

Metal–Microbe Interaction and Bioremediation

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Abstract Bioremediation of heavy metals and radionuclides has shown immense promise as an alternative approach for cleaning up, especially the biosphere component of the environment. The ubiquitous nature of microbes has made them the pioneers in any bioremediation approaches. Understanding the working mechanism of these microorganisms either independently, or as a community in relation to their interaction with heavy metal will enlighten and improve the use of bioremediation for environmental cleanup. The discovery of plants that have metal accumulation potential has opened fresh avenues for bioremediation. Plant-based bioremediation is still at a nascent stage, but it has attracted considerable attention in recent years. A concerted approach of using plants and microbes for bioremediation is another strategy that might work efficiently as both can complement each other through various interactions. Moreover, genetic engineering can be used to improve the bioremediation capacity of both plants and microbes and further improve their capacity in bioremediation of heavy metals and radionuclide.

Keywords Bioremediation · Microbes · Heavy metals · Radionuclides

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1 Introduction

The continuous growth of human population has resulted in the increasing demand for basic life-supporting commodities worldwide. There has also been a concomitant increase in the industrialization process to meet the basic human demands. Furthermore, this has led to the increase in energy demands that are partly met by nuclear energy. Nuclear energy is an important alternative energy source which accounts for 17 % of the overall electricity of the world. As a consequence, mining for radioactive elements like uranium is crucial, which has led to the large amounts of toxic chemicals being released into the environment either deliberately or accidentally. These toxic contaminants include radionuclides like uranium and heavy metals such as lead, cadmium, arsenic, and mercury. Heavy metals like copper, zinc, cobalt, and iron are essential micronutrients for both plants and microorganisms, but if present at higher concentration, they can impair various metabolic processes. Other metals like lead, cadmium, mercury, and uranium have no known biological functions and are toxic even at lower concentrations. Inorganic contaminants like heavy metals and radionuclide are not degradable and tend to accumulate in the environment for a long time (Sarma et al. 2004; Renshaw et al. 2005). Several technologies have been developed to address the problem of heavy metal and radionuclide contamination as they pose serious ecological and health hazards. To compensate the higher cost involved in the newer technologies, alternative methods have been explored to address this issue.

Natural habitats harbor abundant and diverse type of microorganisms which can be explored for bioremediation processes. However, there is a need for the identification and characterization of the microbial strains that reveal high metal accumulation capacity and specificity. Uranium-tolerant bacteria have been isolated from various uranium-mining sites and deposits (Kumar et al. 2011, 2013a; Sarma et al. 2012, 2013). Bacteria from uranium deposits have been characterized for their metal tolerance and uranium binding (Kumar et al. 2011, 2013a; Sarma et al. 2012, 2013). Understanding and exploring the microbe–metal interaction have resulted in an upsurge in the research interest with their importance in various high-throughput biotechnological applications such as biosensor, biofuel cells, and most promisingly in microbe-mediated nanomaterial synthesis (Devi and Joshi 2012). Identification of the microbial ligands/cellular processes involved in metal sequestration has led to the development of engineered organisms with various cell surface displays that facilitate their applications in industrial catalysis, biosorption, bioremediation, biofuel, and biosensor technology (Mandal et al. 2006). Metal nanocrystal synthesis through microbial process is another very promising aspect with importance in metal bioremediation and synthesis of nanoparticles for diverse applications.

2 Metal-Tolerant Microbes

A plethora of microorganisms capable of efficiently degrading toxic compounds in the environment have either been isolated or engineered. One of the prominent bacteria which exhibits extreme radiation resistance is *Deinococcus radiodurans*. It was first discovered in 1956 by Arthur W. Anderson while trying to sterilize meat by irradiating it with gamma radiation (Anderson et al. 1956). For many years, *D. radiodurans* has fascinated biologists by its extraordinary resistance to ionizing radiation. This bacterium, as a polyploid, can withstand radiation exposures of up to ~17,000 gray (Gy) (Daly et al. 1995, 1996, 1997). *D. radiodurans* also dominate the arid environments over other less resilient species by their capacity to regrow after rehydration. Besides UV and IR, *D. radiodurans* is resistant to lethal and mutagenic agents like mitomycin C (Sweet and Moseley 1976), an antibiotic known to cross-link DNA.

