Microbial bioremediation of heavy metals

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Heavy metals are persistent in nature and toxic to all life forms. Increase in industrialization, urbanization and unsafe agriculture practices is constantly adding heavy metals to the environment, and consequently causing heavy metal pollution of water and soil. Considering the negative impacts of heavy metals on the environment, several strategies have been devised to remediate them. However, most of these have their own limitations. Bioremediation of metals by microorganisms is efficient, cost-effective and environment-friendly method of metal detoxification. Microbes can utilize metal contaminants as their energy source and transform them to less toxic forms. When exposed to metals for a considerable period of time, microorganisms interact with them and become tolerant by developing resistance mechanism against them. Metal–microbe interactions can occur in several ways such as biosorption, bioleaching, biomineralization, bioaccumulation and biotransformation. Study of these interactions is important to understand resistance mechanisms against metals which include barriers, efflux system, sequestration and reduction of metals. These mechanisms are encoded by the resistance genes localized in chromosomes and plasmids. Understanding resistance mechanisms against metals in microorganisms becomes crucial for devising strategies for bioremediation of metals.

Keywords: Bioremediation, heavy metals, microorganisms, metal–microbe interactions, resistance mechanisms.

HUMAN activities such as industrialization, urbanization, advancement in technology, and unsafe agriculture practices have increased pollution at an alarming rate and degraded the environment[1]. The resultant degradation of the environment with toxic chemicals and hazardous heavy metals has led to contamination of soil, surface water and groundwater, and is immediately a major threat to all life forms on earth[2,3]. Heavy metals are toxic and cannot be degraded through biological, chemical or physical means to harmless by-products. Therefore, unlike organics, their longevity in the environment can be substantial and can only be transformed to less toxic forms[3–5]. They enter into our body through the food chain. As our body cannot metabolize them, they get accumulated, and become cytotoxic and mutagenic[5,6]. In humans, heavy metal toxicity can cause cancer, cardiovascular and neurological diseases, liver damage as well as central nervous system and sensory disturbances[3]. In plants, heavy metals can cause chlorosis, reduced seed germination and reduced growth because of decreased rate of photosynthesis, mineral nutrition and reduced enzyme activities[7,8]. Besides, heavy metal toxicity also increases reactive oxygen species (ROS) production, which includes oxygen radicals and non-radical derivates of molecular oxygen. Enhanced ROS production decreases antioxidant molecules and can lead to cell death by affecting the normal functioning of the organism[9–11]. Metal toxicity can also be a serious threat to microorganisms. It causes protein and nucleic acid denaturation, inhibition of enzyme activities, disruption of cell membrane and cellular functions, oxidative stress, chromosomal aberrations, mutation, etc. in them[11–13]. Consequently, considering the negative impacts of metal toxicity, immediate actions are needed to address detoxification of heavy metals.

There are several methods used to remove heavy metal from contaminated sites. The most widely used techniques include metal precipitation, filtration, ion-exchange resin and reverse osmosis. Metal precipitation has been proved to be cost-effective and easy to use[14]. However, it may cause secondary environmental issues[15]. Other mentioned techniques proved to be environmentally friendly, but expensive and relatively inefficient in removing heavy metals, and also organic contaminants[16,17]. To solve these problems, bioremediation is an important, attractive, cost-effective and environment-friendly method as this technique utilizes microorganisms which are naturally available in contaminated sites and can readily assist in the removal of heavy metals[3]. Use of microorganisms like algae, bacteria and fungi to detoxify heavy metals in the contaminated sites has emerged as a promising tool (Table 1). Considering microorganisms for bioremediation can be attributed to certain bacterial characteristics. Microorganisms are ubiquitous; they are minute and multiply rapidly, and increase in huge numbers when inoculated to contaminated sites[18]. When they are continuously exposed to pollutants, they become tolerant and exhibit exceptional levels of capability to transform pollutants as their source of energy and raw material. They can also genetically adapt to degrade the contaminants. These attributes can be exploited to make microorganisms an ideal candidate for a low-cost and more environment-friendly biological process[19]. Besides, they are nature's original recyclers.

This article highlights the studies carried out in the last 20 years in the field of heavy metal bioremediation. It

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Table 1. Heavy metal resistant microorganisms as potential tools for bioremediation

