Application of Plant Growth Promoting Rhizobacteria in Bioremediation of Heavy Metal Polluted Soil

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Abstract: The contamination of soil and water with heavy metal pollutants is escalating day by day due to excessive industrialization, waste disposal, agricultural applications and various anthropogenic actions. Accrual of heavy metals, as non biodegradable agents, pose serious environmental concerns for all life forms affecting mostly plants and therefore present a risk to health of humans due to food chain contamination. To avoid heavy metal problems, bioremediation via plant growth promoting rhizobacteria (PGPR) is getting more consideration due to eco friendly nature, less expense and proven efficiency in comparison to physical or chemical remediation methods. Improving growth of plants and conquering the metal toxicity can be enhanced by association of PGPR. These microbes colonize the root or inhabit near root surfaces and involve in mechanisms for plant prevention from toxicity through secretion and production of several regulatory compounds such as phytohormones, siderophores, metal binding proteins etc. The review accentuates the role of PGPR in accelerating phytoremediation for elimination of toxic metals and growth augmentation of plants. Further, explicit spotlight on the exploitation of genetic engineering technology for future PGPR application is highlighted with the aspiration to widen future prospects.

Key words: ACC deaminase, biosorption, phytochelatins, root exudates, sequester

Introduction:
The incessant anthropogenic practices, extensive agricultural activities and rapidly increasing worldwide industrialization has led to many environmental problems by liberation of pollutants such as heavy metals, organic contaminants, toxic wastes, smoke and fumes etc. (Hansda et al., 2014). Heavy metals are the main inorganic pollutants which are water soluble and accumulate in the soil biosphere because of their non degradable property and consequently disturb the environment (mostly plants) and make the food chain contaminated (Rajkumar et al., 2010). The noxious heavy metals in different valence states include zinc (Zn), arsenic (As), chromium (Cr), cadmium (Cd), mercury (Hg), copper (Cu), nickel (Ni) and lead (Pb) (Jing et al., 2007). Some of the heavy metals are required by plants as micronutrients in small quantities but disproportionate accrual of heavy metals is detrimental to the majority of plants. When heavy metal ions are present in elevated levels in the environment, plant roots rapidly absorb and translocate them to shoots and leaves which cause stress leading to disturbed metabolism, reduced growth and even plant death (Figure 1) (Jing et al., 2007). Moreover, high metal concentration in soil decreases its fertility, affect soil microbes and yield losses (McGrath et al., 2001).

![Figure 1. Heavy metal movement in plants](image-url)
a: Heavy metal ion adsorbed at root surface; b: Movement of bioavailable metal into root cells across membrane; c: Vacuole immobilization of a small proportion of the metal; d: Intracellular movement of metal passes membranes into xylem (root vascular tissue); e: Translocation of metal from root to shoot & leaves tissue.

Remediation of heavy metals from soil is difficult for the reason that these cannot be degraded biologically but can only be altered from one oxidation state or organic compound to a different complex or reduced to state of low toxicity (Garbisu and Alkorta, 2001). Many methods including chemical, physical and biological procedures have been applied for the removal of contaminants from polluted areas but the biological processes for remediation i.e. bioremediation offer the best solution as it is less costly, simpler and useful expertise in comparison to physico-chemical detoxifying technologies which are expensive and detrimental to soil characteristics (Quartacci et al., 2006). The phytoremediation method of bioremediation process involves use of plants to sequester, remove and detoxify harmful contaminants and it has been described as successful, in situ, less expensive, ecologically gentle and socially acknowledged technology for pollution eradication (Garbisu et al., 2002).

The phytoremediation competence can be enhanced by developing association of plants with heavy metal resistant bacteria which can amplify the heavy metal mobilization in the soil (Zhuang et al., 2007). The plant growth promoting rhizobacteria (PGPR) are among the soil microbes (rhizobacteria) which are drawn in the plant interactions with metal polluted soil surroundings and need special deliberation because these can directly accelerate the process of phytoremediation by altering the bioavailability of metals via changes in production of phytohormones, siderophores, varying pH and increased release of metal binding chelators (Ma et al., 2011). Heavy metal phytoremediation aided by PGPRs may be one of the quite a few forms: phytoextraction i.e. process in which plants via roots and shoots accumulate metals from polluted soil, rhizofiltration; involves plants to take up metals from effluents and contemplate them within roots (Jing et al., 2007). Phytovolatilization is the process in which plants engage in up taking and liberation of volatile compounds of metals (such as Hg & As) into the atmosphere while phytostabilization process involves plants to lower the bioavailability of heavy metals by lessening the metal mobility through precipitation and absorption (Jing et al., 2007).

