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# Enhancing phytoremediation of chromium-stressed soils through plant-growth-promoting bacteria



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## KEYWORDS

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Plant growth promoting  
bacteria

**Abstract** Chromium, specifically hexavalent chromium is one of the most toxic pollutants that are released into soils by various anthropogenic activities. It has numerous adverse effects not only on plant system but also on beneficial soil microorganisms which are the indicators of soil fertility and health. Recent emergence of phytoremediation as an environmental friendly and economical approach to decontaminate the chromium stressed soils has received wider attention. But major drawback of this process is that it takes long time. Application of multifunctional plant-growth-promoting bacteria (PGPB) exhibiting chromium resistance and reducing traits when used as bio-inoculants with phytoremediating plants, has resulted in a better plant growth and chromium remediating efficiency in a short time span. PGPB improve chromium uptake by modifying root architecture, secreting metal sequestering molecules in rhizosphere and alleviating chromium induced phytotoxicity. The purpose of this review is to highlight the plant-beneficial traits of PGPB to accelerate plant-growth and concurrently ameliorate phytoremediation of chromium contaminated soils.

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## 1. Introduction

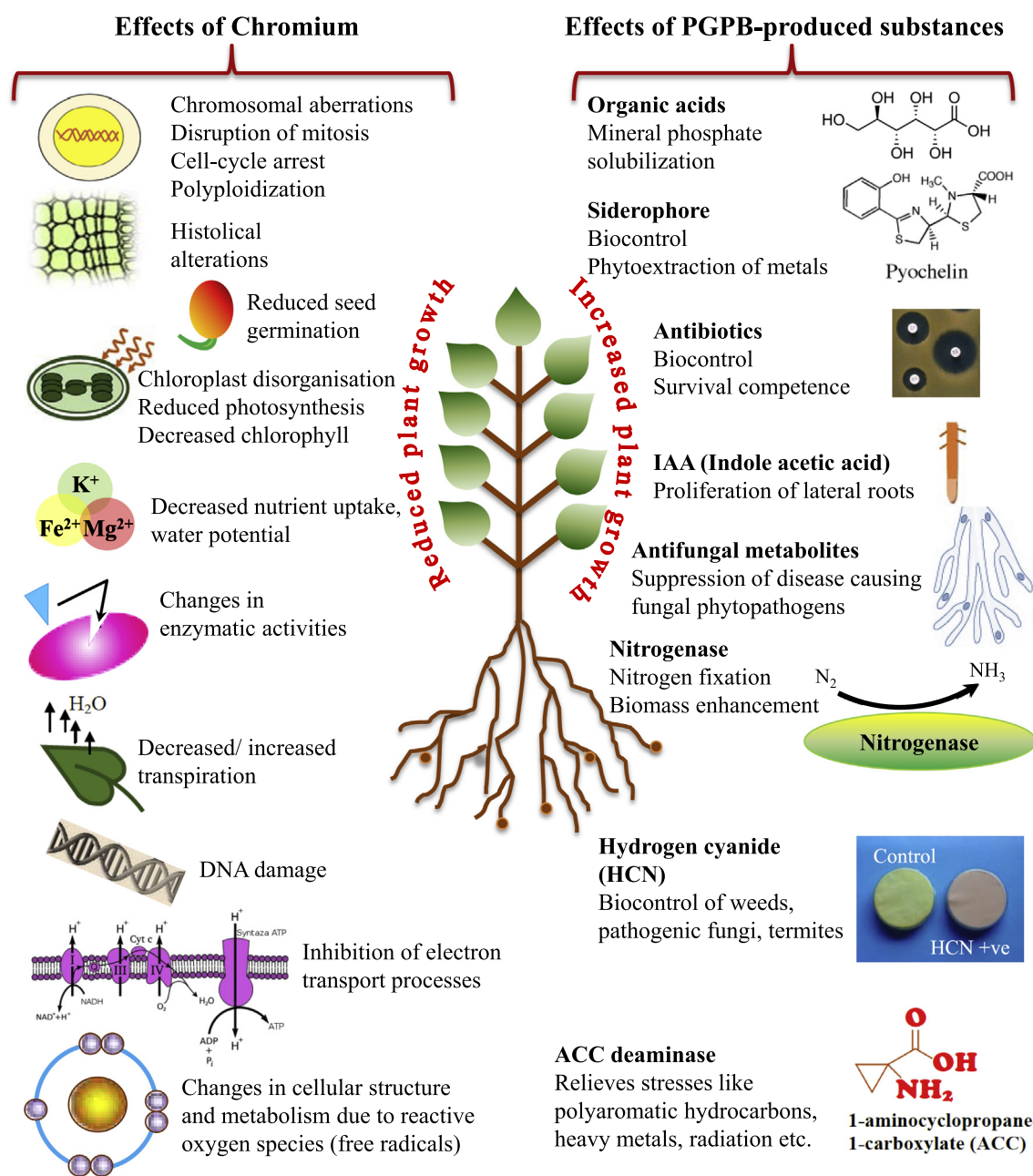
Because of a nutrient-rich resource, soil is a hub of versatile microbiological activities that are indispensable to maintain its fertility and plant productivity by facilitating therein decomposition, mineralization, fixation and immobilization of nutrients and consequently, their recycling [6–8,24,47,79,81]. In addition, soil microbes by their inherent beneficial as well as antagonistic activities sustain the soil ecosystem by supporting/promoting/opposing or inhibiting different biotic and abiotic processes [7,67,78].

