AN OVERVIEW ON POTENTIAL USE OF MICROORGANISMS FOR BIOREMEDIATION OF HEAVY METALS

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Abstract*—Heavy metals are persistent environmental pollutants that are introduced into the environment by natural processes and various anthropogenic activities. Environmental pollution caused by heavy metals has become a severe threat to environment and life causing serious health effects. Removal of heavy metals from the environment is a real challenge and over the years, various physiochemical methods were used for its removal. Recently, bioremediation approach involving microorganisms have gain interest and the new approach is widely used for treating heavy metals from contaminated environment. Bioremediation approach has many advantages over other conventional methods as it is environmental friendly, safe and easy to handle. Microorganisms play a significant role in heavy metal detoxification, however, due to dearth of knowledge on its molecular mechanism their application in the field of bioremediation has been limited. This review describes how bioremediation approach involving microorganisms as agents for elimination of toxic metals from contaminated sites. The present review also focuses on potential adverse effects on human health issues from long term exposure of heavy metals.

***Index Terms*—Bioremediation, heavy metal, detoxification, metal-resistant microorganisms**

I. INTRODUCTION

Indiscriminate industrial activities and increased modernization, have led to large scale contamination of the environment with toxic heavy metals and other xenobiotic compounds affecting every strata of ecosystem including flora and fauna. Heavy metals are non-degradable in nature, and their accumulation in the food chain of the ecosystem is a serious health and environmental concern which needs immediate focus. Many studies have reported the toxic and carcinogenic effects of metals in human and animals [1]. Biro *et al.* [2] proposed that the lethal adverse nature of heavy metal is due to their presence in transition form with incompletely filled *d* orbitals that have the ability to form complex unspecific compounds in the cells. The chronic exposure to the heavy metals such as cadmium, chromium, cobalt, lead and nickel are well reported to cause obstructive airway diseases, emphysema, diabetic and renal complications, deregulated blood pressure, bone disorders, immune suppression and various form of cancers [3]. Existence of diverse metal resistant microorganisms offers opportunity for metal detoxification from contaminated environment by biosorption, bioaccumulation, metal efflux and reduction strategies. Exploiting taxonomically diverse metal resistant microbes particularly bacteria in remediating heavy metals and restoration of polluted environment have been a major focus of research in recent times. Therefore, it is imperative to comprehend the molecular mechanism of detoxification adopted by the biological system.

II. HEAVY METALS

Among 90 different natural elements, there are 21 non-metals, 16 light metals and remaining 53 (with Arsenic) are heavy metals [4][5]. Metals and metal compounds are important constituents of the ecosystem with a wide range of oxidation states and coordination numbers. As suggested by Hawkes [6], heavy metals belong to group 3-16 that are in periods 4 and greater. Heavy metals are elements with atomic weight between 63.5 and 200.6, and a specific density above 5 g/cm^3. These elements are distinct in having partially or incompletely filled *d* orbitals that have ability to form complex compounds. Transition elements such as cobalt, copper, iron, manganese, arsenic, molybdenum, vanadium etc. are referred as heavy metals. These natural elements are released into the environment as a result of geological processes such as volcanic eruption, erosion, spring water, bacterial activity etc. [7][8].

Metals have been categorized into 3 types - essential and non-toxic (e.g. Ca & Mg); essential but harmful (e.g. Fe, Mn, Zn, Cu, Co, Ni & Mo); toxic (e.g. Hg & Cd) [9]. Essential metals such as cobalt, chromium, copper, nickel etc. are known to play significant roles in various cellular processes in prokaryotes and eukaryotes [10]. Metals such as zinc and cobalt are function as cofactors or catalyst for biochemical processes [11][12][13]. Iron, copper, and nickel are essential in catalysis of cellular redox reactions and some metals also have functional role in regulating gene expression and bio-molecular processes [14]. Heavy metals (Zn, Cu, Ni) are known to be associated with antioxidant enzyme involved in superoxide dismutase (SOD) activity [4]. Non-essential heavy metals such as cadmium, lead and manganese have no such important role in physiological processes.

III. TOXIC EFFECTS OF HEAVY METALS

Heavy metals are generally non-toxic however, when concentration exceeds certain threshold limit, they become highly toxic. In recent years, increased anthropogenic activities have resulted in environmental pollution due to increase in utilization and discharge of industrial effluents including heavy metals into the environment [15]. Heavy metals are mostly used in industrial applications such as metallurgical processes, mining, chemical works, welding, and electroplating, etc. Due to their indiscriminate use some of the metals such as lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu) and mercury (Hg) are most frequently released and thus, contaminating the environment (Table 1). Human get exposed to metal contaminants through various routes such as air, water, food, skin etc.

