Evaluating The *In Vitro* Plant Growth Promoting Potential And Antibiotic Susceptibility Of Chromate Tolerant Bacteria.

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Abstract: Heavy metals such as hexavalent chromium Cr (VI) is known to challenge plant health and growth due to its effects in production of free radicals and reactive oxygen species (ROS). This results in alteration of cell components including nucleic acids, proteins, and lipids as well. Plant growth-promoting bacteria (PGPB) seem to pose a more environmentally sounded approach to these effects. These bacteria improve plant growth through biosorption process, bio-reduction and bios-transformation of toxic metal ions. This paper aims at assessing the plant growth promoting ability and antibiotic resistance of two bacterial isolated species, *Bacillus species* and *Corynebacterium species* isolated from arable soil with tolerance to chromate. Plant growth-promoting characteristics of bacterial isolates were identified through in-vitro screening tests including IAA production, HCN production, ammonia production, zinc solubilization, phosphate solubilization and siderophore production. The findings made it clear that both species had high growth-promoting characteristics with stereo typed differences; *Bacillus sp.* was superior to *Corynebacterium sp.* in terms of its P-solubilization, siderophore production, etc. The two isolates were able to produce ammonia hence playing a role in nitrogen availability to plant shoots and elongation. Furthermore, the antimicrobial susceptibility test revealed moderate resistance of the samples which signified their adaptability towards different environment and possible antimicrobial activity against pathogenic microorganisms. The isolates were also characterized by substantial chromium-reducing efficacy, which changed soluble Cr (VI) to the less dangerous Cr (III) species through phenomena such as biosorption and bio-reduction. These bacterial strains hold the bio-remedial potential to combat contaminated

soils and immobilise/re-adsorb heavy metals and enhance the plant health. The study implies the tested PGPB isolates as useful biofertilizer or biocontrol agents for the sustainable means of agriculture, cost-efficient than chemical fertilizer. Therefore, it is advised that more canopy light modification field trials and greenhouse studies be conducted in order to assess the full feasibility of such practices in agricultural ecosystems.

Keywords: Heavy Metal Contamination, Chromate Tolerance, Plant Growth-Promoting Bacteria (PGPB), Bioremediation, Chromium Reduction, Oxidative Stress, Antibiotic Susceptibility, Indole-3-Acetic Acid (IAA), Hydrogen Cyanide (HCN), Ammonia Production, Zinc Solubilization, Phosphate Solubilization, Siderophore Production, Biofertilizers, Sustainable Agriculture.

I. INTRODUCTION

Biological substances such as microbial inoculants or bioinoculants were used in agricultural field to promote plant health and were extensively used within last few years, but many objections have been raised against its uses from the lab to the field [1,2]. Crop plant roots are symbiotically associated with these Plant growth-promoting bacteria (PGPB) which are a group of polyphyletic bacteria in the rhizosphere biotope and the direct or indirect helpful effects of these bacteria in the plant growth promotion. As suggested by [Klopper J], PGPBs are characterized by their capability to become competitive with other microbes in soil microbiome in due respect to multiply, survive, and colonize in the roots of the plant [3,1]. Several mechanisms are also used by Plant growth-promoting rhizobacteria (PGPR) to enhance their growth and colonization in the plant roots. Such PGPR play an important role in different aspects such as they help to tolerate many biotic and abiotic stresses, providing essential nutrients, exogenous plant growth hormones and protecting plants against plant pathogens [4,5,6,7]. To improve crop yields by PGPB or PGPR that serves as an environment-friendly and sustainable approaches with comparison to alternative applications of harmful pesticides and chemical fertilizers [8,9]. Due to these rapid increase in anthropogenic activities, extensive use of chemical fertilizers, and pesticides, and rapid industrialization are the hazardous reasons for increasing environmental pollution [10,11]. And because of these rapid industrialization, lethal toxic metals are released in the environment and are rising at an alarming rate [12]. Such toxic or heavy metals those are recognised with higher level of toxicity and high-density metallic elements, Chromium (Cr), cadmium (Cd), thallium (Th), Mercury (Hg), Lead (Pb) and

Arsenic (As), are considered as the most toxic and carcinogenic heavy metals [12,13]. Among all these hazardous metal chromium (Cr) in the form of Chromate salts (CrO_4^{2-}) is the oxyanion form of hexavalent chromium Cr (VI) are the most perilous metals that enter the environment through a variety of artificial and natural sources and risks the life all living beings, including humans [12]. Chromium is the 24th element which is a silver-coloured hard metal naturally materialized in the rocky soils, and volcanic with molecular weight of 51.1 a.m.u. and a density of 7.19 g/cm^3 [14]. It can also be found in several oxidation states (Cr°, Cr^{1+}, Cr^{2+}, Cr^{3+}, Cr^{4+}, Cr^{5+}, Cr^{6+}), Cr°, Cr^{4+} and Cr^{5+} but can't be obtain naturally [15]. Among those oxidation states Cr^{2+} and Cr^{4+} are generally found in potassium dichromate and chromate salts. With the most toxic form of Chromium is Cr (III) and hexavalent chromium Cr (VI) is the most common and stable form of chromium [16,17], Cr (III) forms ionic compounds with sulfates, hydroxides, and oxides, that was found in the organic matter, soil, and aquatic environment are less mobile [16,17]. Cr (III) and Cr (VI) have different chemical, physical and toxicological properties that vary considerably among them. While in nature Cr (III) is found in the form of ore such as ferro-chromite, And Cr (VI) is mostly produced from anthropogenic activities and is extremely noxious to living organisms [18]. Due to its bioaccumulate capacity it was accepted to be potent and mobile toxin with mutagenic and genotoxic properties, risking the terrestrial and aquatic ecosystems [19]. In a survey the toxic effect of Cr (III) on wheat, microbes and aquatic life was revealed by [20], and scrutinized the impact of higher toxicity of Cr (VI) on the growth of wheat and found the 50 % root length inhibitory concentration (EC_{50}) of Cr (III) was 125 times of Cr (VI) [21]. Excessive Cr levels in plant tissues may arouse several biochemical processes and morpho-physiological in plants [22,23]. Complex series of metal interactions with the signal transduction, genetic processes and pathways and cellular macromolecules in plants are held due to metal toxicity in soil [24,25,26]. Hence, plant growth and essential metabolic processes are affected due to chromium toxicity [27]. In addition, the internal components and cell membrane of plant cell are affected because of its toxic effect, altering the activity of related enzymes in the plant body, and then change the gene expression as well as regulate the bio-synthesis of certain proteins [28]. And, causes oxidative stress because of the production of free radicals which make alterations to plant's biochemical morpho-physiological processes at tissue and cellular levels [29]. To solve these problems due to chromium pollution and other heavy metals, Soil bacteria (PGPB) are used because they are very important in biogeochemical cycles and are used