Consistent proliferation and urbanization of human population have led to extensive advancement in the anthropogenic and technogenic processes e.g. mining, metallurgical

**Table 1** Heavy metals prevailing in soils and their regulatory limits.

Metal	Concentration range (mg kg <sup>-1</sup> )	Regulatory limit (mg kg <sup>-1</sup> )
Lead	1–6900	600
Cadmium	0.1–345	100
Arsenic	0.1–102	20
Chromium	0.005–3950	100
Mercury	0.001–1800	270
Copper	0.03–1550	600
Zinc	0.15–5000	1500

Source: Salt [86].



**Figure 1** Chromium-induced changes and effects of different metabolites/activities of plant growth promoting bacteria (PGPB) on plants.

operations, chemical and automobile industries, and modern agricultural practices involving various agrochemicals [1,20]. Due to these ongoing and incessant processes, prolific release of heavy metals has resulted in substantial contamination of soils [29,48,67]. Metals accumulated in soils above critical levels (Table 1), affect microbial population, their activities and biodiversity and soil fertility; thus disturbing the proper functioning of the soil ecosystem [41,47]. Of different heavy metals, chromium and its various forms have shown more deleterious impact on soil microbial activity and fertility [41,49]. Also, chromium and its derivatives affect the overall development of plants through histological alterations, halting physiological activities and decreasing biomass [64,68,71].

Among diverse soil microbes, plant-growth-promoting bacteria (PGPB) producing plant-growth regulators, mineral solubilizers, phytohormones, and various secondary metabolites have been reported to expedite the plant-growth and development and soothe plants against various environmental stresses including metal stress [5,7,39,60]. Moreover, they have shown excellent results in reducing metal toxicity vis-à-vis promoting plant-growth when used as inoculants [58,67]. With current state of knowledge, this review focuses on recent advancements in functional roles of PGPB in chromium-stressed soils in accelerating growth and development of plants and phytoremediation of chromium in tandem. Other heavy metals have been excluded from this discussion as chromium and its derivatives are the most commonly detected metal contaminants at the polluted sites and produce pronounced mutagenic and carcinogenic effects on organisms even at low concentration ( $100 \mu\text{g kg}^{-1}$ ) [87,88]

## 2. Chromium: forms and toxicity to plants

Being resistant to corrosive agents, chromium (Cr) is used as a protective coating in electroplating industries, manufacturing resistant alloy products, aircrafts and nuclear reactor vessels, electronics, and cement producing plants; its other applications are in tannery, paper industry, negative-film making,

wood preservation, pyrotechnics, glass, ceramics and dye synthesis [9,11,15,44,50]. Due to geological processes as well as the above mentioned anthropogenic activities in addition to spill and dumping of chromium containing wastes, chromium and its compounds get entry into soils. Among two most predominant forms of chromium, trivalent chromium [Cr(III)] naturally occurs in soils owing to its low solubility and has a greater tendency to adsorb on soil particles and is used as a nutrient by organisms for normal growth and development [15,16]. While hexavalent form [Cr(VI)] is a toxin which typically originates from anthropogenic activities and also formed naturally by oxidation of Cr(III) within ultramafic- and serpentine derived soils/sediments [57].

