52.62 mg L\(^{-1}\) after 24, 48 and 72 h of incubation, respectively (Fig. 5). *Serratia* sp. Cr-10 has shown Cr(VI) reduction activity with the highest reduction rate of 0.28 mg L\(^{-1}\) h\(^{-1}\), lower than *Brucella* sp. (0.92 mg L\(^{-1}\) h\(^{-1}\)) (Thacker et al. 2007) and *Pseudomonas* sp. (0.24 mg L\(^{-1}\) h\(^{-1}\)) (McLean et al. 2000).

In the present study, the isolated strain *Serratia* sp. GP01 showed high chromium reduction capacity in comparison to the above mentioned strains reported by different authors. It could be suggested that isolated *Serratia* sp. GP01 could be used for detoxification of Cr(VI) present in effluent from fertilizer industries.
Antioxidants production by *Serratia* sp. GP01

Bacterial crude extract protein samples were estimated to be 2.1 mg mL\(^{-1}\). The assay of bacterial strain G1 (*Serratia* sp. GP01) with 10% (v/v) heavy metals showed a distinct effect of fertilizer industry effluent on superoxide dismutase (SOD), catalase and peroxidase activity (Fig. 6). Increased activity of catalase (72.15 U mL\(^{-1}\)), SOD (57.14 U mL\(^{-1}\)) and peroxidase (62.49 U mL\(^{-1}\)) in *Serratia* sp. GP01 are noticed in response to 10% (v/v) of effluent from fertilizer industry. Heavy metal-induced oxidative stress due to the generation of free radicals enhances the accumulation and activity of detoxifying enzymes (Ilias et al. 2011). Under normal circumstances, the concentration of free radicals remains low due to the activity of protective enzymes which includes SOD, catalase, peroxidase, etc. The expressions of these enzymes are thought to be increased under heavy metals stress to detoxify the reactive oxygen species (Choudhary et al. 2007). An earlier study has shown that the first line of defense against the generation of toxic oxygen species is the induction of SOD activity (Lenartova et al. 1998). Previous study has also reported that hydrogen peroxide may also be involved in peroxidase-mediated reaction of oxidative polymerization strengthening cell wall and formation of barrier anti-stress structures (Cosgrove 1997).

**Bacterial morphology under heavy metals stress**

Morphological assessment and quantification of chromium and other heavy metals absorbed on the surface of isolated bacterial biomass were performed by scanning electron microscopy (SEM) and energy dispersive X-ray analysis.
(EDX) analysis. K2Cr2O7-treated bacterial strain *Serratia* sp. GP01 showed morphological changes. Untreated bacterial strain was rod-shaped and elongated having a smooth surface, whereas K2Cr2O7-treated bacteria were found to be smaller and irregular and associated with extracellular granules. Effluent-treated bacteria have shown drastic changes in morphological features in terms of size and shape like drum (Fig. 7). The changes in cell morphology in response to fertilizer industry effluent might be due to the cumulative toxicity of different heavy metals present in the effluent. The interaction of toxicity of different heavy metals could be the possible factor for creating a highly toxic condition for the isolated bacterial strain (Naik et al. 2012; Jin et al. 2017). Changes in surface morphological features such as roughness, size, shape, and swelling have also been reported on bacteria *Bacillus cereus* IST105, *Bacillus subtilis* and *Escherichia coli* in response to chromium toxicity. Bacterial cells may arrange themselves as groups to reduce exposure to chemicals and this might be the one of the adoptive mechanisms for many microbes (Schembri et al. 2003; Naik et al. 2012; Samuel et al. 2013).

