Role of Heavy-Metal Resistant Bacteria Isolated from Rhizosphere in Bioremediation and Plant Development

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Abstract Heavy metal toxicity of soil and groundwater is a global menace. Bacteria isolated from the rhizosphere often exhibit the dual activity of plant growth promotion and bioremediation (and/or assisted phytoremediation) of heavy metals like arsenic, mercury, copper, cobalt, etc., contaminating the soil. Plant Growth Promoting Rhizobacteria (PGPR) evolve survival mechanisms by expression of different sets of genes or operon under heavy metal and xenobiotic stress. Different molecular pathways are activated within the PGPR for biodegradation, biotransformation, bioaccumulation, bioadsorption and/or biovolatilization of the pollutants. PGPR possess mechanisms to solubilze phosphate and potassium and fix nitrogen, hence, can be used as biofertilizer. They also produce phytohormones, volatile organic compounds and hydrolytic enzymes responsible for promotion of plant growth. Therefore, PGPR have commercial applications in enhancement of agricultural production and reclamation of heavy metal contaminated soils.

1 Introduction

Soil is a rich source of nutrients. It is a hub of various microflora and microfauna, site of diverse metabolic activities and centre of multiple interactions between different forms of life. Metal ions are absolutely essential for various biochemical reactions. Metalloenzymes use metal ions as cofactors. Metabolic pathways like electron transport chain, photosynthesis, transport and storage of different metabolites etc., require metal ions. Metals also play vital role, directly or indirectly, in initiation, activation, regulation and inhibition of various microbiological pathways and interactions between soil, plants and microbes. Heavy metals and metalloids are natural

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Table 1 Symptoms of heavy metal toxicity in plants

Plants	Heavy metal/metals	Symptoms
Barley	Cadmium, mercury	Symptoms similar to water deficiency
Wheat	Arsenic	Reduced seed germination and seedling growth
Helainthus annuus L	Arsenic	Reduced plumule and radical length
Elodea densa	Manganese, copper, cadmium, zinc, nickel	Reduced chlorophyll content, reduced photochemical efficiency of PS(II)
Thalassia hemprichii	Copper, zinc, lead, cadmium	Reduced chlorophyll and carotenoid content, reduced quantum yield
Phaseolus vulgaris	Zinc	Inhibited RUBISCO activity
Erythrina variegate	Cadmium	Reduced RUBISCO activity, reduced CO_2 fixation
Brassica juncea	Arsenic	Altered auxin level

constituents of various compounds that occur in Earth crust in far less amount than their toxic concentration to various life forms. Anthropogenic activities like mining, smelting, application of heavy metals containing compounds like pesticides, herbicides, fungicides, and use of heavy metals in glass, paper, wood and electronics industry, have led to increase in concentration of these heavy metals in soil (Hao et al. 2020; Järup 2003). Heavy metal toxicity in soil has affected the survival and growth of various life forms on Earth including human. Heavy metals like arsenic, mercury, chromium, nickel, cadmium are well known for their carcinogenic and mutagenic effects. These heavy metals have been classified as group 1 carcinogen by International Agency for Research on Cancer. Heavy metal toxicity leads to decline in the species richness and diversity in soil. Plants show symptoms of heavy metal toxicity like reduced growth, chlorosis of leaves, nutrient and water imbalance, root injury, alteration in seed germination, low biomass accumulation, senescence and ultimately death (Singh et al. 2016). Table 1 depicts the symptoms of heavy metal toxicity in plants. The symptoms of heavy metal toxicity in microbes are reduced enzyme activity and cell division, disruption of cell membrane, denaturation of proteins and nucleic acids, etc. (Igiri et al. 2018). We will now discuss the response of different life forms against metal induced stress.

2 Response of Different Organisms to Metal Intoxication and Metal Starvation

Metals cannot be synthesized or degraded. Its concentration can be regulated to an optimum level according to the cellular demands. Metals are indispensable for various

physiological processes involving metalloenzymes. In absence of specific metal, the activity of metalloenzymes is reduced, inhibited or become non-specific. Different metals are used as cofactors for different metalloenzymes. The need of metal in a cell should be carefully regulated by regulating two important parameters-the total concentration of metal in the cell and the labile pool of metal available and accessible for incorporation into various cellular enzymes, proteins and nucleic acids (Hao et al. 2020). Excessive metal outside the cell can alter the morphology, composition, size and growth patterns of microbial community. Metal intoxication within the cell can lead to mis-metalation of various metalloenzymes with non-specific metals leading to physiological imbalance and ultimately stunted growth and reproduction. In case of both metal starvation and intoxication, the first step is to sense the presence of metal inside the cell. Metalloregulatory proteins are specialized proteins regulated by metals. Binding of metals to these regulatory proteins causes allosteric transition in their conformation and alters their DNA binding affinity, modulating transcription of the genes responsible for metal homeostasis. This set of genes includes metallochaperones, metal importers, metal efflux proteins, transporters, etc. (Hao et al. 2020). Regulatory proteins sense and regulate sufficiency, limit and excess of metal, and modulate expression of sets of genes involved in metal homeostasis. Instead of directly sensing the metal, metalloregulators can indirectly sense the direct product of metal homeostasis. These metal regulators are very specific for the metals they bind to. They show high affinity for the specific metal responsible for the allosteric transition in them. The specific sequences of RNA can also act as switch for metal sensing. These riboswitches can sense metal either by directly binding to the metal or indirectly binding to the corresponding metabolic product. They adapt to a specific conformation on metal binding and sequester or present terminator or anti-terminator elements leading to regulation of definite sets of genes (Hao et al. 2020). Metal starvation is generally responded by upregulating the various specific and non specific metal import pathways and alternate pathways, which are either metal independent or do not require the limiting metal. In addition to this, the limiting metal is released from the store during metal starvation. Along with these, the efflux processes that lead to export of metal out of the cell and limiting metal dependent pathways are downregulated. On the contrary, metal intoxication is responded by downregulation of the import system and upregulation of the efflux system, metal sequestration by release of various extracellular polymers and siderophores, metal binding by abundant metabolites within the cell, storage of metal in different compartments of the cell, enzymatic detoxification (oxidation, reduction, methylation and demethylation) etc. (Hao et al. 2020). Excessive heavy metal in the local environment sometimes induces tolerance and resistance in the bacteria. Bacteria employ various strategies to respond to heavy metal toxicity. Bacteria encode for myriad of proteins, chaperones, enzymes and transporters responsible for heavy metal resistance in them. In the next section mechanism of heavy metal resistance in bacteria has been discussed.

3 Molecular Mechanism of Heavy Metal Resistance in Bacteria

Urbanization and modern agricultural activities have increased the use of heavy metal containing compounds in our daily life. Heavy metals are used in medical treatment due to their antimicrobial property, in agricultural field as manure, pesticides, fungicides, weedicides, and in animal husbandry as feed supplement to promote growth and prevent diseases in animal. This has led to the entry of heavy metals from various sources into the food chain and increase toxicity due to its biomagnification in the food chain. The surplus use of heavy metals has destroyed the environmental and human health (Hao et al. 2020; Nascimento and Chartone-Souza 2003). Heavy metals target cellular processes and induce oxidative stress, protein dysfunctioning, DNA damage and also alter membrane integrity. Microbes are also sensitive to environmental changes. Presence of heavy metals in excess induces a selection pressure on the microbe of the local environment. This changes the composition of the microbial community and allows the evolution of heavy-metal resistant microbes. Bacteria employ various survival strategies to cope with the heavy metal toxicity. One bacterium might become resistant to multiple metals if exposed for a prolonged period of time (Hao et al. 2020; Nascimento and Chartone-Souza 2003). We will now discuss some of the heavy metal resistance mechanisms in bacteria.

