

# Chapter 9

## Application of Microorganisms in Bioremediation of Environment from Heavy Metals

Katarzyna Hrynkiewicz and Christel Baum

**Abstract** Heavy metals are important environmental pollutants which belong to the group of non-biodegradable and persistent compounds deposited in plant tissue (e.g. vegetables) which are then consumed by animals and humans. Increased pollution of natural environment with heavy metals, particularly in areas with anthropogenic pressure, also contributes to disorders in the natural balance of microbial populations. Molecular analysis carried out during the past decades revealed that density and diversity of microorganisms significantly correlated with increased contamination of the environment with heavy metals. As a result, a selective promotion of metal-tolerant genera of microorganisms was observed. In general, microorganisms are organisms with relatively high tolerance of unfavourable conditions, and these properties evolved over millions of years. In this chapter, a variety of mechanisms responsible for adaptation of microorganisms to high heavy metal concentrations, e.g. metal sorption, uptake and accumulation, extracellular precipitation and enzymatic oxidation or reduction, will be reported. Moreover, molecular mechanisms responsible for their metal tolerance will be described. The efficiency of accumulation of heavy metals in the microbial cells will be discussed and presented in photos from a reflection electron microscope (REM). The capacities of microorganisms for metal accumulation can be exploited to remove, concentrate and recover metals from polluted sites. This provides the basis for biotechnological solutions for the remediation of contaminated environments. Bioremediation has been regarded as an environment-friendly, inexpensive and efficient means of environmental restoration. Since microorganisms constitute a key factor of this technology, knowledge of the nature and molecular mechanisms of their tolerance of increased heavy metal concentrations is essential.

**Keywords** Pesticides · Environmental and health impact · Bioremediation

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K. Hrynkiewicz (✉)

Department of Microbiology, Institute of Biology and Environmental Protection, Nicolaus Copernicus University, Torun, Poland  
e-mail: hrynk@umk.pl

C. Baum

Soil Science, Faculty of Agricultural and Environmental Sciences, University of Rostock, Rostock, Germany

## 9.1 Environmental Pollution with Heavy Metals and its Influence on the Living Biota: Actual Status and Perspectives

Metals most frequently found at polluted sites are divided into two categories: cationic metals (metallic elements whose forms in soil are positively charged cations) and element compounds (elements whose forms in soil are combined with oxygen and are negatively charged). The most common problem-causing cationic metals are mercury, cadmium, lead, nickel, copper, zinc and chromium, whereas the most common anionic element is arsenic (Olaniran et al. 2013). Analysis of the negative effects of metal toxicity on the environment is complicated since heavy metals may be present in a variety of chemical and physical forms, namely, soil-adsorbed species, soluble complexed species and ionic solutes. Moreover, the effect of environmental conditions such as pH, redox potential of the water phase as well as soil properties, including ion exchange capacity, clay type and content and organic matter content, on the physical and chemical states of the metals can cause further impediments (Olaniran et al. 2013).

The actual status of environmental heavy metal pollution in urban soils, road dusts and in agricultural soils in China was reviewed by Wei and Yang (2010). They revealed the highest heavy metal concentrations (mainly Cr, Ni, Cu, Pb, Zn and Cd) in urban road dusts and identified the traffic and industrial emissions as the major source of this pollution. About 65% of all tested cities in China had high to extremely high levels of contamination. Compared with this, agricultural soils were mainly uncontaminated or slightly contaminated with Cr, Cu, Pb, Zn and Ni. Some agricultural soils were considerably contaminated by Cd and Hg. Worldwide, the main sources of heavy metal pollution in agricultural soils are fertilisation (especially Cd from phosphorus fertilisers) and pesticide applications (especially Cu from fungicides; e.g. Aikpokpodion et al. 2010). Since some heavy metals are characterized by great solubility in water, they can be easily absorbed by the living organisms and are found to be accumulated in humans at the end of the food chain (Charrier et al. 2010). Especially Cd and Zn are known to have high soil-to-plant transfer factors (Olayinka et al. 2011) and can be easily transferred into food products for this reason. Cadmium is, together with Pb and Hg, associated with the main threats to human health from heavy metals (Järup 2003).

