Chapter

Bioremediation of Heavy Metals

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Abstract

Exposure to lead (Pb), zinc (Zn), cadmium (Cd), copper (Cu), and selenite (SeO\(^3^-\)) consider the main heavy metals that threat human health. These heavy metals can interfere with the function of vital cellular components. Soil heavy metal contamination represents risks to humans and the ecosystem through drinking of contaminated groundwater, direct ingestion or the food chain, and reduction in food quality. Bioremediation means cleanup of polluted environment via transformation of toxic heavy metals into less toxic form by microbes or its enzymes. Otherwise, bioremediation by microbes has limitations like production of toxic metabolites. The efflux of metal ions outside the cell, biosorption to the cell walls and entrapment in extracellular capsules, precipitation, and reduction of the heavy metal ions to a less toxic state are mechanisms to metals’ resistance.

Keywords: heavy metals, bioremediation, copper, lead, cadmium, selenite

1. Introduction

Since the industrial revolution, heavy metals’ waste has increased rapidly. Toxic metals’ species are mobilized from industrial activities and fossil fuel consumption and eventually are accumulated through the food chain, leading to both ecological and health problems. Some of these metals are taken up as essential nutrients since they are incorporated into enzymes and cofactors. Some heavy metals exert toxic effects on microbial cells (i.e., mercury, lead, cadmium, arsenic, and silver). Mostly, resistance systems have been found on plasmids, whereas bacterial chromosomes contain genes for resistance to many of the same heavy metals’ cations and oxyanions as do plasmids [1, 2]. To survive under metal-stressed conditions, bacteria have evolved several types of mechanisms to tolerate the uptake of heavy metal ions. These mechanisms include biosorption to the cell walls and entrapment in extracellular capsules, precipitation, the efflux of metal ions outside the cell, reduction of heavy metal ions to a less toxic state accumulation, and complexation of metal ions inside the cell [3, 4].

2. Copper bioremediation

In almost all life forms, copper is a metal essential for the normal function. It acts as a cofactor for a number of enzymes involved in respiration and electron transport proteins in plants, animals, and microorganisms. Copper is toxic to cells at high concentrations mainly due to the disruption of the integrity of cell membranes, its interaction with nucleic acids, interference with the energy transport system, and disruption of enzyme active sites [5–8]. At high cytoplasmic...
concentrations, copper can compete with other metals for their binding sites in proteins that can lead to dysfunctional proteins. Otherwise, the presence of Cu (I) in cells will react with hydrogen peroxide and produce hydroxyl radicals that will damage DNA, lipids, and other molecules [9, 10]. Resistance to copper in microorganisms is dependent mainly on three different systems:

1. The periplasmic plasmid-borne copper (pco) resistance system that encodes for PcoA, a multi-copper oxidase protein responsible for oxidation of Cu(I) in the periplasmic space. This system presents only on plasmids and presents high copper resistance [11–13].

2. The efflux ATPase pump CopA able to throw copper ions outside [10, 14].

3. Cus system (copper sensing copper efflux system) belonging to the resistance-nodulation-cell division (RND) family responsible for heavy metal export (HME-RND) that encodes especially for the CusA protein [10, 13, 15].

In agriculture, copper bactericide is considered one of the most important components in environmental contamination with copper especially in programs practiced worldwide in growing areas with citrus [16]. Many species of plant pathogenic bacteria such as Xanthomonas citri subsp. citri (Xcc) have developed resistance to copper as a consequence of using copper bactericides [5]. Copper resistance genes have taken place from strains of X. alfalfae subsp. citrusmelonis from Florida and Xanthomonas citri subsp. citri from Argentina [17].

In both X. citri subsp. citri and X. alfalfae subsp. citrusmelonis, the long-term use of copper bactericides has led to the development of copper-resistant (Cur) strains. In X. citri subsp. citri A44, open reading frames (ORFs) related to the genes copL, copA, copB, copM, copG, copC, copD, and copF were characterized to be present on a large (~300 kb) conjugative plasmid. The same ORFs, except copC and copD, were also present in X. alfalfae subsp. citrusmelonis 1381 [5, 18]. Via molecular tools, the abundance of the copper resistance genes cusA and copA, encoding, respectively, for a Resistance Cell Nodulation protein and for a P-type ATPase pump, were assessed in Chilean marine sediment cores since, in the impacted sediment, copA gene was more abundant than cusA gene [10]. When Sulfolobus metallicus cells are exposed to 100 mM Cu, proteomic analysis showed that 18 out of 30 upregulated proteins are related to stress responses, the production and conversion of energy, and amino acid biosynthesis [19]. Furthermore, when searching the genome, two complete cop gene clusters encoding a Cu-exporting ATPase (CopA), metallochaperone (CopM), and a transcriptional regulator (CopT) were detected.