3.1 Mechanism of Mercury Resistance in Bacteria

Mercury is used as antibacterial and antifungal agents in agricultural fields. It is also used as catalyst in various industrial processes like amalgam formation during gold extraction. Other anthropogenic activities like burning of coal and petroleum products also add mercury in the environment. Inorganic mercury exists in two forms (Hg^0 and Hg^{2+}). The major form of mercury is Hg^0 that occur naturally in Earth's atmosphere. Mercury vapour (Hg^0) undergoes oxidation in presence of ozone and water to form the mercuric ion Hg^{2+}. Mercuric ion enters the water system and undergoes bacterial conversion into methylmercury. Methylmercury is the most common form of organic mercury. Release of industrial effluents into water body also adds mercury into them, which is again converted to methylmercury and is taken up by fish and other aquatic organisms. Consumption of methylmercury contaminated sea foods cause methylmercury poisoning in human (Foster 1987; Misra 1992; Nascimento and Chartone-Souza 2003). Mercury compounds have strong affinity for sulphur containing biomolecules like enzymes and proteins, which makes it very toxic to the biosystem. *Mer* genes confer mercury resistance in bacteria. These genes are mostly plasmid encoded but can also be present on transposons and bacterial chromosome. *Mer* genes are induced and regulated at transcriptional level and are involved in reduction and detoxification of inorganic and organic mercury. Organic

mercury like methylmercury is detoxified by organomercurial lyase. Mercury resistant bacteria have *mer* operon. *Mer* operon is a cluster of genes responsible for mercury resistance. In most of the bacteria the order of genes in *mer* operon is *merR, merT*, *merP*, *merA*, and *merB*. Some microbes might have *merC* and *merD* genes along with *merT* and *merP* genes. *merR* encodes a metal regulatory protein that senses the presence of mercury and activate the *mer* operon transcription, in presence of inducing concentration of Hg^{2+} ions by binding to the promoter operator region of *mer* operon. In absence of inducing concentration of Hg^{2+} ion, MerR represses the transcription of *mer* genes. MerP and MerT are periplasmic and cytoplasmic proteins, respectively, which transport mercury bound to their cysteine residues. *merA* gene encode for a flavoprotein that is involved in NADPH dependent reduction of Hg^{2+} to Hg^0. This mercury reductase enzyme is activated by substrate inhibitory concentration of mercuric ion and organomercurials. Such type of mercury detoxification is carried out by *Pseudomonas*, *E. coli, Staphylococcus aureus*, etc. (Foster 1987; Misra 1992; Nascimento and Chartone-Souza 2003). Hg^{2+} can easily diffuse across the outer membrane of the bacteria. In the cell, Hg^{2+} binds to the cysteine residues of MerP which then transport it to MerT on the cytoplasmic membrane. MerT transfers the mercuric ion to mercury reductase (MerA). Mercury reductase convert Hg^{2+} to Hg^0. *Mer* operon is present in many gram positive and gram negative bacteria like in transposon Tn21, Tn501, Tn5053 of plasmid NR1, PVS1 and pMR from *Shigella flexneri, Pseudomonas aeruginosa* and *Xanthomonas* sp., respectively. Plasmids pDU1358, pPB and pI258 of *Serratia marcescens*, *Pseudomonas stutzeri* and *Staphylococcus aureus*, respectively, also have *mer* operon. Mercury resistant bacteria are classified into two classes- narrow spectrum and broad spectrum mercury resistant bacteria. Narrow spectrum mercury resistant bacteria can only detoxify inorganic mercury compounds by the mercury reductase enzyme. Broad spectrum mercury resistant bacteria are resistant to both organic and inorganic mercury compounds. In addition to the mercury reductase, these bacteria have enzyme organomercurial lyase encoded by *merB* gene (Foster 1987; Misra 1992; Nascimento and Chartone-Souza 2003). Organomercurial lyase cleaves the C-Hg bond (organometallic linkage) in organomercurials to yield Hg^{2+}. Mercury reductase then converts Hg^{2+} into volatile metallic mercury Hg^0 in a NADPH dependent reduction process. Some bacteria might have a second regulator that downregulate the *mer* operon by binding to the promoter-operator region of the operon very weakly. This regulator is encoded by *merD* gene. Therefore, *mer* operon encodes all the genes required for mercury detoxification. Bacteria also employs other strategies for mercury resistance which include decrease in cell permeability and reduced uptake of Hg^{2+} ion, sequestration of mercury in different compartments of cell, decomposition and inactivation of mercury with H_2S, etc. (Fig. 1, Table 2) (Foster 1987; Misra 1992; Nascimento and Chartone-Souza 2003).

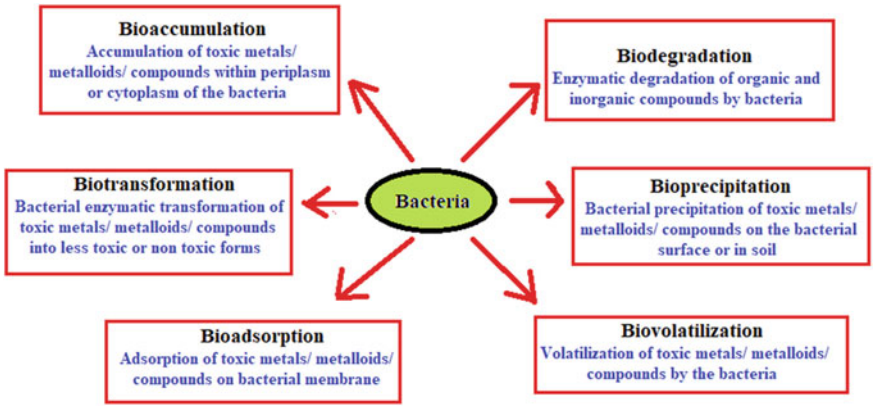

Fig. 1 Different mechanisms of bioremediation employed by heavy metal resistant bacteria

Table 2 Mechanism of heavy metal resistance employed by different bacteria

Bacteria	Mechanism of heavy metal resistance
Pseudomonas putida	Intracellular sequesteration of copper, zinc and cadmium
Rhizobium leguminosarum	Intracellular sequesteration of cadmium
Pseudomonas syringae	Sequesteration of copper in the periplasm
Geobacter spp	Reduction and precipitation of iron
Desulfuromonas spp	Reduction of toxic sulphur compounds
Geobacter sulfurreducens	Conversion of Cr^{6+} to Cr^{3+}
Geobacter metallireducens	Conversion of Cr^{6+} to Cr^{3+}
Klebsiella planticola	Precipitation of cadmium as insoluble sulfides
Vibrio harveyi	Precipitation of soluble lead as complex lead phosphate salt
Bacillus spp	Biomethylation of Hg^{2+} to gaseous methylmercury
Clostridium spp	Biomethylation of Hg^{2+} to gaseous methylmercury
Geobacter metallireducens	Reduction of Mn^{4+} to Mn^{2+} and U^{6+} to U^{4+}