Genome sequence of the highly IR-resistant *D. radiodurans* reveals the same number and types of DNA repair proteins as the IR-sensitive bacteria (Makarova et al. 2001, 2007). Following irradiation with lethal doses of IR, radiation-resistant organisms suffer from similar levels of genomic damage as the sensitive ones. This is due to the ability of the resistant strains to survive the formation of hundreds of IR-induced double-strand breaks (DSBs) per genome (Argueso et al. 2008; Gladyshev and Meselson 2008). Studies have shown that a highly IR-sensitive mutant of *D. radiodurans* that contains a mutated DNA polymerase I gene (*polA*) was fully restored by expression of the corresponding gene of the IR-sensitive *Escherichia coli* (Gutman et al. 1994). The enzymes that mediate DNA repair in *D. radiodurans* are, therefore, probably not unique. Surprisingly, the mechanisms of IR resistance in *Deinococcus* spp. remain unclear along with their unique ability to resist desiccation (Cox and Battista 2005) and UV radiation (Gutman et al. 1994; Minton 1994).

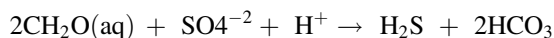
D. radiodurans has been studied for its ability to detoxify or immobilize metallic pollutants. This was made possible by genetic engineering to obtain radiation-resistant organisms that can simultaneously detoxify metals. The *merA* gene encodes mercuric ion reductase that reduces highly toxic, thiol-reactive mercuric ion, Hg(II), to much less toxic and volatile Hg(0) (Schottel 1978; Summers 1986). The *merA* locus from *E. coli* was cloned into *D. radiodurans* to confer both metal resistance and metal-remediating capabilities. In highly irradiating environments, cells that expressed the *merA* protein were better protected from the effects of Hg(II) than the wild-type counterparts. Cr(VI), a potent carcinogen, is another heavy metal commonly found in radioactive waste sites. In this case as well, genetically engineered *D. radiodurans* capable of reducing Cr(VI) to Cr(III) was preferentially used for rendering it non-mutagenic and non-carcinogenic (Brim et al. 2006). In general, the ability of a microorganism to resist the toxic effect of metals is frequently associated with its ability to transform those metals into less toxic chemical states.

The limitations associated with *D. radiodurans* for in situ bioremediation of nuclear waste sites are the requirements to genetically engineer the bacterium to

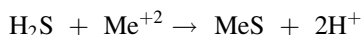
acquire toxic metal resistance and bioremediating capabilities. The culturing of this bacterium also requires a constant supply of carbon nutrient source and is therefore prone to contamination. The highly radiation-resistant eukaryote counterpart of *D. radiodurans* is found in the form of a microalga, *Coccomyxa actinobiotis* (Rivasseau et al. 2013). It can withstand ionizing radiation doses up to 20,000 Gy with half the population able to survive at 10,000 Gy. Metabolic activity of the cell is marginally affected by radiation doses up to 10,000 Gy and a complete recovery of cellular functioning within a few days. This unique microalga also has the capacity to fix radionuclides such as ^{238}U , ^{137}Cs , ^{110}Ag , ^{60}Co , ^{54}Mn , ^{65}Zn , and ^{14}C via metabolically inactive and active processes even in highly radioactive environments. The main advantage of using photosynthetic organisms is the minimal requirement for energy and culture media, which directly alleviates the problem of bacterial contamination. This newly discovered organism therefore offers great potential for the bioremediation of highly toxic radioactive wastes. Detailed studies with respect to elucidating its metabolic activity and its capability to remediate radionuclides are not only necessary but also inevitable.