for the production and growth improvement of crops for decades [30]. The determinants of plant health and soil fertility is dependent on the Plant–bacterial interactions in the rhizosphere [30]. As mentioned above in the text, microorganisms colonize all parts of the plant and the main source of bacteria is the rhizosphere with plant-beneficial activities. These bacteria are termed as plant growth-promoting bacteria (PGPB) or Plant growth promoting rhizobacteria (PGPR) [31]. Due to the root exudation of organic compounds in Plants that provides a habitable surrounding for microorganisms that are necessary for microbial metabolism [32,33]. Such microorganisms can survive on interior and exterior of their host, ample colonizing the plants' rhizosphere or root surface [32,34]. By doing this plant growth promoting rhizobacteria (PGPR or PGPB) regulates the growth promotion, metabolic rate, seed germination and other physiological activities of plants. PGPB decreases toxic effects of Cr (VI) on plant growth through reducing Cr (VI) to Cr (III) [35]. Recent studies and reports narrate the solicitation of heavy metal resistant-PGPBs to increase agricultural yields without cumulation of metal in plant tissues [35]. Various mechanisms are processed by (PGPB) that can boost plant growth through a variety of mechanisms, including biological nitrogen fixation, rhizosphere engineering, production of 1-Aminocyclopropane-1-carboxylate deaminase (ACC), phosphate solubilization, siderophore production, quorum sensing (QS) signal interference and inhibition of biofilm formation, and phytohormone production [36]. Such bacterial strains are termed as plant growth promoting bacteria or rhizobacteria and belonging to genera such as *Bacillus, Azospirillum, Burkholderia, Pseudomonas, Rhizobium, Acinetobacter*, *Erwinia, Serratia, Alcaligenes, Enterobacter, Arthrobacter* and *Flavobacterium* [36]. And, Also the foremost examples of these beneficial bacteria involves species from *Erwinia, Agrbacterium, Arth.robacter, Chromobacterium, Pseudomans, Se.rratia, Azospirillum, Corynebacterium and Burkholde.ria* etc [37]. With the help of these bacterial isolates plant growth and development was increased and also amended the metal contaminated soil being non-pathogenic to plant species and can sustain under stressed conditions [38]. Several academic works suggested that many soil bacteria showed great potential in toxic metal clean-up procedures, and may be used as biofertilizers [39,40], because of their attendant capability to promote plant growth. Other reports also represented that a number of soil dwelling metal-resistant bacteria promoted plant growth by detoxifying heavy metal ions from contaminated soil [41,42]. Reflecting on the importance and tremendous ability offered by heavy metal-resistant bacteria, this study

was carried out to emphasis on isolation, biochemical ability, characteristics, plant growth promotion ability of soil dwelling heavy metal-resistant bacteria [43].

The aim of this article was to study and gather information about heavy metal-resistant bacterial isolates named *Bacillus species* and *Corynebacterium species* those were supplied from Microbiology and Computational Lab in the Botany Department of Raiganj University, Duttar Dinajpur, 733134, and its potential in plant growth promoting with chromate reduction through bacterial isolate's strengths. The provided bacterial isolates were unexplored for plant growth promoting purposes and this makes an excellent opportunity to explore and study the perseverance and expansion of heavy metal resistant bacterial isolates supplied form the lad. Throughout this study, a trailblazer effort was performed to explore and analyse *Bacillus sp.* and *Corynebacterium sp.* isolates with the potential of heavy metal resistance and plant growth promoting potential. During obtaining this study this two bacterial isolated from the arable soil to display or signify the resistance to heavy metals in different parameters. So, based on in-vitro qualitative tests for detecting plant growth promotion ability of those isolates were done to know it's potential to chromate and other metal tolerance. Gradually further studies can be conduct to explore their activities of bioremediation potentiality and plant growth promoting ability to increase the agronomic activities and crop production.

II. Materials and Methods

II.1. Antibiotic Susceptibility Test

Kriby-Bauer test (Antibiotic Susceptiblity Test), also called the disk diffusion test, is a valuable standard tool for measuring the effectiveness of antimicrobics against pathogenic microorganisms. This test was performed upon these bacterial isolates to study their antibiotic resistance and susceptibility conditions. Suitable antibiotic disks for the isolates were provided by consideration according to The European Committee on Antimicrobial Susceptibilty Testing (EUCAST) are mentioned below:

Table 1. Different antibiotic dicks for BP bacterial isolates as for CG bacterial isolates by EUCAST as prescribed by EUCAST for *Corynebacterium*

Bacterial Isolates	**CG *(Corynebacterium sp.)***
Strength	Antibiotics
30 mcg	Amoxicillin (Ax)
30 mcg	Vancomycin (Van)
30 mcg	Linezolid (ZT)
30 mcg	Rifampicin (Rif)
5 mcg	Ciprofloxacin (Cip)

Table 2. Antibiotics for *Bacillus*

Bacterial Isolates	**BP *(Bacillus sp.)***
Strength	Antibiotics
30 mcg	Linezolid (ZT)
30 mcg	Vancomycin (Van)
30 mcg	Amoxicillin (Ax)

5 mcg	Ciprofloxacin (Cip)
10 mcg	Imipenem (Im)

II.2. Procedure

All the glass goods were first sterilised before the experiment. Six plates with Mueller Hinton Agar were prepared and two plates with one control plate for each isolate was labelled and used. After the revive broth with isolated bacterial cultural of *Bacillus sp.* and *Corynebacterium sp.* was provided, under sterile condition an ear-bud full of bacterial suspension was taken by dipping it in *Bacillus sp.* bacterial suspension. After that the ear-bud with *Bacillus sp.* suspension was streaked properly on the solid surface of the three agar plates. As same process was done for isolated bacterial *Corynebacterium sp.* suspension. Then with sterile forceps the antibiotic disks as mentioned in table 1. were placed on surface of the two agar plates with *Bacillus sp.* isolated suspension leaving the control plate and for *Corynebacterium sp.* the antibiotic disks as mentioned in table 2. Were placed on the surface of the two agar plates with *Corynebacterium sp.* isolated suspension leaving the control plate. Finally, the plates were incubated for 48 hours for 37^0 C in the bacteriological incubator and the result was obtained by analysing the zone of inhibition.