In both prokaryotes and eukaryotes including plants, Cr(VI) toxicity is largely attributed to its easy diffusion across the cell membrane, reduction within cells producing free radicals and reactive oxygen species (ROS) which further aggravate its toxicity. Cr(VI) enters into cells as an oxyanion (chromate/dichromate) through nonspecific anion channels in membranes due to the structural resemblance of chromates to sulfates and phosphate ions [56,65]. Within cell, Cr(VI) is formed sequentially by its metabolic reduction to Cr(V), Cr(IV), and finally to biologically stable Cr(III) generating free radicals. Because of weak membrane permeability, intracellularly trapped Cr(III) is unable to cross the cellular membrane. Greater binding efficiency of Cr(III) compared to Cr(VI) allows the formation of stable Cr(III) complexes with proteins and nucleic acids, consequently, leading to a large spectrum of DNA damage including inhibition of DNA replication and RNA transcription [21,56,65]. Toxic effects of Cr(VI) on plant system have been shown in Fig. 1. Excellent reviews are available describing toxic effects of chromium species on plants [22,69,85].

## 3. Plant-growth-promoting bacteria and their plant-beneficial traits

Although PGPB have been isolated from diverse environmental niches, the rhizosphere is the rich source of bacteria exhibit-

**Table 2** Plant-growth-promoting metabolites/activities of chromium resistant/reducing PGPB.

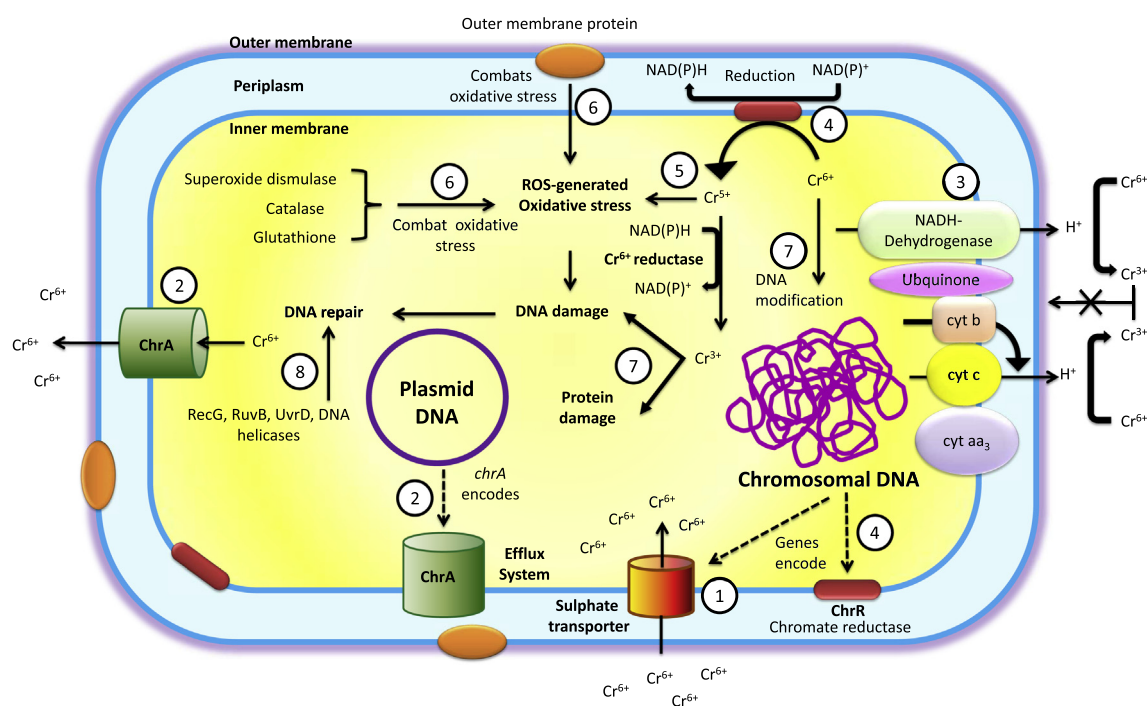
PGPB	Cres/Cred	Metabolite/activity	References
<i>Pseudomonas</i> sp. VRK3	Cres	IAA, phosphate solubilization, siderophore	[40]
<i>Paenibacillus lentimorbus</i> B-30488(r)	Cres	IAA	[46]
<i>Delftia</i> sp. JD2	Cres, Cred	IAA, nitrogen fixation	[54]
<i>Bacillus</i> spp.	Cres, Cred	IAA, phosphate solubilization, HCN, antifungal activity	[45]
<i>Bacillus</i> species PSB10	Cred	–	[82]
<i>Cellulosimicrobium cellulans</i> KUCr3	Cres, Cred	IAA, phosphate solubilization	[23]
<i>Mesorhizobium</i> sp. RC3	Cres, Cred	IAA	[84]
<i>Bacillus</i> spp.	Cres, Cred	IAA, phosphate solubilization, siderophore, HCN, ammonia	[83]
<i>Rhodococcus erythropolis</i> MTCC 7905	Cres, Cred	–	[80]
<i>Ochrobactrum</i> CrT-1, <i>Bacillus cereus</i> S6	Cres, Cred	–	[32]
<i>Pseudomonas</i> sp. PsA4, <i>Bacillus</i> sp. Ba32	Cres	IAA, phosphate solubilization	[61]
<i>Ochrobactrum intermedium</i> C32413	Cres	–	[30]
<i>Ochrobactrum intermedium</i> CrT-3	Cres, Cred	–	[31]
Rhizobacterial strains A3, S32	Cres	IAA, siderophore	[59]
<i>Pseudomonas</i> sp. RNP4	Cres, Cred	IAA, siderophore, phosphate solubilization	[62]
<i>Pseudomonas</i> sp. NBRI 4014	Cres	IAA, phosphate solubilization, siderophore	[37]

