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Implications of Microbial Heavy Metal Tolerance in the Environment

Anne Spain[1], Communicated by: Dr. Elizabeth Alm[2]

Although some heavy metals are essential trace elements, most can be, at high concentrations, toxic to all branches of life, including microbes, by forming complex compounds within the cell. Because heavy metals are increasingly found in microbial habitats due to natural and industrial processes, microbes have evolved several mechanisms to tolerate the presence of heavy metals (by either efflux, complexation, or reduction of metal ions) or to use them as terminal electron acceptors in anaerobic respiration. Thus far, tolerance mechanisms for metals such as copper, zinc, arsenic, chromium, cadmium, and nickel have been identified and described in detail. Most mechanisms studied involve the efflux of metal ions outside the cell, and genes for this general type of mechanism have been found on both chromosomes and plasmids. Because the intake and subsequent efflux of heavy metal ions by microbes usually includes a redox reaction involving the metal (that some bacteria can even use for energy and growth), bacteria that are resistant to and grow on metals also play an important role in the biogeochemical cycling of those metal ions. This is an important implication of microbial heavy metal tolerance because the oxidation state of a heavy metal relates to the solubility and toxicity of the metal itself. When looking at the microbial communities of metal-contaminated environments, it has been found that among the bacteria present, there is more potential for unique forms of respiration. Also, since the oxidation state of a metal ion may determine its solubility, many scientists have been trying to use microbes that are able to oxidize or reduce heavy metals in order to remediate metal-contaminated sites.

Another implication of heavy metal tolerance in the environment is that it may contribute to the maintenance of antibiotic resistance genes by increasing the selective pressure of the environment. Many have speculated and have even shown that a correlation exists between metal tolerance and antibiotic resistance in bacteria because of the likelihood that resistance genes to both (antibiotics and heavy metals) may be located closely together on the same plasmid in bacteria and are thus more likely to be transferred together in the environment. Because of the prevalence of antibiotic resistant pathogenic bacteria, infectious diseases are becoming more difficult and more expensive to treat; thus we need to not only be more careful of the drastic overuse of antibiotics in our society, but also more aware of other antimicrobials, such as heavy metals, that we put into the environment.

INRODUCTION

This paper focuses on the general ways in which microbes interact with metals. Some bacteria have evolved mechanisms to detoxify heavy metals, and some even use them for respiration. Microbial interactions with metals may have several implications for the environment. Microbes may play a large role in the biogeochemical cycling of toxic heavy metals also in cleaning up or remediating metal-contaminated environments. There is also evidence of a correlation between tolerance to heavy metals and antibiotic resistance, a global problem currently threatening the treatment of infections in plants, animals, and humans.

Metal Tolerance Mechanisms

In high concentrations, heavy metal ions react to form toxic compounds in cells (Nies, 1999). To have a toxic effect, however, heavy metal ions must first enter the cell. Because some heavy metals are necessary for enzymatic functions and bacterial growth, uptake mechanisms exist that allow for the entrance of metal ions into the cell. There are two general uptake systems — one is quick and unspecific, driven by a chemiosmotic gradient across the cell membrane and thus requiring no ATP, and the other is slower and more substrate-specific, driven by energy from ATP hydrolysis. While the first mechanism is more energy efficient, it results in an influx of a wider variety of heavy metals, and when these metals are present in high concentrations, they are more likely to have toxic effects once inside the cell (Nies and Silver, 1995).

To survive under metal-stressed conditions, bacteria have evolved several types of mechanisms to tolerate the uptake of heavy metal ions. These mechanisms include the efflux of metal ions outside the cell, accumulation and complexation of the metal ions inside the cell, and reduction of the heavy metal ions to a less toxic state (Nies, 1999). Mergeay et al. (1985) tested the minimal inhibitory concentrations (MICs) of several different metal ions for *Escherichia coli* on agar medium, and the most toxic metal (with the lowest MIC) was mercury, whereas the least toxic metal tested was manganese (Table 1). Three examples of metal ions to which bacteria have evolved well-studied resistance mecha-