3.2 Mechanism of Arsenic Resistance in Bacteria

Arsenite and arsenate, the two inorganic forms of arsenic are toxic to all life forms. Arsenite binds to thiol containing metabolites like reduced glutathione, lipoic acid, etc., and inhibit metabolically significant enzymes. Arsenate being the structural analogue of phosphate interferes with phosphate containing biochemical reactions. Arsenic toxicity shows symptoms of arsenicosis, keratosis, melanosis and cancer of liver, lungs, kidney, etc., in the long run. Arsenic is widely used in manufacturing of pesticides, fungicides, weedicide, herbicide, paints, paper and glass. Plants uptake arsenic from agricultural field and accumulate it in roots (mostly), leaves, stem and grains. Some hyperaccumulating plants accumulate arsenic at high concentration in the vacuoles. They are tolerant to high concentration of arsenic in soil. Various microorganisms also show tolerance and resistance to this heavy metal (Mandal et al. 2017). Arsenite oxyanion resemble the structure of glycerol and thus, is taken up by Glpf aquaglyceroporin, whose natural substrate is glycerol. The arsenic resistance genes in microbes are plasmid or chromosomally encoded. For example, pI258 plasmid of *Staphylococcus aureus* encodes genes for arsenite, arsenate and antimony resistance. Separate sets of genes are involved in resistance of these three chemical species (Silver et al. 1981). R733 plasmid of *E. coli* includes *ars* genes for arsenate reductase, arsenate efflux proteins and regulators (Rosen et al. 1988). Arsenic resistance genes are also present in transposon Tn2502 of pYV plasmid of *Yersinia enterocolitica* (Ye et al. 2007). Chromosomal homolog of arsenic resistance genes work in association with plasmid encoded genes for arsenic resistance. Chromosomal *ars* operon of *Pseudomonas aeruginosa* Dk2 encodes for organoarsenical efflux permease (encoded by *arsJ* gene) that transport *1-arseno-3-phosphoglycerate* (a highly unstable organoarsenical) out of the cell (Chen et al. 2016). Members of the group Eubacteria and Archeae have genes for arsenite oxidation. In *Alcaligenes faecalis* NCIB8687 the region encoding arsenite oxidase enzyme is of 71 kb. The two genes *asoA* and *asoB* along with twenty other genes are involved in arsenite resistance in this bacterium. *asoA* and *asoB* encode for the large molybdopterin containing and the small Rieske subunit of arsenite oxidase. The enzyme arsenite oxidase oxidizes arsenite into arsenate, the less toxic form of arsenic. The other putative genes encode for arsenite ATPase membrane transporter and efflux system. The arsenite oxidase operon *aoxABCD* has been identified in *Centibacterium arsenoxidans,* which also encode for arsenite detoxification and efflux system (Silver and Phung 2005). Arsenate operon in general might contain three, four or five genes, classifying the operon into three types (*arsRBC, arsRABC and arsRDABC*). *ArsR* gene encodes for a repressor protein that represses the transcription of *ars* operon. Binding of arsenic to this trans acting metalloregulatory protein, dissociate it from DNA and initiate the transcription of *ars* genes. ArsD is a metallic chaperone and inducer independent repressor that binds weakly to the promoter operator sequence. The primary role of ArsD is to bind and transfer arsenite to ArsA ATPase. ArsA is stimulated by both arsenite and antimony and interacts with membrane embedded efflux pump ArsB. These two hydrophobic proteins together transport arsenic outside the cell. ArsA also

associates with other membrane proteins. ArsB with twelve transmembrane domains can either use ATPase activity of ArsA or cell membrane potential to extrude arsenic species out of the cell. *ArsC* encode for an arsenate reductase that convert arsenate into arsenite prior to its extrusion. ArsC uses glutathione, redoxin or thioredoxin as electron source for the reduction process (Fekih et al. 2018). *Saccharomyces cerevisiae* have two independent transport systems for arsenic. Acr3p is a plasma membrane transporter involved in arsenite extrusion conferring arsenic resistance to *S. cerevisiae.* Ycf1p is another transporter protein of ABC transporter superfamily involved in storage of arsenite into vacuole in an ATP dependent manner. Chromosome XVI of *Saccharomyces cerevisiae* encodes for Acr1, Acr2 and Acr3, all three of which responds to arsenic stress. Acr1 might be sensitive to both arsenite and arsenate. Acr2 is an arsenate reductase and Acr3 is a plasma membrane embedded arsenic efflux transporter (Ghosh et al. 1999). Bacteria also have genes for detoxification of organic arsenicals. For example *Campylobacter jejuni,* a food borne pathogen is resistant to organic arsenicals like roxarsone (*4-hydroxy-3-nitrobenzene arsonic acid*) which is used as feed additive in poultry farming. *Campylobacter jejuni* consists of *ars* operon with four genes-*arsP, arsR, arsC* and *acr3*. The regulator ArsR, arsenate reductase ArsC, efflux transporter Acr3 and organoarsenical transporter ArsP together confers resistance to arsenic in the pathogen. ArsP have eight transmembrane helix and transport trivalent organoarsenicals mainly (Shen et al. 2014; Chen et al. 2015a). Some bacteria might also contain proteins like ArsH, ArsM, ArsK, ArsI, etc. For example ArsH of *Pseudomonas putida* is an organoarsenical oxidase that converts trivalent methylated and aromatic arsenicals into pentavalent species (Chen et al. 2015b). *ArsM* gene is established to encode for As (III) S-adenosylmethionine methyltransferase which methylate arsenite into volatile trimethyl arsine (Qin et al. 2006). *ArsI* encode Fe^{2+} dependent dioxygenase involved in demethylation of methylarsonic acid (Yoshinaga and Rosen 2014). ArsK is an arsenic efflux transporter that confers resistance to all type of arsenic compounds except pentavalent arsenate. It reduces accumulation of roxarsone, methylarsenite, arsenite, etc. It is induced by arsenite, antimonite, roxarsone and methylarsenite (Shi et al. 2018). Therefore, microbes have developed strategies to resist, tolerate, detoxify and export inorganic and organic arsenic compounds (Fig. 1, Table 2).

3.3 Mechanism of Copper Resistance in Bacteria

Copper is used as bactericide in agriculture field. It is an essential micronutrient for plant growth and development. Excess copper in soil induces stress in plants and microbes. Microbes encode genes that provide resistance to copper. Copper resistance genes are mostly encoded by plasmid. For example pPT23D plasmid of *Pseudomonas syringae pv.* tomato contain copper operon with four genes which are induced by copper only. The operon *copABCD* is under the regulation of copper inducible promoter followed by a constitutive promoter with two regulatory genes *copR* and *copS*. Both copper inducible promoter and the two regulatory genes are

essential for proper expression of copper resistance genes. CopA and CopC are two periplasmic copper binding proteins that limit the copper concentration in periplasm. *CopB* encode for an outer membrane protein that sequester copper outside the outer membrane. CopD is an inner membrane protein involved in copper transport. These proteins function together to reduce the copper concentration within the cytoplasm (Mellano and Cooksey 1988; Lim and Cooksey 1993). Plasmid pRJ1004 of *E. coli* has *pcoABCDRSE* operon that encode for proteins dealing with periplasmic copper toxicity (Rouch et al. 1985). *E. coli* also have two chromosomally encoded copper systems—*cue* system and *cus* system. *Cue* is the main system involved in copper transport. It has three important genes-*cueR, cueO* and *copA*. CueR is a copper responsive regulator and CueO is periplasmic multi-copper oxidase involved in oxidation of Cu^{+} to Cu^{2+}. CopA is an ATPase that transports copper out of the cytoplasm (Bondarczuk and Piotrowska-Seget 2013). *E. coli*, *Enterococcus hirae* and *Mycobacterium tuberculosis* also have two other regulatory proteins CopY and CsoR that negatively regulate the transcription of copper resistance genes under copper limiting condition. After entering into the cell Cu^{2+} can be reduced to Cu^{+}, which is more toxic in nature. Cu^{+} is effluxed out of the cell using the *cus* like *RND* system or it undergoes further oxidation to Cu^{2+} by CopA or PcoA or CueO like periplasmic copper oxidase and ATPase. CopA is a periplasmic copper binding ATPase with eight transmembrane segments. It is mainly involved in transport of copper from cytoplasm by oxidizing Cu^{+} to Cu^{2+} (Rademacher and Masepohl 2012; Bonderczuk and Piotrowska-Seget 2013; Martínez-Bussenius et al. 2017). Chromosomally encoded copper resistance genes are also present in *Acidithiobacillus feroxidans* ATCC23270 and ATCC53993. *Acidithiobacillus feroxidans* ATCC23270 have more than ten genes in its genome that are involved in copper homeostasis. Three genes *copA1, copA2, copB* encode ATPase, which transport copper. Three genes *cusA*, *cusB* and *cusC* encode for inner membrane antiporters that use proton motive force to efflux copper out of the cytoplasm. Two genes *cusF* and *copC* encode for periplasmic metallochaperones. Rus and AcoP are periplasmic copper binding protein (Martínez-Bussenius et al. 2017). Therefore, copper resistance mechanism in bacteria involves cytosolic and periplasmic metallochaperones, outer and inner membrane copper binding proteins, antiporters, transporters, oxidase and regulators (Fig. 1, Table 2).