Sulfate-reducing bacteria (SRB) are nonpathogenic anaerobic prokaryotes known for their non-photosynthetic activity to generate ATP through electron-transfer-coupled phosphorylation. During this process, SRB uses sulfate as the terminal electron acceptor for respiration of hydrogen to produce sulfide. The sulfide produced is highly reactive and toxic, and therefore, SRB are able to cause severe corrosion of metals in a water system by producing enzymes which can accelerate the reduction of sulfate compounds to hydrogen sulfide (Little 1998; Beaton 2007). SRB are used in several in situ technologies like in acid mine drainage (AMD). Extensive mining activity is responsible for changing the basic property of water. When pyrite-containing rocks come into contact with surface water or groundwater, under oxidizing conditions, these rocks produce sulfuric acid and dissolved iron. This acidic water in turn dissolves other metals contained in the rock, resulting in low pH, metal-bearing water known as AMD, or acid rock drainage (ARD). Carbonate minerals may neutralize the acidity and bring the pH to approximately 7.0 to give rise to neutral mine drainage. SRB are used in AMD treatment with the purpose of producing sulfides (for metal sulfide precipitation) and generating alkalinity at the same time.

Microbe-mediated sulfate reduction coupled with organic matter (represented by CH_2O) oxidation forms the chemical basis of SRB remediation



It also involves the chemical reaction of metal (Me) precipitation:



Precipitation of cadmium, copper, iron, lead, mercury, nickel, and zinc is facilitated by the formation of respective metal sulfides. In addition, arsenic, antimony, and molybdenum form more complex sulfide minerals (Figueroa 2005).

Co-precipitation with other metal sulfides can also be achieved for metals such as manganese, iron, nickel, copper, zinc, cadmium, mercury, and lead (Figueroa 2005). Other SRB species are known to reduce certain metals to a more insoluble form, like reduction of uranium (VI) to uranium (IV) (Spear et al. 2000). Sulfate reduction by SRB also consumes acidity, as a result of which there is an increase in the pH. The above precipitation reactions for metal hydroxides are facilitated by increasing the pH (Gadd 2004).

SRB are known to have a major negative economic impact on the petroleum industry because of their involvement in biocorrosion of ferrous metals in anaerobic environments (Hamilton 2003). Hydrocarbons in petroleum (e.g., benzene, toluene, ethylbenzene, xylenes, naphthalene, phenanthrene, and alkanes) may also serve as electron donors in the normal metabolism of SRB resulting in sulfide production. This biogenic sulfide production typically results in metal corrosion and reservoir souring. In addition, it can also result in the acidulation and plugging of petroleum reservoirs and biocorrosion of metal surfaces of pipelines and tanks (Nemati et al. 2001). Due to the explosive nature of the sulfide, this may pose a risk at high concentrations. The accumulation of SRB biomass thus causes a reduction in the oil recovery (Muyzer and Stams 2008). The need to control or inhibit the growth of SRB in petroleum industries is usually achieved by biocide dosage (Korenblum et al. 2010). Though effective, the inherent problems associated with biocides are the occurrence of antimicrobial resistance (Stewart and Costerton 2001; Fraise 2002), the residual concentration, toxicity, and persistence of biocides in industrial effluents. Alternative strategies for SRB control are therefore of great interest to the petroleum industry (Korenblum et al. 2013).

Remediation of radionuclide or radioactive wastes through microbial processes is an emerging field of research. It has been suggested by current researches that improper treatment approaches can lead to negative impacts on environment and biodiversity, which may even increase distribution of radioactive materials (e.g., wind-aided transport of plutonium-contaminated soil) (Whicker et al. 2004). Microbial consortium is a biological tool widely used for the remediation of pollutants, consisting of several species of microorganisms in the form of biofloculant. Biofilms produced by microbes, which exist predominantly in natural environments (~99 %) (Costerton et al. 1995), have the capability to immobilize metals. The different mechanisms adopted by biofilms to immobilize metals or radionuclides are as follows: (1) biosorption to cell components or extracellular polymeric substances (EPS), (2) bioaccumulation, (3) precipitation by reaction with inorganic ligands such as phosphate, and (4) microbial reduction of soluble metal to insoluble form (Gorby and Lovley 1992; Merroun et al. 2003; Renninger et al. 2004). Microbial activity can influence the release of radionuclides by altering bulk pore water chemistry (especially pH and redox reaction), by producing organic complexing ligands or by direct accumulation onto or into cells (West et al. 2002). Microbes can also cause corrosion and hence potentially affect the longevity of the metal waste containers in a repository (Stroes-Gascoyne et al. 2007).