Bacteria	Heavy metals studied	Removal of heavy metals	Reference
Acinetobacter junii	Pb(II)	1071 mg g^{-1}	81
Arthrobacter viscosus	Cr(IV)	1161 mg g^{-1}	142
Geobacillus thermodenitrificans	Cu(II)	57 mg g^{-1}	143
	Pb(II)	53 mg g^{-1}	
	Zn(II)	18 mg g^{-1}	
Bacillus sp.	Cu(II)	44.73%	144
Bacillus thuringiensis	Cd(II)	97.67%	145
Bacterial mixtures			146
(*Sporosarcina soli* B-22,	Cu(II)	5.6%	
Viridibacillus arenosi B-21 and	Cd(II)	85.4%	
Enterobacter cloacae KJ-47 and *E. cloacae* KJ-46)	Pd(II)	98.6%	
Turbinaria vulgaris	Cr(VI)	90.08%	147
Ochrobactrum sp.	Cd(II)	83.3 mg g^{-1}	148
P. aeruginosa	Hg(II)	180 mg g^{-1}	149
	Pb(II)	98%	150
Pseudomonas sp.	Cu(II)	70.4%	151
	Cd(II)	93.5%	
	Pb(II)	97.8%	
	As(III)	34%	152
	Cd(II)	55%	
	Co(II)	53%	
Rhodobacter capsulatus	Zn(II)	164 mg g^{-1}	153
Sporosarcina pasteurii	Pb(II)	98.71%	154
	Cd(II)	97.15%	
	Zn(II)	94.83%	
Bacillus licheniformis	Cu(II)	32%	155
	Cr(VI)	95%	
	Fe(II)	52%	
Staphylococcus sp.	Cd (II)	44%	156
	Cu(II)	34%	
Stenotropho monasmaltophilia	Cu(II)	94%	157
Variovorax boronicumulans	Pb(II)	95.93%	154
	Cd(II)	73.45%	
	Zn(II)	73.81%	
Fungi			
Aspergillus niger	Pb(II)	172 mg g^{-1}	158
	Cd(II)	11 mg g^{-1}	159
Aspergillus fumigatus	Cd(II)	74%	104
	Cr(VI)	48.2 mg g^{-1}	160
Aspergillus flavus	Pb(II)	12.45 mg g^{-1}	76
	Cd(II)	1.3 mg g^{-1}	
Acremonium sp.	Al(III)	–	161
	Cu(II)		
	Fe(II)		
	Mn(III)		
	Pb(II)		
	Zn(III)		
Cellulosimicrobium sp.	Cd(II)	7.65%	77
	Cu(II)	21.21%	
	Fe(II)	70.26%	
	Ni(II)	18.98%	
	Pb(II)	24.12%	
	Zn(II)	22.82%	
Termitomyces clypeatus	Cr(VI)	403 mg g^{-1}	162
Penicillium chrysogenum	Pb(II)	11.55 mg g^{-1}	76
	Cr(VI)	0.10 mg g^{-1}	
	Cd(II)	0.51 mg g^{-1}	
Trichoderma sp.	Cd(II)	21.7 mg g^{-1}	163
Trichoderma viride	Cr(IV)	2.55 mg g^{-1}	76
	Pb(II)	9.14 mg g^{-1}	

(*Contd*)

Table 1. (*Contd*)

Bacteria	Heavy metals studied	Removal of heavy metals	Reference
S. cerevisiae	Cu(II)	16 mg g^{-1}	164
Algae			
Chlamydomonas reinhardtii	As(III)	38.6%	165
Chlorella vulgaris	Pb(II)	72.9%	166
Cystoseira crinitophylla	Cu(II)	160 mg g^{-1}	167
Fucus vesiculosus	Pb(II)	229 mg g^{-1}	168
Sargassum sp.	Cu(II)	49 mg g^{-1}	169
Sargassum filipendula	Cd(II)	7.8 mg g^{-1}	170
Sargassum muticum	Sb(III)	5.5 mg g^{-1}	171
Spirogyra sp.	Cu(II)	87.2 mg g^{-1}	172

also describes metal–microbe interactions and metal resistance mechanisms in microbes which are essential for bioremediation of heavy metals. It also briefly discusses about the resistance mechanisms against mercury, arsenic, cadmium and lead.

Metal–microbe interactions

As mentioned earlier, constant metal exposure help microbes to get acquainted and develop resistance against the metal. Therefore, it becomes necessary to understand the nature of metal–microbe interactions. These interactions can divided into following types.

Biosorption

This is an interactive process in which metal ions bind non-specifically to polysaccharides and proteins present on the cell surface. It is a feature in which both living cells and dead microbial biomass provide binding sites and these binding sites can participate heavy metals even from highly dilute solutions[20].

Bacteria exhibit metal adsorption because of the peptidoglycan layer present in them. Characteristic features of the peptidoglycan layer in Gram-positive and Gram-negative bacteria are different. Gram-positive bacteria exhibit multiple layers of peptidoglycan which consist of teichoic acid (unique to Gram-positive bacteria), amino acids (alanine and glutamate) and meso-diaminopimelic acid, while in Gram-negative bacteria, there is only one layer of peptidoglycan[18]. This layer consists of enzymes, glycoproteins, lipopolysaccharides and phospholipids[3]. These molecules act as ligands and offer active sites for metal-binding[21,22]. Teichoic acid and other acidic groups present in the cell wall are sources of carboxyl groups which play a crucial role in metal uptake[23]. Therefore, Gram-positive bacteria can adsorb more metal ions than Gram-negative bacteria. The cell wall also has complex carbohydrates, lipids, nucleic acids and proteins which constitute to form extra polymer substances (EPS). EPS show great metal-binding ability towards complex heavy metals and prevent their entry into the microbial intracellular environment. Thus, they protect microbes against heavy metal toxicity[24]. Sahmoune found that *Streptomyces rimosus* has good binding affinity for metals such as lead and iron[25]. A study performed by Rahman *et al.*[26], conclusively showed that a lead-resistant bacterium, *Staphylococcus hominis* strain AMB-2 could be effectively used for biosorption of lead and cadmium. Besides, multiple heavy metals could be biosorped by coral-associated solubilizing bacteria *Cronobacter muytjensii* KSCAS2 (ref. 27). Biological activities and applicability of EPS can be modified chemically by acetylation, carboxymethylation, methylation, phosphorylation and sulphonylation[28]. Besides, biofilms can also display adsorption of metals. It was demonstrated that *Staphylococcus aureus* biofilms could bio-precipitate U(IV), and further addition of acid phosphatase contributed to U(IV) remediation[29].