[1]University of Oklahoma; To whom correspondence should be addressed: aspain@ou.edu

[2]Central Michigan University, Dept. of Biology

Heavy Metal	MIC (mM)
Mercury	0.01
Silver	0.02
Gold	0.02
Chromium (Cr(VI))	0.2
Palladium	0.2
Platinum	0.5
Cadmium	0.5
Cobalt	1
Nickel	1
Copper	1
Zinc	1
Thallium	2
Uranium	2
Lanthanum	2
Yttrium	2
Scandium	2
Ruthenium	2
Aluminum	2
Lead	5
Iridium	5
Osmium	5
Antimony	5
Indium	5
Rhodium	5
Gallium	5
Chromium (Cr(II))	5
Vanadium	5
Titanium	5
Beryllium	5
Chromium (Cr(III))	10
Manganese	20

Table 1. Minimal inhibitory concentrations (MICs) of several heavy metals in *Escherichia coli*. The MICs were determined on an agar medium at different acidities, allowing for the dissolution of the metal ions. Minimal inhibitory concentrations refer to the smallest concentration necessary to inhibit growth; thus, lower MIC values indicate more toxic metals and higher MICs indicate less toxicity.

nisms – copper, zinc, and arsenic – are illustrated in this review.

Copper

Copper is used by cells in small quantities in cellular enzymes (e.g., cytochrome c oxidase). However, because copper is so widely used in mining, industry, and agriculture, high levels of copper may exist in some environments. As such, bacteria have evolved several types of mechanisms to resist toxicity due to high copper concentrations. With respect to the prevalence of copper resistance in the environment, Lin and Olson (1995) studied bacteria isolated from a water distribution system experiencing copper corrosion, and 62% were found to be copper resistant. Of these resistant bacteria, 49% had *cop* or *cop*-like gene systems, including both compartmentalization and efflux systems (Cooksey, 1993).

In the plant pathogen *Pseudomonas syringae*, resistance to copper via accumulation and compartmentalization in the cell's periplasm and outer membrane is due to four proteins encoded on the plasmid-borne *cop* operon (Cooksey, 1994). The proteins are found in the periplasm (CopA and CopC), the outer membrane (CopB), and the inner membrane (CopD) and work together to compartmentalize copper away from sensitive cellular functions. In *E. coli*, resistance to copper is based on an efflux mechanism by which copper is removed from the cell. The efflux proteins are expressed by plasmid-bound *pco* genes, which are in turn are dependent on the expression of chromosomal *cut* genes (Cooksey, 1993). Two *cut* genes (*cutC* and *cutF*) were identified by Gupta et al. (1995) and were shown to encode a copper-binding protein and an outer membrane lipoprotein. Cooksey (1993) also states that most bacterial species in the environment have acquired at least one of the aforementioned copper management systems, and that the evolution of copper resistance may have come about through the modification of copper uptake genes found on chromosomes.

Zinc

Zinc is another essential trace element. It is not biologically redox reactive and is thus not used in respiration. It is, however, important in forming complexes (such as zinc fingers in DNA) and as a component in cellular enzymes (Nies, 1999). Bacterial cells accumulate zinc by a fast, unspecific uptake mechanism and it is normally found in higher concentrations (but is less toxic) than other heavy metals (Nies, 1999). Uptake of zinc ions is generally coupled to that of magnesium, and the two ions may be transported by similar mechanisms in bacteria (Nies and Silver, 1995).

Two general efflux mechanisms are responsible for bacterial resistance to zinc. One is a P-type ATPase efflux[1] system that transports zinc ions across the cytoplasmic membrane by energy from ATP hydrolysis. A chromosomal gene, *zntA*, was isolated from *E. coli* K-12 and was found to be responsible for the ATPase that transports zinc and other cations across cell membranes (Beard et al., 1997). The other mechanism involved in zinc efflux is an RND-driven[2] transporter system that transports zinc across the cell wall (not just the membrane) of gram-negative bacteria and is powered by a proton gradient and not ATP (Nies, 1999).