Based on a plate assay, Frankia strains EuI1c, CN3, QA3, and DC12 are tolerant to high levels of copper (MIC values >5 mM), while many other strains tested are very sensitive exhibiting MIC values <0.1 mM [20]. Otherwise, a 24-well growth assay was used to reexamine copper sensitivity of five Frankia strains. Frankia strains EuI1c, CN3, and DC12 showed similar growth patterns. Growth was initially inhibited at low copper concentrations (0.1 mM), but growth yield increased with elevated copper levels reaching a peak at 5 mM (Figure 1).

The cells grown in the elevated copper levels appeared blue which suggest that copper was accumulating inside of Frankia or binding to the cell surface [21]. When observed under phase-contrast microscopy, Cu^{2+}-resistant Frankia EuI1c formed unusual globular structures that were associated with their hyphae [20]. These structures were further investigated at a higher resolution. The increase resolution revealed that the globular structures are composed of aggregates (> 50 um) containing many smaller structures (Figure 2).
These smaller structures were about 5 μm in diameter and were also observed as individual structures throughout the hyphae. At higher magnification, the structures have a grooved pattern and appear connected to the hyphae by amorphous material [21]. Similar globular structures were observed with SEM of other copper-resistant
Frankia strains (e.g., strain DC12). These observations suggest that Frankia may precipitate the Cu\(^{2+}\)-phosphate complex to the hyphae. Acidithiobacillus ferrooxidans will detoxify Cu\(^{1+}\) metal by formatting phosphate granules through stimulation of polyphosphate hydrolysis and formation of metal-phosphate complexes [22].

The elemental composition analysis of these structures was investigated by the use of SEM-EDAX. As expected, these structures exhibited an elevated copper content that was represented by a 73-fold more than the control increase in the intensity but also contained an elevated phosphate content that was about 43.88-fold higher intensity level than the control cells. Furthermore, the oxygen content increased 3.5-fold under copper-stressed condition. All three of these elements had nearly the same intensity values under Cu\(^{2+}\) condition. These results suggest that a copper-phosphate compound forms and binds to Frankia cell surface. The EDAX spectra showed that the bodies present in the cells were mainly composed of phosphorus and oxygen [8, 21]. The highly sensitive MS analysis of excised bands produced peptides such as periplasmic binding protein/LacI transcriptional regulator (E3IXA6; FraEuI1c_7040 gene) with the appropriate protein size (37.6 kDa). Another protein of interest was the sulfate ABC transporter, a periplasmic sulfate-binding protein (E3J029; FraEuI1c_1092; 36.6 kDa) which had 2, 5, and 20 peptides. These data would indicate a tenfold increase in expression under 2 mM Cu\(^{2+}\)-stress, while the extracellular ligand-binding receptor (39.925 kDa) induced up to six- and eightfold under 1 and 2 mM copper, respectively. These proteins may be playing a role in copper resistance through binding and accumulating copper as in the periplasmic binding protein/LacI transcriptional regulator and extracellular ligand-binding receptor or transporting copper outside the cell as in sulfate ABC transporter, periplasmic sulfate-binding receptor. The relative expression of the heavy metal transporter/detoxification gene (FraEuI1c_6308) and copper-translocating P-type ATPase (FraEuI1c_6307) has shown 30- to 35-fold increase in the level of expression compared to the control under Cu\(^{2+}\)-stress for 8 days. These results suggest that these two gene products may play a role in copper tolerance [21].

In some bacteria and algae, it has been proposed that inorganic polyphosphates and transport of metal-phosphate complexes will participate in heavy metal tolerance [23]. After the Frankia grew under copper condition, the level of phosphate in EDAX analysis was high which would support this hypothesis of the formation of a metal-phosphate complex. This complex could be effluxed outside the cell via P-type ATPase or phosphate efflux system [22]. In Enterococcus hirae, CopA functions to import copper when it is deficient [24]. With Pseudomonas syringae, CopA is an outer membrane protein and functions in the sequestration and compartmentalization of copper in the periplasm and outer membrane [25]. The function of the CopB protein in E. hirae is to remove excess copper present in the cytoplasm [24]. The specific function of CopB protein in E. coli and Pseudomonas syringae is not yet defined [26]. With E. hirae, copA and copB are involved in copper transport using ATPases, while copY gene product acts as a copper-responsive repressor. The copZ functions in the transport of intracellular copper.

3. Lead bioremediation

Lead enters the cells through Fe\(^{2+}\) and Ca\(^{2+}\) transporters and then exerts its toxicity by displacing these cations at their binding sites in metalloproteins. Heavy metal resistance systems in many bacterial are based on efflux. Two groups of efflux systems have been recognized in gram-negative bacteria which are chemiosmotic pumps, e.g., the three-component divalent-cation efflux systems of Ralstonia metallidurans (cnr, ncc, and czc [27] and/or P-type ATPases, e.g., the Zn(II), Cu(II),
and Cd(II) ATPases [28, 29]. In both gram-negative and gram-positive bacteria, lead resistance has been reported in lead-contaminated soils. *Bacillus megaterium* demonstrating intracellular cytoplasmic leads to accumulation and *Pseudomonas marginalis* showing extracellular leads to exclusion [30]. Furthermore, the *Staphylococcus aureus* and *Citrobacter freundii* accumulated the metal as an intracellular lead-phosphate [31]. CadA ATPase of *Staphylococcus aureus* and the ZntA ATPase of *Escherichia coli* have been reported as efflux of Pb(II) [32].

