

Phytoremediation enhanced with concurrent microbial plant growth promotion and hexavalent chromium bioreduction

Abstract

Hexavalent chromium (Cr(VI)) is a toxic and carcinogenic heavy metal accumulating in agricultural soils following effluent releases from chromate releasing industries such as tanneries, electroplating, paper and dye manufacturing etc. Cr(VI) is readily taken up by plants and then passed on through the food cycle. Toxic effects of Cr(VI) affect both prokaryotes and eukaryotes primarily by its ability to generate reactive oxygen radicals causing DNA and protein damage. Cr(VI) bioremediation using multifaceted micro-organisms capable of reduction of toxic Cr(VI) to nontoxic derivatives as well as augmented plant growth capable of phytoremediation by production of growth promoting products is currently being exploited.

Keywords: plant growth promoting organisms, chromium reduction, bioremediation, phytoremediation

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Abbreviations: Cr(VI), chromium vi; PGP, plant growth promoting; Hg, mercury; Cd, cadmium; Pb, lead; Zn, zinc

Introduction

Contamination of agriculturally arable land with industry derived pollutants including heavy metals such as chromium has affected soil fertility as well plant, animal and human health by indirectly entering the food chain. Hexavalent chromium is a byproduct of tanning, electroplating, wood, dye, paper, glass, ceramic etc. industries and is usually discharged as industrial effluent. Cr (VI) is a toxic, water insoluble form and Cr (III)/Cr(IV) state is water soluble and less toxic form usually required in the form of micronutrients. Chromium and its derivatives have shown to decrease biomass, affect physiological activity, histological alterations leading to mutagenic and carcinogenic effects for plant and animal cells.¹⁻³ Chromium bioremediation has long been recognized as an economical and ecofriendly solution to the problem.⁴ Recent field and *in situ* studies now provide concrete evidence to the effectiveness of novel bio augmentation strategies that integrate desirable properties to achieve the end goal. One such strategy is to use multifaceted micro-organisms that can concurrently reduce toxic Cr(VI), produce plant growth promoting products that enhance plant growth of species which exhibit phytoremediation properties.^{5,6}

Discussion

Rhizospheric soil serves as a high concentration root exuded nutrition which attracts large diversity of microbial life depending on physicochemical conditions as well as plant species. Many of these root residents have been shown to serve mutualistic association by enhancing plant growth directly or indirectly via plant growth promoting products (PGP).⁷ These PGP include biocontrol agents such as anti phytopathogenic compounds such as hydrogen cyanide, growth inducing phytohormones such as indole acetic acids, nitrogen fixation, organic acids for phosphate and potassium solubilization, iron chelating siderophores as well as stress relieving compounds such as ACC deaminase, superoxide dismutase.⁸ Many of this PGP

producing rhizospheric micro-organisms form association known as biofilms on root surfaces.⁹ Biofilms are communities of organisms adhering onto a substrate encased in an exopolymeric substance. The biofilm existence confers on its residents high levels of resistance to environmental stressors including chromate.¹⁰

Cr(VI) reducing ability of micro-organisms is attributed to several factors including their ability to form biofilms, presence of extracellular and intracellular chromate reductases and chromate efflux pump.¹¹⁻¹³ Microorganisms catalyze redox reactions by combination of non-enzymatic reduction by bacterial surfaces, exopolymeric substances of the biofilm, and enzymatic extracellular/ intracellular reduction of Cr(VI) to non-toxic Cr(III)/Cr(IV) oxidative state.¹³

Plants are also capable of phytoaccumulation, which consists of uptake of Cr(VI) from soil through plant roots, ultimately to shoots where it complexes with organic acids to reduced Cr states.⁶ Phytostabilization involves the conversion of toxic Cr to Cr(III) by plant tissues. Based on their strategies of growing in heavy metal contaminated soils, plants are classified as extruders, accumulators and indicators. Use of Cr tolerant plants whose remediation activity can be enhanced by use of PGP rhizospheric organisms has integrated several approaches for finding optimal solutions to the problem of Cr contamination of agricultural soils.