Non-essential heavy metals are potentially toxic as they interface with important cellular components through covalent and ionic bonding. Previous report suggested that prolonged exposure to high concentration of heavy metals resulted in formation of unspecific complex compounds in the cell, subsequently causing toxic effects [16]. Due to its unique redox property and coordination chemistry, heavy metals escape the control mechanisms of transport, compartmentalization or homeostasis. Heavy metal ions enters cell and interact with enzymes subsequently inhibiting catalysis of biochemical processes. Exposure and accumulation of heavy metals may lead to the disruption of functions of vital organs such as heart, brain, kidneys, bone, liver, etc. [17]. Heavy metals rapidly gets absorbed in the living system causing intoxication particularly neurotoxicity [18], nephrotoxicity [19], genotoxicity [20] or carcinogenicity [21] through the generation of reactive oxygen and nitrogen species.

Excessive amount of these metal pollutants causes oxidative stress in the cell by generating free radicals that can halt enzyme activities, induce DNA breakage, damage cell membrane and disrupt essential functions of cell. Heavy metals such as cadmium, mercury, lead and arsenic have been associated with cancers of skin, lung, prostrate, pancreas, and kidney [22]. It has been reported that hexavalent chromium has structural similarity with sulfate and phosphate at physiological pH, and can enter the body and even reach to the brain [23]. On the other hand, it has been found that arsenic has long-term neurological effects and can cause severe damage to reproductive health. Nickel is tremendously toxic and has potential to generate free radicals which can alter enzyme activities, disruption of cell membrane and often leading to cell death. Chronic contact with nickel was shown to induce structural alterations in chromatin and epigenetic changes [24]. Although few metals are known to have important physiological role in biological system, however, at higher concentration they are reported to cause pernicious effects in human beings, plants, animals and aquatic organisms (Table 2) [25].

IV. HEAVY METAL DETOXIFICATION MECHANISMS

Bioaccumulation of non-biodegradable heavy metals and its excess release into the environment have caused environmental pollution. Inflated level of heavy metals in the environment has become a serious threat as they act as a potent toxicants interfering with the normal biochemical reactions of the human body. Interestingly, organisms which are exposed to harsh environments such as chemical drainage or mining sites, have evolved mechanisms to cope with high concentration of metals that might be toxic to human and other species. Several reports suggested that upon continuous exposure to chronic level of toxicants, organisms develop acclimatization through metabolic reshuffling resulting in increased resistance to toxicants [26][27]. Many microorganisms such as bacteria, algae and yeast have developed different molecular and biochemical processes to overcome such adverse environmental conditions [28]. These mechanisms involves energy dependent efflux of metal as insoluble salts, alteration in the permeability of membrane, production of chelating agents and biochemical transformation of metal ions [29][30][31].

Microorganisms such as bacteria have developed different export mechanisms for efflux of heavy metals. In one such mechanism, energy dependent transporters P-type ATPases or cation/H^+ antiporters have evolved to extrude metal ions from the cytoplasm [32]. In gram negative bacteria, CBA transporters act as chemiosmotic antiporters which are reported to export metals out of the cell. Presence of CBA transporters in microorganisms indicates high resistance to heavy metal ions. Some microorganisms have also evolved membrane transporters such as cation diffusion facilitator (CDF) metal exporter which can act as chemiosmotic ion proton exchangers [33]. Various reports suggest that beside membrane transporters, antioxidant enzymes have potential to prevent metal induced oxidative stress in organisms [34]. Heavy metals are known to affect lipid peroxidation and plasma membrane permeabilization which leads to formation of lipid peroxides. During metal stress conditions, reactive oxidative species (ROS) such as O^{2-}, H_2O_2, OH are generated and to neutralize oxidative radicals enzymes such as glutathione (GSH), ascorbic acid (ASA), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) are expressed in microorganisms [35]. Various studies on microorganisms such as *Bacillus* sp. (PbS6) and *Ochrobactrum* sp. (CdSp9) in response to metal stress displayed increase in the levels of superoxide dismutase (SOD) which converts superoxide radicals to H_2O_2 [36]. Accumulation of H_2O_2 in the cell is prevented by release of antioxidant enzymes such as catalase (CAT), peroxidases (POX) which converts peroxides into H_2O [37]. In a study carried in *Pseudomonas putida* demonstrated that enzymes glutathione reductase (GR) and glutathione peroxidase (GSHPx) are induced in response to arsenic. These enzymes are involved in maintaining glutathione levels in the cell when free thiols are depleted due to metal stress [38].

Algae also have evolved to eliminate heavy metals by producing peptides capable of binding heavy metals and forming organo-metallic complexes. These complexes formed are inside vacuoles controlling the metal concentration in the cytoplasm and thus preventing potential toxic effects [39]. In presence of Cd^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Ag^{+} and Hg^{2+}, synthesis of Mt III complex was observed in microalga *Scenedesmus acutiformis* [40]. Torricelli et al. [41] reported that wild type and tolerant strain (tolerant to Cr^{6+}) of *Scenedesmus acutus* upon exposure to Cd^{2+} displayed elevated levels of reduced glutathione in tolerant strain and MtIII compared with the wild strain.