[1] A P-type ATPase is defined as an ATPase that forms a phosphorylated intermediate while catalyzing a reaction (Nies and Silver, 1995).

[2] RND refers to a family of proteins that are involved in the transport of heavy metals (Nies, 1999).

Arsenic

Arsenic, which is not considered a heavy metal but rather a semi-metal with metallic and non-metallic properties, is toxic to bacteria, as well as other domains of life. For arsenic to have toxic effects, though, it first needs to be in a bioavailable form. Arsenic uptake by bacteria is mediated by phosphate transporters and is generally pumped back out of the cell by an efflux pump (Nies and Silver, 1995).

Several mechanisms for resistance to arsenic have been identified. Chen et al. (1986) proposed a model for the plasmid-mediated mechanism of the efflux of arsenate and arsenite in gram-negative bacteria. The nucleotide sequence of a fragment of DNA containing the *ars* operon[3] was studied, and three genes, *ars*A, *ars*B, and *ars*C, were found to encode for the proteins ArsA, ArsB, and ArsC, respectively. ArsA is a protein with ATPase activity and thus is involved in translocation of the metal ions across the cell membrane. ArsB interacts with ArsA on the inner membrane of the cell, and the two proteins form the arsenite pump. ArsC is a smaller protein that alters the specificity of the arsenite pump to allow for the efflux of arsenate. Thus, ArsC is only required for tolerance to arsenate, and ArsA and ArsB are required for tolerance to both species of arsenic.

Gladysheva et al. (1994) isolated and studied the protein ArsC encoded by the *arsC* gene on plasmid R773 and found that the protein actually catalyzes the reduction of arsenate to arsenite in *E. coli*, using NADPH as the reducing power; this suggests that the arsenite pump is not altered by the ArsC protein, but that it is rather the substrate (arsenate) is altered (or reduced) to fit the arsenite pump. Ji et al. (1994) isolated and purified another arsenate reductase protein; this one, however, was encoded by a gene on plasmid p1258 of *Staphylococcus aureus*, a gram-positive bacterium. This arsenate reductase (ArsR) was found to be active in the presence of thioredoxin and NADPH.

A chromosomal operon homologous to the *ars* operon found on plasmid R773 was identified in *E. coli* (Dioro et al., 1995), and was also responsible for encoding resistance to arsenic. Dioro suggests that this chromosomal operon might have been a precursor to plasmid-mediated arsenic resistance mechanisms involving the reduction of arsenate.

Arsenic Biogeochemistry – An Example of Microbial Interactions with Metals in the Environment

Because arsenic is also toxic to humans and is a known carcinogen, the United States Environmental Protection Agency (US EPA) has established a maximum contaminant concentration level of 50 μg/L of arsenic in drinking water, which is proposed to go down to 10 μg/L within several years (http://www.epa.gov/ogwdw000/ars/ars9.html, visited 1/08/01). Despite these mandates, however, arsenic contamination remains a worldwide threat. Arsenic concentrations are higher in groundwater than in surface water where the presence of arsenic is mainly due to dissolved minerals from weathered rocks and soils. The United States Geological Survey (USGS) found that 10% or more of groundwater in several counties in the Midwest and Northeast U.S. exceeded arsenic concentrations of 50 μg/L (http://co.water.usgs.gov/tace/arsenic, visited 1/08/01). Additionally, in groundwater from the area surrounding and including Hanoi, Vietnam, arsenic concentrations have been found to range from 1-3050 μg/L with an average concentration of 159 μg/L. In highly affected areas, arsenic concentrations averaged over 400 μg/L. Water analyzed after treatment processes had concentrations ranging from 25-91μg/L, but with 50% of wells tested still being over the 50 μg/L arsenic concentration standard (Berg et al., 2001). High arsenic concentrations pose a significant chronic health threat to millions drinking contaminated water, and in some groundwater, concentrations of arsenic are indeed high enough to allow for arsenic resistance mechanisms in microbes to remain ecologically favorable.