**SEM–EDX for Cr and metals biosorption**

SEM–EDX analysis clearly showed the metals disintegration and absorption onto the surface of isolated strain. The isolated strain *Serratia* sp. GP01 showed strong multi-metal absorption potency. EDX analysis revealed that the cells exposed to K2Cr2O7 showed the chromium peaks (with 0.13 wt%) indicating biosorption of Cr and precipitation of chromium inside the bacterial cell. The intensity of the biosorbed peak for different heavy metals was based on the concentration of heavy metals as analyzed through ED-XRF (Supplementary Fig. 3). The potency and efficiency for biosorption of multi-metals could be attributed to the bioreduction of heavy metals through the reductase enzyme. Earlier it has been reported on multi-metal-tolerant strain *Bacillus cereus* FIT10 which could be used for the bioremediation of toxic metals such as Ni(II), Cr(VI), Pb(II), and Cu(II) from aqueous solution (Dhanwal et al. 2018). The biosorption and precipitation of chromium residues on the cell surface of strain *Cellulosimicrobium* sp. has also been studied by EDX analysis (Bharagava and Mishra 2018).

**Study on functional groups**

FTIR analyses of untreated bacteria, K2Cr2O7-treated bacteria and 10% (v/v) effluent-treated bacteria have revealed the status of functional groups as IR spectra pattern (Supplementary Fig. 4). The wave number 2925 cm⁻¹ attributed to the stretching vibration of O–H groups on the surface of untreated bacteria, whereas spectra pattern has shown shifting of O–H group at 2925 and 2930 cm⁻¹ in chromium and effluent-treated bacteria, respectively. Further, the peak at 3416 cm⁻¹ is assigned to stretching of OH groups which enhance the hydration of bacterial cell while there is shifting of O–H at 3424 and 3421 cm⁻¹ under K2Cr2O7 and 10% (v/v) effluent, respectively. Spectroscopic changes at 1394 cm⁻¹ has shown bending vibration of C–H group, whereas similar spectroscopic changes at 1402 cm⁻¹ in the group observed in chromium stress condition. Spectroscopic changes at 1397 cm⁻¹ has shown bending vibration of C–H group, whereas a slight modification in absorbance at 1402 cm⁻¹ was observed in stress condition. Shifting of stretching vibration of N–H group was observed at 1279 and 1235 cm⁻¹, respectively, in response to Cr(VI) and fertilizer industry effluent. A broad band at 1084 cm⁻¹ represented the stretching of C = O of amide groups which brought changes at 1057 and 1235 cm⁻¹ under chromium and effluent stress, respectively.

![Fig. 7 SEM micrographs of a untreated bacteria, b treated bacteria and c 10% (v/v) effluent-treated bacteria](image-url)
FTIR spectroscopy is also used for the study of the position and status of functional groups of potent chromium resistant strain Serratia sp. GP01. The wave number 2925 cm\(^{-1}\) attributed to the stretching vibration of C–H group on the surface untreated bacteria, whereas a higher spectra pattern has shown shifting of C–H group at 2930 cm\(^{-1}\) on chromium and effluent-treated bacteria. There were changes in the functional group of strain Serratia sp. GP01 after treatment of chromium and 10% (v/v) effluent over 24 h of incubation (Supplementary Fig. 4). The peak at 3416 cm\(^{-1}\) is assigned to stretching OH groups which enhance the hydration of bacterial cell. A broad band at 1084 cm\(^{-1}\) represented the stretching of OH groups which enhance the hydration of bacterial cell. The resistance to chromium and metal toxicity is attributed to the presence of chromate reductase gene (ChR) in Serratia sp. GP01. Further antioxidant enzymes such as catalase, SOD and peroxidase were found to be higher as compared to the control condition. FTIR study revealed the changes in the status of functional groups in response to metals and the role of N–H and C=O functional groups on the cell surface of Serratia sp GP01. Hence, the indigenous strain Serratia sp. GP01 could be used for substantial detoxification of Cr(VI) and other heavy metals in fertilizer plant effluent. Further, work on the molecular and biochemical understanding on metal–microbe interaction could augment the treatment of toxic effluent from fertilizer industries by microbial technology using Serratia sp. GP01.