Abbreviations: plant growth promoting bacteria (PGPB), chromium resistance (Cres), chromium (VI) reduction (Cred), indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC), hydrogen cyanide (HCN).

**Table 3** PGPB traits in expediting phytoprotection and phytoremediation from/in metal stress.

Activity/metabolite	Role
Siderophores	Alleviate the suppression of chlorophyll biosynthesis due to metal-induced iron deficiency by providing iron to metal-stressed plants Alleviate the metal induced-stress in plants by supplying iron to plants exposed to metal contaminants Decrease free radical formation around plant roots and shield microbial phytohormones from metal-induced oxidative damage by means of chelation reaction Augment bioavailability and mobility of metals by solubilizing metal-minerals, subsequently enhance metal accumulation, in turn phytoextraction Protect plants from soil-microbial pathogens by limiting iron availability to them
Organic acids	Solubilize and mobilize metal containing inorganic sources
Biosurfactants	Accelerate metal bioavailability by decreasing the tight binding between metals and soil particles Bind preferentially toxic metals with strong affinity than the normal soil metal cations
Indole acetic acid	Enhances plant growth (irrespective of bacterial metal resistance/sensitivity) in metal contaminated soils Promotes absorption of nutrients and metals by proliferating plant roots Facilitates adaptation and tolerance to metals in metal-stressed plants by inducing physiological changes
ACC deaminase	Lowers growth inhibitory levels of ethylene produced in plants exposed to metal stress Improves the effectiveness of metal phytoremediation by facilitating plants to achieve longer roots and greater root density in metal-stressed soils

PGPB: plant-growth-promoting bacteria; ACC: 1-aminocyclopropane-1-carboxylate. Information derived from Burd et al. [19], Neubauer et al. [55], Sharma et al. [70], Singh and Cameotra [74], Idris et al. [42], Kalinowski et al. [43], Belimov et al. [13], Braud et al. [17,18], Arshad et al. [10], Saravanan et al. [66], Dimkpa et al. [26], Bianco and Defez [14], Egamberdieva [28], Dimkpa et al. [27], Gamalero and Glick [34], Rajkumar et al. [58], Glick [35], Ma et al. [53], Hao et al. [38].