In scientific studies, fungi have been extensively employed to carry out heavy metal adsorption. They have exhibited efficient metal-uptake ability and established themselves as good biosorbents[29]. This is because the fungal surface is composed of lipids such as chitins, glucans and mannins, proteins and polysaccharides. Mannan contains negatively charged groups such as amino groups, carboxyls, phosphates and sulphates. Various studies have been undertaken to check the fungal metal-binding ability. Say *et al.*[30] demonstrated that mycelium of filamentous fungi *Phanerochaeta chrysosporium* can act as a biosorbent for cadmium, lead and copper. They further concluded that the mechanism and kinetics of biosorption were based on pH and availability of metal species. *Sacharomyces cerevisiae* has displayed the ability to remove toxic metals from contaminated wastewaters by biosorption[31–33]. *Alternaria alternata* and *Penicillium aurantiogriseum* have also been identified as good biosorbents for the removal of cadmium and mercury[34].

Besides, photosynthetic organisms like algae have been reported to have good heavy metal absorption capabilities. Algal mass surface accumulates heavy-metal ions because it contains cell polymeric substances (peptides and polysaccharides), and polysaccharides such as alginate and cellulose, organic proteins and lipids and functional

groups like carboxyl, amino, hydroxyl, phosphate, imidazole, thiol, sulphonate, etc. in the algal cell wall[35]. Algae also have deprotonated sulphate, laminaran and monomeric alcohols which attract both positive and negative species of different heavy metal ions[36]. Biosorption performance of different microalgal strains such as *Spirulina platensis*, *Chlorella vulgaris*, *Oscillatoria* sp. and *Sargassam* sp. was studied for metal ions[37–39]. Lin *et al.*[40] carried out meta-analysis for heavy metal adsorption potential in different algal phyla and found that phaeophyta had the highest adsorption capacity. They also found that non-living algae were more efficient than living algae in terms of heavy metal biosorption[40]. Thus, algae can also serve as an important candidate in detoxification of heavy metals.

Bioaccumulation

This is a metabolically active process which depends on import-storage system. The system transports heavy metal ions across the lipid bilayer into the cytoplasm or intracellular spaces using transporter proteins. The metal ions are sequestered by metal-binding entities such as proteins and peptide ligands[3], and the heavy metals which are sequestered by these entities can occur in the particulate forms, and insoluble forms and their by-products[41].

In the bacterial membrane, heavy metal bioaccumulation mechanisms can be attributed to endocytosis, ion channels, carrier-mediated transport, complex permeation and lipid permeation[2,42,43]. Ahemad[2] reported bioaccumulation studies of several metals such as mercury, lead, silver, cadmium and nickel. Rani and Goel[44] studied cadmium using transmission electron microscopy, and found that *Pseudomonas putida* 62 BN showed intracellular and periplasmic accumulation of the metal. Sher and Rehman[45] reported that *Monodictys pelagic* and *Aspergillus niger* can accumulate chromium and lead. More recently, Naskar *et al.*[46] demonstrated approximately 20% intracellular accumulation of nickel (II) in growing cells of *Bacillus cereus* M116.

Bioleaching

In bioleaching, microbes like bacteria and fungi which are present in nature, solubilize metal sulphides and oxides from ores deposits and secondary wastes[47,48]. The solubilized metals are then purified using suitable technologies which include adsorption, ion exchange, membrane separation and selective precipitation[49]. It is an economical and environment-friendly processes, as it uses less energy and does not emit harmful gases[50]. It has been used for centuries to leach metals from low-grade ores and currently supports a lucrative global market in the extraction of metals such as copper, cobalt, gold, nickel, uranium, zinc, etc.[51].

Bioleaching can be achieved by contact and non-contact mechanisms. In contact mechanism, a non-random physical contact occurs between the mineral sulphide and bacterial cell[52]. Oxidation to sulphate occurs through several reactions catalysed by enzymes and causes electron transfer from the mineral surface[53]. In non-contact mechanism, there is no physical contact between the bacterial cell and mineral surface[52]. Bacteria generate lixiviant (ferric iron) which chemically oxidizes the sulphide mineral. This reaction occurs only in acidic environment below pH 5.0 (ref. 54). A wide range of microorganisms are involved in bioleaching and acidophiles occupy an important place. Acidophiles are chemolithotrophs which thrive well under low pH conditions, preferably 2.0 or less, and oxidize Fe(II) to Fe(III) and/or reduce sulphur to sulphuric acid. The resultant ferric ions and protons arise from sulphuric acid, and solubilize the metal sulphides and oxides from the ores[55], thereby facilitating metal extraction by separating the metals in solid phase to more water soluble phase. Therefore, on summarizing these chemical reactions achieved by the microbes we find three basic steps[56,57], viz. (i) microbial reduction–oxidation reaction in solution, (ii) formation of acid from inorganic and organic routes and (iii) metal extraction from the matrix.