Remediation of polluted sites can be done on- or off-site. Appropriate remediation strategies for these sites were reviewed by Peng et al. (2009). Bioremediation, in particular, offers valuable strategies for an ecological and economical advantageous on-site remediation. At present, bioremediation of heavy metal-polluted soils, water and sediments is often based on plant uptake during phytoremediation (Peng et al. 2009). However, microbial promotion can significantly increase the efficiency of phytoextraction of metals (e.g. Hryniewicz and Baum 2013, Zimmer et al. 2009).

Since microbes can promote plant growth in heavy metal-polluted areas in various ways (actively or passively), they should be analysed both in relation to particu-

lar taxonomic groups and as a result of different rhizosphere soil conditions including organic matter, pH, temperature, nutrients and pollutants level (Glick 2003; Bais et al. 2006). Among the rhizosphere microorganisms involved in plant interactions with metal-contaminated soil milieu, deserving special attention are plant growth-promoting bacteria (PGPB) and plant growth-promoting fungi (PGPF)—the plant-associated bacteria/fungi which migrate from the bulk soil to the rhizosphere of living plants and colonize the rhizosphere and roots of plants (Hryniewicz et al. 2009, 2012; Elsharkawy et al. 2012). Microorganisms colonizing the internal tissues of plants without causing symptomatic infections or negative effects on their host have been defined as endophytes (Schulz and Boyle 2006). In general, the beneficial effects of endophytes are greater than those of many rhizobacteria (Pillay and Nowak 1997) and might be aggravated when the plant is growing under either biotic or abiotic stress conditions (Hardoim et al. 2008). Endophytic microorganisms may be potential resources of highly efficient biosorbents for heavy metal biosorption since they have the advantage of being relatively protected from the competitive, high-stress environment of the soil (Sturz and Nowak 2000; Xiao et al. 2010).

Direct microbial strategies, besides the promotion of phytoremediation, in remediation of contaminated soils can include the use and stimulation of indigenous microbial populations, bioaugmentation (the addition of adapted or designed inoculants) or addition of genetically modified microorganisms. Particularly, the combination of genetic engineering of bacterial catalysts with judicious eco-engineering of the polluted sites was supposed to be especially important in future bioremediation strategies (Valls and Lorenzo 2002).

## 9.2 Resistance of Microorganisms to Heavy Metals: Mechanisms of Action

In general, metals and metalloids can be categorized as being essential and non-essential to biological life. Some metals, such as calcium, chromium, cobalt, copper, iron, magnesium, manganese, nickel, potassium, sodium and zinc, play an integral role in the life processes of microorganisms and serve as micronutrients which are used for redox processes, stabilization of molecules through electrostatic interactions, as components of various enzymes and for regulation of osmotic pressure (Bruins et al. 2000; Hussein and Joo 2013; Olaniran et al. 2013). Many other metals (e.g. silver, aluminium, cadmium, gold, lead and mercury) have no biological role and are non-essential and potentially toxic to microorganisms (Hussein and Joo 2013; Olaniran et al. 2013). However, all metals according to their concentration have toxicity with respect to living organisms. The minimal inhibitory concentrations of heavy metal/s for *Escherichia coli* are reported in Table 9.1.

Some cations of heavy metals (e.g.  $\text{Hg}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Ag}^{2+}$ ) can bind to sulfhydryl ( $-\text{SH}$ ) groups of enzymes essential for microbial metabolism and inhibit the activity of sensitive enzymes. Moreover, many divalent heavy metal cations (e.g.  $\text{Mn}^{2+}$ ,  $\text{Fe}^{2+}$ ,