Micro-organisms selected for their Cr reducing ability as well as PGP production enhanced phytoremediation of plants can be developed as effective bioinoculants. The effective use of a PGP producing Cr(VI) reducing *Staphylococcus arlette* strain in enhancing *Triticum aestivum* seed germination and *in vivo* plant growth in upto 500µg/kg soil Cr (VI) was reported.¹⁴

Application of Cr- resistant bacteria from rhizosphere and endosphere of *Prosopis juliflora* were used to enhance the phytoremediation of ryegrass. The inoculation of three isolates to ryegrass (*Lolium multiflorum L.*) improved plant growth and heavy metal removal from the tannery effluent contaminated soil.¹⁵ *Rhodococcus erythropolis* MTCC 7905 reduced substantial amounts of Cr(VI) at 10°C and showed plant growth promotion. This isolate

offers promise as a bioinoculant to promote plant growth of pea (*Pisum sativum*) in the presence of toxic Cr(VI) concentration at low temperature.¹⁶

Inoculation of four chromate tolerate rhizobacteria RZB-03 increased *Scirpus lacustris* biomass by 59 and 104%, while total chlorophyll content by 1.76 and 15.3% and protein content increased by 23 and 138% under 2µg/ml and 5µg/ml concentrations of Cr(VI), respectively after 14days as compared to non-inoculated plant. Similarly, Cr accumulation also increased by 97 and 75% in shoot and 114 and 68% in root of inoculated plants as compared to non-inoculated plants at 2 and 5µg/ml Cr(VI) concentrations, respectively after 14days.¹⁷

Microbacterium sp strain SUCR 140 was studied with arbuscularmycorrhizal fungi (AMF-*Glomus mosseae*, *G. aggregatum*, *G. fasciculatum*, and *G. intraradices*) alone or in combination, on *Zea mays* in artificially Cr(VI)-amended soil (Soni, Singh, Awasthi, & Kalra, 2014). Presence of *Microbacterium sp*. SUCR140 reduced the chromate toxicity resulting in improved growth and yields of plants compared to control by lowering bioavailability and mycorrhiza restricting chromate translocation to aerial plant parts.¹⁸ The effects of pretreatment of chromate reducing *Microbacterium sp*. strain SUCR 140 on *P. sativum* plant growth, chromate uptake, bioaccumulation, nodulation, and population of *Rhizobium* was studied. 15days pretreatment before sowing showed maximum increase in growth and biomass in terms of root length (93%), plant height (94%), dry root biomass (99%), and dry shoot biomass (99%). Coinoculation of *Rhizobium* with SUCR140 showed further 117, 116, 136, and 128% increase, respectively, in root length, plant height, dry root biomass, and dry shoot biomass. The bioavailability of Cr(VI) decreased significantly in soil (61%) and in uptake (36%) in SUCR140-treated plants; the effects of *Rhizobium*, however, either alone or in the presence of SUCR140, were not significant.¹⁹

Chromium reducing and plant growth promoting *Mesorhizobium* strain RC3 and *Bacillus species* PSB10 significantly improved growth, nodulation, chlorophyll, leghemoglobin, seed yield and grain protein of chickpea crop grown in the presence of different concentrations of chromium compared to the plants grown in the absence of bioinoculant.^{20,21}

Bioremediation studies with other heavy metals such as mercury (Hg), cadmium (Cd), lead (Pb), zinc (Zn) have also been well studied and reviewed.⁹ Many Cr(VI) reducing bacteria are cross tolerant to other heavy metals such as mercury (Hg), lead (Pb), cadmium (Cd), zinc (Zn), nickel (Ni), cobalt (Co) and arsenic (As). Chromate remediation has a higher success rate in comparison to many other heavy metals in that micro-organisms are capable of reducing a toxic Cr(VI) to non toxic Cr(III) and Cr(IV) oxidation states in addition to separation of Cr(VI) from environment by bioadsorption and bioaccumulation.¹¹ Commercial production and application of biofertilizers or PGP bio formulations for sustainable agriculture are already available.⁷ Many of the *in situ* plant bioassay and field experiments with PGP producing Cr(VI) remediating micro-organisms enhanced phytoremediation case studies cited in this review provide promising pilot studies. Development of heavy metal bioremediation bio formulations for commercial applications and marketing of such products to agriculturists as well as industries would be enhanced with academia-user collaborations. Augmenting the scale of phytoremediation methods such that it can be adopted by the chromate pollution causing industries as part of its effluent treatment protocols would be enormous value.

Conclusion

The chromate tolerant rhizobacteria which play an important role in chromium uptake and growth promotion in plant may be useful in development of microbes assisted phytoremediation systems for decontamination of chromium polluted sites. Intensive research is essential in understanding compatibility parameters affecting rhizospheric colonization as well as factors affecting chromium availability for PGP organisms in plants growing in Cr(VI) polluted soils. Role of genetic engineering in creating custom made bioinoculant consortia to increase efficacy of remediation process as well as allowing effective monitoring of biological activity are currently being explored. Effective bioformulations and carriers for increased life shelf of bioinoculants are also areas requiring further investigations. An in depth understanding of the underlying mechanisms wherein PGP producing organisms facilitate chromate uptake and evasion by plants can provide a strong basis for using PGP organism assisted phytoremediation of chromate.

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Conflict of interest

The author declares no conflict of interest.

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