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Engineering Bacteria for Bioremediation

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1. Introduction

Bioremediation is a process that uses microorganisms or their enzymes to promote degradation and/or removal of contaminants from the environment. The use of microbial metabolic ability for degradation/removal of environmental pollutants provides an economic and safe alternative compared to other physicochemical methodologies. However, although highly diverse and specialized microbial communities present in the environment do efficiently remove many pollutants, this process is usually quite slow, which leads to a tendency for pollutants to accumulate in the environment and this accumulation can potentially be hazardous. This is especially true for heavy metals. Heavy metal contamination is one of the most significant environmental issues, since metals are highly toxic to biota, as they decrease metabolic activity and diversity, and they affect the qualitative and quantitative structure of microbial communities. For treating heavy metal contaminated tailings and soils, bioremediation is still the most cost-effective method, although various heavy metals are beyond the bioaccumulation capabilities of microorganisms. Perhaps, because of the toxicity of these compounds, microorganisms have not evolved appropriate pathways to bioaccumulate them; populations of microorganisms responsible for this bioaccumulation are not large or active enough to remove these compounds completely, or complex mixtures of pollutants resist removal by existing pathways. The pathway used to accumulate these compounds is adsorption, where metals are taken up by microbial cells (biosorption). Biosorption mechanisms are numerous and are not yet fully understood. However, biosorption capacity often varies with test conditions, such as initial metal concentration, solution pH, contact time, biomass dosage, processing method, and so on. Accordingly, populations of microorganisms that are able to promote metal adsorption and accumulate them are not large or active enough to support these compounds by existing pathways. Furthermore, there are several strategies that optimize the bioremediation process of pollutants. One approach to enhance populations of microorganisms capable of pollutant removal is the addition of exogenous microorganisms in order to expand indigenous populations. This process, commonly known as bioaugmentation, can be performed either by adding microorganisms that naturally contain catabolic genes or those that have been genetically modified (GMOs). This strategy can also result in the transfer of plasmids containing the necessary genetic material between the different populations. Recent advances in the molecular biology field have been applied to microorganisms in order to produce novel strains with desirable properties for the
bioremediation processes. These include the construction or adaptation of catabolic pathways; redirection of carbon flux to prevent the formation of harmful intermediates; modification of catabolic enzyme affinity and specificity; improvement of the genetic stability of catabolic activities; increasing the bioavailability of pollutants; and enhancement of the monitoring, yield, control, and efficiency of processes. Despite the many advantages of GMOs with regards to bioremediation, their use is still limited in the environment because of the instability of the inserted genetic material. There are two major reasons for this: first, the efficiency of GMOs is dependent on their ability to carry the genetic material in a stable manner; second, the transfer of genetic material to the indigenous organisms is perceived to be a negative attribute, despite the fact that this transfer is a common phenomenon among native organisms. These factors have incentivized the study of survival, competition and persistence of GMOs in the environment, as well as the potential risks involved in their use. Besides the significant advances that have already been made with regards to the development and utilization of GMOs for bioremediation of contaminants in the environment, many more challenges still remain. In this chapter, we will detail how genetic engineering may improve bioremediation through the engineering of bacteria. Several genetic approaches have been developed and used to optimize enzymes, metabolic pathways and organisms that are relevant to biodegradation. New information on metabolic routes is still being accumulated, thus the available toolbox is continuously being expanded. With molecular methods enabling the characterization of microbial community structure, metabolic pathways and enzyme activities, the performance of microorganisms under in situ conditions can be improved by making heavy metal bioremediation a much more efficient process. The present review also highlights the current situation pertaining to biosorbents, their mechanisms and advantages and disadvantages. Thus, this article reviews the achievements and current status of biosorption technology, which exploits natural biodiversity and molecular tools, in order to engineer microorganisms and provide new information about this research frontier.