**Table 9.1** Minimal inhibitory concentrations (MIC) of heavy metals for *Escherichia coli* (Nies 1999)

| MIC (mM) | Heavy metals                                                                                                                                                                                                                     |
|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 0.01     | Hg <sup>2+</sup>                                                                                                                                                                                                                 |
| 0.02     | Ag <sup>2+</sup> , Au <sup>3+</sup>                                                                                                                                                                                              |
| 0.2      | CrO <sub>4</sub> <sup>2-</sup> , Pd <sup>2+</sup>                                                                                                                                                                                |
| 0.5      | Pt <sup>4+</sup> , Cd <sup>2+</sup>                                                                                                                                                                                              |
| 1.0      | Co <sup>2+</sup> , Ni <sup>2+</sup> , Cu <sup>2+</sup> , Zn <sup>2+</sup>                                                                                                                                                        |
| 2.0      | Tl <sup>+</sup> , UO <sub>2</sub> <sup>2-</sup> , La <sup>3+</sup> , Y <sup>3+</sup> , Sc <sup>3+</sup> , Ru <sup>3+</sup> , Al <sup>3+</sup>                                                                                    |
| 5.0      | Pb <sup>2+</sup> , Ir <sup>3+</sup> , Os <sup>3+</sup> , Sb <sup>3+</sup> , Sn <sup>2+</sup> , In <sup>3+</sup> , Rh <sup>2+</sup> , Ga <sup>3+</sup> , Cr <sup>3+</sup> , V <sup>3+</sup> , Ti <sup>3+</sup> , Be <sup>2+</sup> |
| 10.0     | Cr <sup>2+</sup>                                                                                                                                                                                                                 |
| 20.0     | Mn <sup>2+</sup>                                                                                                                                                                                                                 |

MIC minimal inhibitory concentrations

Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup> are structurally very similar. To differentiate structurally similar metal ions, microorganisms have evolved two types of uptake mechanisms: fast—unspecific, constitutively expressed and driven by the chemiosmotic gradient across the cytoplasmic membrane of bacteria—and inducible (slower)—with high substrate specificity, with the use of ATP hydrolysis as the energy source and applicable by the cell in times of starvation or a special metabolic situation (Bruins et al. 2000; Nies 1999; Olaniran et al. 2013). Regardless of specific microbial uptake systems, high concentrations of non-essential metals can be transported into the cells by a constitutively expressed unspecific system and (similarly as non-essential metals) damage cell membranes, alter enzyme specificity, disrupt cellular functions, damage the structure of DNA and impose oxidative stress on microorganisms (Bruins et al. 2000; Kachur et al. 1998; Nies 1999). Since metal ions (unlike other toxic pollutants) cannot be degraded or modified (Orhan and Buyukgungor 1993), some microorganisms develop metal ion homeostasis factors and metal-resistance determinants (Bruins et al. 2000; Nies 1999; Ji et al. 1995). One (or more) from the following six metal-resistance mechanisms allow microorganisms to function in metal-contaminated environments: (1) exclusion by permeability barrier, (2 and 3) intra- and extracellular sequestration, (4) active transport efflux pumps, (5) enzymatic detoxification and (6) reduction in the sensitivity of cellular targets to metal ions (Bruins et al. 2000; Nies 1999; Ji et al. 1995; Carine et al. 2009; Rensing et al. 2009; Silver and Misra 1988; Olaniran et al. 2013).

High concentrations of heavy metals reduce biomass of sensitive soil microorganisms and can lead to changes in their structure, diversity and enzymatic activities (Hartmann et al. 2005; Frey et al. 2006; Lazzaro et al. 2006). Several results revealed decreased bacterial and fungal diversity in heavy metal-polluted soils (e.g. Brandt et al. 2006; Hu et al. 2007, Del Val et al. 1999; Li et al. 2012). Metal-sensitive microbial populations are reduced or disappear, and are replaced by other indigenous populations, which can better tolerate and adapt to high concentrations of heavy metals in the environment (Lazzaro et al. 2008). Changes in bacterial and fungal diversity of heavy metal-polluted soils can be useful for environmental mon-

itoring (Turnau et al. 2006; Demoiing and Baath 2008). The diversity of microbial population at metal-polluted sites has been investigated already by several scientists using culture-dependent and molecular techniques. Microorganisms naturally inhabiting metal-polluted soils can belong to gram-positive bacteria such as *Bacillus* and *Arthrobacter* and gram-negative bacteria such as *Pseudomonas* and *Burkholderia* (Kozdrój and van Elsas 2001; Ellis et al. 2003; Héry et al. 2003; Hryniewicz et al. 2012). However, in the group of *Proteobacteria*, bacteria with high tolerance as well as those very sensitive to increased heavy metal concentrations can be present (Tsai et al. 2005). Mechanisms of adaptation of microorganisms are correlated with the time of their exposure to increased heavy metal concentrations. In short term, naturally inhibiting microbial populations can survive the pollution, and in long term, the surviving microorganisms may adapt to unfavourable conditions using phenotypic or genetically based adaptation mechanisms (Lazzaro et al. 2008). To date, several genes coding metal resistance of bacteria have been discovered; however, a detailed understanding of the key indigenous organisms able to tolerate heavy metal pollution is still lacking (Naz et al. 2005; Lazzaro et al. 2008). Tolerance mechanisms are often plasmid encoded, but, in some instances, the genes are found on the chromosome, suggesting an important evolutionary pressure to keep these genes, e.g. mercury ( $\text{Hg}^{2+}$ ) resistance in *Bacillus*, cadmium ( $\text{Cd}^{2+}$ ) efflux in *Bacillus* and arsenic efflux in *E. coli* (Carlin et al. 1995; Shrivastava et al. 2003).