II.3. IAA production

The production of IAA was determined by inoculating bacterial suspension into YEM/NB broth supplemented with tryptophan (0.01%) for 3 days. Then centrifuged at 10000 rpm for 20 mins and with the 2 ml of supernatant a few drops of ortho-phosphoric acid and 4 ml of Salkaowski reagent (1 ml of 0.5 M $FeCl_3$ solution in 50 ml of 35% perchloric acid (perchloric available commercially as 70%)) are added and was kept in dark for 30 mins at room temperature Presence or absence of pink colouration indicates positive IAA production.

II.4. HCN production

HCN production was evaluated by streaking the bacterial isolates on YEMA/NA medium amended with glycine (4.4 g/L) and by placing the sterilized Whatman No.1 filter paper soaked in sterilized picric acid solution (0.05% solution in 2% sodium carbonate) on the lid of petri-plate. Then the plate was sealed and incubated for 48 hrs at 30^0 C. If colour

changes on the filter paper from deep yellow to reddish brown was considered as an indicator of HCN production.

II.5. Ammonia production

The production of ammonia was screened for bacterial isolates in peptone water. Freshly growing culture was inoculated in 10 ml peptone water (2%) and incubated for 48-72 hrs at 28 C. Then Nessler's (0.5 ml) (commercially available) was added. Whether or not development of brown to yellow colour indicates positive or negative results for ammonia production respectively.

II.6. Solubilization activity

Modified Pikovskayas media supplemented with zinc oxide (glucose 10 g/L, $MgSO_4.7H_2O$ 0.1 g/L, $(NH_4)_2SO_4$ 1g/L, KCL 0.2 g/L, K_2HPO_4 2g/L, yeast 5 g/L, ZnO 1g/L, agar 20g/L) was used to determine Zn solubilizing activity of the isolates and the inoculated plate was incubated for 7 days at 30 C. Clear zone around the colony can be evaluated as Zn solubilizer.

II.7. NBRiP

To Prepare NBRiP plate we mixed solution A (TCP 5g/L, bromophenol blue 0.0.25 g/L) and solution B (glucose 10 g/L, $MgCl_2$. $6H_2O$ 5 g/L, $MgSO_4.7H_2O$ 0.25 g/L, $(NH_4)_2SO_4$ 0.1 g/L, Agar 20 g/L). Streaking was done or pour plate can be used for freshly culture media and incubate for 7 days. If blue plates changes into colourless after digestion by bacteria indicates phosphate solubilizing activity.

II.8. Siderophore test

We prepared CAS-Agar plate and inoculate freshly grown bacteria by streaking and was incubated for 7 days. If Green colour plate turned into colourless by appearance of halo around bacteria, this indicates positive test for siderophore. The following chart is meant to prepare CAS-Agar.

II.9. CAS medium

II.9.a. Aqueous solution of $Fe.CL_3.6H_2O$: We Prepared 1 mM of Ferric chloride solution by dissolving it in 10 mM HCl, for 100 ml we need 0.016 Ferric chloride, 99.914 ml Double Distilled Water (DDW) and 86 µl of HCL.

II.10.b. Aqueous solution of CAS: For 50 ml of CAS, we added 60.5 mg (0.0605 g) CAS.

II.12.c. Aqueous solution of HDTMA: For 40 ml of HDTMA we added 72.8 mg (0.0728 g) HDTMA.

II.10. CAS-Agar

The above stock was added to N.A in ratio of 1 CAS solution: 9 N.A. (E.g. For 500 ml we need 50 ml of CAS solution and 450 ml N.A.).

III. Review of Literature

III.1. Cr pollution in soil

The most toxic heavy metal chromium (Cr) which is found naturally and is widely used in industrial processes. It is found in the natural Cr-sources, especially from the earth's crust [46-44], especially in trivalent chromium Cr (III) and hexavalent chromium Cr (VI) form [45]. Chromium is responsible for environmental threat, critically impacting the environment and natural resources, mostly affecting water and soil [44]. Infiltrated water waste, weathering and leaching chromite from mines was reported as a serious cause of soil and water pollution [47]. Excess chromite mining has deteriorated soil, landscape and worsen water quality. The amount of chromium found in chromite mines was estimated to be ranging from 10 to 4000 mg/kg in air, water, and soil [48]. The concentration of Cr range varies with natural conditions in soil ranges between 10 and 50 mg/kg, but it can also reach much optimum concentrations depending on the nature of the substrate [49]. also, the concentration of Cr (III) in soils and sediments are an outcome of silicate modification in minerals comparative to chromite, <200 mg/kg of Cr concentration is found in ultramafic rocks and serpentinites of ophiolite complexes [49]. Due to weathering Cr are liberated, which are drawn in clay minerals and precipitated as homogeneous solids or with Al (III)/Fe (III)-hydroxides [50]. The primary source of Cr is Chromite [$FeCr(III)_2O_4$] in ultramafic and serpentinite rocks, but manifest low solubility, and there are a small number of naturally occurring oxidants of Cr(III). Mn (VI/III) oxides and Hydrogen peroxide (H_2O_2) are the only naturally formed oxidants of aqueous Cr (III) at pH <9 [50]. Other soil contaminating common compounds of chromium are $HCrO_4$ and CrO_4^{2-} those are found easily in soil and are absorbed by plants [51-52]. $HCrO^{-4}$, CrO_2^{-4}, and $Cr_2O_2^{-7}$ are mainly soluble oxygen containing anions those are forms of hexavalent Chromium Cr (VI) [53]. But, if Compared with Cr (VI) and Cr (III) they are more toxic because it has greater solubility, stronger activity, and is mobile in soil [53-54]. Both of these forms of chromium contamination have detrimental effects on the environment such as reduces plant growth, decreases soil fertility and alters microbial activity [55].