3 Bioremediation: Plant–Microbe Interaction Perspective

The concept of using plants to clean up the environment has generated considerable interest in the last few decades. With the discovery of some plant that has high metal-accumulating capacity (hyperaccumulators) such as *Thlaspi caerulescense* and *Alyssum murales*, cleaning up of metal-contaminated sites using these plants seems like a promising strategy. Metal accumulation in plant biomass constitutes a subclass of phytoremediation called phytoextraction (Raskin and Eansley 2000). Approximately 400 plants have been identified so far which have potential for phytoextraction. Plants species such as *Thlaspi* sp and *Alyssum* spp. from the family Brassicaceae, *Viola calaminaria* and *Astragalus racemosus* from Violaceae and Leguminosae have been found to accumulate high concentration of heavy metals and radionuclides (Negri and Hinchman 2000; Reeves and Baker 2000). Metal-accumulating phenotype in plants is a complex mechanism that requires a concerted effort of tolerance, translocation, and sequestration of targeted metal/s (Hall 2002; Eapen and D'Souza 2005). Understanding the working mechanism of these areas will help enable or improve the metal-accumulating property in hyperaccumulator or even non-accumulating plants. There have been efforts to understand the mechanism of metal accumulation in potential candidates like *T. caerulescense* and other members of Brassicaceae (Baker et al. 1994; Kramer et al. 1996; Salt et al. 1999; Bert et al. 2000; Zhao et al. 2002; Milner and Kochian 2008). However, naturally occurring hyperaccumulators lack certain qualities such as large biomass, fast growth, and habitat incompatibility which limit their use in phytoremediation (Eapen and D'Souza 2005; Kotrba et al. 2009). The success of hyperaccumulation as a mean of cleaning up metal contaminated soil relies on the ability of these plants to tolerate high concentrations and wider metal resistant properties, possessing efficient transport mechanism for metal uptake to accumulation in deep or wide spread roots and aerial portions of the plant that can be easily harvested (Eapen and D'Souza 2005; Kotrba et al. 2009).

Another important aspect of phytoremediation is the relationship of plants with microorganisms existing in the rhizosphere or within the plants itself (endophytes) (Glick 2003, 2010; Kavamura and Esposito 2010; Ma et al. 2011). Plant–microbe interactions are well-known relationships which have been studied thoroughly in laboratory and field studies. Hence, there is no surprise that plants and microbes existing in metal-contaminated site also use this relationship to thrive in stressful environment (Tokala et al. 2002; Gray and Smith 2005). Microorganisms like bacteria are specialists in dealing with metals as they have existed together long before any other higher life forms. Hence, microbes are better adapted and possess well-organized mechanism to deal with the presence or invasion of toxic metals. The various strategies that bacteria utilize in order to negate the presence or increase concentrations of metals include the efflux of metal by different transporters (Nucifora et al. 1989; Solioz and Odermatt 1995), complexation inside the cell (Silver 1996; Robinson 2008), bioprecipitation, and reduction to a less toxic state (Bosecker 1997) (Fig. 1). Bacteria existing in metal-contaminated site can

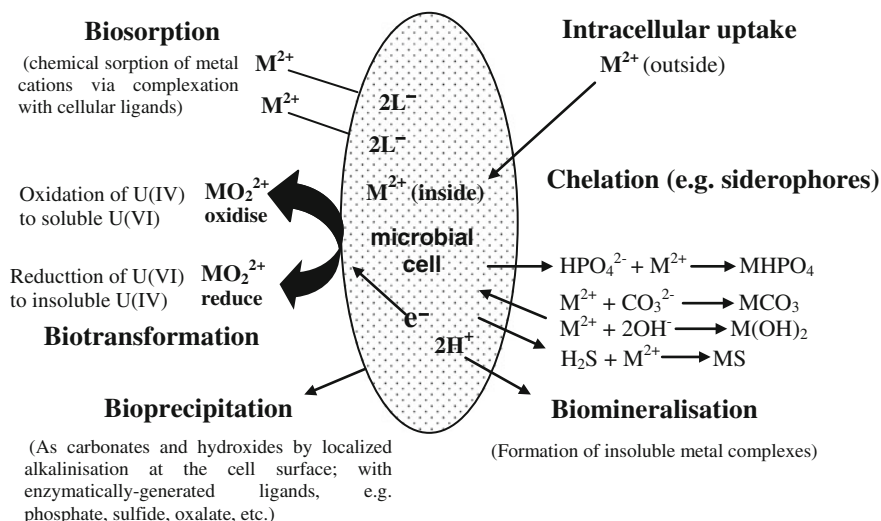


Fig. 1 Diagrammatic representation of the mechanisms of metal/radionuclide–microbes interaction (adapted from Geissler 2007)