3.4 Mechanism of Cobalt Resistance in Bacteria

Cobalt is a naturally occurring element in the Earth's crust. Airplane exhaust, burning of coal, volcanic eruption, etc., adds more cobalt to the environment. Cobalt is an important cofactor of various enzymes present in microbes, plants, animals and human. It is a component of vitamin B_{12}. Cobalt toxicity affects iron-sulphur proteins like succinate dehydrogenase, sulphide reductase, nitrate reductase, aconitase B, etc. It misbalances the iron homeostasis and induces sulphur assimilation. It generates oxidative stress in various life forms. Cobalt competes for iron at different sites and

replaces iron from the active sites of various enzymes. Cobalt also target proteins with cysteine or thiol groups, due to its affinity for sulphur atoms. Altogether, cobalt interferes with homeostasis of other metals (Nies 1992; Barras and Fontecave 2011). Cobalt has similar coordination property like iron and nickel. Therefore, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and Mg^{2+} are usually transported into cell by divalent cation uptake system with broad specificity. Co^{2+} is imported by importers like FeoB (import Fe^{2+} mainly), CorA (Mg^{2+} importer), ZnpT (Zn^{2+} transporter) and by NikABCDE nickel uptake system. As cobalt is imported non-specifically by various divalent cation importers, mere downregulation of these importers won't be a solution to cobalt toxicity. Therefore, bacteria evolved plasmid encoded metal resistance genes to deal with various metal intoxications. These genes encode for efflux system inducible under stress condition to reduce the accumulation of metal inside the cytosol (Nies 1992; Barras and Fontecave 2011). For example, *Alcaligenes eutrophus* encodes for *czc* system with *czcA, czcB, czcC, czcD* and *czcR* genes. Zn^{2+} is the main cation exported by this system. CzcA is a cation proton antiporter involved in the efflux of Co^{2+}, Cd^{2+} and Zn^{2+}. CzcB is the ancillary protein with cation binding subunit. CzcC is the modifier, which changes the substrate specificity of the system when required. CzcD and CzcR are the regulators of the *czc* system (Nies 1992). *E. coli* chromosome have *rcnRAB* gene cluster, which control cobalt and nickel efflux. RcnR is the regulator controlling the homeostasis of copper by regulating CsoR (copper repressor) and sulphur by regulating CstR (presulphide sensing transcriptional repressor). RcnA is a cobalt efflux pump which work in conjugation with RcnB (periplasmic protein). Binding of cobalt to transacting metalloregulatory protein RcnR leads to its dissociation from DNA and initiate the transcription of *rcnA* and *rcnB* genes. RcnRAB system is also present in *Salmonella enterica* and many other bacteria. Cobalt stress also induces the expression of *nfuA gene*, and *iscRSUA* and *suFABCDSE* operons, all of which are involved in iron-sulphur biogenesis under normal and stress condition (Barras and Fontecave 2011). Therefore, efflux pumps, antiporters, metalloregulatory proteins, periplasmic proteins, etc., are activated in response to cobalt toxicity (Fig. 1, Table 2).

4 Application of Heavy Metal Hypertolerant Bacteria in Bioremediation of Heavy Metal Toxicity

Heavy metal pollution is a serious and complex environmental issue. Various physical and chemical methods are applied to remove heavy metals from the contaminated site. Common methods like ion-exchange, precipitation, membrane filtration, chemical extraction, adsorption, etc., are employed to remove heavy metals from groundwater, drinking water and wastewater. But these methods have some disadvantages like high cost of implementation, constant maintenance, high learning curve, etc. Also, they are unsuitable for removal of very low concentration of heavy metals, often add other toxic chemicals to the environment and are affected by various physico-chemical

factors like pH, temperature, organic matter content, nature of sample, etc. Bioremediation is the biological removal, reduction, detoxification and degradation of toxic compounds into less toxic or non toxic forms. Many microbes have adapted to heavy metal stress by evolving heavy-metal resistant genes which encode detoxification systems and resistant mechanisms in them, as discussed in the previous section (Fig. 1). These heavy metal resistance mechanisms can be exploited for bioremediation, which is an effective, cheap, eco-friendly and low input technology. Microbes can be used for bioadsorption, biotransformation, bioprecipitation, bioaccumulation and biovolatilization of toxic heavy metals and xenobiotics. These methods involve surface adsorption of heavy metals on the active groups of bacterial membrane, enzymatic transformation of toxic heavy metallic compounds into less toxic forms, precipitation of heavy metals on bacterial surface, accumulation of toxic metal within the bacterial cell and volatilization of toxic metals from the local environment, respectively (Tarekegn et al. 2020). Many heavy-metal resistant bacteria have been used for removal of heavy metals from the contaminated sites. For example *Acinetobacter* sp. and *Arthrobacter* sp. reduce concentration of chromium by 78% if applied in consortium (De et al. 2008). *Micrococcus luteus* could reduce lead concentration significantly (Puyen et al. 2012). *Bacillus megaterium* and *Bacillus subtilis* also showed reduction in lead concentration from 2.13 to 0.03 mg/L and 0.04 mg/L, respectively, in tannary effluents after 20 days. In the same study, *Bacillus subtilis* also reduced the concentration of cadmium from 0.4–0.03 mg/L (Table 2 and 3) (Abioye et al. 2018). Therefore, heavy-metal resistant microbes could be exploited for bioremediation of heavy metal toxicity. This biological technique can be easily applied on a large

Table 3 Bioremediation efficiency exhibited by different heavy metal hypertolerant bacteria

Bacteria	Reduction in heavy metal concentration	Bioremediation efficiency
Pseudomonas aeruginosa	Cadmium-100–17.4 mg/L	75% after 72 h
Brevibacterium iodinium	Lead-100–2 mg/L	Greater than 87% after 96 h
Alcaligens faecalis	Cadmium-100–19.2 mg/L	70% after 72 h
Immobilized *Bacillus subtilis*	Chromium-570–2 mg/L	99.6%
Pseudomonas aeruginosa	Lead-100–1.8 mg/L	98% after 96 h
Pseudomonas aeruginosa and Bacillus subtilis (in consortium)	Chromium-570–2 mg/L	99.6%
Bacillus megaterium	Lead-2.13–0.03 mg/L	98.6% after 20 days
Bacillus subtilis	Lead-2.13–0.04 mg/L	98% after 20 days
Bacillus subtilis	Cadmium-0.4–0.03 mg/L	92.5% after 20 days
Bacillus megaterium	Cadmium-0.4– 0.06 mg/L	85% after 20 days
Immobilized *Pseudomonas aeruginosa*	Chromium-570.4–4 mg/L	99.3%
Alcaligens faecalis	Copper-100–19.2 mg/L	70%
Pseudomonas aeruginosa	Copper-100–17.4 mg/L	75%

scale in a cost effective manner, even for very low metal concentration with minimal monitoring and exhibit some advantages over physical and chemical techniques.