The potential of selected microorganisms to survive and grow in soils with high concentrations of heavy metals may be a useful feature for risk assessment and bioremediation of polluted sites (Lazzaro et al. 2008). The application of microorganisms to extract or immobilize heavy metal contaminants is considered as a biotechnological approach to clean up polluted environments (Cristiani et al. 2012). Microorganisms can improve phytoextraction by altering the solubility, availability and transport of heavy metals and nutrients by reducing soil pH, release of chelators, P solubilization, or redox changes (Ma et al. 2011). Among different metabolites produced by microorganisms, siderophores may play a very important role in metal mobilization and accumulation (Rajkumar et al. 2010). These microbial compounds may be synthesised by both mycorrhizal fungi and rhizosphere bacteria. Siderophores solubilize unavailable forms of heavy metal-bearing Fe but form complexes with bivalent heavy metal ions that can be assimilated by root-mediated processes (Braud et al. 2009).

### 9.3 Microorganisms as Biosorbents of Heavy Metals

Since degradation and metabolism of heavy metals are not possible, microorganisms have evolved coping strategies to either transform the element to a less-harmful form or bind the metal intra- or extracellularly, thereby, preventing any harmful interactions in the host cell (Monachese et al. 2012). Moreover, they are able to actively transport the metal out of the cell cytosol (White and Gadd 1998).

Bacterial strains can be specific to accumulate one or several heavy metals (Mejare and Bulow 2001). They can bind high levels of heavy metals according to a variety of mechanisms: accumulate metals by either a metabolism-independent (passive) or a metabolism-dependent (active) process and can remove heavy metals through bioaccumulation or biosorption (Cristani et al. 2012). In the bioaccumulation process, metals are transported from the outside of the microbial cell through the cellular membrane into the cell cytoplasm, where the metal is sequestered (Cristani et al. 2012). Metals adsorption is determined by the sorptivity of the cell envelope and influenced by differences in the cell wall construction of gram-positive and gram-negative bacteria (Jiang et al. 2004; Cristani et al. 2012). The factors responsible for metal adsorption of bacterial cells include the presence of phosphoryl groups, lipopolysaccharides, carboxylic groups and teichoic and teichuronic acids (parameters characteristic for specific groups of bacteria) as well as toxicity, composition and total content of metals in the environment (Haferburg and Kothe 2007; Cristiani et al. 2012).