PGPR interaction for heavy metal removal:

The possession of many characteristics by PGPR have enabled them to vary bioavailability of heavy metals via release of chelating substances, acidifying the microenvironment and by inducing modifications in redox potential (Whiting et al., 2001; Lasat, 2002). The specificity of heavy metal resistant PGPR interaction is dependent on soil environment, the type of plant, bioavailability of the metal contaminants, composition of root exudates and the level of nutrients. Apart from that, the metabolic necessities for removal of heavy metals may reveal the type of association as specific or non specific PGPR interaction (Jing et al., 2007). Interaction limiting factors which may result in halting plant growth under metal stress includes low water supply, lack of soil structure, arid conditions, unfeasible nutrient exchanges or its deficiencies. Sequestration ability and sensitiveness towards heavy metals of soil microbes interacting with plants make them prospective mediators for bioremediation (Hansda et al., 2014). PGPR can vary plant cellular functions so that upon contact to heavy metal toxicity, the plants are capable to cope with elevated levels of metals and thus can resist the metal stress (Welbaum et al., 2004). The day by day increasing metal contamination of soil and water for plants can be best coped by interaction of metal resistant PGPR. This has also started new studies and findings for finding potential PGPR having effective role in phytoremediation. Some studies of PGPR involved in heavy metal bioremediation in association with various plants are abridged in Table 1.