influence the physicochemical properties of their habitat including metal(s) in more than one ways.

Bacterial communities have been known to solubilize or precipitate metals in soil, which in turn affects the bioavailability of metals. The bacterial capacity to change the availability of metal in soil can affect plants either by solubilizing the metals so that plant can easily absorb the metals or by precipitating the metals, affecting the survival of plants. Besides changing the bioavailability of metal(s), bacteria can influence the efficiency of phytoextraction by nitrogen fixation and secreting plant growth-promoting hormones such as IAA, siderophore, etc. (Glick 2010; Ma et al. 2011). Plants on the other hand secrete nutrients such as amino acids, sugars, and other metabolites from the roots, which nourish different bacterial species in the rhizosphere.

4 Metagenomics of Metal-Contaminated Sites

The existence of plant–microbe interactions in metal-contaminated site is irrefutable, and various studies have been carried out to understand this relationship (Sriprang et al. 2003; Kuiper et al. 2004; Wu et al. 2006). Moreover, the success of phytoremediation does not depend on plant alone but also on its interaction with the microorganisms in the rhizosphere (Whiting et al. 2001). Understanding the microbial communities existing in metal-contaminated sites is a prerequisite for understanding this relationship. Culturable bacteria have been studied in metal- and

radionuclide-contaminated site where diverse groups of bacteria have been isolated and identified (Roane and Kellogg 1996; Selenska-Pobell et al. 2001a, b; Shelobolina et al. 2004; Nedelkova et al. 2007; Islam and Sar 2011; Kumar et al. 2013a). However, culture-dependent methods do not give the true diversity and types of microorganism as ~99 % of bacteria cannot be cultured in laboratory (Pace 1997; Torsvik and Øvreås 2002). With the improvement in metagenomic studies, total bacterial communities existing in metal- and radionuclide-contaminated sites have been explored (Selenska-Pobell et al. 2001a, b; Satchanska et al. 2004; Islam and Sar 2011; Kumar et al. 2013a). This method allows a better understanding on the existing relationship of these bacterial communities with the plant species in that habitat. Comparative metagenomics have been carried out to study the effects of heavy metals on the diversity of bacterial communities. Bacterial communities' structure in uranium deposits has also been studied using both culture-dependent and culture-independent techniques to obtain baseline knowledge on the bacterial communities prior to any mining activities (Kumar et al. 2013b). Similarly, bacterial communities have been explored in uranium-mining sites using both culture-dependent and culture-independent methods. The rhizosphere tends to harbor diverse group of microorganisms as compared to the bulk soil. Hence, a comparative study of bacterial species between the rhizosphere and the bulk soil provides an idea about different bacterial species that contribute to the well-being of the plant(s). Bacterial species in the rhizosphere of hyperaccumulators plants such as *T. caerulescence*, *A. murales*, etc., have been studied and identified (Gremion et al. 2003). Besides the rhizosphere of hyperaccumulators, bacterial communities in the rhizosphere of pioneer plants in metal-contaminated sites have also been explored (Navarro-Noya et al. 2010). Metagenomics has also been used to compare the effects of different metals on the diversity of bacterial communities (Sobolev and Begonia 2008; Gołębiewski et al. 2013). Besides understanding the community structure, metagenomics has also been in use to understand the different metabolism, evolution, and adaptation of microbial communities in different habitats. Functional metabolic markers such as *nirS*, *nirK*, *dsrAB*, *amoA*, *pmoA*, etc., have been used to understand the dominant metabolic activities in radionuclide contamination sites (Hemme et al. 2010). Similarly, genes encoding for metal resistance were found to be prevalent and disseminated among those microorganism in metal-contaminated sites (Coombs and Barkay 2004; Martinez et al. 2006; Nongkhlaw et al. 2012) An in-depth and comprehensive study on the microbial communities and its function is very important before any bioremediation practice is established, and metagenomics is an important approach to achieve that goal.