III.2. Chromium Toxicity in Plants

From the last three decades researchers showed that chromium, especially the oxyanion chromate Cr (VI) act as a lethal substance polluting the environment that vigorously damages plants affecting its metabolism, represses growth and development, oxidative stress, disruption of nutrient uptake, photosynthesis [56]. Chromium toxicity in plants is due to its valence states: Cr (III) that is less toxic and Cr (VI) that is highly toxic and is mobile [57]. There is no proper transport system for Cr in plants, but is accumulated by carriers of essential ions such as sulfate or iron [57]. Various physiological, morphological, and metabolic traits are adversely affected due Cr exposure in plant body that gradually lead to plant death [58]. The development of plant development, photosynthesis, and nutrient absorption are greatly affected due to Cr toxicity and increasing the production of reactive oxygen species (ROS) and altering antioxidant activities [59]. Different ROS variants, such as hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2), and superoxide anions (O_2•), due to Cr toxicity that led causes serious complications in plants, including lipid peroxidation and inhibition of enzyme activities, growth retardation, degradation of photosynthetic pigments, and chromosomal aberrations [58]. Moreover, oxidative damage is caused due to ROS generation because of Cr accumulation [60]. And the disarray of membrane function and structure are generated due to ROS that results in the peroxidation of membrane lip, as well as arousing the oxidation of proteins and nucleic acids, resulting in cellular component damage and eventually cell death [61]. Additionally, ROS alters in biochemical processes of plant leading to harmful changes in their architecture and morphology [62].

The various production of agricultural crops such as pulses, cereals, vegetables, etc., are affected due to Cr toxicity that have effect on our sustainable agriculture and food security [59,63,64,65]. In recent studies it was reported that high Cr accumulation in plants damages the chlorophyll (Ch l) content (Ch l a, b, and total), leading to the hindrance of photosynthesis [58]. Previous studies have represented inhibition of cell cycle, nitrogen assimilation, the antioxidant system, water and mineral balance, enzyme activity and metabolic processes are damaged due to surfeit of Cr deposited in plant tissues [58-66]. Furthermore, studies showed Cr deposition in plant occurs because of the creation of Cr-organic ligand complexes [67-68]. Various oxidation state of the Cr ions in plant species influence the distribution and translocation of Cr within plants due its Cr concentration in

growth media [69]. Both Cr (III) and Cr (VI) can be accumulated through the epidermal root cell of plant, but there is notable variance in the pathways and regulation of their entry into cells [67]. Between them Cr (VI) is easily accumulated by plants rather than Cr (III) because of higher transmembrane efficiency and higher water solubility [70]. Accumulation of Cr (III) in plants is proceeded or processed through passive mechanism [71,72], and Cr (VI) is absorbed across the plasma membrane, that is regarded as an active cycle involving convey of essential anions such as sulphate [71-73-74]. After accumulation Cr (VI) is transformed into Cr (III) inside the plant tissue and binds with cell wall, which obstruct the further transport of Cr within plant tissues [71-75]. Cr (III) is deposited in the cytosol inside the plant cell [76-77]. This leads to inhibition of cell division and inhibits the plant growth by inducing chromosomal divergence [76-78]. It also affects photosynthesis in terms of photophosphorylation, CO2 fixation, enzyme activities and electron transport [79]. With oxidative potential of Cr (VI) affecting photosynthesis, that may support the sinks for electrons could have been intensify by reduction of molecular oxygen and defines the oxidative stress brought about by Cr (VI) [80], And Various report suggests that Cr is the most potent inhibitor of plant photosynthesis [81]. Cr was also reported to cause several effects on biochemistry and physiology of crops plants [82]. Also affecting plant germination, root growth and length, stem growth and leaf development due to inducing its toxic effects [82].

Table 3. Few of such effects on physiology of plant due to Chromium uptake are listed below:

Plant species	Physiological changes	Reference
Oryza sativa	Expansion of POD activity	Ma et al. [83]
Oryza sativa	Increases SOD and CAT activity	Zhang et al. [84]
Triticum aestivum	Increases lipid peroxidation	Zhang et al. [84]
Zea mays	Increases GPX and SOD activities	Maite et al. [85]
Vigna radiata	Decreased glutathione level	Shanker et al. [86]
Chamomilla recutita	Increases MDA level	Kováčik et al. [87]
Ocimum tenuiflorum	Increases proline level	Rai et al. [88]

III.3. Role of PGPB in reducing Chromium toxicity in crop plants

Chromium contamination is a major concern that affects the plant growth in drastic ways leading to death of the particular plant species. As discussed earlier that Cr is among the toxic HMs (Heavy metals) released because of excess use of agrochemicals, involving pesticides, along with several reckless human activities, has results discriminating extensiveness of pesticides and HMs in crop plants and the environment [89]. Because of these pollutions heavy metals in soil expand its bioavailability of metals like hexavalent chromium Cr (VI), eventually restricting plant growth and decreasing the regulation of phytoremediation [90]. But recent studies says that Plant Growth-Promoting Bacteria

(PGPB) have the significant capability to increase plant growth with its tolerance to metal stress [90]. Therefore, in this context, PGPB is represented as the best eco-friendly perspective for phytoremediation would be one of the best choices to increase crop productivity and to diminish heavy metal problems [91-92-93-94-95]. PGPB helps the plant by reducing the efficiency of Cr (VI) and decreases the Cr absorption by the plants [90]. Several mechanisms such as bioremediation, biosorption, and biotransformation processes are used by these soil microbes to convert more mobile and toxic Cr (VI) into less toxic form Cr (III) [96-97-98]. Mechanisms such as biocontrol and growth promotion are also used to improve the growth and resistance of plants to Cr(VI), And other mechanisms including production of antioxidant enzymes to scavenge ROS; production of ammonia, phytohormones stimulation, decrease stress-induced ethylene production by synthesized enzyme ACC (1-aminocyclopropane-1-carboxylate) deaminase; siderophores; phosphate solubilization; nitrogen fixation; and bacterial secretion of extracellular polymeric substances (EPS) [98-99-100-101]. By using bioremediation process the isolated indigenous soil microbes reduces the toxicity of HMs like Cr by converting its toxic form into less toxic one by releasing the organic acids and manipulating pH [102-103]. This states that soil microbes are useful in using oxidation–reduction of transition metals [102-104]. Metals such as Cr, Cd and as are reported to be resisted by many soil microbes through energy-dependent and plasmid-encoded metal efflux systems i.e. chemiosmotic ion/proton pumps and ATPases [104-105]. According to new insights exchange of protons and its characteristics were quantified and the metals were accumulated on EPS free bacterial cells so that they can detect the relative significance of EPS molecules of bacterial cell in metal degradation [106]. Several other insights also refers that the chemical compounds in bacterial cell like cysteine, sulphite, glutathione and thiosulfates might reduce Cr (VI) into Cr (III) [107-108]. Reduction of Cr (VI) might occur because of the enzymatic activities of bacteria due its soluble and membrane-bound reductases that exist in several of aerobic, facultative and anaerobic bacteria [107-109]. Hence, PGPB is capable to reduce Cr (VI) and have the capability to support for plant growth improvement as well as for Cr (VI) bioremediation [110].