Fungi possess various molecular mechanisms for metal detoxification. Metal chelating compounds such as siderophores and organic acids are produced by fungi to remove and sequester toxic metal ions from cytosol [42]. To counter metal toxicity, over excretion of oxalic acid in fungi occurs and their production is associated with immobilization of toxic metals through formation of insoluble compounds [43]. It is reported that for arsenate detoxification, *Aspergillus niger* showed significant changes in the level of certain enzymes and higher level of oxalic acids as compared to other organic acids such as malonic acid, citric acid etc. [44].

V. BIOREMEDIATION AS A TOOL FOR HEAVY METAL DETOXIFICATION

Heavy metals are natural elements released into the environment by various geochemical processes. Overexploitation and anthropogenic activities such as mining and smelting have also resulted in excess of these elements in the environment. Over the years, remediation of heavy metals from contaminated aquatic and terrestrial environments has been the most concerned global issue [45]. Previously, various physiochemical methods including electrochemical treatment, ion-exchange, precipitation, reverse osmosis, evaporation and oxidation/reduction, were used for removal of heavy metal from waste streams Although these methods were widely used for metal detoxification, however, these methods had several disadvantages such as high operating cost and low efficiency in detoxification [46]. In recent years, bioremediation has emerged as better alternative for heavy metal detoxification with several advantages such as eco-friendly, cost effective, ease of use and high efficiency in detoxification [47] [48].

Bioremediation is one of the most intensively studied technologies which uses microbes capable of removing contaminants present in soils, sediments and water. Metal detoxification by microbes involves degradation of toxic pollutants either through intracellular accumulation or via enzymatic transformation to reduce into non-toxic compounds. Selective removal of heavy metals involves a complex process which depends on factors such as pH, temperature, ionic composition, moisture content, contaminant concentrations, redox potential, cell wall composition and physiology [49]. Presently, different strategies are implemented for in situ and ex situ bioremediation which includes biostimulation, bioaugmentation, biotransformation, bioaccumulation, biosorption, natural attenuation etc. Biostimulation involves addition of nutrients to the contaminated sites to stimulate naturally occurring microorganisms capable of bioremediation whereas in bioaugmentation, microorganisms are additionally incorporated to biodegrade or biotransform a particular contaminant. The intrinsic bioremediation that occurs in situ and depends on the existing natural biological processes are known as natural attenuation [50].

Microorganisms particularly bacteria use numerous means to control or tolerate high levels of variety of heavy metals and developed tolerance which is often plasmid-borne with capability to be transferred throughout the bacterial community by the lateral gene transfer [51]. Analysis has revealed that continuous exposure to toxic components causes gene alterations which contribute to microbial adaptation, ensuring their survival. Gene conferring resistance is transferred to next generations and to other organisms through lateral gene transfer [52]. The genetic and molecular mechanisms involved in regulating levels of metals are essential factors determining the role of microbes in natural and metal contaminated environment. These microbes might contribute in the bioremediation of heavy metal contaminated water or soil and help in restoration of polluted environment [53]. Development of resistance in metal resistant microbes is a spontaneous process. It remains challenging to understand the alterations in microbial genome controlling adaptation to environmental stress. Further, more efforts should be made to explore diverse microorganisms and provide insights into molecular mechanism involved in metal detoxification.

Table 1 Industrial application of heavy metals

Heavy metal	**Industrial applications**
Cadmium (Cd)	stabilizers in PVC products, color pigment, paints, several alloys, phosphate fertilizers, fungicides, re-chargeable nickel–cadmium batteries
Mercury (Hg)	electrochemical processes, dental amalgam fillings thermometers, barometers and other instruments
Lead (Pb)	paint, batteries, glass, petroleum, smelting alloying, melting operations, coal-based thermal, power plants, ceramics
Arsenic (As)	batteries, pesticides, wood preservatives, smelting operations, thermal power plants, fuel burning and semiconductors,
Chromium (Cr)	leather tanning, mining, coolants, electroplating, metal finishing and chromate preparation processes
Nickel (Ni)	Ni-Cd batteries, pigments for ceramics, dental materials, as catalyst for hydrogenation processes in the food, petroleum and petrochemical industries

Table 2Toxic effects of heavy metals on human health

Heavy metal	**Toxic effects in humans**
Cadmium	acute pulmonary disorders, anemia, emphysema, growth impairment, hypoglycemia, reproductive disorders, schizophrenia, stroke, cerebral hemorrhage, cirrhosis, cancer
Mercury	contact eczema, nervous system damage, visual and auditory difficulties, depression, autoimmune diseases, insomnia, depression
Lead	encephalopathy, nervous system damage, cancer, acute psychosis, anemia, impairment in learning and coordination
Arsenic	bone marrow depression, hemolysis, hepatomegaly, melanosis, polyneuropathy and encephalopathy, peripheral vascular disease, cancer, disturbances of cardiovascular and central nervous systems
Chromium	respiratory problems, cancer, causes allergy
Nickel	Cancer, contact dermatitis, others are lung fibrosis, cardiovascular and kidney diseases

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