Furthermore, 27 isolates were isolated from some abandoned mining areas in Morocco and found to belong to *Streptomyces* and *Amycolatopsis* genera. The minimum inhibitory concentration (MIC) recorded was 0.1 mg·mL$^{-1}$ for both Zn and Cu, 0.55 for Pb, and 0.15 for Cr. Chemical precipitation assay revealed that the 27 isolates have a strong ability to accumulate Pb (up to 600 mg of Pb/g of biomass for *Streptomyces* sp. BN3) [33].

Interplay between CBA transporters and P-type ATPases in *Cupriavidus metallidurans* CH34 for zinc and cadmium resistance is reported [34]. The pbrTRABCD gene cluster from *Cupriavidus metallidurans* CH34 revealed that export of Zn$^{2+}$, Cd$^{2+}$ and Pb$^{2+}$ was via the main transporter component of the operon P-type ATPase PbrA, whereas PbrB, the second component of the operon, was shown to be a phosphatase that increased lead resistance. P-type ATPase that removes Pb$^{2+}$ ions from the cytoplasm and a phosphatase that produces inorganic phosphate for lead sequestration in the periplasm represent the new lead resistance model in *Cupriavidus metallidurans* CH34. In several different bacterial species and when searching databases, gene clusters containing neighboring genes for P-type ATPase and phosphatase were detected which suggest that Pb$^{2+}$ detoxification via active efflux and sequestration may be a widespread mechanism of resistance [34]. In *Pseudomonas putida* KT2440, two P-type ATPases and two CBA transporters exhibited that resistance mechanisms for Zn$^{2+}$ and Cd$^{2+}$ are somewhat different than for Pb$^{2+}$ since Zn$^{2+}$ and Cd$^{2+}$ cannot be sequestered as insoluble compounds easily [32, 34].

A group of transporters, the cation diffusion facilitator family (CDF), can catalyze heavy metal influx or efflux in both prokaryotes and eukaryotes. All characterized CDF proteins to date can transport metals only (such as Fe$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Zn$^{2+}$ and Cd$^{2+}$), in contrast to other protein families, such as P-type ATPases or CBA transporters. In *C. metallidurans*, CDF family of chemiosmotic efflux systems with the CzcD Cd$^{2+}$ and Zn$^{2+}$ efflux system was first described [34]. CDF transporters provide very low resistance level, but it plays a main role in heavy metal buffer at low concentration of the metal in the cell cytoplasmic [34].

Detoxification mechanism for Pb$^{2+}$ can also be achieved by sequestration. In several bacterial species and via the use of intra- and extracellular binding of Pb$^{2+}$, they can avoid toxicity as in *S. aureus*, *Citrobacter freundii* [35, 31], and *Vibrio harveyi* [36] by precipitating lead as a phosphate salt. Mainly through exopolysaccharides (EPSs), binding of heavy metals can take place. EPS could act as a biosorbent of free metal ions, but it cannot be considered as inducible resistance mechanism in response to metals [34].

Nine candidate core biomarker genes might be tightly correlated with the response or transport of heavy metals. These genes, namely, *NILR1, PGPS1, WRKY33, BCS1, AR781, CYP81D8, NR1, EAP1*, and *MYB15*. The same expression trend and response to different stresses (Cd, Pb, and Cu) by experimental results have been shown [37].

The mechanism of lead resistance in *Frankia* sp. strain EAN1pec has been reported which include cells’ accumulated Pb$^{2+}$ with saturation kinetics (**Figure 3**). The Cu$^{2+}$-ATPase and cation diffusion facilitator (CDF) in addition to several hypothetical transporters were upregulated under lead stress that may indicate
metal export. Furthermore, a potential transcription factor (DUF156) binding site associated with several proteins was identified with heavy metals [38]. The EDAX results showed much high proportion of phosphate in Frankia cultures exposed to higher Pb$^{2+}$ concentrations which could indicate different Pb$_x$(PO$_4$)$_x$ compounds formed, which bind to Frankia cell surface [38].

4. Cadmium bioremediation

Cadmium (Cd$^{2+}$), the heavy metal, is toxic in its ionized form to microbes and humans. It is found in the biosphere and often associated with zinc ores at concentrations approaching 0.01–1.8 ppm. It can enter the bacterial cell normally by essential divalent cations via transport systems. Cadmium toxicity has effect by inhibiting respiration via binding to essential proteins’ sulphydryl groups and can also cause single-strand breakage of DNA in E. coli [39].