5 Transgenic Metal-Tolerant Plants

The inherent property of some plants to accumulate metal(s) in their biomass has generated and opened new scope for heavy metal remediation. Plants with the capacity to accumulate 50–500 times metal in their biomass as compared to their

counterpart growing in the same habitat are termed as hyperaccumulators and considered potent candidates for use in phytoremediation.

The success of hyperaccumulation as a means of cleaning up metal contaminated soil depends on various factors like metal tolerance capability, efficient transport mechanism, high biomass in aerial portion of the plant etc. So far, such ideal plants have not been discovered or do not exist. However, genetic engineering can be used to optimize the existing potential of hyperaccumulators or non-accumulators by introducing new traits from other sources.

With the increased knowledge in plant genetics procedures and metal-tolerant plants with established genetic makeup, *B. juncea*, *Helianthus annuus*, and *Nicotiana glaucum* appear good candidates for genetic engineering for the purpose of phytoextraction (Eapen and D'Souza 2005; Kotrba et al. 2009). Understanding the mechanism that involves in metal accumulation is very important before genetic manipulation. The ability of plants to accumulate metal(s) in their biomass involves well-organized mechanism that includes metal uptake from the soil, translocation to target compartments, and sequestration. Genetically engineered plants for phytoremediation usually involve the introduction of metal-tolerating genes from other plants, but genes from human, animal, fungi, and bacteria have also been used to improve metal accumulation (Eapen and D'Souza 2005; Kotrba et al. 2009). Genes that encode metal sequestration factors such as metallothioneins (Misra and Gedamu 1998; Evans et al. 1992) and phytochelatins (Zhu et al. 1999a, b; Harada et al. 2001) from other sources have been introduced in plants. Constitutive expression of genes encoding metallothioneins from mouse, human, and Chinese hamster in *Nicotiana tabacum*, *Brassica oleracea*, and *Arabidopsis thaliana* showed increased Cd^{2+} tolerance but reduced metal accumulation in shoots. Similarly, genes from bacteria and fungi have been cloned and expressed in plants cells for obtaining metal-tolerant phenotype (Table 1). Expression of yeast *CUP1* increased Cd^{2+} tolerance in *B. oleracea*, but there is no increased accumulation. However, expression of yeast *CUP1* increased Cu^+ but not Cd^{2+} accumulation in leaves of *N. tabacum*. A more promising Cd^{2+} accumulation in transgenic line was seen with recombinant HisCUP. Improved Hg^{2+} accumulation was also seen in *A. thaliana* transgenic expressing bacterial Hg^{2+} binding protein, MerP. In addition to the improvement of metal accumulation trait, plants also need to combat the oxidative stress known to be induced by heavy metals. Hence Glutathione synthesis genes from bacteria and fungi have been introduced and overexpressed in plants with the aim of increasing metal tolerance and sequestration (reviewed in Eapen and D'Souza 2005; Kotrba et al. 2009). Maintenance of metal homeostasis carried out by various metal-transporting proteins is another important aspect for metal tolerance and accumulation. Similarly, metal transporters from bacteria and fungi have been cloned and expressed in plants. Zinc/Lead/Cadmium metal-transporting P_{IB} -ATPase (ZntA) from *E. coli* has been successfully expressed in *A. thaliana* (reviewed in Eapen and D'Souza 2005; Kotrba et al. 2009). Similarly, mercury-resistant genes such as *merA*, *merB*, and *merC* have been successfully cloned in plant species