Zhang and Gu[58] reported that arsenic bioleaching is possible with both individual and mixed culture of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. It was observed that mixed culture of the strains yielded higher bioleaching.

Biomineralization

Biomineralization of metal ions is a natural process of mineral formation. It occurs through natural synthesis of minerals such as carbonates, oxides, silicates, sulphates and phosphates, and involves different mechanisms attributed to the activities of living organisms[59]. Presence of highly variable and highly reactive interfaces such as cell wall and additional organic layers (EPS and S-layer) with different hydration, composition and structure are crucial factors for mineral formation. Besides, there are organic ligands such as amine, carboxyl, hydroxyl, phosphoryl and sulphur which deprotonate and impart net negative charge on the microbial surface with increase in pH[60]. Potential toxic metals having positive charge precipitate non-uniformly into more stable and compact mineral products[61].

Metal immobilization or complexation can be achieved by phosphate precipitation, carbonate precipitation, oxalate precipitation, etc.[62–65]. Recently, numerous studies have demonstrated that remediation of toxic heavy metal ions such as copper, cadmium, lead, manganese, nickel, uranium and zinc can be achieved by biomineralization[65–67]. Zhang *et al.*[61] also reported that *Bacillus* sp. could release

free inorganic phosphate and trap toxic metal ions by the formation of an insoluble metal phosphate coat.

Biotransformation

This is the process by which the structure of a chemical compound is modified, leading to the synthesis of a molecule with relatively more polarity[68,69]. In other words, by this metal–microbe interaction process metal and organic compounds are modified from toxic form to a relatively less toxic form. This mechanism has been evolved in microbes to help them acclimatize with changes in the environment. Microbial cells have high surface–volume ratio, high growth rate, high rate of metabolic activity and maintenance of sterile condition is easy. Therefore, they are ideal for biotransformation. This process can be achieved through condensation, hydrolysis, formation of new carbon bonds, isomerization, introduction of functional groups, oxidation, reduction and methylation. These reactions may lead to volatization of metals and reduce their toxicity[70].

Microbial transformation is being used widely for the transformation of various pollutants, including hydrocarbons, pharmaceutical substances and metals[71]. There are reports where microbes have been used to transform metals. *Acinetobacter* sp. and *Micrococcus* sp. oxidized toxic As(III) into harmless and less soluble As(III) and decreased its toxicity[72]. Thatoi *et al.*[73] also provided evidence that Cr(VI)-resistant *Bacillus* sp. SFC 500-1E can reduce toxic Cr(VI) to less toxic Cr(III) by NADH-dependent reductase. Highly soluble and mobile U(VI) was transformed into highly insoluble U(IV)[74].

Microbial resistance mechanisms against heavy metals

In heavy metal stress conditions, microbes either die of metal toxicity or survive by developing resistance mechanisms against metals. To be selected as potential agents of bioremediation, microbes must exhibit resistance mechanisms against metal toxicity. They can achieve resistance against heavy metals by five main mechanisms[75]: (i) extracellular barriers, (ii) active transport of metal ions (efflux), (iii) extracellular sequestration, (iv) intracellular sequestration and (v) reduction of heavy metal ions.

Extracellular barriers

Cell wall, plasma membrane and other surface structures (EPS and biofilms) can act as barriers and prevent the entry of heavy metals inside the bacterial cells. Microbial cell surfaces exhibit a wide range of characteristics as discussed in metal–microbe interactions. They prevent the entry of metal ions by adsorbing them on their surface, indicating that they can act as barriers. For example, Kumar *et al.*[76] demonstrated that fungal and bacterial isolates can act as biosorbents of heavy metals such as chromium, copper and lead. Gram-positive bacteria, *Cellulosimicrobium* sp. also showed resistance against multiple heavy metals such as copper, cadmium, iron, zinc, lead and nickel. This resistance mechanism was mediated by chemisorption sites[77].

Microbes produce biofilms which act as barriers. Biofilms constitute extracellular polymers which can accumulate metal ions and consequently safeguard cells inside them. Biofilm cells of *Pseudomonas aeruginosa* demonstrated resistance against copper, lead and zinc ions[78]. In *Rhodotorula mucilaginosa*, biofilms increased metal removing efficiency from 91.71% to 95.35% (ref. 79).

Besides cell wall and biofilms, there are studies which show that EPS can also act as a barrier to metals. Adsorption of lead ions was reported in *P. aeruginosa*, *Acinetobacter junii* L. Pb1 and *Azotobacter chroococcum* XU1 (refs 80–82). Kazy *et al.*[83] observed that EPS provided copper resistance to *P. aeruginosa*. Copper-resistant strain produced twice the amount of EPS than the sensitive strain. In addition, entry of metal ions into the cells can be prevented by changing the permeability of the plasma membrane.