III.4. Role different of bacterial isolates for Cr (VI) reduction

In this article as two PGPB bacterial isolates BP and CG were experimented for different in-vitro qualitative tests for detecting plant growth promotion ability of the studied isolates.

The in-vitro qualitative tests were conducted to result out the potential of bioremediation, phytoremediation, bio-reduction, biosorption, and biocontrol of those PGPB isolated microbes to chromate ion Cr (VI). Microbial detoxification of Cr (VI) and their mechanisms are known as biosorption, bioaccumulation, complexation, or bio-reduction [111]. Cr (VI) solubility and toxicity is much more compared to Cr (III), bio-reduction of Cr (VI) to Cr (III) is the main necessary condition in may studies, and through various enzymatic and nonenzymatic reduction the bioremediation of Cr (VI) have been categorised [111]. And in this article, we are trying to survey the reduction potential of Cr (VI) in those provided isolated strains and with their respected species which were identified to be strains of *Bacillus sp.* and *Corynebacterium sp*. as per provided information. These bacterial isolated species are reported for bioremediation of heavy metals capability in various studies and among many such isolated species are *Flavobacterium sp., Pseudomonas sp., Bacillus sp., Arthrobacter sp., Corynebacterium sp., Methosinus sp., Rhodococcus sp., Mycobacterium., Stereum hirsutum, Nocardia sp., Methanogens sp., Aspergilus niger , Pleurotus ostreatus , Rhizopus arrhizus , Azotobacter sp., Alcaligenes sp., Phormidium valderium , Ganoderma applantus* [104]. Among such strains of *Bacillus sp*. one strain named CRB-1 was able to completely reduce 50 mg/L of Cr (VI) within 24 h under temperature 24–42 °C with provided aerobic condition manifests considerable Cr (VI) deduction potentiality in the pH ranging from 7.0 to 9.0, Cr (VI) reduction occurs in cytoplasm because of permeabilized cells, and subcellular fractions [111]. CRB-1 reduction potential of Cr (VI) is enzymatically mediated and mainly transpire in the cytoplasm [111]. The cell of Bacillus sp. has bioaccumulation mechanism that can accumulate Cr (VI) up to 99% [112]. Bioaccumulation at 34.5 and 32.0 mg of Cr/g dry weight was reported in *Bacillus circulan and Bacillus megaterium*, and brought optimum concentration of Cr (VI) to the low limit within 24 h when the initial concentration was 50 mg Cr (VI)/L [112]. The oxidation states Cr (VI) into Cr (III) by approximately 60% and 98% was also delineate in *Pseudomonas* sp. and *Aeribacillus pallidus* through bio-reduction mechanism [113]. Bio-reduction of Cr (VI) by producing Plant Growth Promoting (PGP) substance such as Ammonia, Indole-3-Acetic Acid and solubilized phosphate was seen in three rhizosphere bacteria, and resulted bio-reduction potentiality was analysed to be 13.7% by *Bacillus cereus*, which was much higher Cr (VI) reduction compared to *Bacillus. aerius* (4.4%) and *Exguobacterium profundum* (3.6%) [115]. Cr (VI) more than 90% was observed in gram-positive bacteria generally include

Bacillus, Staphylococcus, Pseudomonas and others, due to their resistance potential towards heavy metal pollutants [114]. Gram-positive bacteria *Bacillus aerius* with almost 100% of Cr (VI) reduction within five days at 37 °C [115]. Cr (VI) reduction in bacterial cell takes place due to chemical reactions associated with compounds present in intra or extracellular compounds such as amino acids, nucleotides, sugars, vitamins, organic acids, or glutathione [116].

Different isolated bacteria from chromium polluted environments such as tannery effluent and chromite mining sites have the most important role in chromium bioremediation [117-118]. Excavating in chromite mining environment of Orissa, India by researchers reported that during their survey for chromium resistant and reducing bacteria they discovered four efficient chromite reducing strains namely, *Pseudomonas putida* SKPD 1202 (MTCC 8729), *Corynebacterium paurometabolum* SKPD 1204 (MTCC 8730), *Arthrobacter* sp. SUK 1201 (MTCC 8728, GenBank accession number JQ 312665), and *Arthrobacter* sp. SUK 1205 (MTCC 8731, GenBank accession number JQ 312666) [119-120]. Among all these bacterial isolates *Corynebacterium paurometabolum* SKPD 1204 reduced 62.5% of 2 mM Cr (VI) during growth in Vogel bonner broth with decolourization in the medium, and after 8 days incubation the Cr (VI) reduction rate was reduced from 0.075 to 0.0037 mM Cr (VI)/mg of cell/h [121]. During incubation 90% of 100 μM Cr (VI) was reduced by isolates *Pseudomonas putida* SKPD 1202 and *Corynebacterium paurometabolum* SKPD 1204 [122]. *Corynebacterium paurometabolum* reduces Cr (VI) with its best carbon source glycerine [123]. Other bacterial strains with maximum tolerance and reduction of Cr (VI) after stored at −20 °C in glycerol/Luria Bertani (LB) broth (1:1) were *Corynebacterium kutscheri* FL108Hg (17 mM), *Rhodococcus sp.* AL03Ni (10 mM), *Pseudomonas aeruginosa* CA207Ni (12 mM) and *Burkholderia cepacia* AL96Co (6 mM) [124]. *Corynebacterium vitaeruminis* LZU47-1 with highest Cr (VI) reduction efficiency (96.46%) than any other *Corynebacterium sp.* strains above [125].

