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# Heavy metals resistance by bacterial strains

# Khosro Issazadeh<sup>\*</sup>, Nadiya Jahanpour, Fataneh Pourghorbanali, Golnaz Raeisi and Jamileh Faekhondeh

Department of Microbiology, Faculty of Basic Sciences, Lahijan Branch, Islamic Azad University, Lahijan, Iran

#### ABSTRACT

Microorganisms are ubiquitous in nature and are involved in almost all biological processes of life. With rapid urbanization and natural processes, heavy metals have been found in increasing proportions in microbial habitats. Metals have been known to play a major role either directly or indirectly in almost all metabolic processes, growth and development of microorganisms. Bacteria that are resistant to such heavy metals and have the ability to grow in high concentrations of these metals play an important role in their biological cycling which has great potential in bioremediation of poorly cultivable soil high in heavy metal content. This review describes the workings of known metal-resistance systems in microorganisms.

Keywords: Heavy metals, Metal resistance genes, bacteria.

# INTRODUCTION

Microbes may play a large role in the biogeochemical cycling of toxic heavy metals also in cleaning up metalcontaminated. Heavy metals are often defined as a group of metals whose atomic density is greater than 5g cm<sup>-3</sup> [1]. Cobalt, copper, manganese, nickel, and zinc in trace amounts are essential for growth of microorganisms, but at high concentrations they have noxious effects on various organisms and on human health [2]. On the other hand some other heavy metals have no biological role and are detrimental to the organisms even at very low concentration (cadmium, mercury, lead etc.) [3]. Heavy metals exist both in bioavailable and non-bioavailable forms. Their mobility depends on two factors: (I) the metallic element that precipitates as positively charged ions and (II) the one, which makes up negatively charged component of salt [4].Generally, the higher concentration of these metals above threshold levels has deleterious impact on the functional activities of microbial communities in Environment. Otherwise, microorganisms exposed to the higher concentrations of toxic heavy metals may develop resistance against the elevated levels of these metals. In addition, microorganisms inhabiting in metal polluted soils have evolved various strategies to resist themselves against metal stress. Such metal resistant microorganisms can be used as successful bioremediation agents [4, 5]. 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 complication of the metal ions inside the cell, and reduction of the heavy metal ions to a less toxic state [6]. Various metal-resistant bacteria have been previously reported. They were isolated from contaminated soils, waters, and sediments. This review describes the workings of known metal-resistance systems in microorganisms.

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#### METAL RESISTANCE MECHANISMS

When the bacterial cells are exposed to the high concentrations of heavy metals, the metals react within cells with various metabolites and form toxic compounds. Mechanisms for uptake of these metal species are present in the bacterial cell through which heavy metals enter the cell. Generally, there are two types of uptake mechanisms for heavy metals: one of them is quick and unspecific which is driven by a chemiosmotic gradient across the cell membrane and consequently, does not require ATP. In contrast, the second process of metal uptake is comparatively, slower and more substrate-specific and is dependent upon the energy released from ATP hydrolysis [4, 7]. Heavy metal, copper is utilized by bacterial cells in small quantities in biosynthesis of metabolic enzymes like, cytochrome c oxidase. However, bacteria in different ecosystems including soil and water, are exposed to very high concentration of this metal as high levels of copper exists in soil ecosystem due to its wide application in mining, industry processes, and agricultural practices. Consequently, bacteria have evolved several types of mechanisms to defend against the high copper concentration and copper induced biotoxicity. Regarding the prevalence of copper resistance in bacteria, Lin and Olson [8] isolated copper resistant bacteria from a copper corroded water distribution system and studied resistance pattern against copper. They observed that 62% of the total isolates exhibited substantial resistance against copper. Among these resistant bacteria, 49% isolates had cop or cop-like gene systems as well as both compartmentalization and efflux systems. In other study, Cooksey [9] reported that resistance against copper in the plant pathogen Pseudomonas syringe was because of the copper accumulation and compartmentalization in the cell's periplasm and the outer membrane and concluded that the protective mechanism against copper in P. syringae was due to four types of proteins (CopA, CopB, CopC and CopD). These proteins are encoded by the cop operon present on bacterial plasmid and proteins are found in the periplasm (CopA and CopC), the outer membrane (CopB), and the inner membrane and work together to compartmentalize copper away from bacterial cells. In contrast, copper resistance in E. coli is dependent upon efflux mechanism to overcome copper stress. The efflux proteins are expressed by plasmid-borne pco genes, which are in turn rely upon the expression of chromosomal cut genes. Moreover, two cut genes (cutC and cutF) encode a copper binding protein and an outer membrane lipoprotein. Most bacterial species in the metal stressed environment have acquired at least one of the abovementioned protective mechanisms. In addition, the evolution of the bacterial copper resistance occurred through the modification of copper uptake genes found on chromosomes [7]. Zinc, an essential trace element is not biologically redox reactive. Hence, it is not used in cellular metabolisms like respiration. However, it is structurally, a vital constituent of several cellular enzymes. Furthermore, it also forms complexes in cells for instance, zinc fingers in DNA [7]. In addition, zinc actually, displays comparatively less toxicity to bacterial cells than other heavy metals and it is generally occurs in higher concentrations within bacterial cells. That is why bacteria in heavy metal polluted environment accumulate zinc by a fast but unspecific uptake mechanism. Generally, uptake of zinc ions by bacterial cells is coupled with magnesium, and both ions may be transported by similar mechanism [9]. The zinc resistance in bacteria is achieved through the two general efflux mechanisms: (I) mediated by a P-type ATPase efflux system and (II) mediated by an RND-driven transporter system [9]. As a matter of fact, a P-type ATPase catalyzes the reactions by ATP hydrolysis forming a phosphorylated intermediate whereas, the term RND belongs to a family of proteins involved in the heavy metal transport. The P-type ATPase efflux system transports zinc ions across the cytoplasmic membrane by the energy released from ATP hydrolysis. In this regard, Beard et al. [10] isolated a chromosomal gene, zntA, from E. coli K-12 and inferred that the gene zntA might be accountable for the zinc and other cations transporting ATPase across cell membranes. In contrast to P-type ATPase efflux system, the RND-driven transporter system does not derive energy through ATP hydrolysis to transport zinc within the bacterial cells. As an alternative, it is powered by the proton gradient across the cell wall specifically, in gram-negative bacteria [7, 9]. Various metal-resistant bacteria have been previously reported. Mergeay et al. [11] 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.