The full resistance to Cd$^{2+}$ required the interplay of a P-type ATPase that exported cytoplasmic ions to periplasm and a CBA transporter that further exported periplasmic ions to the outside. Furthermore, membrane transport pumps export metal ions from the cell and binding factors involved in creating tolerance to heavy metal ions through detoxify metals by sequestration (i.e., cell wall components (exopolysaccharides) and intracellular binding proteins (like metallothioneins and metallochaperones)) [34]. As cytoplasmic metal cation-binding proteins, metallothioneins can lower the concentrations of free ion in the cytoplasm. SmtA from Synechococcus PCC 7942 was the first metallothionein characterized in bacteria and can sequester and detoxify Zn$^{2+}$ and Cd$^{2+}$. Otherwise, SmtB is a repressor which can dissociate from DNA in the presence of metals [40–43].

In Streptococcus thermophilus Strain 4134, two genes (cadCSt and cadASt) were confirmed to constitute in cadmium/zinc resistance. P-type cadmium efflux ATPases are the proposed product of the cadA open reading frame (CadASt), whereas ArsR-type regulatory proteins are the predicted proteins encoded by cadCSt (CadCSt) [39]. The plasmid-encoded cad system in S. aureus is the best characterized Cd(II) resistance efflux system. CadA functions as an efflux pump that exports Cd(II) from the cell interior [44–46]. The gene product of cadC binds Cd(II) as it proposed inside the organism since cadC can bind two Cd(II) ions via a pair of cysteine residues. It is proposed that cadA takes Cd(II) from cadC in the cytoplasmic membrane [47]. cadD, the cadmium resistance gene, has been identified in a two-component operon which contains the resistance gene cadD and an inactive regulatory gene, cadX, from the Staphylococcus aureus plasmid pRW001.
[48]. ZntA, the metal-dependent ATP hydrolysis activity which exports Cd(II), Pb(II), and Zn(II) from *Escherichia coli*, is a cation-translocating ATPase. ZntA expression is mediated by transcriptional regulator protein ZntR, belonging to the MerR family. Based on in vitro molecular cloning analysis and in silico studies, P\textsubscript{cadR} and CadR are active in the presence of Cd with the highest binding affinity between the CadR protein and P\textsubscript{cadR} [45, 49].

The *Frankia* strain ACN14a and EuI1c genome was first searched for cadmium-binding motifs in COG and Pfam databases. A BLASTP analysis was performed on the *Frankia* ACN14a and EuI1c genome using the known CadA proteins as a query sequence. Blasting the published *Frankia* genomes against functionally identified CadA and putative cobalt-zinc-cadmium resistance amino acid sequences has revealed two possible genes (FRAAL0989 and FRAAL3628).

The identified gene which is *CadA* (FRAAL0989) in *Frankia* ACN14a is predicted to encode cation-transporting P-type ATPase A that possess cadmium and zinc outside the cells (Figure 4). Moreover, the putative cobalt-zinc-cadmium resistance (FRAAL3628) in the same strain is expected to work as transmembrane protein and consider cobalt-zinc-cadmium efflux system protein.

5. Selenite reduction

Selenium, in the form of selenocysteine or selenomethionine, is found in several stress proteins including glutathione peroxidase, alkyl hydroperoxidase, and multiple disulfide reductases. The deprotonated electrons of selenium cofactors make the selenoproteins’ reduction–oxidation reactive, explaining why many identified selenoproteins are involved in thiol and oxidative stress resistance. Since selenite generates these stresses in the cell, the stress-related selenoproteins may function doubly in detoxification and removal of free selenite ions from the cytoplasm. About 20% of sequenced bacteria contain selenoproteins [50].

The detoxification mechanism of selenite reduction in aerobic condition by microorganisms is not yet fully elucidated. Previously, it has been reported that selenite reduction may be catalyzed by a periplasmic nitrate reductase as in a selenate reductase, a periplasmic nitrate reductase in *Thauera selenatis* [51], a molybdenum–dependent membrane-bound enzyme of *Enterobacter cloacae* SLD1a-1 [52], *Thiosphaera pantotropha* [53], a periplasmic cytochrome B in *Thauera selenatis* [54], or a hydrogenase 1 of *Clostridium pasteurianum* [55]. Recent studies have indicated that NADPH-/NADH-dependent selenate reductase enzymes bring about the reduction of selenium (selenite/selenate) oxyanions. Selenite can be reduced to inert elemental selenium, which occurs in the selenite-resistant *Frankia* strains CN3, EuI1c, EUN1f, and DC12 [20].

However, all of the *Frankia* genomes contained synthase proteins for small thiols like mycothiol (MSH), which may substitute for glutathione for metal resistance.
Selenium can also be reduced enzymatically either using thioredoxin and its reductase (TrxA and TrxB) or other oxyanion reductases, whereas selenite reduction by fumarate reductase (FccA) in the periplasm was identified in *Shewanella oneidensis* MR-1 [56]. *Frankia* CN3 has a second type of nitrate reductase (NasC) located with the nitrite reductases (NirBD) that may also contribute to its greater selenite resistance.