Conclusions

In the present study, we have characterized potent indigenous bacterial strain Serratia sp. GP01 having multi-metal resistance capacity. The strain has shown the efficacy of removing toxic chromium from the culture amended with fertilizer industry effluent and K\(_2\)Cr\(_2\)O\(_7\) over 48 h of incubation. The biosorption of chromium and other heavy metals by Serratia sp. GP01 as analyzed by SEM–EDX has revealed the bioremediation potency of the isolated strain. The resistance to chromium and metal toxicity is attributed to the presence of chromate reductase gene (ChR) in Serratia sp. GP01. Further antioxidant enzymes such as catalase, SOD and peroxidase were found to be higher as compared to the control condition. FTIR study revealed the changes in the status of functional groups in response to metals and the role of N–H and C=O functional groups on the cell surface of Serratia sp GP01. Hence, the indigenous strain Serratia sp. GP01 could be used for substantial detoxification of Cr(VI) and other heavy metals in fertilizer plant effluent. Further, work on the molecular and biochemical understanding on metal–microbe interaction could augment the treatment of toxic effluent from fertilizer industries by microbial technology using Serratia sp. GP01.

Acknowledgements We would like to acknowledge SERB, Govt. of India, for financial support for developing instrumentation facilities. Financial assistance to the Centre of Excellence in Environment and Public Health by Higher Education Department of Government of Odisha under OHEPEE is also gratefully acknowledged (HE-PTC-WB-02017). We are very much thankful to the technical staffs of AIRF, JNU for SEM–EDX analysis and CIF, Institute of Life Sciences at Bhubaneshwar, India for sequencing of genomic DNA.

Author contribution Umesh Chandra Naik designed, wrote and reviewed the manuscript. Hrudananda Sahoo and Sushama Kumari executed the experiments, analyzed the data and wrote the manuscript. All the authors were involved in the critical review on writing the manuscript.

Funding No funding was received for conducting this study.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126
Aguilar-Barajas E, Palusco E, Cervantes C, Rensing C (2008) Expression of chromate resistance genes from Shewanella sp. strain ANA-3 in Escherichia coli. FEMS Microbiol Lett 285:97–100
Ashok AH, Mizuno Y, Volkow ND, Howes OD (2017) Association of stimulant use with dopaminergic alterations in users of cocaine, amphetamine, or methamphetamine: a systematic review and meta-analysis. JAMA Psychiatry 74:511–519
Avudainayagam S, Megharaj M, Owens G, Kookana RS, Chittleborough D, Naidu R (2003) Chemistry of chromium in soils with emphasis on tannery waste sites. Rev Environ Contam Toxicol 178:53–91
Bagchi D, Stofs SJ, Bernard WO, Bagchi M, Preus HG (2002) Cytotoxicity and oxidative mechanism of different forms of chromium. Toxicol 180:5–22
Baruthio F (1992) Toxic effects of chromium and its compounds. Biol Trace Elem Res 32:145–153
Bharagava RN, Mishra S (2018) Hexavalent chromium reduction potential of Cellulosimicrobium sp. isolated from common effluent treatment plant of tannery industries. Ecotoxicol Environ Saf 147:102–109
Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
Camargo FAO, Okeke BC, Bento FM (2003) In vitro reduction of hexavalent chromium by a cell-free extract of Bacillus sp. ES29 stimulated by Cu (II). Appl Microbiol Biotechnol 62:569–573
Chance B, Maehly AC (1955) Assay of catalases and peroxidases. Methods Enzymol 2:773–775
Choudhary M, Jetley UK, Khan MA, Zutshi S, Fatma T (2007) Effect of heavy metal stress on proline malondialdehyde and superoxide dismutase activity in the cyanobacterium Spirulina platensis-S5. Ecotox Environ Saf 66:204–209
Cosgrove DJ (1997) Relaxation in a high-stress environment: the molecular bases of extensible cell walls and cell enlargement. Plant Cell 9:1031–1041
Das S, Mishra J, Das SK, Pandey S, Rao DS, Chakraborty A, Sudadaran M, Das N, Thatoi H (2014) Investigation on mechanism of Cr