#### BIOSENSORS

Metal resistance trait in microorganisms is regulated by genes which are organized in operons. Generally, the genes for heavy metal resistance are found on extra chromosomal circular DNA i.e. plasmid carried by metal resistant bacteria. The heavy resistance genes are expressed and induced in the presence of specific metals in the vicinity of bacterial niche. Since the regulation of the metal resistant gene expression is specific for each heavy metal and is dependent upon metal species concentration, the promoters and regulatory genes from the bacterial operons responsible for resistance attribute can be used to create metal-specific biosensors (promoter-reporter gene fusions). The metal specific bacterial sensors coupled with tools for chemical analyses can be used to differentiate the bioavailable metal concentration from the total metal concentration of the samples. Various metal-specific sensor strains have been developed and applied in many laboratories. These sensor strains are all based on the same concept: a metal responsive regulation unit regulates the expression of a sensitive reporter gene. Reporter genes include those that code for bioluminescent proteins, such as bacterial luciferase (*luxAB*) and firefly luciferase (*lucFF*) or for  $\beta$ -galactosidase, which can be detected electrochemically or by using chemiluminescent substrates. The light produced can be measured by a variety of instruments, including illuminometers, photometers and liquid-scintillation counters [12].

#### **BIOSORPTION AND BIOACCUMULATION OF HEAVY METALS**

Fundamentally, biosorption of heavy metals by bacterial cells is based on non-enzymatic processes such as, adsorption. Adsorption is characterized by the non-specific binding of metal ions to extracellular/ cell surface associated polysaccharides and proteins. In a nut shell, biosorption is defined as an attribute of the inactive or dead microbial biomass to bind and concentrate heavy metals even from highly dilute solutions [13]. The metal uptake by the microbial biosrobent may be an active or passive process or exhibit both active and passive processes depending upon the microbial species. Moreover, passive uptake is a rapid and reversible process and is independent of cellular metabolisms, physical conditions such as pH and ionic strength. However, the passive process is relatively nonspecific with respect to the metal species. Conversely, the active process is comparatively slow method and depends on the cellular metabolism. In this process, heavy metals form complexes with specific proteins like metallothionins. However, it is affected by metabolic inhibitors, uncouplers and temperature. Both the active and passive mode may occur simultaneously [14]. Generally, many microbial species with high cell wall chitin contents act as an effective biosorbent in addition to the chitosan and glucans. Furthermore, the walls of fungi, yeasts, and algae, are also efficient metal biosorbents. Moreover, the cell walls of the Gram-positive bacteria attach higher concentrations of metals than that of the Gram-negative bacteria. Therefore, bacteria, waste fungal biomass derived from several industrial fermentations are considered the cost-effective and efficient sources of biosorptive materials. These biosorbents loaded with charged metal species may be regenerated with the treatment of acid or some chelating agents [15]. Bioaccumulation is an active process dependent upon metabolic energy of microorganisms. In other words, bioaccumulation is an energy-dependent heavy metal transport system. Besides, potential bioaccumulation mechanisms of heavy metal influx across the bacterial membranes include ion pumps, ion channels, carrier mediated transport, endocytosis, complex permeation, and lipid permeation. This active mechanism has been reported to be associated with the transport of heavy metals like, mercury, lead, silver, cadmium and nickel. Assessment of heavy metal accumulation in the microbial cells can be done by transmission electron microscopy (TEM). In a study, TEM analysis of P. putida 62BN demonstrated intracellular and periplasmic accumulation of cadmium [14, 15]. Similarly, heavy metal transport through bioaccumulation has been reported in many bacterial genera like, Citrobacter sp. (lead and cadmium), Thiobacillus ferrooxidans (silver), Bacillus cereus (cadmium), Bacillus subtilis (chromium), Pseudomonas aeruginosa (uranium) Micrococcus luteus (strontium) Rhizopus arrhizus (mercury), Aspergillus niger (thorium), Saccharomyces cerevisiae (uranium) [15, 16, 17, 7].

### CONCLUSION

Heavy metals are metals with a density above 5 g/cm3, thus the transition elements from V (but not Sc and Ti) to the half-metal As, from Zr (but not Y) to Sb, from La to Po, the lanthanides and the actinides can be referred to as heavy metals. This review describes the workings of known metal-resistance systems in microorganisms. Some microorganisms under heavy metals stress may develop resistance against the elevated levels of these toxic metals and evolve various strategies to resist against the metal stress. Therefore, the metal resistant microorganisms including bacteria can be exploited as bioremediation, biosorption and bioaccumulation processes.

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