Many studies have been done on microbial metabolism of arsenic in aquatic environments and the effects microbes have on the speciation and mobilization of arsenic. Since aquatic sediments can be anaerobic, and because arsenic concentrations in sediments can range from 100-300 μg/L, microbe-mediated arsenic reduction may be common. Brannon and Patrick (1987) found that the addition of arsenate to an anaerobic sediment resulted in the accumulation of arsenite, indicating the reduction of arsenate to arsenite by microbes. Ahmann et al. (1997) further showed that native microorganisms from the Aberjona watershed were, in fact, responsible for the arsenic flux in the anoxic contaminated sediments. Harrington et al. (1998) also demonstrated the ability of microbes in sediments from Coeur d'Alene Lake to reduce arsenate. In reducing conditions, it was found that arsenite was the dominant form of arsenic. They also found that dissimilatory iron-reducing bacteria (DIRB) and sulfate-reducing bacteria (SRB) are capable of both arsenic reduction and oxidation and thus may contribute to the cycling of arsenic in sediments.

Microbial reduction of arsenate in aquatic sediments is important because arsenite (the reduced form) is more toxic and more soluble (and thus, more mobile) than arsenate, which forms relatively insoluble, non-bioavailable compounds with ferrous oxides and manganese oxides. Speciation of arsenic is affected or controlled by not only oxidation and reduction processes by microbes, but also by methylation by microbes, and adsorption to other particles (Aurilio et al., 1994). It was found that DIRB responsible for the dissolution of iron oxides bound to arsenic can also free soluble arsenic into the sediment (Cummings et al., 1999). Another study done on arsenic biogeochemistry in Lake Biwa Japan showed that arsenic concentration and speciation may also depend on eutrophication[4] (Sohrin et al, 1997).

[3] *ars* operon refers to the operon found on plasmid R773 in gram-negative bacteria that encodes for the efflux of arsenate and arsenite

Many scientists have sought microbial community members responsible for arsenate reduction. Hoeft et al. (2002) found that, in the anoxic water of Mono Lake (California), two subgroups (Sulfurospirillium and Desulfovibrio) of the Proteobacteria lineage were present and most likely using arsenate as an electron acceptor for growth. They also found an interesting cycling of arsenic occurring; the presence of nitrate rapidly re-oxidized any arsenate that had been produced. Thus, in some environments, both oxidation and reduction of arsenic may occur. In another study of aerobic contaminated mine tailings, it was found that members of the *Caulobacter*, *Sphingomonas*, and *Rhizobium* families may be responsible for the reduction and mobilization of arsenic (Macur et al., 2001).

While it has been shown that microbes are capable of arsenic reduction, the question remains whether microbes reduce arsenate for detoxification purposes (as described in the section on arsenic tolerance mechanisms) or for growth during anaerobic respiration. In a sample of agricultural soil, it was determined that the reduction of arsenate was not involved in respiration because rates of arsenate reduction did not contribute to microbial growth (Jones et al., 2000). Thus, arsenate reduction in this case is probably due to intracellular detoxification by mechanisms, similar to those described in *E. coli* and *S. aureus*. Conversely, Laverman et al. (1995) showed that the bacterial strain SES-3 could grow using a diversity of electron acceptors, including Fe(III), thiosulfate, and arsenate coupled to the oxidation of lactate to acetate. Another study reported the growth of strain MIT-13 (isolated from the Aberjona watershed) by using arsenate as an electron acceptor, and the inhibition of arsenate reduction by molybdate (Ahmann et al., 1994). Additionally, an organism from the genus *Desulfitobacterium* isolated from lake Coeur d'Alene was shown to reduce arsenate, but it was not determined whether this reduction supported growth (Niggemeyer et al., 2001). From arsenic-contaminated mud from Australia, however, a *Bacillus* strain was isolated and characterized as being able to respire with arsenate (Santini et al., 2002).