5 Phytoremediation of Heavy Metal Pollution

Plants require nonmetals and compounds like ammonia nitrogen, phosphate, borate, sulfate, etc., for their growth and development. Metals like copper, zinc, magnesium, iron, calcium, potassium, manganese, molybdenum, etc., also play a vital role in various physiological processes of plants. These nutrients must be in aqueous phase to be absorbed by plant roots. The uptake and transport mainly involved the apoplasm (root cells) of the plant. However, the symplastic pathway (from cell to cell, crossing the root cell membrane) is also involved in transport of root minerals to the upstream of the plant. Nonessential minerals and contaminants like arsenic, selenium, chromium, mercury and other micronutrients like copper, zinc, cadmium, lead, cobalt, etc., also easily enter the plant roots, when present in easily soluble form. The minerals and contaminants can become an environmental concern when present in excess. These contaminants are also taken up through passive channels or transport proteins in addition to the water uptake system (Tsao 2003). Heavy metals are cytotoxic, mutagenic and carcinogenic in nature (Rajkumar et al. 2009). Heavy metals hamper the normal physiological processes of plants. Plants employ various mechanisms to deal with these toxic metals. Plants release various biochemical root exudates that facilitate the precipitation, sequesteration or complexation of metals at the rhizosphere. Plant roots can irreversibly bind the contaminant and prevent its entry. They can also uptake the contaminant through roots and sequester it into vacuole, the storehouse of the cell. This will prevent the transport of the contaminant into other plant parts. Plants also might release some enzymes in order to reduce the toxicity of inorganic substance or change its speciation making it available for incorporation into organometallic compounds (Tsao 2003). In spite of these strategies, plants may fail to reduce the toxicity of heavy metals, when present in excess amount. Some plants can accumulate heavy metal contaminants at a concentration much greater than the toxic concentration of the metal. These plants, also known as hyperacuumulators can store a specific metal up to 1% of their dry weight (that is 10,000 mg per kg) depending on the type of inorganic element. Members of genus *Brassica*, *Pinus*, *Salicornia*, *Thlaspi*, *Atriplex*, *Helianthus*, *Kochia*, and *Pelargonium* are hyperaccumulators of various metals. Hyperaccumulators often produce root exudates that react and transform toxic compounds into less toxic or nontoxic forms (phytotransformation). These are then taken up by plant roots and stored in vacuoles (phytosequesteration). The root exudates can also sequester, immobilize and precipitate contaminants in soil or on the root surface. Toxic metals can also be sequestered within root tissues (Tsao 2003). Sulfate, hydroxides, oxides, carbonates and carboxylates are often released from roots to precipitate or form metal complexes in soil. Root exudates can also change the soil pH, which leads to precipitation of various ionic species. Root exudates can convert the oxidation state of the metals and can

alter their solubility, bioavailability and bioadsorption by roots. Secondary metabolites like terpenoids, flavonoids, alkaloids, etc., are also released by roots for defense against pathogens. Plants also secrete chemicals against other plants for their defense. These biochemicals alter the microenvironment of the soil. Mucigel secreted by plant protect the plant root, increases the root penetration in soil and promote plant growth (Tsao 2003). Plants also produce proteins and enzymes that can degrade complex compounds into simpler ones. For instance, nitroreductase enzyme when released by plant, can break nitroaromatics like *trinitrotoluene*, *hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine*, etc. Similarly, dehalogenases, phenoloxidases, nitrilases and phosphatases released as root exudates, can degrade halogen components of contaminants, phenolic compounds, pesticides, herbicides and insecticides, respectively. Degradation of organic and inorganic contaminants by plant root exudates is called phytodegradation (Tsao 2003). Phytovolatilization is the process of volatilizing organic compounds and certain metalloids by plants. Contaminants present in water soluble form are taken up by plant roots. Water and the solutes form a continuous path in the plant from soil to root to leaves. Water along with some metalloids and volatile organic compounds are transpired out through the leaves. In addition to phytosequestration, phytotransformation, phytodegradation and phytovolatilization, plants can also use phytoextraction and phytostabilization as strategies to combat heavy metal stress. Plants can extract and subsequently remove the contaminants into terrestrial plant tissues (phytoextraction). Root exudates can also stabilize the contaminants in the soil preventing its uptake by plants (phytostabilization). Phytoextraction, phytostabilization, phytovolatilization, phytotransformation, phytodegradation and phytosequestration, are the mechanism of phytoremediation, where plants are used to remediate and revitalize metal/metalloid contaminated soil (Tsao 2003) (Fig. 2, Table 4). Phytoremediation is an eco-friendly and cost effective method with intangible benefits to soil ecosystem. Phytoremediation can not only remove the toxic metals and metalloids but also improve soil quality by enhancing sequestration of soil carbon,

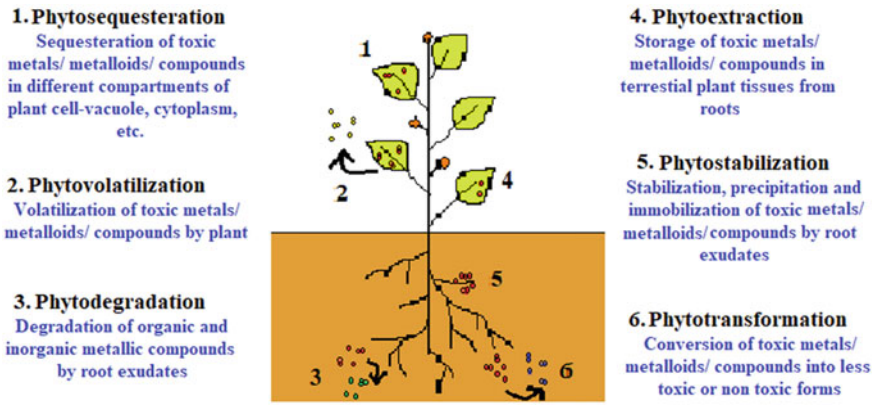

Fig. 2 Different strategies of phytoremediation of heavy metal toxicity

Table 4 Proteins involved in heavy metal uptake, accumulation and translocation in different compartments of hyperaccumulating plants

Hyperaccumulating plants	Increase in heavy metal uptake/accumulation/tolerance/resistance	Proteins involved
Thlaspi caerulescens	Zinc	Over-expression of ZIP (Zinc regulated transporter, iron regulated transporter protein): ZTN1, ZTN2 for zinc uptake
Arabidopsis halleri	Zinc	Over-expression of ZIP6, ZIP9 for uptake of zinc
Pteris vittata	Arsenic	Over-expression of phosphate/arsenate transporter in roots for uptake of arsenate
Astragalus bisulcatus	Selenium	Over-expression of sulphate transporters for uptake of selenium
Arabidopsis thaliana	Cadmium	Involvement of AtMRP1 and AtMRP2 transporters in transport of Phytochelatin-cadmium complex in vacuole
Arabidopsis halleri	Zinc, cadmium, cobalt, nickel	Over-expression of MTPs (Metal Tolerance Proteins): MTP1, MTP8, MTP11 for transport of heavy metals from cytosol to vacuole
Arabidopsis thaliana	Manganese	Over-expression of MTP8 and MTP11 for vacuolar transport of manganese
Stanleya pinnata	Selenium	Over-expression of sulphate transporters for uptake of selenium
Arabidopsis thaliana	Iron, manganese	Over-expression of NRAMP1 (Naturally resistant associated macrophage protein) for iron and manganese uptake
Thlaspi caerulescens	Zinc, cadmium, cobalt, nickel	Over-expression of MTPs (Metal Tolerance Proteins): MTP1, MTP8, MTP11 for transport of heavy metals from cytosol to vacuole