Many bacteria including *Enterobacter cloacae* SLD1a-1, *Bacillus megaterium*, *Comamonas testosteroni* S44, *Thauera selenatis*, *Rhodopseudomonas palustris* Strain N, and *Bacillus cereus* form nanospheres of Se° during selenite or selenate reduction [57–63], whereas under aerobic conditions, *B. cereus* reduces selenite to Se° nanospheres in the size range of 150–200 nm [64]. *Rhodospirillum rubrum* is postulated to efficiently transport elemental selenium out of the cell [65]. This hypothesis is supported by the results of ultracentrifugation experiments showing that the buoyant density of cells increases in the presence of selenite during the reduction phase.

With *Desulfovibrio desulfuricans*, selenium-containing particles are postulated to be formed in the cytoplasm. However, the red elemental selenium that accumulates in the media during the stationary growth phase is released, the result of cell lysis. On the surfaces of *E. cloacae* cells grown in the presence of selenite, more or less spherical protrusions were observed [66]. Selenium-containing particles were observed in the culture medium, but intracellular Se° was not detected in this study. Selenite reduction was suggested to occur via a membrane-associated reductase that was followed by rapid expulsion of the Se particles. Our data shows extra- and intracellular nanosphere particles which may be transported through the membrane. My hypothesis is that the small particles are transported out of the cell and then form the large particles observed in the culture medium by extracellular aggregation. This postulated mechanism of transport would require an extremely large amount of energy.

In summary, selenite resistance may result from oxidation of selenite to the less toxic selenate using SorA. *Frankia* selenite resistance is likely due to alternate sulfate transporters (CysPUWA) that prevent sulfur starvation. The selenite reduction observed in resistant strains could occur through several mechanisms including NasC/NirBD or mycothiol, TrxA/B, and YedY.

*Frankia* strain EuI1c showed a pattern of resistance to selenite. Growth steadily decreased as selenite levels elevated reaching a plateau at 3 mM that remained constant up to 8 mM. Strain EuI1c showed a MTC value of <0.1 mM, while the MIC was 3 mM. Strain CN3 showed a different overall pattern and a modest level of selenite resistance. This strain was more sensitive to 0.1 mM levels than higher levels (1–5 mM). Both of these strains formed a reddish cell suspension in the 24-well plates. These results indicate the reduction of the toxic, soluble, and colorless sodium selenite (Na$_2$SeO$_3^{2-}$) to the nontoxic, insoluble, and red-colored elemental selenium form (Se°). The red color development started to appear in these cultures after the 48-h incubation [67]. Visual observation of the cultures implies that *Frankia* will reduce colorless selenite to red-colored elemental selenium, which is nontoxic and insoluble [60]. Cells exposed to 0.1 mM selenite reduced completely all of the selenite at day 5. At 0.5 mM selenite, the Se° production initiated at day 3 and ended at day 8. However, at 1 mM culture, Se° production initiated at day 3 and reached saturation at day 7.

When examined under scanning electron microscope, selenite-resistant *Frankia* EuI1c formed spherical nanospheres (Figure 5). These nanospheres were associated with the hyphae outside the cell as free deposits and also appeared as aggregates attached to the hyphae mass. Furthermore, these spherical particles also appear to be located inside the hyphae. The mentioned nanospheres may composed from reduced formed Se°. Since the reduction may occur in the cytoplasm, the nanoparticles would be exported outside. These nanospheres were observed in different sizes in the nanometer range [67, 68].
The elemental composition analysis of these nanospheres was investigated by the use of SEM-EDAX. As predicted, these nanospheres exhibited an elevation.

Three absorption peaks in EDAX analysis at 1.37 keV (peak SeLα), 11.22 keV (peak SeKα), and 12.49 keV (peak SeKβ) can be produced from selenium absorption. The first peak is related to 1.37 keV (peak SeLα) (keV = kilo electron Volt), whereas the second peak is met with 11.22 keV (peak SeKα) [61, 64].

6. Conclusion

Heavy metals are harmful to human health via interference with the function of vital cellular components. Lead (Pb), cadmium (Cd), copper (Cu), and selenite (SeO₃²⁻) are metals and metalloids that are widespread in the environment. P-type ATPase system that exported cytoplasmic ions to the periplasm and a CBA transporter that further exported periplasmic ions to the outside are general mechanisms in resistance Co, Pb, and Cd. Furthermore, in metals detoxification by sequestration, binding factors will be involved in creating tolerance to heavy metal ions.

