Improved Sprouting and Growth of Mung Plants in Chromate Contaminated Soils Treated with Marine Strains of *Staphylococcus* Species

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Abstract Marine bacteria possess a wide variety of bioremediation potential which is beneficial environmentally and economically. In this study, bacterial isolates from marine waters were screened for tolerance and growth in high concentrations of chromate (Cr^{6+}). Two isolates, capable of tolerating Cr^{6+} concentrations $300 \mu\text{g mL}^{-1}$ or higher, and found to completely reduce $20 \mu\text{g mL}^{-1}$ Cr^{6+} were grown in Cr^{6+} (50 and 100 mg kg^{-1}) spiked garden soil. Notably, both facilitated normal germination and growth of mung (*Vigna radiata*) seeds, which could hardly germinate in Cr^{6+} spiked garden soil without either of these bacteria. In fact, large percent of mung seeds failed to sprout in the Cr^{6+} spiked garden soil and could not grow any further. Apparently, chromate detoxification by marine bacterial isolates and the ability of mung plants to deal with the reduced form appear to work complementarily. This study provides an insight into marine bacterial abilities with respect to chromium and potential applications in promoting growth of leguminous plants-similar to mung in particular-in Cr^{6+} contaminated soil.

Keywords Marine bacteria · Hexavalent chromium (Cr^{6+}) · Chromate · *Staphylococcus* sp. · *Vigna radiata* · Mung seed

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Introduction

Chromium and its compounds are used in many industrial applications. Their use is large in metallurgy for the production of refractory steel, in electroplating, as manufacturing catalysts and in leather processing. They are also used in the preparation of speciality chemicals. The current scale of these anthropogenic activities and subsequent mismanagement of untreated waste discharge lead to widespread Cr contamination in the environment, notably of soil and water [1–5].

In nature, Cr exists in two stable forms, viz. Cr^{6+} and Cr^{3+} . Higher solubility and permeability through biological membranes and subsequent interactions with nucleic acids and proteins render chromate more toxic than the trivalent form which, in general, is biologically ‘inert’. Notably, Cr^{6+} is mutagenic [6], carcinogenic and teratogenic [7].

To circumvent the deleterious effects, conventional treatment of Cr^{6+} contaminated soil and groundwater involves sorption, precipitation, containment, reduction or removal in addition to other physico-chemical processes, which are expensive and also run the risk of further contamination [4, 8]. Biological methods [9–13] based on microbial reduction are thus viable alternatives with a potential of providing techniques for Cr treatment in an eco-friendly, cost-effective manner. As a result, bacterial reduction of Cr^{6+} through either enzymatic transformation or interaction with metabolites can therefore be used to evaluate their Cr^{6+} bioremediation potential.

To exploit this feature, a number of Cr^{6+} reducing bacteria, indigenous to several environments particularly from tannery wastewaters are examined [14–16]. However, studies screening marine bacteria for such potential are

scarce except for those by Cheung et al. [17–19]. It is thus worthwhile to examine the dual advantages of their halophilic trait and ability to reduce/detoxify Cr^{6+} as is reported for mercury [20] and arsenite resistant as well as detoxifying marine bacteria [21]. The purpose of this study was to isolate Cr^{6+} resistant marine bacteria (CRMB), to select, identify and to test the ability of a select set to reduce Cr^{6+} . It was also to evaluate the capability of a few CRMB strains to check if they facilitated sprouting and growth of mung (*Vigna radiata*) seeds in autoclave-sterilized garden soil spiked with Cr^{6+} vis a vis those seeds in Cr^{6+} spiked treatments without CRMB.

Materials and Methods

Sample Collection

Water samples were collected in sterile screw capped bottles from a downstream location along the Mandovi Estuary (Lat- 15°30' 19.206" and Lon-73°50' 9.5748") at low tide. Some physicochemical parameters of seawater viz., temperature (°C), pH and salinity (PSU) were measured [22].