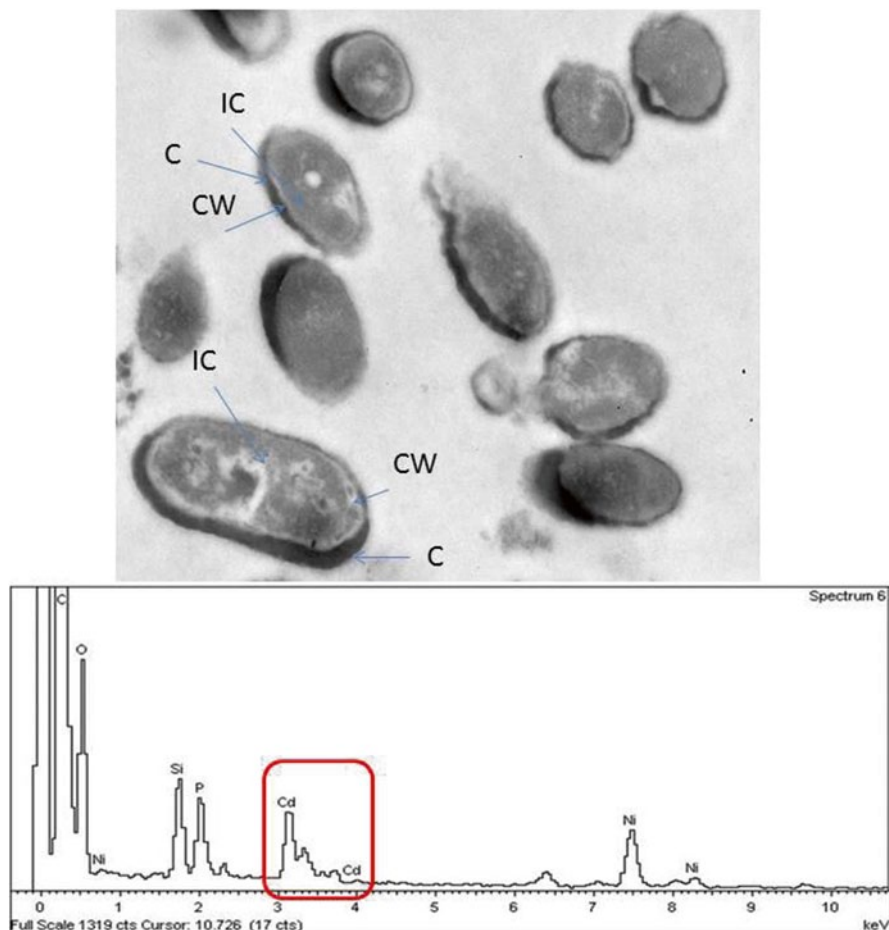
The metal-accumulating capacity of microorganisms can be exploited to remove, concentrate and recover heavy metals from mine tailings and industrial effluents (Malekzadeh et al. 2002). Several reports of aerobic bacteria accumulating metals like Ag, Co, Cd, Cu, Cr and Ni are available (e.g. Adarsh et al. 2007; Karelová et al. 2011).

A multi-metal-resistant endophytic bacterial strain *Bacillus* sp. L14 (EB L14) was isolated from the cadmium hyperaccumulator *Solanum nigrum* L (Guo et al. 2010). The hormesis of EB L14 was observed in the presence of divalent heavy metals (Cu, Cd and Pb) at a relatively lower concentration (10 mg/L). This observation was the effect of abnormal activities of increased ATPase which provided energy to help EB L14 reduce the toxicity of heavy metals by exporting the cations. It was revealed that within a 24-h incubation period, EB L14 can specifically take up about 76, 80, and 21 % of Cd, Pb and Cu from a pre-given solution, respectively (Guo et al. 2010). Subcellular fractionation studies revealed that almost 81 and 76 % of the Cd and Pb taken up by the cells are found in the membrane fraction, whereas the presence of Cd and Pb in the cytoplasm is only about 5 and 7%, and on the cell wall is 14 and 16% of the total uptake, respectively (Guo et al. 2010).

Opposite results were obtained in our laboratory during observation of rhizosphere bacteria *Pseudomonas* sp. (Fig. 9.1). Microscopic analysis revealed the highest concentrations of Cd in external components (capsule) and cell walls of the bacterial cells, cultivated in the presence of Cd ions (not published data). The internal spaces of bacterial cells possessed lower concentrations of Cd.

The results shown in Fig. 9.1 were similar to other reports (Bai et al. 2008; Kumar and Upreti 2000) in which bacterial cell walls were always responsible for almost all heavy metal uptakes. These kinds of uptakes seem to depend on the intrinsic surface properties of the cells wall which involves the contribution of diffusion, sorption, chelation, complexation or micro-precipitation mechanisms (Guo et al. 2010).

Bacteria can interact with a range of toxic metals through differences between the net negative charge of bacteria and the cationic charge of many metals. It is



**Fig. 9.1** Spatial distribution of Cd in bacterial cells (*Pseudomonas* sp.). Qualitative and quantitative analyses of the elemental composition and location of Cd in bacterial cells of *Pseudomonas* sp. Analyses were performed using a JEM 1400 electron microscope (JEOL Co., Japan 2008) equipped with an X-ray microanalyser (EDS INCA Energy TEM, Oxford Instruments) and tomography system as well as a charge-coupled device (CCD) camera MORADA (SiS-Olympus). *CW* cell wall, *IC* inside the cell, *C* capsule

