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

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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. Potent strain *Serratia* sp. GP01 treated with 100 mg L^{-1} of $\text{K}_2\text{Cr}_2\text{O}_7$ 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 $\text{K}_2\text{Cr}_2\text{O}_7$ 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.

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 $\text{O}_2^{\cdot-}$, $\text{OH}^{\cdot}$, and H_2O_2 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 prosperity of Cr (VI) as it can easily seep into the groundwater. Cr (VI) can easily pass through the semi-permeable biological membrane and interacts 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 also is 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. 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* (Pattanapitpaisal 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 bio-sorption, 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 metabolising pathway. Extracellular, intracellular and membrane bound reductases are involved in the reduction of Cr (VI) into Cr (III) (Joutey 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. Side location map is depicted in 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 μ L) 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 h to 48 h for the growth of bacteria. Based on the morphological features such as size, colour, 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 3 days 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 resistance bacteria

Genomic DNA was isolated from the potent chromium resistance bacteria strain (G1) by QIAamp DNA Mini Kit from Qiagen. The 16S rDNA was amplified using primer sets of forward primer 27F (5'-AGAGTTT GATCMTGGCTCAG-3') and the reverse primer 1492R (5'-CGGTTACCTTGTTACGACTT-3'). The PCR reaction mixture of 25.0 μ L contains 0.5 μ M of each primer, Taq Buffer (1X), 1.5 mM of MgCl₂, 500 μ M of DNTP's mix, 1U 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 sec, primer annealing at 50°C for 30 sec, elongation at 72°C for 90 sec 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 amplified product (approximately 1500bp) was used for 16SrDNA sequencing with Genetic Analyser-3500 (Applied Biosystems, USA), using BDT v3.1 ready reaction mixture. The sequences were assembled and compared with nucleotide BLAST search, by using the existing gene bank database, 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 the table 2 (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 sec, primer annealing at 53°C (for *ChR*) and 56°C (for *ChrT*) for 30 sec, elongation at 72°C for 60 sec 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 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.

Table 2 Details of the primers used for the amplification of chromate reductase genes.

Chromate reductase		Primers/Oligos sequence (5'-3')	Reference
<i>ChR</i>	Forward	TCACGCCGGAATATAACTAC	Patra et al. 2010
	Reverse	CGTACCCTGATCAATCACTT	
<i>ChrT</i>	Forward	ATCATGTCAGATACCTTGAAAGTGG	Zhou et al. 2017
	Reverse	TGCTTTAACCCGCCGAATATA	

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 h, 48 h and 72 h followed by centrifugation at 12000 ×g for 10 min at 4°C. Then about 1 mL of diphenylcarbazide solution (0.25gm 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 colour 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 refers to initial concentration and residual concentration respectively.

Antioxidant Assay

Equal volume (10 mL) of effluent treated and untreated bacteria culture were centrifuged at 10000 × g 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 MgCl₂, 0.1 mM EDTA Na₂, 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 sec on and 5 sec off for 15 cycles at 4°C. The lysate was spun in a pre-chilled centrifuge for 15 min at 20000 × g 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 H₂O₂ buffer and absorption was measured at 240 nm using a UV-vis spectrophotometer for 60 sec at interval of 15 sec. The activity was calculated by decrease in absorbance of H₂O₂ 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) of hydrogen peroxide 30% solution and absorption was measured at 420 nm using UV-VIS spectrophotometer per 34 sec 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), (Kowalczyk 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 shown in table 1. 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).

Table 1 XRF elemental analysis of fertilizer industry effluent at Paradeep, India.

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	0.730	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 $K_2Cr_2O_7$ stress

The bacterial population was analyzed from the effluent site near to 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, colour, texture, the bacterial strains were isolated into different morphological communities. Bacterial isolates G1 and G2 incubated in MSM amended with 50 to 500 mg L⁻¹ of $K_2Cr_2O_7$, 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 h, 48 h 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-products 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 21×10² cfu mL⁻¹ than strain G2 having 11×10² 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. 2011). 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 neighbour-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 figure 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 is shown in figure 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 figure 2). In the present study nucleotide sequences of chromate reductase

gene (*ChrR*) 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 (*ChrR*), 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 exhibit 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 mg L⁻¹, 69.05 mg L⁻¹, 52.62 mg L⁻¹ after 24 h, 48 h 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 enhance the accumulation and activity of detoxifying enzymes (Ilias et al. 2011). Under normal circumstances, the concentration of free radicals remain 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 in 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. $K_2Cr_2O_7$ treated bacterial strain *Serratia* sp. GP01 showed morphological changes. Untreated bacterial strain was rod-shaped and elongated having a smooth surface whereas $K_2Cr_2O_7$ 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 $K_2Cr_2O_7$ 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. 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, $K_2Cr_2O_7$ treated bacteria and 10% (v/v) effluent treated bacteria have revealed the status of functional groups as IR spectra pattern are shown in supplementary figure 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 cm^{-1} 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 cm^{-1} and 3421 cm^{-1} under $K_2Cr_2O_7$ and 10% (v/v) effluent respectively. Spectroscopic changes at 1394 cm^{-1} have 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 cm^{-1} 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 cm^{-1} and 1235 cm^{-1} under chromium and effluent stress respectively.

FTIR spectroscopy is also used for the study of the position and status of functional groups of potent chromate 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 hr of incubation (supplementary figure 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 C=O of amide groups which showed shifting at 1057 cm^{-1} and 1235 cm^{-1} under Cr (VI) and effluent respectively. The phenomenon of absorption on bacterial cell surface might be occurring due to modification of functional groups like amide, ether, aromatic groups in response to chromium and effluent from the fertilizer industry. Moreover, the heavy metal affects the expression and suppression of certain functional groups by interacting on the bacterial surface which might help the bacterial strain to tolerate toxicity of the heavy metal (Naik et al. 2012).

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 $\text{K}_2\text{Cr}_2\text{O}_7$ 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.

nology using *Serratia* sp GP01.

Declarations

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

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Figures

Figure 1

Location map and sampling site of industrial effluent at Paradeep region in India.

Figure 2

Growth pattern of potent isolated bacteria strain G1 under different $K_2Cr_2O_7$ concentrations.

Figure 3

Growth pattern of potent isolated bacteria strain G1 under different $K_2Cr_2O_7$ concentrations.

Figure 4

Phylogenetic tree constructed using neighbour-joining and 1000 bootstrap probabilities showing the relationship between chromate reductase genes ChR of *Serratia* sp. GP01 and ChR of other bacteria groups.

Figure 5

Removal of chromium (%) by *Serratia* sp. GP01 over different incubation periods.

Figure 6

Production of antioxidants such as catalase, SOD and peroxidase by isolated strain *Serratia* sp. GP01.

Figure 7

SEM micrographs of (a) untreated bacteria, (b) treated bacteria and (c) 10% (v/v) effluent treated bacteria.

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