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-021-02523-z.
(VI) reduction and removal by *Bacillus amylovorans*, a novel chromate tolerant bacterium isolated from chromite mine soil. Chemosphere 96:112–121

Dhanwal P, Kumar A, Dudeja S, Badgjur H, Chauhan R, Kumar A, Dhull P, Chhokar V, Beniwal V (2018) Biosorption of heavy metals from aqueous solution by bacteria isolated from contaminated soil. Water Environ Res 90:424–430

Dharmendra S, Kumar MR, Chinnamaye A, Ranjan SD, Ranjan PC (2020) Assessment of marine sediment contamination and detection of their potential sources at Paradip port, East Coast of India. Res J Chem Environ 24:6

Elangovan R, Abhipsa S, Rohit B, Ligy P, Chandraraj K (2006) Reduction of Cr (VI) by a *Bacillus* sp. Biotechnol Lett 28:247–252

Ewing JF, Janero DR (1995) Microplate superoxide dismutase assay employing a nonenzymatic superoxide generator. Anal Biochem 232:243–248

Faisal M, Hasnain S (2004) Comparative study of Cr (VI) uptake and reduction in industrial effluent by *Ochrobactrum intermedium* and *Brevibacterium* sp. Biotechnol Lett 26:1623–1628

Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156:609–643

Gimeno-Garcia E, Andreu V, Boluda R (1996) Heavy metals incidence in the application of inorganic fertilizers and pesticides to rice farming soils. Environ Pollut 92:19–25

Gutierrez AM, Cabriales JJ, Vega MM (2010) Isolation and characterization of hexavalent chromium-reducing rhizospheric bacteria from a wetland. Int J Phytoremediation 12:317–334

Ilias M, Rafiquillah IM, Debnath BC, Mannan KS, Hoq MM (2011) Isolation and characterization of chromium (VI)-reducing bacteria from tannery effluents. Indian J Microbiol 51:76–81

Jin Y, Wu S, Zeng Z, Fu Z (2017) Effects of environmental pollutants on gut microbiota. Environ Pollut 222:1–9

Joutey NT, Sayel H, Bahafid W, El Ghachtouli N (2015) Mechanisms of hexavalent chromium resistance and removal by microorganisms. Rev Environ Contam Toxicol 233:45–69

Juhinke S, Peitzsch N, Hubener N, Grobe C, Nies DH (2002) New genes involved in chromate resistance in *Ralstonia metallidurans* strain CH34. Arch Microbiol 179:15–25

Kalsoom A, Batool R, Jamil N (2021) Highly Cr (vi)-tolerant *Staphylococcus simulans* assisting chrome evacement from tannery effluent. Green Process Synth 10:295–308

Khater AE (2012) Uranium and trace elements in phosphate fertilizers–Saudi Arabia. Health Phys 102:63–70

Kim OS, Cho YJ, Lee K et al (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Kowalczik D, Pituscha M (2019) Application of FTIR method for the assessment of immobilization of active substances in the matrix of biomedical materials. Materials 12:2972

Kumar S, Stecher G, Tamura K (2016) MEGA7 molecular evolutionary analysis version 7.0 for bigger dataset. Mol Biol Evol 33:1870–1874

Kwak YH, Lee DS, Kim HB (2003) Vibrio harveyi nitratreductase is also a chromate reductase. Appl Environ Microbiol 69:4390–4395

Lenartova V, Holovska K, Javorsky P (1998) The influence of mercury on the antioxidant enzyme activity of rumen bacteria *Streptococcus bovis* and *Selenomonas ruminantium*. FEMS Microbiol Ecol 27:319–325