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Conflict of interest

The authors declare no conflict of interest.
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References

[1] Igiri BE, Okoduwa SIR, Idoko GO, Akabuogu EP, Adeyi AO, Ejiogu IK. Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: A Review. Journal of Toxicology. 2018;2018:16

[2] Mohamed RM, Abo-Amer AE. Isolation and characterization of heavy-metal resistant microbes from roadside soil and phylloplane. Journal of Basic Microbiology. 2012;52(1):53-65

[3] Nies DH. Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiology Reviews. 2003;27(2-3):313-339

[4] Nies DH. Microbial heavy-metal resistance. Applied Microbiology and Biotechnology. 1999;51(6):730-750

[5] Behlau F, Canteros BI, Jones JB, Graham JH. Copper resistance genes from different xanthomonads and citrus epiphytic bacteria confer resistance to Xanthomonas citri subsp. citri. European Journal of Plant Pathology. 2012;133(4):949-963

[6] García-Horsman JA, Barquera B, Rumbley J, Ma J, Gennis RB. The superfAMILY of heme-copper respiratory oxidases. Journal of Bacteriology. 1994;176(18):5587-5600

[7] Cervantes C, Gutierrez-Corona F. Copper resistance mechanisms in bacteria and fungi. FEMS Microbiology Reviews. 1994;14(2):121-137

[8] Rehan M. Microbial bioremediation: A Review. Journal of Agricultural and Veterinary Sciences. 2017;10(2):147-162

[9] Harrison MD, Jones CE, Soliz M, Dameron CT. Intracellular copper routing: The role of copper chaperones. Trends in Biochemical Sciences. 2000;25(1):29-32

[10] Besaury L, Bodilis J, Delgais F, Andrade S, De la Iglesias R, Ouddane B, et al. Abundance and diversity of copper resistance genes cusA and copA in microbial communities in relation to the impact of copper on Chilean marine sediments. Marine Pollution Bulletin. 2013;67(1-2):16-25

[11] Brown NL, Barrett SR, Camakaris J, Lee BT, Rouch DA. Molecular genetics and transport analysis of the copper-resistance determinant (pco) from Escherichia coli plasmid pRJ1004. Molecular Microbiology. 1995;17(6):1153-1166

[12] Gittins JR. Cloning of a copper resistance gene cluster from the cyanobacterium Synechocystis sp. PCC 6803 by recombineering recovery. FEBS Letters. 2015;589(15):1872-1878

[13] Staehlin BM, Gibbons JG, Rokas A, O’Halloran TV, Slot JC. Evolution of a heavy metal homeostasis/Resistance Island reflects increasing copper stress in Enterobacteria. Genome Biology and Evolution. 2016;8(3):811-826

[14] Rensing C, Fan B, Sharma R, Mitra B, Rosen BP. CopA: An Escherichia coli Cu(I)-translocating P-type ATPase. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(2):652-656

[15] Piddock LJ. Multidrug-resistance efflux pumps—Not just for resistance. Nature Reviews. Microbiology. 2006;4(8):629-636

[16] Leite RP, Mohan SK. Integrated management of the citrus bacterial canker disease caused by Xanthomonas campestris pv. Citri in the state of Paraná, Brazil. Crop Protection. 1990;9(1):3-7

[17] Behlau F, Hong JC, Jones JB, Graham JH. Evidence for acquisition of copper resistance genes from
different sources in citrus-associated xanthomonads. Phytopathology. 2013;103(5):409-418

[18] Behlau F, Canteros BI, Minsavage GV, Jones JB, Graham JH. Molecular characterization of copper resistance genes from Xanthomonas citri subsp. citri and Xanthomonas alfalfae subsp. citrumeloni. Applied and Environmental Microbiology. 2011;77(12):4089-4096

[19] Orell A, Remonsellez F, Arancibia R, Jerez CA. Molecular characterization of copper and cadmium resistance determinants in the biomining Thermoacidophilic archaeon Sulfolobus metallicus. Archaea. 2013;2013:16

[20] Richards JW, Krumholz GD, Chval MS, Tisa LS. Heavy metal resistance patterns of Frankia strains. Applied and Environmental Microbiology. 2002;68(2):923-927

[21] Rehan M, Furnholm T, Finethy RH, Chu F, El-Fadly G, Tisa LS. Copper tolerance in Frankia sp. strain EuI1c involves surface binding and copper transport. Applied Microbiology and Biotechnology. 2014;98(18):8005-8015

[22] Alvarez S, Jerez CA. Copper ions stimulate polyphosphate degradation and phosphate efflux in Acidithiobacillus ferrooxidans. Applied and Environmental Microbiology. 2004;70(9):5177-5182

[23] Remonsellez F, Orell A, Jerez CA. Copper tolerance of the thermoacidophilic archaean Sulfolobus metallicus: Possible role of polyphosphate metabolism. Microbiology. 2006;152(Pt 1):59-66

[24] Solioz M, Stoyanov JV. Copper homeostasis in enterococcus hirae. FEMS Microbiology Reviews. 2003;27(2-3):183-195

[25] Mellano MA, Cooksey DA. Nucleotide sequence and organization of copper resistance genes from pseudomonas syringae pv. Tomato. Journal of Bacteriology. 1988;170(6):2879-2883

[26] Masami N, Masao G, Katsumi A, Tadaaki H. Nucleotide sequence and organization of copper resistance genes from pseudomonas syringae pv. Actinidiae. European Journal of Plant Pathology. 2004;110(2):223-226

[27] Taghavi S, Mergeay M, Nies D, van der Lelie D. Alcaligenes eutrophus as a model system for bacterial interactions with heavy metals in the environment. Research in Microbiology. 1997;148(6):536-551