known that nucleation sites on the bacterial cell surface have the ability to bind metals of opposite charge (Monachese et al. 2012). However, analysis of potentiometric titration showed that changes in the pH of the environment can alter the cell surface charge and affect the ability of bacterial species to bind metal in solution (Fein et al. 2001). Fein and co-workers proposed (2010) that a neutral pH 7 has the optimum binding potential of heavy metal cations, since at this pH, reactive functional groups are not ionized. However, this is not valid for all bacterial species or all interactions with metals, since in many environments (e.g. acid mine tailings), bacterial species exist with the ability to not only survive in extreme pH conditions but also cope

with high metal concentrations that are toxic to humans and the majority of other species. It means that unique microbes have the ability to cope with metals through a variety of mechanisms but most notably through the precipitation of metal particles and active efflux (Monachese et al. 2012). The potential of specific/selected microorganisms to survive and grow in extreme heavy metal-polluted soils may be a useful feature for risk assessment and bioremediation of polluted sites (Lazzaro et al. 2008).

## 9.4 Microbial Technologies of Remediation

The potentials of microbes in metal remediation were reviewed by Rajendran et al. (2003). Microbial metal bioremediation can be an efficient strategy due to its low cost, high efficiency and eco-friendly nature. Microbes can effectively sequester heavy metals. They can partly tolerate high heavy metal concentrations and can develop high heavy metal-binding capacity. They can produce heavy metal-binding proteins in response to toxic heavy metal concentrations. Microbial bioimmobilization of metals in terrestrial and aquatic environments is promoted by the high surface to volume ratio of microorganisms. Furthermore, metal-related reactions catalysed by bacteria allow altering the physicochemical conditions in polluted substrates (Valls and Lorenzo 2002).

Microorganisms can be used as biosurfactants in metal-contaminated soils. The biosurfactant technology can be an effective and non-destructive method for bioremediation of soils polluted with Cd and Pb (Das et al. 2009). The efficiency of bacterial extraction of metals from polluted wastewater using metal-resistant bacteria was tested for strains of *Pseudomonas aeruginosa* by Nezhad et al. (2010). In this investigation, the strains were subjected to mutation to increase the inhibitory concentration of Cd for their growth. Nezhad et al. (2010) concluded from their results that the biomass of *P. aeruginosa* strains can be used for bioremediation of Cd-polluted industrial waste.

## 9.5 Engineering of Microorganisms in Remediation of Heavy Metals

The use of autochthonic microorganisms found in the environment, e.g. soil and water, pioneered the field of bioremediation and is still a main object of further improvements of this technology. However, the use of genetically engineered microorganisms (GEM) can significantly increase capabilities of bacteria to degrade environmental toxins and bind heavy metals (Monachese et al. 2012). Sayler and Ripp (2000) designed the *Pseudomonas fluorescens* strain KH44 which was able to sense toxic polycyclic aromatic hydrocarbons and degrade them. Application of GEM to increase heavy metal remediation in contaminated sites was based on the trans-



formation and expression of metallothionein by bacterial cells. Valls et al. (1998) successfully engineered metallothionein to be expressed on the surface of *E. coli* as an attempt to increase metal-binding sites, leading to increased Cd accumulation.

Recombinant bacterial sensors (biosensors) have been constructed and used for the determination of a broad range of toxic parameters, including, e.g. heavy metal pollutants. In the main mechanism of action, the toxic compound crosses the cell wall and cell membrane and then triggers a sensing element (in most cases, a promoter linked to a reporter gene), leading to the production of easily measurable reporter proteins. The most commonly used reporter proteins for optical detection in microbial systems are green fluorescent protein for fluorescence and bacterial luciferase for luminescence. For the construction of metal-sensing strains, the operons for metal resistance that some naturally occurring bacteria possess are often used as promoters (Woutersen et al. 2011). Biosensors based on luminescent bacteria provide a rapid, easily measurable response in the presence of relevant toxic (mixtures of) compounds and may be valuable tools for monitoring the chemical quality and safety of soil and drinking water. The genes used most often for construction of recombinant bacterial strains are luciferase genes *lux* (Woutersen et al. 2011). Bacterial biosensors with *lux* genes can detect contaminations ranging from milligrams per litre to micrograms per litre. The sensitivity of *lux* strains can be enhanced by various molecular manipulations; however, most reported detection thresholds are still too high to detect levels of individual contaminants. Bacterial *lux* strains sensing specific toxic effects may also respond to mixtures of contaminants and, thus, could be used as a sensor for the sum effect (Woutersen et al. 2011).