McLean JS, Beveridge TJ, Phipps D (2000) Isolation and characterization of a chromium-reducing bacterium from a chromated copper arsenate-contaminated site. Environ Microbiol 2:611–619

Mekki A, Sayadi S (2017) Study of heavy metal accumulation and residual toxicity in soil saturated with phosphate processing wastewater. Water Air Soil Pollut 228:215

Morais PV, Branco R, Francisco R (2011) Chromium resistance strategies and toxicity: what makes *Ochrobactrum tritici* Svbl1 a strain highly resistant. Biometals 24:401–410

Morgan R (2013) Soil, heavy metals, and human health. In: Brevik EC, Burgess LC (eds) Soils and human health. CRC Press, Boca Raton, pp 59–82

Naik UC, Srivastava S, Thakur IS (2012) Isolation and characterization of *Bacillus cereus* IST105 from electroplating effluent for detoxification of hexavalent chromium. Environ Sci Pollut Res 19:3005–3014

Patra RC, Malik S, Beer M, Megharaj M, Naidu R (2010) Molecular characterization of chromium (VI) reducing potential in Gram positive bacteria isolated from contaminated sites. Soil Biol Biochem 42:1857–1863

Pattanapipaitaisal P, Brown NL, Macaskie LE (2001) Short contribution: chromium reduction and 16S rRNA Identification of bacteria isolated from a Cr (VI)-contaminated Site. Appl Microbiol Biotechnol 57:257–261

Rajkumar M, Nagesh Babu JS, Lee KL, Lee WH (2005) Characterization of a novel Cr6+ reducing *Pseudomonas* sp. with plant growth-promoting potential. Curr Microbiol 50:266–271

Ramirez-Diaz MI, Diaz-Perez C, Vargas E, Riveros-Rosas H, Campos-Garcia J, Cervantes C (2008) Mechanisms of bacterial resistance to chromium compounds. Biometals 21:321–332

Sahoo H, Senapati D, Thakur IS, Naik UC (2020) Integrated bacterial- algal bioreactor for removal of toxic metals in acid mine drainage from iron ore mines. Bioreosur Technol Rep 11:100422

Samuel J, Pulimi M, Paul ML, Maura Y, Chandrasekaran N, Mukherjee A (2013) Batch and continuous flow studies of adsorptive removal of Cr (VI) by adapted bacterial consortia immobilized in alginate beads. Bioreosur Technol 128:423–430

Schembri MA, Hjerrild L, Gjermansen M, Klemm P (2003) Differential expression of the *Escherichia coli* autoaggregation factor antigen 43. J Bacteriol 185:2236–2242

Stuckebrandt E, Goebl BM (1994) Taxonomic note: a place for DNA–RNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Evol Microbiol 44:846–849

Sultan S, Hasnain S (2005) Chromate reduction capability of a gram positive bacterium isolated from effluent of dying industry. Bull Environ Contam Toxicol 75:699–706

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Thacker U, Parikh R, Shouche Y, Madamwar D (2007) Reduction of chromate by cell-free extract of *Brucella* sp. isolated from Cr (VI) contaminated sites. Bioreosur Technol 98:1541–1547

Thompson MR, Ver Berkmoes NC, Chourey K, Shah M, Thompson DK, Hettich RL (2007) Dosage-dependent proteome response of *Shewanella oneidensis* MR-1 to acute chromate challenge. J Proteome Res 6:1745–1757

Zahoor A, Rehman A (2009) Isolation of Cr (VI) reducing bacteria from industrial effluents and their potential use in bioremediation of chromium containing wastewater. J Environ Sci 21:814–820

Zhang L, Liu C, Li D, Zhao Y, Zhang X, Zeng X, Yang Z, Li S (2013) Chromium resistance strategies and toxicity: what makes *Ochrobactrum tritici* Svbl1 a strain highly resistant. Biometals 24:401–410

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.