Active transport of metal ions

This is a key mechanism for heavy metal resistance in microbes. Efflux pumps remove heavy metals and maintain homeostasis. Metals can enter inside the cell through the same transport channel system from where essential elements enter. For example, in *Ralstonia metallidurans*, chromium enters through the sulphate transport system and facilitates ions of cadmium, zinc, cobalt and nickel, while manganese enters through the magnesium transport system[84]. Uptake of arsenate and arsenite by arsenic-resistant microbes (*Escherichia coli*) mediated by GlpF and phosphate transporters (Pst and Pit pumps) is also a good example[85]. Excessive concentration of these heavy ions is removed by the energy-dependent efflux system. For instance, Cu efflux in *E. coli* is controlled by a RND CusCBA multiprotein complex[86]. *Bacillus* sp. SFC 500-1E carries out efflux of toxic chromate ions Cr(VI) by a chromate ion transporter protein known as ChrA[87].

In *R. metallidurans* CH34, efflux of metal ions is mediated by the CzcCB2A complex[88]. In some bacteria, the efflux system works with other heavy metal resistance mechanisms for removing metals[89]. In Gram-positive and Gram-negative bacteria, the *ars* operon system encodes for ATPase pump, ArsA/ArsB and ArsC (arsenic reductase). ArsC is known to reduce arsenate to arsenite in the cytoplasm, and the efflux mechanism involves export of

arsenite outside through the plasma membrane[90]. *Shewanella oneidensis* MR1 also demonstrated tolerance mechanisms of metal efflux and biotransformation. The host cell removed and decreased the concentration of intracellular chromium by the efflux system and consequently allowed cells damaged by chromium to grow and detoxify it over extended periods of time[91].

Extracellular sequestration

In extracellular sequestration cellular components present in the periplasm or the outer membrane form a complex with metal ions. Besides, the mechanism can also involve formation of insoluble compounds due to metal-ion complexation[92]. *Pseudomonas syringae*, a copper-resistant strain synthesizes copper-inducible proteins. These proteins bind with copper ions and accumulate them, and turn bacterial colonies blue[93]. Copper-tolerant *Pseudomonas pickettii* US321 strain also exhibits the same phenomenon. Gilotra and Srivastava[94] observed that the resistant strain accumulated copper as a complex and transported it into the cytoplasm, while the sensitive strain accumulated copper in a free ionic form which is highly toxic to the cell.

It was demonstrated that under aerobic condition, *Klebsiella planticola* produces hydrogen sulphide from thiosulphate and precipitates cadmium ions as insoluble sulphides[95]. Under carbon-limiting conditions multi-resistant *Pseudomonas putida* S4 strain forms an insoluble precipitate composed of copper ions, hydroxyl and phosphate residues[96]. *Desulfovibrio desulfuricans* produces hydrogen sulphide in the extracellular environment which protects the host cell from heavy metal toxicity by metal-ion precipitaion[97].

Intracellular sequestration

Metal ions are harmful to sensitive cellular components at toxic levels. Therefore, these metal ions are sequestered inside the cytoplasm and prevented from reaching toxic levels. Intracellular sequestration involves formation of a complex between metal ions and metal-binding peptides in the cytoplasm[75].

Metal-binding peptides in eukaryotes are of two types – metallothioneins and phytochelatins. Both are cystein-rich and metal ions bind by sulfhydryl groups[98]. Metallothioneins are metalloproteins which have high metal-binding affinity. They are induced in the presence of heavy metal ions[99]. Cysteine residues in metallothioneins may serve as a sink when toxic metal ions are in excess[97]. The ability to synthesize metallothionein was demonstrated by the cyanobacterium *Synechococcus* sp. PCC 7942, in which the *smtA* and *smtB* genes were responsible for its synthesis[100]. Consequently, it resulted in the sequestration of cadmium and zinc by methionein binding[101]. In microalgae, metallothioneins are highly diverse and potential novel forms help them survive in heavy metal-contaminated environments. Few examples of such microalgae genera include *Chlamydomonas*, *Chlorella* and *Scenedesmus*[102].

Phytochelatins are peptides which have low molecular weight and are found in fungi and plants. They are synthesized from glutathione and have 5–11 amino acid residues[103]. Ianieva[75] observed that glutathione in *Rhizobium leguminosarum* cells could sequester cadmium ions intracellularly. Talukdar *et al.*[104] reported that the fraction of chromium (IV) ions that enters into the *Aspergillus* sp. are sequestered by glutathione and converted into less toxic form, and ultimately thrown out of the system through the efflux system.

Reduction of heavy metal ions

Metals and metalloids have a wide range of high oxidation states which can be reduced by enzymes to form more stable and less toxic metal forms[105]. Some bacteria use metals and metalloids as electron donors or acceptors to generate energy. In bacteria, during anaerobic respiration, the oxidized form of metals can act as terminal acceptors of electrons. Several studies report reduction of heavy metals by bacteria. Smirnova[106] isolated bacteria from different ecological niches which reduced chromate, molybdate and vanadate. Joshi *et al.*[107] reported that *Geobacter sulfurreducens* uses naturally abundant iron (III) minerals and produces magnetic iron (II)-bearing nanoparticles. These bio-nanoparticles have the potential to reduce mobile and toxic chromium (VI) to soluble and less toxic chromium (III). Ma *et al.*[108] reported that chromium (IV) was reduced to less toxic form by bacterial consortium. Detoxification of mercury ions by mercuric reductase encoded by *mer* operon also serves as a good example.