[28] Silver S, Nucifora G, Phung LT. Human Menkes X-chromosome disease and the staphylococcal cadmium-resistance ATPase: A remarkable similarity in protein sequences. Molecular Microbiology. 1993;10(1):7-12

[29] Borremans B, Hobman JL, Provooost A, Brown NL, van Der Lelie D. Cloning and functional analysis of the pbr lead resistance determinant of Ralstonia metallidurans CH34. Journal of Bacteriology. 2001;183(19):5651-5658

[30] Roane TM. Lead resistance in two bacterial isolates from heavy metal-contaminated soils. Microbial Ecology. 1999;37(3):218-224

[31] Levinson HS, Mahler I. Phosphatase activity and lead resistance in Citrobacter freundii and Staphylococcus aureus. FEMS Microbiology Letters. 1998;161(1):135-138

[32] Rensing C, Sun Y, Mitra B, Rosen BP. Pb(II)-translocating P-type ATPases. The Journal of Biological Chemistry. 1998;273(49):32614-32617

[33] El Baz S, Baz M, Barakate M, Hassani L, El Gharmali A, Imziln B, et al. Resistance to and accumulation of
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DOI: http://dx.doi.org/10.5772/intechopen.88339

heavy metals by Actinobacteria isolated from abandoned mining areas. The Scientific World Journal. 2015;2015:14

[34] Hynninen A. Zinc, cadmium and lead resistance mechanisms in bacteria and their contribution to biosensing. 2010

[35] Levinson HS, Mahler I, Blackwelder P, Hood T. Lead resistance and sensitivity in Staphylococcus aureus. FEMS Microbiology Letters. 1996;145(3):421-425

[36] Mire CE, Tourjee JA, O'Brien WF, Ramanujachary KV, Hecht GB. Lead precipitation by Vibrio harveyi: Evidence for novel quorum-sensing interactions. Applied and Environmental Microbiology. 2004;70(2):855-864

[37] Niu C, Jiang M, Li N, Cao J, Hou M, Ni D-a, et al. Integrated bioinformatics analysis of As, Au, Cd, Pb and Cu heavy metal responsive marker genes through Arabidopsis thaliana GEO datasets. PeerJ. 2019;7:e6495

[38] Furnholm T, Rehan M, Wishart J, Tisa LS. Pb2+ tolerance by Frankia sp. strain EAN1pec involves surface-binding. Microbiology. 2017;163(4):472-487

[39] Schirawski J, Hagens W, Fitzgerald GF, Van Sinderen D. Molecular characterization of cadmium resistance in Streptococcus thermophilus strain 4134: An example of lateral gene transfer. Applied and Environmental Microbiology. 2002;68(11):5508-5516

[40] Olafson RW, McCubbin WD, Kay CM. Primary- and secondary-structural analysis of a unique prokaryotic metallothionein from a Synechococcus sp. cyanobacterium. The Biochemical Journal. 1988;251(3):691-699

[41] Turner JS, Morby AP, Whitton BA, Gupta A, Robinson NJ. Construction of Zn2+/Cd2+ hypersensitive cyanobacterial mutants lacking a functional metallothionein locus. The Journal of Biological Chemistry. 1993;268(6):4494-4498

[42] Turner JS, Glands PD, Samson AC, Robinson NJ. Zn2+—sensing by the cyanobacterial metallothionein repressor SmtB: Different motifs mediate metal-induced protein-DNA dissociation. Nucleic Acids Research. 1996;24(19):3714-3721

[43] Naz N, Young HK, Ahmed N, Gadd GM. Cadmium accumulation and DNA homology with metal resistance genes in sulfate-reducing bacteria. Applied and Environmental Microbiology. 2005;71(8):4610-4618

[44] Oger C, Berthe T, Quillet L, Baray S, Chiffoleau JF, Petit F. Estimation of the abundance of the cadmium resistance gene cadA in microbial communities in polluted estuary water. Research in Microbiology. 2001;152(7):671-678

[45] Prabhakaran R, Rajkumar SN, Ramprasad T, Selvam GS. Identification of promoter PcadR, in silico characterization of cadmium resistant gene cadR and molecular cloning of promoter PcadR from Pseudomonas aeruginosa BC15. Toxicology and Industrial Health. 2018;34(12):819-833

[46] Oger C, Mahillon J, Petit F. Distribution and diversity of a cadmium resistance (cadA) determinant and occurrence of IS257 insertion sequences in staphylococcal bacteria isolated from a contaminated estuary (Seine, France). FEMS Microbiology Ecology. 2003;43(2):173-183

[47] Bruins MR, Kapil S, Oehme FW. Microbial resistance to metals in the environment. Ecotoxicology and Environmental Safety. 2000;45(3):198-207

[48] Crupper S, Worrell V, Stewart G, Iandolo J. Cloning and expression of
cadD, a new cadmium resistance gene of *Staphylococcus aureus*. Journal of Bacteriology. 1999;181(13):4071-4075