Table 1 Examples of transgenic plants expressing bacterial/fungal genes

Transgenic plant	Source	Gene name	Altered phenotype	References
Cauliflower	Yeast	CUP-1	Cd tolerance	Hasegawa et al. (1997)
Tobacco	Yeast	CUP-1	Cu tolerance	Thomas et al. (2003)
Indian Mustard	<i>E. coli</i>	γ -Glutamylcysteine synthetase	Cd tolerance	Zhu et al. (1999b)
Tobacco	Yeast	FRE1 and FRE2	Fe content	Samuelsen et al. (1998)
Rice	Bacteria	Citrate synthase	Al tolerance	De la Fuente et al. (1997)
Indian Mustard	Bacteria	Arsenate reductase g-glutamylcysteine synthetase <i>gshI</i> and <i>arsC</i>	As tolerance	Dhankher et al. (2002)
<i>Arabidopsis thaliana</i>	<i>E. coli</i>	<i>zntA</i>	Cd and Pb tolerance	Lee et al. (2003)
<i>Arabidopsis</i>	Yeast	YCF-1	Cd and Pb tolerance	Song et al. (2003)
<i>B. juncea</i>	<i>E. coli</i>	<i>gshI</i>	Cd accumulation	Zhu et al. (1999b)
<i>Populus</i>	<i>E. coli</i>	<i>gshI</i>	Cd, Zn, Cu, and Pb accumulation	Bennett et al. (2003)
<i>A. thaliana</i>	<i>Acidithiobacillus ferrooxidans</i>	<i>merC</i>	Hg ²⁺ accumulation	Sasaki et al. (2006)
<i>A. thaliana</i>	<i>S. cerevisiae</i>	GSH1	Cd and As accumulation	Guo et al. (2008)
<i>Lycopersicon esculentum</i>	<i>Enterobacter cloacae</i>	ACC deaminase	Cd, Co, Cu, Ni, Pb, and Zn accumulation	Grichko et al. (2000)
Tobacco	<i>Neurospora crassa</i>	Zn transporter gene	Zn accumulation	Dixit et al. (2010)
Tobacco	<i>Neurospora crassa</i>	Cu transporter (<i>ctu-1</i>)	Cu acquisition	Singh et al. (2011)
<i>A. thaliana</i>	Bacterial	<i>merC</i>	Cd accumulation	Kiyono et al. (2012)

resulting in increased tolerance and accumulation of mercury by the transformed plant. Very few reports are available on the use of metal transporters from bacterial origin for making metal-tolerant transgenic plants except for those mentioned above.

Negri and Hinchman (2000) have reported the use of the plants for treatment of ^3H , U, Pu, ^{137}Cs , and ^{90}Sr . Phytoextraction removes radionuclides from soil without destroying the soil structure having limited impact on soil fertility for the treatment of large areas of low-level contamination, and its success depends on the bioavailability of radionuclides in soil, on the rate of uptake by plant roots and transportation efficiency of the vascular system of plants (Slavik Dushenkov 2003).

6 Conclusion

Microbial bioremediation is the process by which microbes degrade or transform hazardous organic compounds into non-toxic products. Since the plethora of microorganisms teeming in nature are not capable of degrading all toxic compounds, especially xenobiotics, the use of genetically modified organisms forms an indispensable part of bioremediation approaches with the advancement in genetic manipulation. Although genetically engineered microbes are quite promising, their implementation for in situ bioremediation still requires additional routes for developing safe steps to environmental cleanup. One of the major challenges is to optimize conditions and procedures for sustained and effective bioremediation in the presence of toxic metals and organic compounds. Conditions are created to enhance microbial activity for in situ biostimulation or bioaugmentation which may disrupt the natural microbiota. Various issues are to be dealt with to enhance the metabolic activity while maintaining the required growth conditions such as pH, temperature, the levels of contaminants and nutrients, etc. Due to the complex nature of interactions between microorganisms and radionuclides, it is far from easy to understand the wide range of environments these organisms inhabit. To study the molecular mechanisms and identify novel genes, proteins, and enzymes involved in the bioremediation of radionuclides necessitates the study toward the structural and functional interactions between proteins and other metabolites. Therefore, identification of potential genes and proteins involved in the metabolism of radionuclides can be achieved by advanced genomics and proteomics techniques. With the recent advances in next-generation sequencing, genomics, and proteomics, it has become possible to check for the expression of proteins and enzymes of interest with the potential for radionuclide resistance. Genome-wide transcriptome analysis can further provide detailed insight into better understanding of the metabolic pathways and the physiology of the microorganisms.

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