Genetic determinants of heavy metal resistance in bacteria

Nanda *et al.*[18] reported that bacteria can grow almost everywhere and their ubiquitousness has provided them with an opportunity of being exposed to a wide range of metal toxicity. Over the course of evolution, they have tolerated and developed resistance mechanisms against heavy metals such as arsenic, copper, cadmium, chromium, mercury, nickel, lead, etc. Metal resistance in bacteria and fungi is due to resistance genes found on the chromosomes and plasmids. However, the resistance mechanisms mediated by the chromosomes and plasmids vary. The resistance mechanisms encoded by chromosomes are more complex than those of plasmids.

The genetic determinants responsible for metal resistance were first discovered in plasmids[109]. Operon

systems in plasmids can serve as heavy metal genetic determinants. For example, the *czc* operon in *R. metalliduran*s CH34 plasmid PMOL30 acts as a genetic determinant for resistance against cadmium, zinc and cobalt. Another plasmid, PMOL28 is also present in these bacterial systems which localizes the *cnr* operon that serves as a genetic determinant for cobalt, nickel and chromium[110]. Both plasmids in the bacterium also confer resistance against mercury and titanium. According to Nies[111] and Cooksey[112] the genetic determinant, *cop* operon for copper resistance is found in *Pseudomonas* sp. plasmid. This operon is induced by the presence of copper and encodes four proteins, namely CopA, CopB, CopC and CopD. The Cop proteins accumulate copper ions and simultaneously form compartments in the cell periplasm and outer membrane. There are also instances where genetic determinants for metal resistance are localized in the chromosome. One example of such a case is the ATP-driven active transport system involving efflux of zinc ions across the plasma membrane. Here, the *zntA* gene encodes chromosomal regulation of P-type ATPase[113].

It has been reported that several bacteria may have similar metal resistance mechanisms in both plasmids and chromosomes. For example, *ars* operons in the chromosomes of *E. coli*, *P. aeruginosa* and *B. subtilis* are structurally similar to genetic determinants, *ars* operons found in the plasmids[90]. However, the systems may be different as the rule suggests that essential metal ion homeostasis genes should be localized in the chromosomes, while toxic metal resistance genes should be localized in the plasmids[114,115].

Metal resistance mechanisms are attributed to resistance gene systems located in the plasmids or/and chromosomes. Therefore, identification and characterization of these genetic determinants become crucial for the understanding and characterization of tolerance, which could be essential to devise an efficient bioremediation strategy.

Mercury resistance mechanism and bioremediation

Mercury is a heavy metal with the strongest toxicity; so, it has no beneficial functions. Bacteria being ubiquitous are likely to confront toxic Hg(II) concentrations[111]. The *mer* operon, mercury resistance determinant is widespread in bacteria[116]. In Gram-positive and Gram-negative bacteria, it is located in the plasmids. It comprises *merR* and *merD* genes (regulatory genes), *merT* and *merP* genes (transport genes) and *merA* gene (gene encoding mercuric reductase)[117].

In Gram-positive bacteria periplasm, Hg(II)-binds with Hg(II)-binding protein, MerP. This helps in preventing Hg(II) toxicity in periplasmic proteins. MerP delivers Hg(II) ions to another transporter protein, MerT which transports the toxic metal ions into the cytoplasm. In contrast, Hg(II) ions are transported inside the cytoplasm via specific uptake systems in Gram-negative bacteria. It is to be noted that there is another transport system by MerC, which can be either addition or substitution to MerT[118]. As Hg(II) ions enter inside the cell, NADPH-dependent mercuric reductase (MerA) reduces them to Hg(0)[119]. Organomercurials are more toxic than Hg(II). To detoxify them, the *mer* operon must encode a MerB organomercurial lyase along with other Mer proteins. The MerB protein first cleaves organomercurials into Hg(II), and then it is reduced by MerA[117,120]. Barkay *et al.*[105] reported that there are five different mechanisms of mercury resistance. These include: (i) reduction in mercuric ions uptake, (ii) conversion of demethylated methylmercury to compounds of mercuric sulphide, (iii) sequestration of methylmercury, (iv) methylation of mercury and (v) reduction of Hg(II) to Hg(0).

A Chang *et al.*[121] reported that mercury resistance in filamentous fungus, *Penicillium* sp. DC-F11 obtained from a heavy metal-contaminated site is a multisystem collaborative process. Extracellular sequestration such as adsorption and precipitation help the fungal cells to detoxify Hg(II) ions. The intracellular response to Hg(II) stress according to comparative transcriptome analysis includes thiol compound metabolism, *mer*-mediated detoxification system, defense against oxidative stress and metabolism for damage repair. They further claimed to have reported for the first a fungus using detoxification system for Hg(II) volatization through the *mer* operon.