**Figure 2** Schematic depiction of chromium resistance and toxicology in bacterial cell: (1) chromate due to the structural similarity with sulfate enters the bacterial cell through *sulfate transporter* encoded by the *chromosomal DNA*. (2) *Plasmid DNA* encoded efflux systems are used to expel the intracellular chromates outside the bacterial cell to resist the chromate toxicity. (3) Aerobic  $Cr^{6+}$  reduction into  $Cr^{3+}$  involves soluble reductase which requires  $NAD(P)H$  as an electron donor while anaerobic  $Cr^{6+}$  reduction occurs in the electron transport pathway by cytochrome b (*cyt b*) or cytochrome c (*cyt c*) along the respiratory chains in the inner membrane;  $Cr^{3+}$  cannot pass the bacterial cell membranes due to the insolubility of  $Cr^{3+}$  derivatives. (4) Membrane-embedded chromate reductase which is encoded by the *chromosomal DNA*, reduces  $Cr^{6+}$  anaerobically in the presence of electron donors. (5)  $Cr^{5+}$  produced during the redox cycle of  $Cr^{6+}$  produces oxidative stress by the production of reactive oxygen species (ROS). (6) To combat the *ROS generated oxidative stress*, protective metabolic enzymes *superoxide dismutase*, *catalase* and *glutathione* are secreted. Some *outer membrane proteins* are also involved to counter the *oxidative stress*. (7)  $Cr^{6+}$  and principally  $Cr^{3+}$  not only negatively affects DNA replication and RNA transcription by damaging DNA but also alters gene expression. In addition,  $Cr^{3+}$  also damages proteins by impairing their functions. (8) *DNA repair* system is activated in order to repair the damaged DNA (Source: Ahemad [3]).

ing enormous degree of plant-growth promoting (PGP) activities [12]. PGPB have been successfully implicated in promoting plant growth and concurrently mitigating the degree of toxicity or damage to plants exposed to stress generated by different heavy metals including chromium in metalliferous soils [7]. They enhance the growth of plants under both normal and stressed environment either by stimulating them or through biocontrol activities (Fig. 1) by various PGP traits e.g. production of indole-3-acetic acid, siderophores, hydrogen cyanide, ammonia, nitrogen fixation and phosphate solubiliza-

tion (Table 2). Many of these traits not only facilitate growth promotion and protection of plants from deleterious impact of environmental stresses including phytopathogens, and metal toxicity but also help in remediation of metalliferous soils when PGPB expressing these traits are used as bioinoculants (Table 3). Recently, several critical reviews have been published by authors describing comprehensively the mechanisms of PGPB in protecting plants from phytopathogens, nutrient deficiency and various stresses and concurrently, accelerating the plant growth [2,4,7,36,58].