Total Viable Counts

For enumerating total viable counts (TVC), aliquots of 0.1–0.3 mL water samples were spread plated onto seawater nutrient agar (SWNA with composition $[\text{L}^{-1}]$: peptic digest of animal tissue 5.0 g, sodium chloride 5.0 g, beef extract 1.5 g, yeast extract 1.5 g, agar 15 g, distilled water 1000 mL, pH 7.4 $\pm$ 0.2) as well as SWNA containing 25, 50, 75 or 100 $\mu\text{g mL}^{-1}$ Cr^{6+} , prepared by mixing appropriate amounts of filter sterilized potassium dichromate [$\text{K}_2\text{Cr}_2\text{O}_7$, A.R grade from Merck] stock solution and NA media with 50% aged seawater, in triplicate. Plates were incubated at 28 °C for 24–48 h and final counts of colony forming units (CFU) mL^{-1} were noted for both normal and Cr^{6+} amended SWNA.

Isolation of Cr Resistant Marine Bacteria (CRMB)

For isolation of CRMB, 5 mL of water sample was filtered through 0.22 μm pore size filters (Millipore India Pvt. Ltd.). These were placed on SWNA plates containing 100 $\mu\text{g mL}^{-1}$ Cr^{6+} and incubated at 28 °C for 48 h. As a result, isolation of Cr^{6+} resistant bacterial strains was done under selective stress of at least 100 $\mu\text{g mL}^{-1}$ Cr^{6+} . They were then streaked on to SWNA containing 150 $\mu\text{g mL}^{-1}$ Cr^{6+} , and isolates that grew were designated as CRMB and used in further studies. Purified CRMB were maintained at 4 °C on SWNA slants containing 100 $\mu\text{g mL}^{-1}$ Cr^{6+} .

Biochemical Characterization and Genomic DNA Extraction for 16S rRNA Gene Sequencing

The CRMB were characterized morphologically and biochemically according to Holt et al. [23]. Total genomic DNA was extracted directly from pelleted bacterial cultures [24] as described by Ausubel [25] following “CTAB method” and used as template for 16S rRNA gene amplification by PCR using two general bacterial 16S rRNA primers 27F (5'-AGAGTTTGATCACTGGCTCAG-3') [26] and 1492R (5'-CTACGGCTACCTTGTACGA-3') [27]. The PCR product purified using GenElute PCR Clean-Up Kit was sequenced by the Applied Biosystems 16-capillary 3130xl Genetic Analyzer at the CSIR-NIO, Goa.

Phylogenetic Analysis

The nucleotide sequences of the isolates obtained were compared with closely related validly published type strains retrieved from the database of EzTaxon Server (<http://eztaxon-e.ezbiocloud.net>) [28], and the 16S rRNA sequence similarities were $\geq 97\%$.

These 16S rRNA gene sequences (and published reference strains from EzTaxon database) were aligned and compared using the Clustal_X 1.81 program [29]. MEGA 5 [30] was used to assess the molecular evolutionary distances following the Maximum Composite model and phylogenetic trees were constructed using neighbour joining (NJ) algorithm [31]. The bootstrap analysis was performed to evaluate the stability of relationship by performing 1000 replications for the tree topology [32]. The sequences were submitted to NCBI-GenBank and accession numbers were assigned from KY672997 to KY673003 (Table 1).

Determination of Minimum Inhibitory Concentration (MIC) of Cr^{6+}

The MIC of Cr^{6+} at which no growth occurred was determined by broth dilution method [33]. Test-tubes with 5 mL nutrient broth (NB, containing $[\text{L}^{-1}]$ 5.0 g peptone and 3.0 g yeast extract) supplemented with different concentrations of Cr^{6+} were inoculated with CRMB in exponential growth phase (0.1% (v/v) of primary inocula ($\sim 1 \times 10^8$ cells mL^{-1}), to ensure that inoculum size and growth phase of inoculated cells were similar/identical). The cultures, in triplicate, were incubated on a shaker at 200 rpm for 24–48 h at 28 °C. Minimum concentration of Cr^{6+} with no observed turbidity was considered the MIC. Two CRMB isolates, NIOMR 3 and NIOMR 8 showed high tolerance ($\geq 300 \mu\text{g mL}^{-1}$ Cr^{6+}) and based on 16S rRNA sequencing, were expressed as *Staphylococcus xylosus*, and as, *Staphylococcus gallinarum* respectively.