Table 4. Different bacterial isolates with Cr (VI) resistance (Cres) and reduction (Cred) potential in different growth media prepared for in-vitro qualitative tests:

PGBP	**Cres/Cred**	**Metabolite/ Actibity**	**Reference**
Pseudomonas sp. VRK3	Cres	IAA, phosphate solubilization, siderophore	126
Delftia sp. JD2	Cres, Cred	IAA, nitrogen fixation	127
Bacillus spp.	Cres, Cred	IAA, phosphate solubilization, HCN, antifungal activity	128
Pseudomonas sp. PsA4, Bacillus sp. Ba32	Cres	IAA, phosphate solubilization	129
Pseudomonas sp. RNP4	Cres, Cred	IAA, siderophore, phosphate solubilization	130
Rhizobacterial strains A3, S32	Cres	IAA, siderophore	131
Bacillus spp.	Cres, Cred	IAA, HCN, Siderophore, ammonia	132

IV. Results:

IV.1. Antibiotic Susceptibility Test

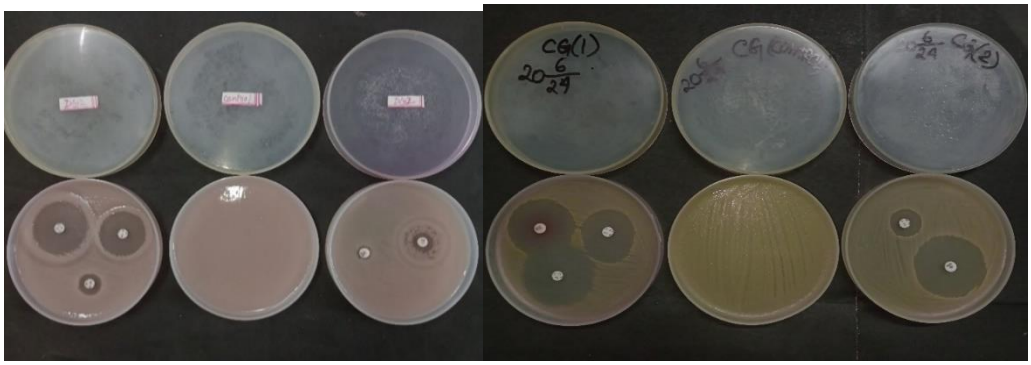


Figure 1. a. *Figure 2. b.*

Figure 1. Antibiotic susceptibility or Kriby-bauer test representing cultured plates of (a) *Bacillus sp.* and (b) *Corynebacterium sp.* bacterial culture grown with different antibiotic dicks that forms a clear zone of inhibition.

IV.2. IAA production test

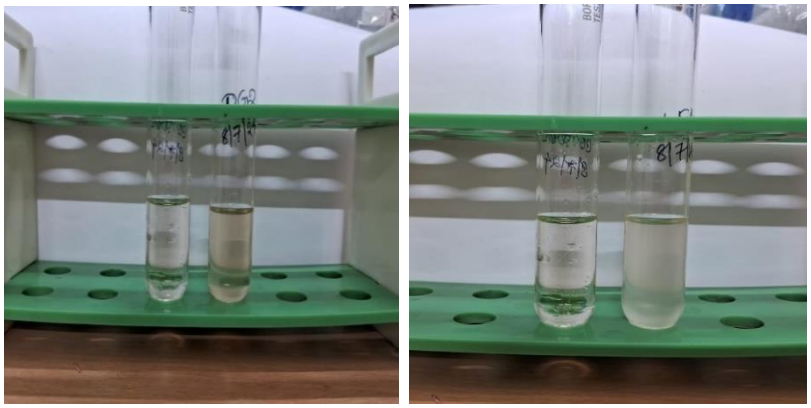


Figure 3. a. *Figure 2. a.*

Figure 2. Indole Acetic Acid (IAA) production test representing cultured tubes of (a) *Bacillus sp.* and (c) *Corynebacterium sp.* bacterial suspended culture in YEM/NB broth supplemented with tryptophan (0.01%).

Table No 5. *Bacillus sp.* and *Corynebacterium sp.* isolates representing negative result under IAA production test

		Appearance of pink colouration	
Sl. No.	Bacterial isolates	Culture tube 1	Culture tube 2
1	*Bacillus sp.*	Negative	Negative
2	*Corynebacterium sp.*	Negative	Negative

IV.3. HCN production test

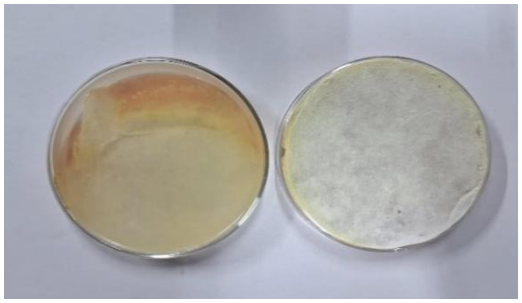


Figure 4. a. & b.

Figure 3. HCN production test represent cultured plates of (a) *Corynebacterium sp.* and (b) *Bacillus sp.* bacterial culture on YEMA/NA medium amended with glycine (4.4 g/L) and by placing the sterilized Whatman No.1 filter paper soaked in sterilized picric acid solution (0.05% solution in 2% sodium carbonate) on the lid of petri-plate.

Table No 6. *Corynebacterium sp.* and *Bacillus sp.* isolates representing positive and negative result under HCN production test

		Appearance of reddish-brown colouration	
Sl. No.	Bacterial isolates	Petri plate 1	Petri plate 2
1	*Corynebacterium sp.*	Positive	Positive
2	*Bacillus sp.*	Negative	Negative

IV.4. Ammonia production test:

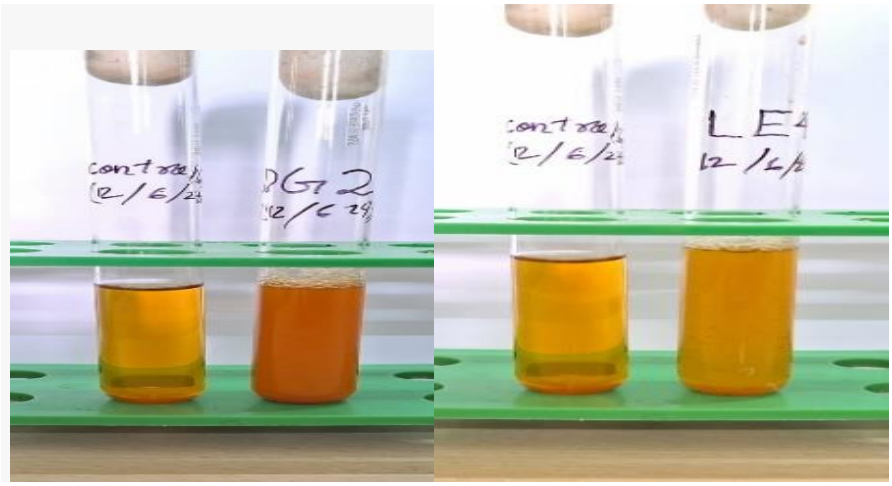


Figure 4. Ammonia production test representing cultured tubes of (a) *Bacillus sp.* and (b) *Corynebacterium sp.* bacterial suspended culture grown in 10 ml peptone water (2%) and incubated for 48-72 hrs at 28 C. Then Nessler's (0.5 ml) (commercially available) was added.