Arsenic resistance mechanism and bioremediation

Arsenic is a toxic heavy metal which enters inside the microbes through phosphate transport system and gets accumulated. However, its presence can be toxic; therefore microbes have developed resistance mechanism against it. Arsenic resistance is attributed to the *ars* operon which is present in plasmids or/and chromosomes[111]. For example, in *E. coli* it is found in both plasmids and chromosomes whereas in *S. aureus* it is found only in the plasmids. In *E. coli*, plasmid R773 consists of five genes, viz. *arsR*, *arsD*, *arsA*, *arsB* and *arsC*. Therefore, the whole gene sequence in the cluster is known as arsRDABC. The *ars* operon in *S. aureus* plasmid pI258 is different from that of *E. coli* and it has three genes, viz. *arsR*, *arsB* and *arsC* (refs 111, 122). In arsenic-resistant microbes, different genes encode for different protein molecules. *arsR* encodes for the repressor protein which is induced by the presence of arsenate, arsenite, antimonite and bismuth. *arsB* encodes for the arsenic efflux pump. *arsC* encodes for the intracellular enzyme known as arsenate reductase. Arsenate reducatase reduces As(V) to As(III). *arsA* encodes for intracellular ATPase protein and *arsB* plays an important role in chemiosmosis[122–124].

Once arsenate reaches inside the microbial cell, it may accumulate and cause toxicity. Therefore, it should be removed from the cell. However, it is difficult to remove the metal ion as it shares structural similarity with phosphate which is high in concentration. It becomes difficult to export arsenate effectively[125]. Therefore, there should be a mechanism to distinguish it from phosphate, and here comes the role of arsenate reductase. It reduces As(V) to As(III), and the microbial cell exits As(III) through the efflux pump. Interestingly, the efflux pump specifically for arsenate still remains unknown[45]. In *E. coli*, the ArsC protein couples with glutathione via glutaredoxin to reduce arsenate and in *S. aureus*, the electron donor is thioredoxin[111,126].

Wu *et al.*[127] proposed that *Alishewanella* sp. WH16-1 resistance against As(III) is mediated by *RuvRCAB*. As(III) enters inside the cells through transporter systems and causes rearrangement of DNA. The *RuvR* gene positively regulates expression of *RuvCAB* and repairs the DNA damage. This DNA repairing mechanism against heavy metals by *RuvCAB* may be largely confined to Gram-negative bacteria. In another study on arsenic resistance mechanism, complete genome analysis of *P. putida* was performed. It was discovered that 61 ORFs (open reading frames) were involved in metal resistance suggesting multiple metal resistance, with seven ORFs being most crucial in metal resistance mechanisms. It included two arsenic resistance systems, two *cadA*, two operons for copper chelation and one for chromate ions. Some proteins were also involved in multiple metal resistance[128].

Cadmium resistance mechanism and bioremediation

Cadmium is the best known toxic heavy metal. It may enter inside the microbial cells (*R. metallidurans* CH34 and *S. aureus*)[129,130] and accumulate via the magnesium transport system. It may also enter inside other microbial cells through any manganese uptake system. However, molecular-level understanding of Cd(II) uptake is still lacking[111]. Cadmium efflux forms the basis of Cd(II) resistance in bacteria. Cd(II) resistance in Gram-positive bacteria is attributed to CadA protein, which is also known as Cd(II) efflux P-type ATPase. The synthesis of CadA protein is induced by the presence of Cd(II) ions. The CadA protein from *Staphylococcus* plasmid pI258 was the first to be sequenced, and it is 727 amino acids long. The basic model of CadA protein proposed by Silver and Walderhaug[131] hypothesized that it has three cytoplasmic domains: The first domain is homologous to MerP and MerA proteins of mercury resistance and involves in Cd(II) binding. The second domain functions as a channel to funnel out Cd(II) ions and prevent initial binding of microbial membrane. It also functions as phosphatase and removes phosphate Asp415-Pi. The third domain consists of an ATP-binding motif and a heptapeptide aspartyl kinase. It is most widely distributed among Gram-positive bacteria which include *Bacillus, Listeria* and *Staphylococcus*[115].

In *S. aureus*, Cd-mediated resistance is imparted by *cadA* and *cadB* operons which are present in the plasmids. The *cadA* operon present in plasmid pI258 contains two genes, *cadA* and *cadC*. *cadA* encodes for a protein which is 727 amino acids long and shows sequence similar to the P-class of ATPases. Cd enters inside *S. aureus* cells through an active transport system which is Mn^{2+}-specific, and gets accumulated to toxic levels[132]. These accumulated Cd(II) ions are then transported outside by CadA protein, consequently leading to their removal from the bacterial cell. *cadC* encodes for the CadC protein which is 122 amino acids long and regulates transcription of cadmium operon. Thus, products of *cadA* and *cadC* genes are integral for cadmium resistance[115]. The *cadB* operon differs significantly from the *cadA* operon. It resides in an incompatibility group II in *S. aureus* plasmid (pII147) and consists of two genes, *cadB* and *cadX*. In *S. aureus* cells (pII147 containing), there is no intracellular bioaccumulation of Cd(II) ions despite Mn^{2+}-specific transport system being active. Moreover, it has also been proposed that *CadB* could bind with Cd ions in the membrane, but does not promote their efflux[133].