Table 1 Biochemical characteristics of Cr^{6+} resistant marine bacteria termed in this study as CRMB (bacterial isolates capable of growing in NA with $150 \mu\text{g mL}^{-1} \text{Cr}^{6+}$)

Characteristics	Isolates						
	NIOMR 3	NIOMR 4	NIOMR 6	NIOMR 8	NIOMR 13	NIOMR 15	NIOMR 17
Colony shape	Round	Round	Round	Round	Round	Round	Round
Colony elevation	Convex	Flat	Flat	Flat	Flat	Flat	Flat
Colony color	Dull yellow	Yellow	Yellow	Yellow	White	Yellow	White
GS	+	+	+	+	–	+	–
CT	+	+	+	+	+	+	+
NRT	–	–	+	+	–	+	+
CUT	–	–	–	–	+	–	+
GHT	+	–	–	+	–	+	+
SHT	–	–	–	–	–	–	+
CHT	–	–	–	+	–	–	–
IT	–	–	–	–	–	–	–
MR	–	–	–	–	–	–	–
VP	–	–	–	–	–	–	–
TSIT	AB&S	AB	AB&S	AB&S	–	AB&S	AB&S

The isolates originated from the Mandovi Estuary and were designated as NIOMR (abbreviated for NIO Mandovi Estuary) along with a serial number

GS gram staining, CT catalase test, NRT nitrate reduction test, CUT citrate utilisation test, GHT gelatin hydrolysis test, SHT starch hydrolysis test, CHT casein hydrolysis test, IT indole test, MR methyl red test, VP voges proskauer test, TSIT triple sugar iron test, + positive, – negative, AB acidic butt (glucose fermenter), AB & S acidic butt and slant (lactose and sucrose fermenter)

Effect of Cr^{6+} on Growth of NIOMR 3 and NIOMR 8

NIOMR 3 and NIOMR 8 were grown in NB with ($100 \mu\text{g mL}^{-1}$) and without Cr^{6+} . For each isolate, medium was taken in one set (100 mL in triplicate) autoclaved and inoculated with freshly prepared inoculum. Cultures were incubated at 28°C in a shaker at 110 rpm. Aliquots of culture were taken at hourly intervals and optical densities measured with non-inoculated control used as the blank.

Determining Optimum Growth Conditions of NIOMR 3 and NIOMR 8

For examining optimum growth conditions of NIOMR 3 and NIOMR 8, two physiological parameters, i.e., temperature and pH were considered [34]. For determination of optimum temperature, six sets of flasks (in triplicate) were inoculated with fresh inoculum. Each set (consisting of 100 mL NB, with or without $100 \mu\text{g mL}^{-1} \text{Cr}^{6+}$, in triplicates) was incubated at 20, 24, 28, 32, 37 and 42°C , respectively in a temperature controlled orbital shaker. After an incubation period of 24 h, optical density was measured at 600 nm.

Similarly, to determine optimum pH, 100 mL NB was prepared into seven sets. The pH of each set was adjusted to 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 or 10.0 using concentrated HNO_3 or 1 N NaOH. The flasks were inoculated with fresh

inoculum and incubated for 24 h, after which optical density was measured.

Reduction of Cr^{6+} by NIOMR 3 and NIOMR 8

Diphenylcarbazide method of Fulladosa et al. 2006 [35] was followed to estimate Cr^{6+} reduction under both shaken (110 rpm) and static cultures condition. The experiment was conducted in 250 mL flasks containing 50 mL of medium with the initial pH set at 7 and temperature maintained at 28°C . Aliquots (1 mL) from cultures, taken at regular intervals and spun down at 14,000 rpm ($6500\times g$) for 5 min were used to estimate Cr^{6+} remaining in the medium. 100 μL of cell free supernatant was added to 10 mL glass-distilled water in a test-tube, followed by the addition of 1 mL of diphenylcarbazide solution (prepared by dissolving 0.25 g diphenylcarbazide in 100 mL acetone) and 2 drops of phosphoric acid. The mixture was kept at room temperature for 10 min for colour development, and then the absorbance read at 540 nm. All experiments above were conducted in duplicate with non-inoculated controls.