Table No 7. *Bacillus sp.* and *Corynebacterium sp.* isolates representing positive result under Ammonia production test

		Appearance of yellow colouration	
Sl. No.	Bacterial isolates	Culture tube 1	Culture tube 2
1	*Bacillus sp.*	Positive	Positive
2	*Corynebacterium sp.*	Positive	Positive

IV.5. Zn solubilization activity test

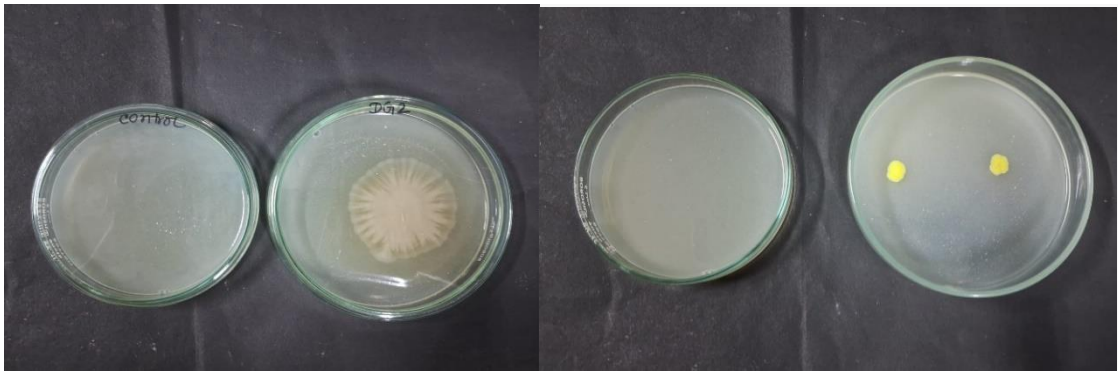


Figure 5. a. *Figure 5. b.*

Figure 5. Zinc solubilization activity test representing cultured plates of (a) *Bacillus sp.* and (b) *Corynebacterium sp.* bacterial isolates grown in modified Pikovskayas media supplemented with zinc oxide (glucose 10 g/L, $MgSO_4.7H_2O$ 0.1 g/L, $(NH_4)_2SO_4$ 1g/L, KCL 0.2 g/L, K_2HPO_4 2g/L, yeast 5 g/L, ZnO 1g/L, agar 20g/L) was used to determine Zn solubilizing activity of the isolates.

Table No 8. *Bacillus sp.* and *Corynebacterium sp.* isolates representing negative result for clear zone in zinc solubilizing activity test.

		Clear zone around the colonies	
Sl. No.	Bacterial isolates	Culture plate 1	Culture plate 2
1	*Bacillus sp.*	Negative	Negative
2	*Corynebacterium sp.*	Negative	Negative

IV.6.NBRiP

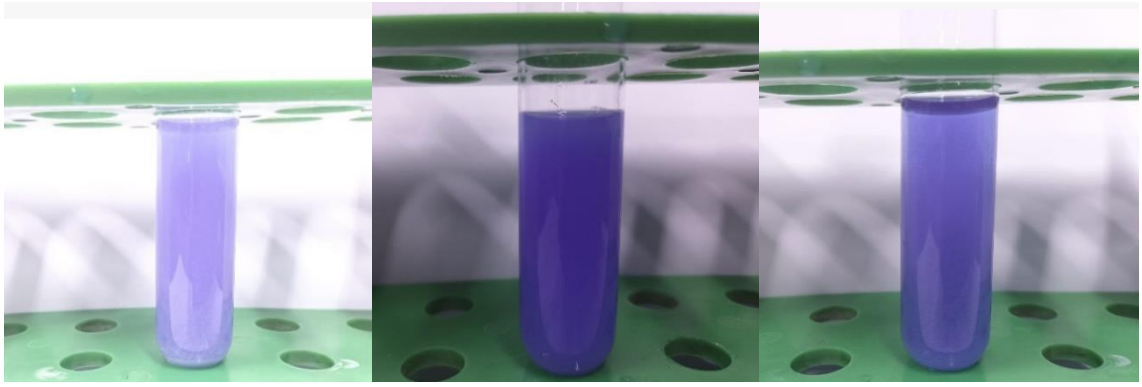


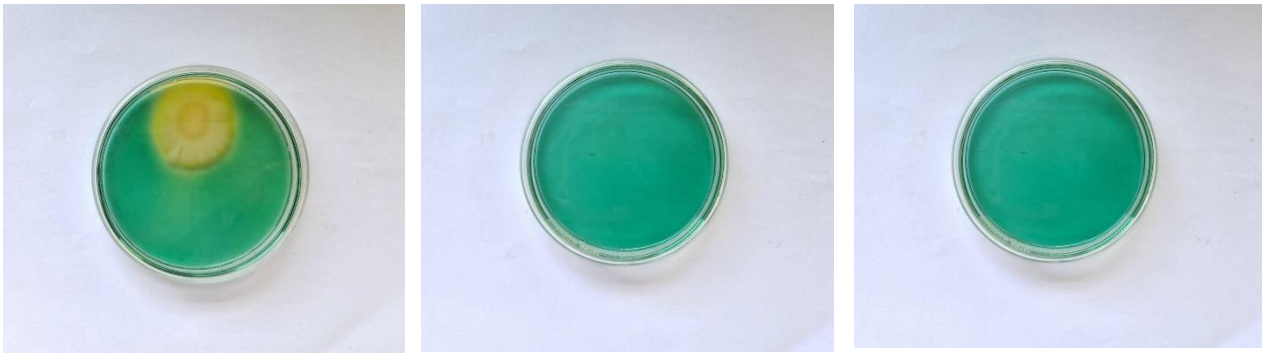
Figure 6. (a) *Figure 6. Control* *Figure 6. (b)*

Figure 6. NBRiP test representing cultured tubes of (a) *Bacillus sp.* and (b) *Corynebacterium sp.* bacterial isolates grown in culture broth that is a mixed solution of A (TCP 5g/L, bromophenol blue 0.0.25 g/L) and solution B (glucose 10 g/L, $MgCl_2$. $6H_2O$ 5 g/L, $MgSO_4.7H_2O$ 0.25 g/L, $(NH_4)_2SO_4$ 0.1 g/L, Agar 20 g/L) for determining phosphate solubilizing activity.