[49] Binet MR, Poole RK. Cd(II), Pb(II) and Zn(II) ions regulate expression of the metal-transporting P-type ATPase ZntA in Escherichia coli. FEBS Letters. 2000;473(1):67-70

[50] Zhang Y, Gladyshev VN. General trends in trace element utilization revealed by comparative genomic analyses of Co, Cu, Mo, Ni, and Se. The Journal of Biological Chemistry. 2010;285(5):3393-3405

[51] Schroder I, Rech S, Krafft T, Macy JM. Purification and characterization of the selenate reductase from *Thauera selenatis*. The Journal of Biological Chemistry. 1997;272(38):23765-23768

[52] Watts CA, Ridley H, Condie KL, Leaver JT, Richardson DJ, Butler CS. Selenate reduction by Enterobacter cloacae SLD1a-1 is catalysed by a molybdenum-dependent membrane-bound enzyme that is distinct from the membrane-bound nitrate reductase. FEMS Microbiology Letters. 2003;228(2):273-279

[53] Berks BC, Richardson DJ, Robinson C, Reilly A, Aplin RT, Ferguson SJ. Purification and characterization of the periplasmic nitrate reductase from Thiosphaera pantotropha. European Journal of Biochemistry. 1994;220(1):117-124

[54] Krafft T, Bowen A, Theis F, Macy JM. Cloning and sequencing of the genes encoding the periplasmic-cytochrome B-containing selenate reductase of *Thauera selenatis*. DNA Sequence. 2000;10(6):365-377

[55] Yanke LJ, Bryant RD, Laishley EJ. Hydrogenase I of clostridium pasteurianum functions as a novel selenite reductase. Anaerobe. 1995;1(1):61-67

[56] Li D-B, Cheng YY, Wu C, Li W-W, Li N, Yang Z-C, et al. Selenite reduction by Shewanella oneidensis MR-1 is mediated by fumarate reductase in periplasm. Scientific Reports. 2014;4:3735

[57] Javed S, Sarwar A, Tassawar M, Faisal M. Conversion of selenite to elemental selenium by indigenous bacteria isolated from polluted areas. Chemical Speciation & Bioavailability. 2015;27(4):162-168

[58] Kora AJ. *Bacillus cereus*, selenite-reducing bacterium from contaminated lake of an industrial area: A renewable nanofactory for the synthesis of selenium nanoparticles. Bioresources and Bioprocessing. 2018;5(1):30

[59] Mishra RR, Prajapati S, Das J, Dangar TK, Das N, Thatoi H. Reduction of selenite to red elemental selenium by moderately halotolerant bacillus megaterium strains isolated from Bhitaranika mangrove soil and characterization of reduced product. Chemosphere. 2011;84(9):1231-1237

[60] Yee N, Ma J, Dalia A, Boonfueng T, Kobayashi DY. Se(VI) reduction and the precipitation of Se(0) by the facultative bacterium Enterobacter cloacae SLD1a-1 are regulated by FNR. Applied and Environmental Microbiology. 2007;73(6):1914-1920

[61] Zheng S, Su J, Wang L, Yao R, Wang D, Deng Y, et al. Selenite reduction by the obligate aerobic bacterium Comamonas testosteroni S44 isolated from a metal-contaminated soil. BMC Microbiology. 2014;14(1):204

[62] Debieux CM, Dridge EJ, Mueller CM, Splatt P, Paszkiewicz K, Knight I, et al. A bacterial process for selenium nanoparticles assembly. Proceedings of the National Academy of Sciences. 2011;108(33):13480

[63] Li B, Liu N, Li Y, Jing W, Fan J, Li D, et al. Reduction of selenite
Bioremediation of Heavy Metals
DOI: http://dx.doi.org/10.5772/intechopen.88339

To red elemental selenium by Rhodopseudomonas palustris strain N. PLoS One. 2014;9(4):e95955

[64] Dhanjal S, Cameotra SS. Aerobic biogenesis of selenium nanospheres by Bacillus cereus isolated from coalmine soil. Microbial Cell Factories. 2010;9:52

[65] Kessi J, Ramuz M, Wehrli E, Spycher M, Bachofen R. Reduction of selenite and detoxification of elemental selenium by the phototrophic bacterium Rhodospirillum rubrum. Applied and Environmental Microbiology. 1999;65(11):4734-4740

[66] Losi ME, Frankenberger WT. Reduction of selenium oxyanions by Enterobacter cloacae SLD1a-1: Isolation and growth of the bacterium and its expulsion of selenium particles. Applied and Environmental Microbiology. 1997;63(8):3079-3084

[67] Rehan M, Alsohim AS, El-Fadly G, Tisa LS. Detoxification and reduction of selenite to elemental red selenium by Frankia. Antonie Van Leeuwenhoek. 2019;112(1):127-139

[68] Gonzalez-Gil G, Lens PNL, Saikaly PE. Selenite reduction by anaerobic microbial aggregates: Microbial community structure, and proteins associated to the produced selenium spheres. Frontiers in Microbiology. 2016;7:571