Ivask et al. (2002) constructed recombinant luminescent bacterial sensors for the determination of the bioavailable fraction of cadmium, zinc, mercury, and chromium in soil. The sensors carried the firefly luciferase gene as a reporter under the control of zinc-, chromate- and mercury-inducible units. The specificity of the above sensors was determined by using different heavy metal compounds. They have revealed that the zinc and mercury sensors were not completely specific to the target metals. The zinc sensor was co-inducible with cadmium and mercury and the mercury sensor with cadmium. The chromate sensor was inducible not only by chromate but also with  $\text{Cr}^{3+}$ . In another experiment (Rasmussen et al. 2000), the *mer-lux* gene fusion in *E. coli* was applied for the estimation of the bioavailable fraction of mercury in soil. The *mer*-promoter was activated when  $\text{Hg}^{2+}$ , present in the cytoplasm of the biosensor bacterium, binds to *merR*, resulting in transcription of the *lux* genes and subsequent light emission. The luminescence-based bacterial sensor strains *P. fluorescens* OS8 (pTPT11) for mercury detection and *P. fluorescens* OS8 (pTPT31) for arsenite detection were used in testing their application in detecting heavy metals in soil extracts (Petanen and Romantschuk 2002). The sensor strain with pTPT31 appeared to have a useful detection range similar to that of chemical methods.

Strict regulatory guidelines of the Environmental Protection Agency make the use of GEM difficult, and a better understanding of how these microbes work and their safety and environmental containment is needed before they are used for bioremediation (Monachese et al. 2012).

In the end, it is worth mentioning that many plants have been genetically modified with microbial catabolic genes and specific transporters for increased remedial activities (Abhilash et al. 2012). For example, poplar plants carrying g-glutamyl-cysteine synthetase from *E. coli* accumulate higher concentrations of cadmium and copper (Bittsanszky et al. 2005).

## 9.6 Potentials and Limitations of Microbial Bioremediation of Heavy Metals

The food and water we consume are often contaminated with a range of heavy metals (e.g. lead and cadmium) that are associated with numerous diseases. The ability to prevent and manage this problem is still a subject of much debate, with many technologies being ineffective and others too expensive for practical large-scale use, especially for developing nations where major pollution occurs (Monachese et al. 2013).

Microbial-assisted phytoremediation is a reliable and dependable process. Microorganisms, especially rhizosphere and endophytic, can accelerate phytoremediation in metal-contaminated soils, e.g. by promoting plant growth/health and increasing the bioavailability of metals by plants. However, there are still many areas of poor understanding or lack of information. First of all, basic molecular mechanisms of microbial-assisted phytoremediation have to be still elucidated in order to speed up this process and to optimize the rate of mobilization/absorption/accumulation of pollutants by microorganisms. Many functional properties (e.g. adapting strategies, production of various metabolites, metal-resistant, biosorption as well as mobilization/immobilization mechanisms) of bacterial isolates, the factors required by bacteria to colonize the rhizosphere and/or interior tissues of the plant, promote plant growth, and metal uptake should be identified (Ma et al. 2011). The other problem is related with colonization and survival of microbial inoculums at natural sites and under different environmental conditions (Hryniewicz and Baum 2013). Moreover, implementation of microbial-assisted phytoremediation in the field level needs intensive future research on understanding the diversity and ecology of plant-associated microorganisms in multiple-metal-contaminated soils. Explanation of the role of naturally adapted indigenous microbes that have been cultured and enriched in the laboratory in the phytoremediation potential of various plants in multiple-metal-contaminated soils can also improve this technology (Ma et al. 2011).

New insight into ability of microorganisms to bind metals suggests that species such as *Lactobacillus*, present in the human mouth, gut and vagina and in fermented foods, have the ability to bind and detoxify pollutants. The gut microbiota have key roles in regulating digestion by providing enzymes required for metabolic breakdown by processing and metabolizing compounds as they enter the host through normal diet. However, so far we do not know what effect the gut microbiota may have on binding and sequestering metals, thus imparting protection to the host.

This is why understanding of detoxification mechanisms of lactobacilli and how, in the future, humans and animals might benefit from these organisms in remediating environmental contamination of food is, so far, a big challenge (Monachese et al. 2013).

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