Table No 9. *Bacillus sp.* and *Corynebacterium sp.* isolates representing of positive results by forming blue broth into colourless after digestion by bacteria that indicates phosphate solubilizing activity.

		Colourless change of Blue Nutrient Broth	
Sl. No.	Bacterial isolates	Culture tube 1	Culture tube 2
1	*Bacillus sp.*	Positive	Positive
2	*Corynebacterium sp.*	Positive	Positive

IV.7. Siderophore test



(a) *Bacillus sp.* Control plate (b) *Corynebacterium sp.*

Figure 7. Siderophore test representing cultured plates of (a) *Bacillus sp.* and (b) *Corynebacterium sp.* bacterial isolates grown in CAS-Agar nutrient media

Table No 9. (a) *Bacillus sp.* and (b) *Corynebacterium sp.* isolates representing of positive and negative results by forming clear halo and non-colourless appearance around the bacterial colonies after digestion by bacteria.

		Clear halo zone around the colonies	
Sl. No.	Bacterial isolates	Culture plate 1	Culture plate 2
1	*Bacillus sp.*	Positive	Positive
2	*Corynebacterium sp.*	Negative	Negative

V. Discussion

Day by day soil contamination due to bioavailability of heavy metals in high concentration is affecting the plant growth by causing production of free radicals, reactive oxygen species, uncontrolled oxidation, initiation of chain reaction with cellular biomolecules like nucleic acids, proteins, and lipids, ultimately causing oxidative stress and cellular damage [133]. To overcome these effects in plants Plant growth promoting bacteria (PGPB) are widely as biofertilizers used to reduce or to control the toxicity of such heavy metals.

Generally, the microorganisms are found to colonize in every part of the plants with beneficial activities and are generally defined as plant growth-promoting bacteria (PGPB) [134]. PGPB uses various resistant mechanisms to bioremediate those hazardous metals specially chromium. As discussed above the PGPB uses its environmentally techniques known as phytoremediation to decontaminate the chromium stress in soils [135]. To study all other potentiality of PGPB the antibiotic susceptibility test and in-vitro screening tests was conducted for the bacterial isolates from arable soil to study their plant growth promoting potentiality. In antibiotic susceptibility test both *Bacillus sp.* and *Corynebacterium sp.* isolates showed moderate results that illustrates they can resist different antibiotic substances and can produce strong antibiotics substance to inhibit other pathogenic microbes affecting the plant body. Next *Bacillus sp.* and *Corynebacterium sp.* showed negative result for Indole 3-Acetic Acid (IAA) production which is a plant growth hormone such as auxin, class phytohormone that influence root architecture, nutrient uptake, cell division and supports plant development. For Hydrogen cyanide (HCN) test only *Corynebacterium sp.* showed positive result and *Bacillus sp.* showed negative. *Corynebacterium sp.* bacterial species may inhibit cytochrome c oxidase and several other metalloenzyme [136]. HCN producing PGPB helps promote plant growth, biocontrol of pathogens, insects, termites and nematodes [137]. In Ammonia production test, both *Bacillus sp.* and *Corynebacterium sp.* showed positive result that means both have plant growth promotion abilities. PGPB producing ammonia supports with shoot elongation of plant, promote root development and nitrogen supply to the plant body biomass [138]. *Bacillus sp.* and *Corynebacterium sp.* isolates both showed negative result for the 5th experiment which was Zinc solubilization test, Zinc solubilization in soil by PGPB helps to covert insoluble zinc into accumulating form that increases zinc bioavailability in soil, and is alleviated to different parts of the plant [139]. Zinc is also important for processing many metabolic activities, cellular functions, enzyme function, and ion transport in plants [140]. National Botanical Research Institute's phosphate growth liquid medium (NBRiP) is a microbiological growth medium use to screen phosphate solubilizing microorganisms. In this particular study *Bacillus sp.* and *Corynebacterium sp.* showed positive results that means both isolates were capable to dissolve tricalcium-phosphate and increase the amount of phosphorus in soil that helps in promoting plant growth [141]. Because phosphorus is necessary for cell division, development of growing tips of the plant and is a constituent of plant cell. And in Siderophore test *Bacillus sp.* showed positive result and

Corynebacterium sp. with negative result represents that *Bacillus sp.* can liberate siderophore as secondary metabolite that helps in plant growth through iron uptake under iron stressed condition [142]. Therefore, the invitro screening tests are an essential step to identify and characterize plant growth-promoting bacteria.

VI. Conclusion

Chromate pollution due to industrial exposure and naturally deposition of Cr (VI) in soil was affecting the growth of the plants through many ways. Hence, the removal of chromium from the environment is necessary by using bioremediation methods. Such as soil dwelling microbes especially Plant Growth Promoting Bacteria (PGPB) are seen to remediate the Cr (VI) in the environment by its reduction potential. They reduce Cr (VI) to Cr (III) through bioremediation, detoxification of Cr (VI), biotransformation, biosorption, and other cellular processes such as electrocoagulation, membrane separation, chemical precipitation, etc. Helping the plants to grow in arable lands and to find out their potentiality, Different tests are performed such as antibiotic susceptibility and in-vitro screening tests were performed to detect plant growth promotion ability of the supplied bacterial isolates. In this study it was found that provided bacterial isolates were able to exhibit plant growth promotion activity. Different heavy metals such as Cd, Cu, Zn, Ni, Fe, Pb, Cr, etc., are bioremediated by PGPB and Cr (VI) was reported to be most abundantly bio-transformed into less toxic substances. During the in-vitro screening tests *Bacillus sp.* showed the highest potential compared to *Corynebacterium sp.* in plant growth promotion activity. These isolates were reported to use their plasmid-encoded chromate resistance genes for the reduction of Cr (VI) in many studies. They can tolerate high Cr concentrations and resist Cr toxicity. Through various enzymatic and non-enzymatic processes, the cell converts Cr (VI) to Cr (III) and makes it less toxic. The process of bio-reduction and biosorption mechanism are used by PGPB for bioremediation and reduction of Cr (VI). They can trap Cr (VI) and make it immobile for other biological uptake that indirectly supports the plant with less exposure toxic heavy metals. Hence, the supplied heavy metal-tolerant plant growth promoting bacterial strains will be a beneficial, eco-friendly, cost effective and sustainable approach for converting lethal substances into less toxic form and can be further evaluated in greenhouse and field trails to assess their potential as biofertilizers or biocontrol agents. This process will help in reducing the chemical fertilizers and promoting sustainable agriculture.

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