Evaluating Cr^{6+} Remediation of Garden Soil by NIOMR 3 and NIOMR 8

To test the ability of the two chosen CRMB to remediate Cr contamination, a locally collected garden soil was used.

Autoclaved garden soil was spiked with stock solution of $K_2Cr_2O_7$ to achieve final concentrations of 50 or 100 mg Cr^{6+} kg^{-1} . Soil (50 g) was mixed thoroughly and dispensed into different sets of Petri dishes to treat with CRMB individually as follows-

Group I (a) NIOMR 3 + *V. radiata* + Cr^{6+} (50 $\mu g\ mL^{-1}$) and **(b)** NIOMR 8 + *V. radiata* + Cr^{6+} (50 $\mu g\ mL^{-1}$).

Group II (a) NIOMR 3 + *V. radiata* + Cr^{6+} (100 $\mu g\ mL^{-1}$) and **(b)** NIOMR 8 + *V. radiata* + Cr^{6+} (100 $\mu g\ mL^{-1}$).

Group III (control group) bacterial free set of **(a)** *V. radiata* without Cr^{6+} **(b)** *V. radiata* + Cr^{6+} (50 $\mu g\ mL^{-1}$) and **(c)** *V. radiata* + Cr^{6+} (100 $\mu g\ mL^{-1}$).

Freshly prepared inocula of NIOMR 3 and NIOMR 8 were added as per the treatments mentioned above. Their initial OD_{600} was adjusted to ~ 1.2 by suspension in sterilized nutrient broth, to ensure $\sim 10^9$ cells in each dish. To facilitate bacterial growth in all the treatments listed, 1 mL of sterile 0.2% yeast extract (w/v) as an additional source of C and N was added daily for 5 days. On day five, for each treatment listed, triplicate sets of ten mung seeds (*Vigna radiata*, a legume) were sown evenly after being washed in 0.5% NaOCl for 15 min and rinsed thoroughly in sterile distilled water. The experiment was conducted under uniform light conditions (in the laboratory) and moistened once every 48 h. On germination, shoot lengths of all sprouts were recorded daily.

PCR Amplification of Cr Resistance and Reduction Genes in NIOMR 3 and NIOMR 8

NIOMR 3 and NIOMR 8 was examined for the presence of *ABC transporter*, *ChrA*, *ChrB* and *NfsA* genes that encode for either Cr resistance or reduction using specific PCR primers (Supplement Table 1). The PCR conditions for *ABC transporter* were 94 °C for 5 min for initial denaturation, followed by 30 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 40 s with a final extension of 72 °C for 10 min [36]. *ChrA* and *ChrB* were amplified using the same conditions but with annealing temperatures at 59 and 52 °C [37] respectively. For

NfsA, an initial denaturation at 95 °C for 7 min was followed by 30 cycles of 95 °C for 60 s, 55 °C for 60 s and 72 °C for 60 s with a final extension of 72 °C for 5 min [38].

Resistance to Heavy Metal Ions

The resistance of NIOMR 3 and NIOMR 8 to other heavy metals was determined by broth dilution method in NB amended with appropriate stock solutions of metal salts (mercury chloride, cadmium chloride, copper sulphate, zinc sulphate, arsenic trioxide, nickel chloride and chromium trichloride).

Results

Total Viable Counts

The percent of TVC (expressed as a fraction of counts from non supplemented SWNA) on SWNA supplemented with 100 $\mu g\ mL^{-1}$ Cr^{6+} (2 CFU mL^{-1}) was lower than that on unamended SWNA (654 CFU mL^{-1}). The CFU decreased substantially with an increase in Cr^{6+} concentration. In media with 25, 50 and 75 $\mu g\ mL^{-1}$ Cr^{6+} , the TVC respectively were 231, 67 and 18 mL^{-1} .