**Table 4** PGPB-assisted plant-growth promotion vis-à-vis phytoremediation of chromium-stressed soils.

Cr(VI) resistant/reducing PGPB	Plant	Role of PGPB	References
<i>Brucella</i> sp. K12	Okra ( <i>Hibiscus esculentus</i> L.)	Improved plant-growth and yield with a significant reduction in Cr(VI) concentration (more than 50% over control) both in soils and plant parts	[52]
<i>Microbacterium</i> sp. SUCR140	<i>Pisum sativum</i>	Increased the overall plant-growth and <i>Pisum sativum</i> – <i>Rhizobium</i> symbiosis; decreased Cr(VI) toxicity to plants by minimizing its soil bioavailability and uptake in SUCR140-inoculated plants	[76]
<i>Microbacterium</i> sp. SUCR140	<i>Zea mays</i>	Improved plant-growth, decreased Cr(VI) toxicity to plants by lowering soil bioavailability and its plant uptake through increased mycorrhizal colonization	[77]
<i>Ochrobactrum intermedium</i> , <i>Brevibacterium</i> sp., <i>Bacillus cereus</i>	<i>Lens esculenta</i>	Increased root and shoot lengths, number and weight of grains/pod, number and weight of grains/plant	[51]
<i>Agrobacterium tumefaciens</i>	<i>Zea mays</i>	Enhanced plant biomass and Cr(VI) uptake	[63]
<i>Paenibacillus lentimorbus</i> B-30488(r)	Chickpea ( <i>Cicer arietinum</i> L.)	Promoted growth and reduced Cr(VI) uptake by plants	[46]
<i>Delftia</i> sp. JD2	Alfalfa, clover	Helped rhizobia to perform better	[54]
<i>Bacillus</i> species PSB10	Chickpea ( <i>Cicer arietinum</i> L.)	Significantly improved growth, nodulation, chlorophyll, leghemoglobin, seed yield and grain protein; reduced the uptake of chromium in roots, shoots and grains	[82]
<i>Cellulosimicrobium cellulans</i> KUCr3	Chilli	Significantly increased growth parameters and reduced Cr uptake in plants	[23]
<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas fluorescens</i> , <i>Ralstonia metallidurans</i>	Maize	Promoted plant growth, facilitated soil metal mobilization, enhanced Cr uptake	[17]
<i>Mesorhizobium</i> sp. RC3	Chickpea ( <i>Cicer arietinum</i> )	Increased the dry matter accumulation, number of nodules, seed yield, grain protein, N in roots and shoot	[84]
<i>Rhodococcus erythropolis</i> MTCC 7905	Pea ( <i>Pisum sativum</i> )	Promoted plant growth at low temperatures	[80]
<i>Pseudomonas</i> sp. PsA4, <i>Bacillus</i> sp. Ba32	Indian mustard ( <i>Brassica juncea</i> )	Stimulated plant growth and decreased Cr(VI) content	[61]
<i>Ochrobactrum</i> CrT-1, <i>Bacillus cereus</i> S6	Mungbean	Lowers the toxicity of chromium to seedlings by reducing Cr(VI) to Cr (III)	[32]
<i>Brevibacterium</i> sp.	Sunflower ( <i>Helianthus annuus</i> )	Increased plant height, fresh and dry weight, auxin content, and seedlings growth	[33]
Rhizobacterial strains A3 and S32	Indian mustard ( <i>Brassica juncea</i> )	Promoted the plant growth	[59]
<i>Pseudomonas</i> sp. RNP4	Black gram, Indian mustard, pearl millet	Significantly promoted plant growth	[62]
<i>Ochrobactrum intermedium</i> C32413	Sunflower ( <i>Helianthus annuus</i> )	Increased seedling length, fresh weight, dry weight, fresh weight, phosphatase and auxin contents; decreased Cr(VI) uptake	[30]
<i>Ochrobactrum intermedium</i>	Sunflower ( <i>Helianthus annuus</i> )	Increased seed germination and plant height and decreased Cr(VI) uptake	[31]
<i>Pseudomonas</i> sp. NBRI 4014	Soybean ( <i>Glycine max</i> PK 564)	Promoted root and shoot elongation of plants	[37]

PGPB: plant growth promoting bacteria.

#### 4. PGPB-assisted plant growth promotion in chromium-stressed soils

Many *in situ* bioremediation approaches to clean up the metal polluted soils have been proposed and practiced delineating encouraging results. Of them, phytoremediation, exploiting the metal-accumulating plants to remediate metalliferous soils, is an economical and ecologically healthy approach. But this process is very slow and time-consuming and also requires increased plant biomass, root growth and metal mobility in soils to decontaminate expeditiously the metal stressed soils [25,58]. In this context, application of PGPB in phytoremediation (which may be directed chiefly to either accumulating toxic metal species in plant tissues through phytoextraction in moderately contaminated soils or to mitigate the metal-generated toxic effects on plants through phytostabilization in extremely polluted sites) has gained wider acceptability due to their excellent performance in augmenting the remediation efficiency as well as growth of plants [25].