Table 1. Examples of PGPR utilized for detoxification of heavy metals in plants

<table>
<thead>
<tr>
<th>PGPR</th>
<th>Heavy metals</th>
<th>Plants</th>
<th>PGPR function</th>
<th>Experiment conditions</th>
<th>Investigate d by</th>
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</thead>
<tbody>
<tr>
<td><em>Achromobacter</em> xyllosidans strain Ax10</td>
<td>Cu</td>
<td><em>Brassica juncea</em></td>
<td>Significantly enhanced Cu uptake by plants and increased the root/shoot length and fresh/dry weights</td>
<td>Pots</td>
<td>Ma et al. (2009b)</td>
</tr>
<tr>
<td><em>Azotobacter chroococcum</em> HKN-5</td>
<td>Pb</td>
<td><em>Brassica juncea</em></td>
<td>Prevented from Pb toxicity &amp; stimulated growth of plant</td>
<td>Greenhouse</td>
<td>Wu et al. (2006)</td>
</tr>
<tr>
<td><em>Bacillus licheniformis</em> NCCP-59</td>
<td>Ni</td>
<td><em>Oryza sativa</em></td>
<td>Improved seed germination under Ni stress and safeguarded against toxicity</td>
<td>Pots</td>
<td>Jamil et al. (2014)</td>
</tr>
<tr>
<td><em>Bacillus megaterium</em> HKP-1, <em>Bacillus</em> mucilaginosus HKK</td>
<td>Zn, Pb</td>
<td><em>Brassica juncea</em></td>
<td>Plant growth stimulation and prevention from metal stress</td>
<td>Green house</td>
<td>Wu et al. (2006)</td>
</tr>
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<td>PGPR</td>
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<td><em>Bacillus</em> PSB10 sp.</td>
<td>Cr</td>
<td><em>Cicer arietinum</em></td>
<td>Prominently enhanced growth, nodulation, seed yield and grain protein. Reduced the uptake of Cr in roots, shoots and grains</td>
<td>Pots</td>
<td>Wani and Khan (2010)</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> SJ-101</td>
<td>Ni</td>
<td><em>Brassica juncea</em></td>
<td>Facilitated Ni accrual</td>
<td>Growth chamber</td>
<td>Zaidi et al. (2006)</td>
</tr>
<tr>
<td><em>Bacillus weihenstephanensis</em> is Strain SM3</td>
<td>Ni, Cu, Zn</td>
<td><em>Helianthus annuus</em></td>
<td>Improved plant biomass and the accretion of Zn and Cu in the root and shoot systems, also enhanced the concentrations of soluble Ni, Zn and Cu in soil with their metal mobilizing potential</td>
<td>Pots</td>
<td>Rajkumar et al. (2008)</td>
</tr>
<tr>
<td><em>Bradyrhizobium</em> sp.750, <em>Pseudomonas</em> sp., <em>Ochrobactrum cytisi</em></td>
<td>Cu, Cd, Pb, Zn</td>
<td><em>Lupinus luteus</em></td>
<td>Increased plant biomass, Nitrogen (N₂) content, bettered phytostabilization potential</td>
<td>Fields</td>
<td>Dary et al. (2010)</td>
</tr>
<tr>
<td><em>Brevibacillus</em> sp.</td>
<td>Zn</td>
<td><em>Trifolium repens</em></td>
<td>Improved plant growth and nutrition and reduced Zn content in plant tissues</td>
<td>Pots</td>
<td>Vivas et al. (2006)</td>
</tr>
<tr>
<td><em>Kluyvera ascorbata</em> SUD165</td>
<td>Ni, Pb, Zn</td>
<td><em>Brassica napus, Solanum lycopersicum</em></td>
<td>No augmentation of metal uptake in comparison to non-inoculated plants. Reduction in decrease of metal stress</td>
<td>Growth chamber</td>
<td>Burd et al. (2000)</td>
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<tr>
<td><em>Mesorhizobium</em> sp. RC3</td>
<td>Cr (VI)</td>
<td><em>Cicer arietinum</em></td>
<td>Increased the nodules number, dry matter content, grain protein and seed yield up by 86%, 71%, 16% &amp; 36% correspondingly in comparison to non-inoculated treatments. N₂ in roots and shoots improved by 46% and 40%</td>
<td>Pots</td>
<td>Wani et al. (2008)</td>
</tr>
<tr>
<td><em>Microbacterium oxydans</em> AYS09223 (RS)</td>
<td>Ni</td>
<td><em>Alyssum murale</em></td>
<td>Aided in phytoretaction of Ni</td>
<td>Pots</td>
<td>Abo shanab et al. (2006)</td>
</tr>
<tr>
<td><em>Ochrobactrum sp., Bacillus cereus</em></td>
<td>Cr (VI)</td>
<td><em>Vigna radiata</em></td>
<td>In seedlings, Cr toxicity was lowered by reduction of Cr (VI) to Cr (III)</td>
<td>Pots</td>
<td>Faisal and Hasnain (2006)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> Strain MKRh3</td>
<td>Cd</td>
<td><em>Vigna munga</em></td>
<td>Plants elaborated lessened accretion, increased rooting and stimulated plant growth</td>
<td>Pots</td>
<td>Ganesan (2008)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa, Pseudomonas fluorescens, Ralstonia metallidurans</em></td>
<td>Pb, Cr</td>
<td><em>Zea mays</em></td>
<td>Supported plant growth, aided soil metal mobilization and increased Pb and Cr uptake</td>
<td>Pots</td>
<td>Braud et al. (2009)</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em> KNP9</td>
<td>Cd, Pb</td>
<td><em>Vigna radiata</em></td>
<td>Reduction of Cd and Pb uptake and enhanced plant growth</td>
<td>Greenhouse</td>
<td>Tripathi et al. (2005)</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp.</td>
<td>Cr, Cd, Ni</td>
<td><em>Glycine max, Vigna radiata, Triticum vulgare</em></td>
<td>Improvement of plant growth in all the species under the applied metal stress</td>
<td>Pots</td>
<td>Gupta et al. (2002)</td>
</tr>
<tr>
<td><em>Psychrobacter</em> sp. SRS8</td>
<td>Ni</td>
<td><em>Helianthus annuus, Ricinus communis</em></td>
<td>Enhanced plant growth and Ni accretion in both plant species with improved plant biomass, content of proteins and chlorophyll</td>
<td>Pots</td>
<td>Ma et al. (2011)</td>
</tr>
<tr>
<td><em>Rhizobium</em> sp. RP5</td>
<td>Ni</td>
<td><em>Pisum sativum</em></td>
<td>Improved the dry biomass, nodule numbers, seed yield and grain protein by 19%, 23%, 26% and 8% correspondingly at 290 mg Ni/kg.</td>
<td>Pots</td>
<td>Wani et al. (2007)</td>
</tr>
<tr>
<td><em>Sinorhizobium</em> sp. Pb002</td>
<td>Pb</td>
<td><em>Brassica juncea</em></td>
<td>Effectiveness of Pb phytoextraction improved</td>
<td>Microcosms</td>
<td>Di Gregorio et al. (2006)</td>
</tr>
<tr>
<td><em>Variovox paradoxous, Rhodococcus</em> sp.</td>
<td>Cd</td>
<td><em>Brassica juncea</em></td>
<td>Elongation of root stimulated</td>
<td><em>In vitro</em></td>
<td>Belimov et al. (2005)</td>
</tr>
<tr>
<td><em>Xanthomonas</em> sp. RJ3, Azomonas sp. RJ4, Pseudomonas sp. RJ10, Bacillus sp. RJ31</td>
<td>Cd</td>
<td><em>Brassica napus</em></td>
<td>Cd accretion elevated and stimulation of growth of plant</td>
<td>Pots</td>
<td>Sheng and Xia (2006)</td>
</tr>
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</table>
PGPR counteracting mechanisms towards heavy metal:

PGPR play a vital role in adaptation of the host plant to a varying environment due to heavy metal contamination. The mechanism of plant development stimulation comprises synthesis of phytohormones, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, siderophores, biocontrol agents and uptake of nutrients (Figure 2). Phytohormones such as indole acetic acid (IAA) released by PGPR, induce plant growth and are also accountable for metal uptake as well as activating plant defense response against heavy metal stress (Zaidi et al., 2006, Spaepen and Vanderleyden, 2011). PGPR produce ACC deaminase, which function in lowering the production of stress hormone ethylene. Levels of ethylene released by plants diminish under heavy metal toxicity only when associated with PGPR and thereby plant development remains unaffected (Ahemad and Kibret, 2013). Microbial siderophores aid to lessen the stresses forced on plants due to elevated soil content of heavy metals. Rhizospheric bacteria are recognized to influence the metal mobility and accessibility to plants via production of siderophores, iron chelators and initiation of redox changes along with acidification mechanisms for guaranteeing the availability of iron and assembling of metal phosphates (Burd et al., 2000). Siderophores help to lessen the stresses imposed on plants by making stable complexes with toxic metals of environmental concern such as Cd, Cu, Cr, Pb and Zn (Rajkumar et al., 2010). The PGPR can also facilitate in reducing the metal phytotoxicity by the method of biosorption and larger quantities of heavy metals can also be adsorbed by microbial cells either by metabolism dependent or metabolism independent methods (Khan et al., 2009; Zaidi et al., 2006). The plant growth promoting features in connection to the bacterial bioaccumulation/biosorption mechanisms involving ACC deaminase and phytohormones production accounts for enhanced plant development in polluted environments of various heavy metals (Zaidi et al., 2006).

Figure 2. Plant growth enhancement with influence of PGPR under heavy metal stress

Genetic engineering strategies for PGPR:

Plant growth promoting rhizobacteria implicated in metal bioremediation of a particular environment or associated with a specific host plant cannot be essentially utilized in another polluted environment or with another plant species for the same purpose but it is probable that PGPR is modified according to the polluted locale or it can be practicable that the less effective existing microbes interacting with plants in an environment of metal pollution can be modified via genetic engineering advancements. Certain criteria has been suggested in order to choose an appropriate PGPR strain for gene cloning and inoculating into the plant rhizosphere, these are: first, the strain must be stable subsequent to cloning and the expression of target gene should be high, second, the PGPR strain ought to be resistant or non sensitive to the heavy metal pollutant and third, as PGPR strains contribute only in specific plant rhizosphere therefore such environment should be provided (Huang et al., 2004). Although the genetic manipulation for PGPR improvement needs continuous research and developing molecular
techniques for finding out effective remediation role of PGPR in natural environment also but nevertheless it has beneficial consequences for protecting and promoting development of plants under stresses.

The efficient expression of target genes or the production of target enzymes or proteins by PGPR involved in metal bioremediation can be enhanced and among these proteins, phytochelatins (PC) and metalloproteins (MT) are of major focus. PC and MT bind a broad range of heavy metals with large affinity (Ma et al. 2009b). In bacterial and plant (root) cells, MT at the outer membrane combine with metal ions and ensure their carrying in the cytosol where particular protein chelators i.e. metallochaperons relocate these combined metals to the related receptor proteins. Both the approaches can be effective against heavy metal tolerance i.e. genetically modifying PGPR for enhanced metal bioremediation or by developing transgenic plants with genes acquired from heavy metal resistant bacteria. Sriprang et al., (2003) had shown that by inserting new PC synthesizing genes into Mesorhizobium huakuii subsp. regei strain B3, the bacterium was able to produce more phytochelatins, which were functional for accumulation of Cd ions after establishing symbiosis with the plant Astragalus sinicus.

**Future prospects:**

Controversies may arise due to release of genetically modified PGPR into the nature and their field investigation may be stopped until safety issues and the potential for environmental reparation are cleared (Wacket, 2004). But PGPR application may be favorable alternate way for enhancing plant growth (mostly of crop plants) due to existing unwillingness all over the world to accept food produced by genetically modified crops. The broad scale applications of PGPR are believed to decrease heavy metal contamination in rhizosphere and PGPR application technology is easily accessible to growers in both developing and developed countries (Gamalero et al., 2009). In future, PGPR are expected to substitute artificial growth regulators, chemical fertilizers and insecticides/pesticides which have many adverse effects on large scale agriculture. Finding new perceptions and research about the mechanisms of PGPR-Plant association would cover the approaches to discover more competent strains of rhizobacteria which may facilitate heavy metal bioremediation under various agro-environmental circumstances.

**Conclusion:**

Heavy metal polluted soils can be remediated by use of environmental friendly and less costly methods of PGPR application. The use of PGPR for plants is presently receiving substantial worldwide attention and the latest successive PGPR researches exhibit luminous prospects for bioremediation of polluted soil environments. Studies on PGPR entail that it is now possible to develop innovative strategies by inoculating plants with or without genetically modified rhizospheric microbes for the purpose of removal of metals from polluted soils and also enhancing plant growth effectively. In future, PGPR application would be the most favored technology keeping in view the advantages of PGPR usage over physical and chemical remediation approaches.

**References:**


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