production of biomass and biofuel and maintenance of biodiversity (Teng et al. 2015). Effective phytoremediation require well developed root system in contact with soil. Rhizosphere is the soil region of 1–3 mm surrounding the individual roots. Rhizosphere is the site of high biological activities. It is the habitat of large population of microbes, some of which enhance the growth of plant. The rhizosphere surface should be large enough in order to have greater surface area for phytoremediation. Some plants like *Poa* sp. have shallow but dense fibrous root system that extends only few inches within the soil. Clovers (*Trifolium* sp.) and grasses (like *Lolium* sp.) have roots that can reach 1–4 feet below the soil surface. Larger surface area of rhizosphere is more advantages in phytoremediation than longer roots. Phytoremediation is a type of bioremediation with higher public acceptance, eco-friendly nature, low cost and low learning curve (Tsao 2003). Phytoremediation involves application of heavy metal hyperaccumulating plants at the contaminated site. But hyperaccumulators are mostly small and slow growing as heavy metals could affect their growth and metabolic rates. Also, the nature of phytoremediation employed by a plant depends upon the type of contaminant, ability of the contaminant to pass through plant root membrane, properties of contaminant, process of decontamination, type of plant species, surrounding microflora and fauna, soil type, etc. Phytoremediation technique predominantly includes plants but also involves interaction between plants, soil, contaminants, microflora and fauna. Therefore, significant change at any stage can affect the efficiency of plants to remove, detoxify, sequester immobilize, volatilize and extract toxic metal and metalloid from soil (Tsao 2003; Rajkumar et al. 2009). Rhizosphere often has heavy-metal resistant bacteria that can tolerate or resist heavy metal toxicity. These bacteria can assist and speed up the phytoremediation process of plants and can promote the growth of plant as discussed in the following sections.

Phytoremediation was used for integrated waste management in the town of Arcata, situated along the northern coast of California. The wastewater including the sewage of this town was treated in two stages. After the conventional sedimentation, filtering and chlorine treatment lots of dangerous pollutants, including toxic metals were still present in the wastewater. In the second stage, the wastewater was passed through six connected marshland containing suitable plant, algae, fungi and bacteria for phytoremediation, rhizoremediation and bioremediation resulting in neutralization and absorption of the pollutants present in the wastewater. These marshlands possess rich biodiversity of flora and fauna and constitute a wild life sanctuary. This is a real time example of phytoremediation in action.

6 Role of Rhizospheric Heavy-Metal Resistant Bacteria in Enhancement of Plant Growth

The A Horizon of the soil usually has 10^5–10^8 microbial cells per gram of dry soil. There is a decrease in the population of microbes with increasing depth of

the soil. The B and C horizon have 10^3–10^6 microbial cells per gram of dry soil, whereas groundwater usually has 10^5 cells per cubic milliliter of water. Rhizosphere is the zone surrounding the plant roots and is under its direct influence. The rhizosphere soil shows 10–100 fold higher population of microbes than the bulk soil (Tsao 2003). Rhizosphere is a highly competitive ecosystem where all species fight to colonize the best root zones. It is the habitat of variety of bacteria that have the ability to degrade different type of contaminants. Some rhizospheric bacteria are resistant to heavy metals, organic pollutants and amide herbicides. Heavy-metal resistant bacteria isolated from rhizosphere can sequester heavy metals and decompose organic and inorganic compounds (bioremediation). They can decrease metal phytotoxicity and accumulation in plant, thereby promoting plant growth (Khatoon et al. 2020). They can fix atmospheric nitrogen, mineralize and solubilize insoluble potassium and phosphate for plant (biofertilization), suppress phytopathogens, induce plant resistance mechanism (bioprotection) and promote production of phytohormones in plants (biostimulation). Plants release sugars, amino acids, flavonoids, proteins and organic acids. These molecules serve as messenger for rhizospheric bacteria and promote their activity (Khatoon et al. 2020). For example *Azospirillum brasilense* can promote growth of plant by biostimulation, biofertilization and bioremediation. It can produce *indole-3-acetic acid*, fix atmospheric nitrogen and alter heavy metal uptake in rice and other cereals. *Bacillus subtilis* show biostimulation, bioprotection, bioremediation and biofertilization in maize, chickpea, tomato etc. It produces *indole-3-acetic acid*, cytokinin, catalase and lipopeptides, and degrades xenobiotics for plants. *Azospirillum* sp. supplies sufficient amount of nitrogen in crop field which improves the yield and productivity of the land. *Acetobacter diazotrophicus* also plays the role of biofertilizer by fixing atmospheric nitrogen (Khatoon et al. 2020). *Rhizobiales* is an order of gram negative bacteria having agronomic importance. Some species of this order undergo symbiotic relationship with leguminous plant and provide the advantage of nodulation, atmospheric nitrogen fixation and plant growth in absence of external sources of nitrogen. *Rhizobia* can remove multiple types of organic pollutants like hydrocarbons, chlorinated compounds, phenolic compounds, pesticides, etc., from the environment (Teng et al. 2015). Members of genus *Rhizobium* degrade toxic compounds and therefore, make the order *Rhizobia*, important tool for heavy metal bioremediation. Members of *Rhizobia* confer heavy metal resistance by various mechanisms which include volatilization, adsorption, accumulation and sequestration of heavy metals. *Rhizobium* species with metal resistance genes can encode for efflux, detoxification and sequestration system in order to respond to the heavy metal toxicity. These genes are upregulated in presence of toxic metal and metalloids and confer heavy metal resistance to the bacteria (Teng et al. 2015). For example, *Mesorhizobium amorphae* CCNWGS0123 encode for CusA and CusB protein which participate in efflux of copper out of the cell. Similarly, arsenic resistance genes responsible for detoxification and resistance to arsenic, is present in *S. meliloti,* a member of *Rhizobiales*. Another rhizospheric bacteria *Pseudomonas putida* KT 2440 with *arsM* gene is involved in arsenic methylation and volatilization leading to arsenic removal. *Rhizobia* promote phytoextraction, phytotransformation, phytostabilization and phytovolatilization by the adjacent plants.