In this regard, current upsurge in the recovery of novel PGPB with Cr(VI) reducing-potential has contributed in reducing chromium toxicity and enhancing plant biomass in chromium-stressed soils [52,76,77]. Many bacterial genera of Cr(VI) reducing PGPB like, *Ochrobactrum* [31], *Delftia* [54], *Pseudomonas* [62], *Bacillus* [45,82,83], *Cellulosimicrobium* [23], *Mesorhizobium* [84], and *Rhodococcus* [80] have been isolated from soils. Bacterial trait of reductive immobilization of chromium has a special significance as through mechanisms of Cr(VI) reduction, toxic chromium derivatives are converted into environmentally less harmful products (Fig. 2) [23,75].

Plants inoculated with PGPB exhibiting Cr(VI) reducing property have shown better adaptation while growing in chromium-stressed soils as the these beneficial bacteria induce changes in plant metabolism (e.g. extensive proliferation in roots for better nutrient absorption, increased bacterial siderophore-mediated iron uptake, and upregulation of genes involved in stress mitigation etc.) thereby they become more tolerant to chromium stress. Recently, various studies, using different plants inoculated with different genera of Cr(VI) reducing-PGPB, have been conducted by many authors across the world to phytoremediate the chromium-contaminated or spiked soils with concurrent promotion of plant health and growth (Table 4). It is evident from Table 4 that both methods of phytoremediation, phytoextraction and phytostabilization have been successfully attempted to overcome the chromium stress in soils. In fact, the phytoextraction approach is applicable in soils with moderate concentration of contaminants and the phytostabilization method is tried in soils with such high concentrations of contaminants which can never be easily remediated through phytoextraction. For PGPB-assisted phytoextraction of chromium, its bioavailability in soils is a major factor while considering other factors: plant type, bacterial inoculum showing PGP traits and their colonization, soil type and edaphic conditions [7,34,53]. In this regard, PGPB increase the bioavailability chromium in soils for phytoextraction by producing various primary and secondary metabolites like, siderophores and organic acids [17,27,34]. In addition, bacterial biosurfactants also increase the phytoavailability of metals including chromium as the bacterial biosurfactant helps in releasing metals that are strongly bound to soils [34,72,73]. In contrast, various bacterial chromium-resistant mechanisms

(Fig. 2) decrease phytoavailability of chromium in soils so that phytostabilization process is operated smoothly in chromium stressed soils.

#### 5. Conclusions and perspectives

Evidently, PGP traits along with chromium detoxifying property showed that PGPB have great potential to improve plant-growth and concurrently, phytoremediation of chromium-stressed soils. Since, performance of specific PGPB varies significantly according to the soil type and environmental conditions; an intensive research is needed to enhance the rhizosphere colonization and chromium phytoavailability by PGPB in chromium-stressed soils. Using consortia (more than one ecologically distinct PGPB) instead of mono-inoculant is a better strategy in order that plant-beneficial bacterial activities are expressed continuously in soils. Moreover, unravelling the exact mechanisms of PGPB in facilitating chromium uptake and evasion by plants would further consolidate the PGPB-assisted phytoremediation of chromium in diverse niches.

In addition, efficiency of phytoextraction/phytostabilization is measured by translocation factor (TF) and bio-concentration factor (BF). Therefore, compatibility of chromium remediating plants with the appropriate PGPB is required in a specific soil type so that the maximum efficiency can be achieved in terms of TF and BF.

Further, most of the studies of PGPB-assisted phytoextraction/phytostabilization have been conducted in chromium-spiked soils under controlled environment (pots/greenhouse/gnotobiotic conditions); field trials would reveal the actual practicability of chromium-reducing PGPB in accelerating the pace of remediation vis-à-vis plant-growth amelioration because field environment is exposed to several constraints including biotic (biological antagonism by indigenous microflora and phytopathogens) and abiotic (acidity, salinity, drought, temperature, and radiation etc.) factors.

Recently, new biotechnological and genetic engineering tools have revolutionized the bioscience as new traits can be produced in the recipient organism by inserting/modifying specific genetic sequence of desired traits from host organisms. By this approach, PGPB can be genetically modified in order to increase the phytoextraction or mobilization of chromium. But releasing such PGPB strains in natural environment would encounter legal and ethical problems which must be addressed prior to their application.

Considering the above scenario, plant-growth promotion with chromium-stress evasion and simultaneously expediting phytoremediation through PGPB can be realized with full potential.

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