CRMB Characterization and Identification

The morphological and biochemical characteristics of CRMB isolates are given in Table 1. Seven morphologically distinct Cr^{6+} resistant bacterial isolates were isolated based on their resistance to 150 $\mu g\ mL^{-1}$ of Cr^{6+} . Molecular identifications based on 16S rRNA gene sequence comparison with reference strains from EzTaxon are shown in Table 2 and comparison of the 16S rRNA sequence in the form of a phylogenetic tree is shown in Fig. 1.

Minimum Inhibitory Concentration (MIC) of Cr^{6+}

The Cr^{6+} tolerance limit of the two CRMB isolates; *Staphylococcus xylosus* (NIOMR 3) and *Staphylococcus*

Table 2 16S rRNA gene identity of CRMB along with the minimum inhibitory concentrations (MIC) of Cr^{6+} ($\mu g\ mL^{-1}$)

Sr. no.	Bacterial isolate	Bacteria identified by 16S rRNA gene	Accession. No.	Similarity %	MIC ($\mu g\ mL^{-1}$)
1	NIOMR 3	<i>Staphylococcus xylosus</i>	KY672997	99.86	300
2	NIOMR 4	<i>Staphylococcus kloosii</i>	KY672998	99.75	200
3	NIOMR 6	<i>Staphylococcus saprophyticus</i>	KY672999	100	200
4	NIOMR 8	<i>Staphylococcus gallinarum</i>	KY673000	99.87	400
5	NIOMR 13	<i>Psychrobacter aestuarii</i>	KY673001	99.34	200
6	NIOMR 15	<i>Staphylococcus sciuri</i>	KY673002	99.86	200
7	NIOMR 17	<i>Vibrio rumoiensis</i>	KY673003	99.87	200

Fig. 1 Phylogenetic tree of selected Cr^{6+} resistant marine bacteria and related type strains based on 16S rRNA gene sequences, constructed by the neighbor-joining method. Bootstrap percentages (>50%) based on 1000 replications are shown at branch nodes. Bar 0.02 substitutions per nucleotide position. Assigned accession numbers on submission to NCBI GenBank are provided in the phylogenetic tree beside the isolates (in bold) from this study

gallinarum (NIOMR 8) was comparatively high ($\geq 300 \mu\text{g mL}^{-1}$, Table 2). All the following experiments were therefore conducted using these two isolates.

Growth of CRMB

Growth patterns of both isolates in Cr^{6+} were significantly different from those of control with a prominent second lag-phase (Supplement Fig. 1 and 2). Although NIOMR 8 grew better in both Cr^{6+} free and Cr^{6+} supplemented media in comparison to NIOMR 3, NIOMR 3 has a shorter second lag-phase. Both isolates could grow in a wide range from 24 to 37 °C, 24 °C apparently is the most suitable temperature for both isolates in Cr^{6+} free medium (Supplement Fig. 3). In media with Cr^{6+} , both grew well at 37 °C. Isolate NIOMR 3 grew well at pH 6 and, NIOMR 8 at pH 8 in control flasks. Both grew better in alkaline pH of (8–9) in broth amended with $100 \mu\text{g mL}^{-1}$ Cr^{6+} supplemented media (Supplement Fig. 4).

Reduction of Cr^{6+} by NIOMR 3 and 8

While growth was significantly higher when agitated, Cr^{6+} reduction was observed only in static condition (Supplement Fig. 5). It is important to note that both cultures/ isolates completely reduced $20 \mu\text{g mL}^{-1}$ in about ten days. In the flasks amended with 50 or $100 \mu\text{g mL}^{-1}$, the isolate NIOMR 3 reduced the Cr^{6+} by 46 and 27% and NIOMR 8 by 51 and 34%.

Evaluating Cr^{6+} Remediation of Garden Soil by CRMB

Mung seeds germinated within 16 h in garden soil spiked with Cr^{6+} (50 or $100 \mu\text{g mL}^{-1}$), (Fig. 2). Sprouting and germination shoot lengths were significantly higher in all sets of CRMB treated Cr^{6+} contaminated soil (Fig. 3). Barring Group III (a) and (b), leaves appeared in all

Fig. 2 Percentage germination in garden soil amended with Cr^{6+} inoculated with *Staphylococcus xylosus* (NIOMR 3) or *S. gallinarum* (NIOMR 8)

conditions by day 4 with shoots-attaining up to 1.4 cm. About 40% of shoots/leaves showed signs of wilting by day 8 and 90% by day 9. By day 10, shoots/leaves in all conditions had effectively wilted. Group III (a) and (b) characteristically exhibited shoots that were thin, dry and yellow.