Rhizobia secrete enzyme like ACC (*1-aminocyclopropane-1-carboxylate*) deaminase, siderophores or organic acids to sequester and trap toxic pollutants in the soil, thereby reduce the symptoms of heavy metal stress in plants. It can also alter the redox state of metals and increase its complexation and bioavailability. Toxic metals can be adsorbed on the bacterial surface or can accumulate within the bacterial cell (Teng et al. 2015). Bacteria can volatilize or transform the toxic compounds into simpler ones by cytosolic or periplasmic or membrane embedded metal binding proteins. They can also fix atmospheric nitrogen and solublize phosphorus, thereby making them available to plants. They can induce plants to synthesize phytohormones. *Rhizobium* sp. RP5 secretes siderophores and increases the bioavailability of nickel and zinc to plants. *Bradyrhizobium* sp. relieves the stress of cadmium, zinc and nickel in *Vigna radiata*. *Cupriavidus taiwanesis* can overcome the low availability of metals and remove metal and metalloids for its symbiont *Mimosa pudica*. Therefore, Rhizobia and other rhizospheric bacteria aid in phytoremediation of metal by the symbiont plants and increase the plant biomass and soil fertility, as well as decrease the concentration of the metal in the local environment (Teng et al. 2015). The phenomenon of assisted phytoremediation (where microorganisms facilitate phytoremediation by plants) is a low input biotechnology technique that does not require addition of bacterial inoculants repeatedly at the contaminated site. But the effectiveness of this technique can be influenced by other competitive native bacteria present at the contaminated site. These bacteria can reduce the survival and bioremediation ability of *Rhizobium* and other rhizospheric bacteria. In addition, changing environmental condition like limitation of nutrients, change in pH, etc., can also affect the efficiency of these bacteria (Teng et al. 2015). Bacteria residing in the rhizosphere and promoting plant growth are also termed as plant growth promoting rhizobacteria (PGPR). These bacteria often exhibit tolerance or resistance to heavy metals when isolated from contaminated soil. They can convert, absorb, precipitate, accumulate or efflux heavy metals. These bacteria are of particular importance for their bioremediation potential and plant growth promotion. Serpentine soils with high pH contain high concentration of heavy metals like nickel, cadmium, cobalt and low concentration of calcium and other macronutrients. Presence of nickel at very high concentration leads to significant toxicity in plants growing in serpentine soil. Hyperaccumulating plants thriving in serpentine soil are mostly nickel hyperaccumulators. Serpentine soils are model for studying evolution of metal resistance in plants and plant growth promoting microorganisms. Hyperaccumulating plants like *Thlaspi goesingense, Thlaspi caerulescens, Alyssum bertoloni and Alyssum murale*, *Sebertia acuminate*, etc., are usually observed in serpentine soil. Serpentine soil is the habitat of various heavy-metal resistant bacteria that are hypertolerant to nickel and zinc toxicity (Rajkumar et al. 2009). Many heavy-metal resistant bacteria have been isolated from rhizosphere of *A. murale* and other hyperaccumulating plants. Most of the isolated strains are resistant to copper, cobalt, nickel, zinc, cadmium, chromium, arsenic, mercury and lead. Examples of heavy-metal resistant bacteria isolated from rhizosphere of plants are *Arthrobacter rhombi, Clavibacter xyli, Microbacterium arabinogalactolyticum, Rhizobium mongolense, Variovorax paradoxus,* etc. Such heavy-metal resistant rhizospheric bacteria also promote growth of plants at

different metal contaminated sites (Rajkumar et al. 2009). For example, inoculation of siderophore producing *Pseudomonas* sp. and *Bacillus megaterium* increased plant growth and enhanced nickel hyperaccumulation in *Brassica juncea,* without any visible symptoms of nickel toxicity. Other plant growth promoting bacteria like *Pseudomonas* sp. and *Pseudomonas jessenii* isolated from rhizosphere of serpentine soil protected *Ricinus communis* against heavy metal toxicity. These bacteria were applied at the site of nickel, copper and zinc contamination. They promote plant growth by producing and utilizing IAA as sole nitrogen source, and solubilizing phosphate and making it available for plants (Rajkumar et al. 2009). Interaction between PGPR like *Pseudomonas* sp. with *Rhizobium* indicated towards a synergistic process with potential nodule formation and better nitrogen fixation. Horizontal transfer of genes important for nodule formation and nitrogen fixation might have taken place between *Rhizobia* and *Pseudomonas* and *Burkholderia* which allowed them to form nodules in roots of *Robina pseudoacasia.* Therefore, combined application of rhizobacterial species is advantageous over application of a single species of nitrogen fixing bacteria at the contaminated site (Khatoon et al. 2020). Application of heavy-metal resistant plant growth promoting bacteria can reduce the cost of agricultural production by reducing the need for chemical fertilizers and increasing the bioavailability of nutrients. Once added as inoculant they increase their population within a short period of time due to their short doubling time. They often trigger weak defense response in plant than fungal elicitors and might facilitate sustainable and balanced relationship between bioremediation partners. These bacteria might also change the constitution and amount of root exudates and increase the availability of nutrients to the plants (Teng et al. 2015). Leguminous plants and nitrogen fixing bacteria often associate together in a symbiotic relationship, which gives various advantages to plants and bacteria. Plants provide nutrients like carbohydrates, inorganic minerals, etc., to the rhizospheric bacteria. Rhizospheric bacteria form a protective sheet around the plant and prevent contact between toxic contaminants and plant. The symbiotic relationship between plants and microbes is highly effective in removing environmental contaminants and promoting ecological sustainability (Fig. 3). However, the effectiveness of this method depends on the type of plant species, nature, diversity and richness of microbial community, toxicity, bioavailability of contaminants, physical and chemical properties of soil, organic matter content, pH, texture, etc. Plant growth promoting rhizospheric bacteria must be able to enhance the growth, development and yield of the plant. It must have broad spectrum of action and should be able to suppress the pathogenic infection in plants. It should have low doubling time, high rhizosphere competence and compatibility with other *Rhizobium* species (Teng et al. 2015; Khatoon et al. 2020). The next section describes few mechanisms employed by heavy metal resistant plant growth promoting bacteria for enhancement of plant growth.

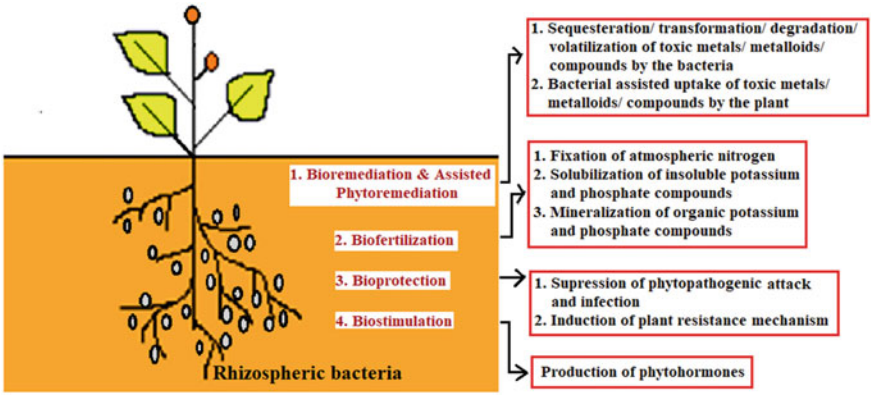

Fig. 3 Enhancement of plant growth and bioremediation of heavy metal pollutants by plant growth promoting Rhizobacteria (PGPR)

7 Mechanism of Action of Heavy-Metal Resistant Plant Growth Promoting Bacteria

7.1 Phosphate Solubilization

Phosphorus is one of the essential macronutrient essential for growth and development of plants. Phosphate participates in many metabolic pathways like photosynthesis, electron transport chain, respiration, root and seed growth and development, etc. Adsroption and chemical precipitation make phosphate less soluble or insoluble in soil. Though phosphate is present in high concentration in the soil, it is not available to the plants. Heavy metals in the soil also interfere with the uptake of phosphate by plants leading to reduced growth. Application of chemical fertilizers increases the agricultural production cost and changes the structure of soil ecosystem. Plant growth promoting bacteria that produce enzymes like phosphatase and phytase are involved in mineralization of complex organic phosphate compounds. Phytic acid which is the major component of organic phosphorus compound is broken down by the enzyme phytase. Phosphatase enzyme use organic phosphorus as a substrate and transform it into inorganic forms. Plant growth promoting bacteria also produce various organic acids that lower the pH of the soil and chelate mineral ions. These organic acids solublize the insoluble phosphate and make the phosphorus available for plant, without the application of chemical fertilizers (Rajkumar et al. 2009; Khatoon et al. 2020).