In Gram-negative bacteria, cadmium resistance is mediated by RND-driven zinc exporter, viz. the Czc system[111,117] and a nickel exporter, Ncc[134]. In *R. metallidurans* plasmid pMOL30 the *czc* operon has been reported, where it stands for cadmium, zinc and cobalt. It consists of three structural genes: *czcA*, *czcB* and *czcC*. *czcA* is essential for the three above-mentioned heavy metals. It encodes for an anti-porter cation carrier which resides inside the membrane. *czcB* encodes for CzcB protein which plays an ancillary role in cation transportation. Deletion of this gene will result in complete loss of resistance against Cd(II) and Zn(II). *czcC* encodes for the outer membrane CzcC protein. These three proteins form a complex membrane cation efflux pump (CzcABC complex) for Cd(II) detoxification. Besides, *czcR* and *czcD* genes are also present which are involved in *czc* operon expression[135].

In yeast *S. cerevisiae*, Cd resistance is mediated by glutathione. It has been reported that incoming Cd(II) ions were bound by glutathione to form cadmium–bisglutathionato complex. This complex is later transported into the vacuole by the YCF1p transporter, which is an ABC transporter[136,137]. In cyanobacteria, cadmium resistance is conferred by *smt* operon which consists of two genes, *smtA* and *smtB*. The former encodes for Mts protein which is crucial for Cd tolerance[138], while the latter regulates *smtA* gene expression and the operator–promoter region that exists between the two genes[139].

Lead resistance mechanism and bioremediation

The first lead-specific resistance determinant system was described by Borremans *et al.*[140] in *R. metallidurans*

CH34 plasmid pMOL30. Pb resistance is conferred by the *pbr* operon. For Pb(II) detoxification, it combines metal uptake, accumulation and efflux of Pb(II). The *pbr* operon consists of several resistance genes such as *pbrT*, *pbrA*, *pbrB*, *pbrC*, *pbrD* and *pbrR*. Pb(II) uptake into the cytoplasm is achieved with the help of PbrT protein. Once inside the host cytoplasm, Pb(II) is excreted out through the *pbrA* gene-encoded P-type ATPase, or it may bind to the PbrD protein which is capable of being a chaperone molecule. However, it should be noted that the PbrD protein is not a necessity for Pb(II) resistance, but cells without PbrD protein may show reduced bioaccumulation. This internal sequestration of Pb(II) ions may protect against those metal ions which are exported freely, and prevent the uptake–export futile cycle of metal ions. The P-type ATPase efflux system may appear sufficient for Pb(II) resistance but for complete Pb(II) resistance, PbrB and PbrC proteins are also essential. PbrB and associated integral membrane protein are considered to be part of transporter-assisting resistance proteins, and may transport Pb(II) ions from the periplasm to the outer membrane. This would decrease Pb(II) uptake by PbrT protein. The *pbrC* gene encodes for prolipoprotein signal peptidase and is a crucial entity in the *pbr* operon. *pbrB* and *pbrC* genes belong to the same transcriptional unit; therefore, it can be hypothesized that the PbrC prolipoprotein signal peptidase is essential for PbrB prolipoprotein processing, and vice versa.

In *R. metallidurans* CH34 chromosome, *pbrR* and its homologues show remarkable affinity towards Pb(II) ions and are considered to be the only characterized natural metalloproteins. A computational study was carried out by Tolbatov *et al*.[141] on the affinity of Pb(II) towards the pbrR protein. It was found that pbrR may regulate its affinity towards Pb(II) ions by changing its conformation and protonation states. Hence, it is able to initiate metal sequestration or release the ions in response to external stimuli. Studies of such evolutionarily developed microbial resistance mechanisms are of utmost importance to devise novel strategies to carry out metal isolation and bioremediation.

Pd resistance in *P. aeruginosa* N6P6 is mediated by expression of *bmtA* genes. These genes encode for metallothionein which maintains intracellular homeostasis of essential metal ions. It is interesting to note that with increase in Pb(II) ion concentration, the expression of *bmtA* genes also increases. Besides, *bmtA* genes were also induced by other heavy metals which include mercury, copper, cadmium and arsenic[118].

Future prospects and conclusion

This article shows that use of microorganisms for heavy metal bioremediation is environment-friendly, efficient and cost-effective. Microbes are ubiquitous and show fast growth. In heavy metal-contaminated sites, they become accustomed to heavy metal ions. They interact with the metal ions and consequently develop strategies of tolerance and resistance against them. Knowledge of genetics and resistance mechanisms against heavy metals has helped in finding solutions to heavy metal pollution. More research should be directed towards finding newer strategies that can be adopted for metal detoxification and restoring heavy metal-contaminated sites. Microbial remediation is further made efficient by genetically engineered microorganisms (GEMs), but they do have some legal, ethical and biosafety issues. Besides, efficiency of GEMs in field conditions is always a matter of concern. Therefore, extensive research on GEMs in accordance with biosafety guidelines is necessary.

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Received 25 July 2019; revised accepted 20 November 2020

doi: 10.18520/cs/v120/i6/1013-1025