Detection of Gene ABC Transporter, *ChrA*, *ChrB* and *NfsA* in NIOMR 3 and NIOMR 8 by PCR Amplification

Using the primers (Supplement Table 1) Cr specific genes were amplified from the genome of NIOMR 3 and NIOMR 8 and were obtained at the expected sizes (~ 900 , 950, 750 and 400 bp) for *ABC transporter*, *ChrA*, *ChrB* and *NfsA* respectively (Fig. 4). Both isolates gave an intense signal when amplified for *ABC transporter*. No amplification was obtained from NIOMR 8 for *ChrA*, *ChrB* and *NfsA*.

Fig. 3 Mean shoots length of mung seeds grown in presence and absence of Cr^{6+} , either inoculated with *Staphylococcus xylosus* (NIOMR 3) or *S. gallinarum* (NIOMR 8)

Fig. 4 Agarose gel electrophoresis (1%) of PCR products of total genomic DNA from NIOMR 3 and NIOMR 8 with primer pairs for genes *ABC transporter*, *ChrA*, *ChrB* and *NfsA*. Lanes M, 100 bp DNA ladder (Marker); N, Negative (for NIOMR 8 with primer pairs for *NfsA*). 1–5, amplified PCR product of 1, *ABC transporter*_NIOMR 3; 2, *ABC transporter*_NIOMR 8; 3, *ChrA*_NIOMR 3; 4, *ChrB*_NIOMR 3; 5, *NfsA*_NIOMR 3

Table 3 Resistance profile of NIOMR 3 and NIOMR 8 to different heavy metals

Isolate	Toxic metals ($\mu\text{g mL}^{-1}$) ^a						
	Hg	Cd	Cu	Zn	As	Ni	Cr^{3+}
NIOMR 3	20	40	120	50	700	300	800
NIOMR 8	20	40	140	60	750	300	850

^a Salts of different metals dissolved to attain concentrations of toxic metal ion. Data shown here represents the tolerance of NIOMR 3 and NIOMR 8 to different toxic metals

Resistance to Heavy Metal Ions

Although NIOMR 3 and NIOMR 8 showed elevated resistance to Cr^{6+} , they were less tolerant of other heavy metals (Table 3). Hg, Cd and Zn seemed to be the most toxic of the

metals tested. Both isolates were less sensitive to the effects of Cu and Ni and were found to be highly tolerant of As. The order of resistance to metals for both isolates was $\text{As} > \text{Cr} > \text{Ni} > \text{Cu} > \text{Zn} > \text{Cd} > \text{Hg}$ in NB, with both isolates being most tolerant of As. Trivalent chromium (CrCl_3 , Cr^{3+}) was found to be much less toxic in comparison to Cr^{6+} .

Discussion

Most prokaryotes in general, and copiotrophs in particular possess adequate genetic potential to sense, undergo transduction and integrate external stimuli essential for their ability to rapidly respond to changing environmental conditions [39]. As a result, nutrient influx or excretion, as well as toxic metal expulsion are regulated rather effectively, making them ecologically resilient. In marine environments, varying pH, salinity, sea surface temperature, water currents, precipitation, wind speed, etc., govern the physiological adaptations of marine biota. Many marine bacteria have been reported to transform, degrade or accumulate hydrocarbons, heterocyclic compounds, pharmaceuticals, radionuclides and/or toxic metals [40]. Additionally, their need for and endurance to high salinity enables them to proliferate in hazardous wastewaters such as tannery effluents (Das et al. submitted) without any genetic manipulation. Exploiting this bioremediative potential judiciously is useful in environmental pollution mitigation [9, 11]. In this regard, isolation and examination of CRMB in this study, which could grow and reduce chromate and facilitate both germination and growth of mung seeds, is indeed a novel approach.