7.2 Potassium Solubilization

Potassium is also an essential macronutrients required for plant growth and development. It is essential for root hair development, growth of pollen tube, management of cellular osmotic balance, etc. Potassium also sometimes becomes unavailable to plants. Potassium solubilizing plant growth promoting bacteria produces organic acid like citrate, oxalate, acetate, etc. These acids cause extensive degradation and transformation of insoluble potassium containing compounds like clay silicates, mica, feldspar, granite, calcite, etc., into soluble forms, which is then taken up by the plants. For example, *Bacillus* sp. produces carboxylic acid that can solubilize potassium containing compounds (Khatoon et al. 2020).

7.3 Nitrogen Fixation

Nitrogen is an essential element for plant growth. However, plants cannot use the atmospheric nitrogen directly. There are examples of rhizobacteria which can fix atmospheric nitrogen in the soil as ammonia or release the nitrogen stored in decaying biomass as ammonia in the soil (ammonification). Further, the ammonia could be converted into nitrites and nitrates by bacterial action. Symbiotic rhizobacteria like *Rhizobium* sp. could fix nitrogen in the form, which could be utilized by the plant. Free living bacteria like *Azotobacter*, *Azospirillum*, etc. found in the rhizosphere could also fix nitrogen efficiently. *Rhizobium*, *Azotobacter* and some Cyanobacteria are used as biofertilizers to increase the nitrogen content of the soil.

7.4 Siderophore Production

Iron is the most important nutrient that participates in various physiological processes of the plant. Plants become deficient in iron supply under stress condition. Siderophores are organic molecules that show high affinity for Fe^{3+} ions. Siderophores can also form complexes with other bivalent heavy metal ions. Plant growth promoting bacteria produce siderophores of various types. For example, bacillibactins, pyoverdines, and cephalosporins are some of the common siderophores produced by these bacteria to chelate iron and make it available to plants. Siderophores decreases the free radical formation and protect phytohormones from oxidative damage. It increases the bioavailability and mobility of metals. It protects plants from pathogen by making iron unavailable to them. Therefore, siderophore producing plant growth promoting bacteria can reduce metal induced toxicity in plants. Such bacteria can also be used as biocontrol agent as they can reduce bacterial and fungal infection in plants (as antibacterial and antifungal agents) (Ahemad 2015; Khatoon et al. 2020).

7.5 *Production of Phytohormones*

Phytohormones are messenger molecules produced by plants participating in various physiological processes at very low concentration. Cell elongation, apical dominance, tissue differentiation, cell division, intracellular communications, etc., involves the action of phytohormones. Plants produce auxin in the form of *indole-3-acetic acid*. When the production of *indole-3-acetic acid* is low then it promotes primary root elongation. Higher production of *indole-3-acetic acid* inhibits primary root growth and promotes lateral and adventitious root formation. Phytohormones can alleviate biotic and abiotic stress condition. Plant growth promoting bacteria mainly produces auxin, which is involved in various physiological processes like cell elongation, division, differentiation, etc. *Indole-3-acetic acid* producing bacteria promote absorption of nutrients by proliferating plant roots. They also reduce metal adsorption and assist adaptation to heavy metal stress. They improve the antioxidant system and induce physiological changes promoting plant growth. For example, members of genus *Rhizobium*, *Pantoea*, *Agrobacterium*, *Bacillus, Pseudomonas*, etc., produce auxin and promote plant growth. Four tryptophan dependent pathways are involved in microbial *indole-3-acetic acid* biosynthesis. These four pathways engage *indole-3-acetamide*, *indole-3-pyruvic acid*, *indole-3-acetonitrile* and *indole-3-tryptamine* as intermediates. *Indole-3-pyruvic acid* pathway is the main pathway of *indole-3-acetic acid* production in plant growth promoting bacteria. The major precursor of *indole-3-acetic acid* is tryptophan and the production is catalyzed by aminotransferase and flavin containing monooxygenase (Rajkumar et al. 2009; Ahemad 2015; Khatoon et al. 2020).

7.6 *Production of* 1-Aminocyclopropane-1-Carboxylate *(ACC) Deaminase*

Ethylene is a gaseous plant hormone produced by all parts of plant in small amount. Ethylene production increases in plant during fruit development, ripening and under stress conditions like draught, salinity and metal induced stress. ACC is the immediate precursor of ethylene. Plant secretes ACC as one of its root exudates. ACC deaminase transforms ACC into ammonia and α-ketobutyrate. ACC deaminase is also synthesized by plant growth promoting bacteria. These bacteria utilize the ammonia after the degradation process and promote the secretion of more ACC from plant roots, which decreases the ACC concentration in plant and subsequently reduces the symptoms of adverse environmental stress in plants. ACC deaminase also improves the metal phytoremediation ability by facilitating longer root and greater root density in plants experiencing metal stress. These bacteria also increase metal mobility and bioavailability by producing varieties of organic acids, iron chelators and enzymes for plants. *Pseudomonas fluorescens* YsS6 is a free living ACC deaminase producing bacteria that alters the ethylene level and promotes nodulation in plants. *Pseudomonas* sp.

UW4 also show ACC deaminase activity and promote plant growth (Rajkumar et al. 2009; Ahemad 2015; Khatoon et al. 2020).

7.7 Production of Volatile Organic Compounds

Some plant growth promoting bacteria produce volatile organic compounds like *hydrogen cyanide*, *N, N-dimethyl hexa decyclamine*, *dimethyl disulfide*, etc., which can promote growth of host plant either by protecting it from harmful microbes or by increasing availability of minerals. For example *Arthrobacter agilis* UMCV2 produces *N, N-dimethyl hexa decyclamine* that protects the host plant from the attack of *Botrytis cinerea* and *P. cinnamomi*. *Dimethyl disulphide* act as elicitor of defense response in plants and show antagonistic action against *Botrytis cinerea.* Hydrogen cyanide also has an antagonistic role against various pathogens. It also increases the bioavailability of phosphate in rhizosphere. *Bacillus* spp. and *Pseudomonas* spp. are examples of volatile hydrogen cyanide producing bacteria (Khatoon et al. 2020).

7.8 Production of Hydrolytic Enzyme

Some plant growth promoting bacteria produce hydrolytic enzyme like cellulase, pectinase, etc. Cellulase when secreted from bacteria degrades cellulose (components of dead plant parts) into glucose which adds carbon to soil and promote plant growth (Khatoon et al. 2020).

7.9 Miscellaneous Actions of Plant Growth Promoting Bacteria

Brucella sp. K12 improved growth and yield of *Hibiscus esculentus* L. and reduced the Cr^{6+} concentration in soil and plant tissues. Other bacteria like *Microbacterium* sp. SUCR140 decreases Cr^{6+} toxicity in *Pisum sativum* and *Zea mays* and increased the overall growth of the plant. Vesicular–arbuscular mycorrhiza (VAM) is a symbiotic association between fungi and plant. This association also protects plant by restricting the uptake of toxic element like cadmium, nickel, lead, etc. VAM sequester toxic metals into their tissues, increases water uptake and provide resistance to plants against drought, salinity, etc. Fungi can extend their hyphae beyond the rhizosphere and collect nutrients from distant soil, increasing the nutrient uptake by plants.

8 Conclusion

Heavy-metal resistant plant growth promoting rhizobacteria is a potentially significant tool to combat the dual issue of metal contamination of soil and groundwater and low agricultural yield. The knowledge gathered about PGPR by different research groups all over the world could be translated into field application. Application of different PGPR as biofertilizers according to the need of the soil could supplement or substitute the use of harmful chemical fertilizers. Further, the bioremediation and the assisted phytoremediation potential exhibited by the rhizospheric bacteria could be exploited for construction of bio-filters and mitigation of heavy metal toxicity in soil, groundwater and wastewater.

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Conflicts of Interest On behalf of all authors, the corresponding author states that there are no conflicts of interest/competing interests.

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