Uranium Reduction – An Example of Microbial Metal Bioremediation

Because of radionuclides present in soils and groundwater due to nuclear waste during the Cold War Era, much effort has been put forth to see whether microbes can contribute to remediation by reducing and immobilizing toxic metals, such as uranium (Francis and Dodge, 1998). It has been shown that DIRB in the family *Geobacteraceae* are involved in uranium reduction in contaminated aquifer sediments (Holmes et al., 2002) and also in technetium reduction (Lloyd et al., 2000). Certain sulfate reducers have also been shown to be capable of reducing uranium; in *Desulfovibrio vulgaris*, it was shown that cytochrome c_3 was responsible and necessary for uranium reduction activity (Lovley et al., 1993). Additionally, members of *Clostridium* species have been shown to reduce uranium under anaerobic conditions (Francis et al., 1994). This has implications in the bioremediation of uranium contaminated aquifers and sediments, as soluble and mobile uranium poses much more of a threat to public health and the environment than an insoluble precipitate.

Correlation of Metal Tolerance and Antibiotic Resistance

Bacterial resistance to antibiotics and other antimicrobial agents is an increasing problem in today's society. Resistance to antibiotics is acquired by a change in the genetic makeup of a bacterium, which can occur by either a genetic mutation or by transfer of antibiotic resistance genes between bacteria in the environment (American Academy of Microbiology, 2000).

Because our current antibiotic are becoming less useful but used more heavily against antibiotic resistant pathogenic bacteria, infectious diseases are becoming more difficult and more expensive to treat. The increased use of antibiotics in health care, as well as in agriculture and animal husbandry, is in turn contributing to the growing problem of antibiotic-resistant bacteria. Products such as disinfectants, sterilants, and heavy metals used in industry and in household products are, along with antibiotics, creating a selective pressure in the environment that leads to the mutations in microorganisms that will allow them better to survive and multiply (Baquero et al., 1998).

According to Jeffrey J. Lawrence's (2000) discussion of the Selfish Operon Theory, clustering of genes on a plasmid, if both or all genes clustered are useful to the organism, is beneficial to the survival of that organism and its species because those genes are more likely to be transferred together in the event of conjugation. Thus, in an environment with multiple stresses, for example antibiotics and heavy metals, it would be more ecologically favorable, in terms of survival, for a bacterium to acquire resistance to both stresses. If the resistance is plasmid mediated, those bacteria with clustered resistance genes are more likely to simultaneously pass on those genes to other bacteria, and those bacteria would then have a better chance at survival. In such a situation, one may suggest an association with antibiotic resistance and metal tolerance. For example, Calomiris et al. (1984) studied bacteria isolated from drinking water and found that a high percent of bacteria that were tolerant to metals were also antibiotic resistant.

CONCLUSIONS

Although some heavy metals are important and essential trace elements, at high concentrations, such as those found in many environments today, most can be toxic to microbes. Microbes have adapted to tolerate the presence of metals or can even use them to grow. Thus, a number of interactions

[4] Eutrophication is a condition in which the presence of large amounts of biomass/organic matter in surface waters due to high nutrient loads results in low oxygen concentrations, poor water quality, and fish kills.

between microbes and metals have important environmental and health implications. Some implications are useful, such as the use of bacteria to clean up metal-contaminated sites. Other implications are not as beneficial, as the presence of metal tolerance mechanisms may contribute to the increase in antibiotic resistance. Overall, it is most important to remember that what we put into the environment can have many effects, not just on humans, but also on the environment and on the microbial community on which all other life depends.

ABOUT THE AUTHOR

Anne Spain carried out research related to the material in this article at Central Michigan University in Mt. Pleasant, Michigan from the fall of 2000 until the spring of 2002. Her work focused on finding a correlation between heavy metal tolerance and antibiotic resistance in *Escherichia coli* that had been isolated from central Michigan recreational waters. She also worked on trying to characterize the microbial community of arsenic-contaminated aquifers from several counties in Southeastern Michigan by amplification of community 16S rDNA and analysis by polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis. Due to complications with PCR, however, and limited time at the university, the project was not completed. Anne is currently enrolled as a graduate student at the University of Oklahoma where she is studying environmental microbiology and microbial ecology. She hopes to obtain her PhD after several years and continue in academia, conducting research and eventually teaching.

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