In water samples of the lower reaches of the Mandovi Estuary, *Staphylococcus* was the dominant CRMB with isolates belonging to *Psychrobacter* and *Vibrio* spp forming a minor component. Despite not being known widely from marine habitats, there are many reports of Cr^{6+} tolerant *Staphylococcus* sp. indigenous to soil habitats [41–45] and it is therefore not uncommon to find them waterborne. NIOMR 3 and NIOMR 8, both staphylococci, showed high resistance to Cr^{6+} both in liquid and solid media and exhibited diauxic growth in medium amended with $100 \mu\text{g mL}^{-1}$ Cr^{6+} . Being facultative oligotrophs, both isolates behaved like copiotrophs [46] growing rapidly in nutrient Cr^{6+} containing medium and also displayed mesophily like any other marine bacteria. Lag in growth ~ 10 – 12 h in NIOMR 3 and ~ 8 – 12 h in NIOMR 8 implies their adaptation to toxic milieu, as has also been observed by De and Ramaiah [47] for MRB and by Yin et al. [48]. As Egler et al. [49] suggested, such a lag may be for switching on relevant metabolic genes.

Both isolates contained the *ABC transporter* gene homologous to ABC sulphate transporters with functions in

sulphur limitation response, since the uptake of Cr is closely associated with that of the structurally similar sulphate [36]. NIOMR 3 possessed *ChrA* and *ChrB* genes both of which are part of CHR transporters that efflux Cr^{6+} [37]. The presence of these genes, in particular the *ABC transporter* gene appear to confer Cr^{6+} tolerance in these two isolates, although there might be other factors that influence it or Cr^{6+} reduction. Both isolates reduced chromate (initial concentration of $20 \mu\text{g mL}^{-1}$) completely, with NIOMR 8 bearing a slightly higher reduction rate ($0.09 \mu\text{g mL}^{-1} \text{ h}^{-1}$) than NIOMR 3 ($0.08 \mu\text{g mL}^{-1} \text{ h}^{-1}$). The two were tested with multiple primers designed to identify the presence of a chromate reductase. Although no such gene was found, NIOMR 3 contained an *NfsA* gene encoding a Nitroreductase which has been shown by Kwak et al. [38] to act functionally as a chromate reductase. While observations from this study may not conclusively demonstrate whether Cr^{6+} is intracellularly or extracellularly transformed, they do provide an idea on how other proteins may bring about Cr^{6+} reduction.

Studies on metal tolerant terrestrial bacteria by Abbasi et al. [50], Bishoni et al. [51] and Jamal et al. [52] report of facilitating the growth of mung [53, 54] common wheat [55] and/or other plants [6, 56, 57] in contaminated soils. No study so far is available on evaluating bacterial isolates of marine origin for this purpose. Mung seeds were chosen for this study based on their rapid germination (<24 h at 28 °C) and high sensitivity to Cr, a biologically non-essential metal to plants [10]. In fact, Cr^{6+} had a detrimental effect on germination and growth which exacerbated with increased Cr^{6+} concentration. It is known that accumulation of Cr can reduce growth, induce chlorosis in young leaves, reduce pigment content, alter enzymatic function, damage root cells and cause ultra-structural modification of the chloroplast and cell membrane [58, 59]. The fading leaves, shrinking shoots and roots in soil with $100 \mu\text{g mL}^{-1}$ in this study indicate Cr^{6+} uptake by mung seeds. Both NIOMR 3 and NIOMR 8 enabled mung plants to achieve substantial shoot lengths compared to the completely stunted growth in Cr^{6+} spiked garden soil without either isolate. In all likelihood, reduction of toxic Cr^{6+} by metal sequestration and immobilization or, direct reduction of Cr^{6+} to less harmful Cr^{3+} , or promotion of plant growth through their rhizospheric colonization facilitated both germination and growth [60]. This study highlights factors such as the presence of Cr transporter genes/Cr reduction that contribute to Cr resistance and/or reduction. Importantly, CRMB tested in this study do possess the bioremediation potential to reduce Cr^{6+} and facilitate normal growth of mung plants in Cr^{6+} contaminated soil.

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