1.1 Heavy metals and toxicity

Heavy metals are considered to be chemical elements with an atomic mass greater than 22 and a density greater than 5g/mL. This definition includes 69 elements, of which 16 are synthetic. Some of these elements are extremely toxic to human beings, even at very low concentrations (Roane & Pepper, 2000; Wang & Chen, 2006). The main heavy metals associated with environmental contamination, and which offer potential danger to the ecosystem, are copper (Cu), zinc (Zn), silver (Ag), lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd), chromium (Cr), strontium (Sr), cesium (Cs), cobalt (Co), nickel (Ni), thallium (Tl), tin (Sn) and vanadium (V) (Wang & Chen, 2006). In general, metal ions can be classified as: 1) Essential and important for metabolism (Na, K, Mg, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Mo and W); 2) Toxic heavy metals (Hg, Cr, Pb, Cd, As, Sr, Ag, Si, Al, Tl), which have no biological function (in ecotoxicology terms, hexavalent forms of Hg, Cr, Pb and Cd ions are the most dangerous); 3) Radionuclides (U, Rn, Th, Ra, Am, Tc), which are radioactive isotopes and, although toxic to cells, they are nonetheless important in nuclear medicine procedures; 4) Semi-metals or metalloids (B, Si, Ge, As, Sb, Te, Po, At, Se), which exert distinct biological effects on metals. However, metals are predominantly present in the environment in cationic and anionic forms in semimetals, and As is often classified as heavy metal (Roane & Pepper, 2000; Ahluwalia & Goyal, 2007). In the environment, metals can be
divided into two categories: 1) bioavailable (soluble, non-absorbed, mobile); and 2) non-bioavailable (precipitated, complexed, sorbed, non-mobile). The ionic form (speciation) of a metal determines its bioavailability and its destination. Most heavy metals are cations and this determines their sorption to negatively charged functional groups that are present in: cell surfaces, which are generally anionic at a pH of between 4 and 8; surfaces with residual hydroxides (OH\(^-\)) or thiol (SH\(^-\)) and anionic salts, such as PO\(_4\)\(^-\) and SO\(_4\)\(^-\), humic acid, and clay minerals (Roane & Pepper, 2000). Heavy metal ions possess great electrostatic attraction and high binding affinities to the same sites that essential metal ions normally bind to various cellular structures, causing destabilization of the structures and biomolecules (cell-wall enzymes, DNA and RNA), thus inducing replication defects and consequent mutagenesis, hereditary genetic disorders and cancer. This occurs, for example, with arsenate, which competes with phosphate, and cadmium, which competes with zinc. By employing microarray technology, Kawata et al. (2007), found that six heavy metals (arsenic, cadmium, nickel, antimony, mercury and chromium) induce gene expression patterns that are very similar to the pattern induced by DMNQ (2,3-dimethoxy-1, 4-naphthoquinone), the reactive oxygen species (ROS) chemical generating agent, which causes "oxidative stress", leading to deleterious effects (membrane damage or other cellular lipid structures, modification of proteins, fragmentation and cross-links, changes in DNA that can induce mutations or be repaired by repair mechanisms). Therefore, the ions of heavy metals cause oxidative damage, both directly, by producing ROS, and indirectly, by inactivating the cellular antioxidant system, thus leading to cell damage (Mannazzu et al., 2000; Liu et al., 2005).

1.2 Heavy metals and the environment
Among the different contaminants, heavy metals have received special attention due to their strength and persistence in accumulating in ecosystems, where they cause damage by moving up the food chain to finally accrue in human beings, who are at the top of this chain (Figure 1) (Voilesky, 2001; Ahluwalia & Goyal, 2007; Machado et al., 2008).

![Fig. 1. The destiny of heavy metals released into the environment and their accumulation throughout the chain food. Adapted from Voilesky (2001).](image-url)
need to be controlled by mandating waste treatment at the sources of pollution. The
development of new treatment technologies is required at these sources; however, even
though there is awareness of this problem, sustainable solutions are not easily accessible. In
general, the conventional treatment methods used to remove metals from wastewater are
inefficient and cost-prohibitive.

1.3 Conventional technologies for treating environments contaminated by heavy
metals
Environments contaminated by heavy metals are treated by means of conventional
technologies based on physicochemical princi