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Biotransformation of Heavy Metals by Plant Growth Promoting Endophytic Bacteria: An Assessment

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A B S T R A C T

As a consequence of urbanization and industrialization, the environment is progressively polluted with heavy metals which is a problem of great concern. In plants it can create oxidative stress, ethylene production, chlorosis, Necrosis, stunted growth, inhibits various physiological processes, and decrease biomass. Due to their persistent and non-degradable nature, they enter the food chain leading to biomagnification and causing carcinogenic, mutagenic, and lethal effects on humans and animals. Bioremediation, phytoremediation, biotransformation, non-host inoculation, and other methods are used to treat heavy metals in the environment. Endophytic bacteria have gained attention for their potency to remove or immobilize heavy metals. Bacterial bioremediation is an effective and reliable technique to degrade, detoxify, mineralize, transform, or reduce the concentration of pollutants. This review helps in understanding the endophytic bacterial activity on bioremediation along with its plant growth-promoting properties.

INTRODUCTION

Industrial operations such as mining, smelting, combustion of fossil fuels, electroplating, modern agricultural practices, and considerable anthropogenic activities have led to a significant increase of metallic materials in the biosphere, which has a long-term detrimental effect and also consequently result in biomagnification in the food chain (Tiwari & Lata, 2018, Rajkumar et.al., 2009). Many scientists agree that plants play an important role in decomposing heavy metals with the help of microbes. However, the thought-provoking aspect is that bacteria found endophytically in plants would not only destroy the heavy metals but will also promote their degradation. Heavy metals are a major source of concern for humans due to their cytotoxicity, mutagenicity, and carcinogenicity (Lim & Schoenung, 2010). Excessive heavy metal accumulation in the soil has affected the microbial community their structure, function, and diversity (Etesami, 2018). The deposition of heavy metals in agricultural soil is a key environmental constraint that leads to lower crop production and food/feed safety (Maleki et.al., 2017).

Many physicochemical procedures for heavy metal removal have been developed, including filtration, electrochemical treatment, oxidation/ reduction, chemical precipitation, reverse osmosis, ion exchange, membrane technology, and evaporation recovery (Xiao et.al., 2010). Most techniques are expensive, labor-intensive, ineffective, or lack specificity in the treatment (Chen et.al., 2008, Tang et.al., 2008). To resist heavy metals stress, plants have evolved detoxifying mechanisms which include vascular sequestration of chelated complexes, activation of enzymatic and nonenzymatic antioxidant systems, and phytochelation of metals (Kumar & Trivedi, 2016). The utilization of bacteria with metal-detoxifying properties as well as plant-beneficial features is a cost-effective and environmentally benign metal bioremediation technique for increasing biomass output as well as plant tolerance to heavy metals (Ahemad, 2019 & Tirry et.al., 2018). The isolation and employment of microbial populations for the bioremediation of heavy metals have attracted researchers and metal-detoxifying plant growth-enhancing bacteria became an entity of particular importance (Wenzel, 2009, Aafi et.al., 2012 & Yang et.al., 2012). The main objective of this study is to review how different heavy metals may be detoxified or degraded by plant growth-promoting endophytic bacteria.

MATERIALS AND METHODS

Since the study relies on secondary data, the data were collected from research articles, journals, and web-based information. Physical and chemical methods and other viable approaches have been primarily studied in prior research to reduce or detoxify heavy metal concentrations. Among them, the application of plant growth-promoting endophytic bacteria for heavy metal concentration reduction or degradation is considered an eco-friendly technique. Inoculation of endophytic bacteria in non-host plants, genetic modification of bacteria, and application of nanotechnology help in better biodegradation of heavy metals.

RESULTS AND DISCUSSION

Heavy Metals Stress in Plants

Plants are naturally subjected to a variety of environmental stresses, which can be biotic or abiotic. Despite other stresses, the plants are subjected to heavy metal stress, which has a significant negative impact on plant development and productivity (Gill, 2014). Increasing concentrations of both essential and non-essential heavy metals have a negative impact on plant growth and host legumes, as well as legume-rhizobium symbiosis, owing to increased reactive oxygen species (ROS) (Stambulska & Bayliak, 2020). Heavy metals create oxidative stress in plants, which is a key contributor to pathological disorders (Fryzova et.al., 2017). Heavy metals cause oxidative stress in cells by a.) directly transferring electrons in single-electron reactions that produce free radicals b.) disrupting metabolic pathways, c.) inactivating antioxidant enzymes (peroxidases, catalases, and superoxide dismutase) and d.) depleting low molecular weight antioxidants, including glutathione (Dietz et.al., 1999 & Shaw et.al., 2004). Plants exposed to heavy metal stress, directly or indirectly, commonly disturb the chemical or physical structures of DNA and cause cytotoxic or genotoxic stresses, resulting in genomic instability and severely impacting plant health (Dutta et.al., 2018). Crop productivity was reduced by the interference of heavy metals with various physiological processes such as photosynthesis, seed germination, accumulation, and remobilization of seed reserves during germination (Shahid et.al., 2015). Leafy vegetables are typically dwarf plants, closer to the ground, making them more susceptible to contamination of the soil (Huang et.al., 2007, Sharma et.al., 2008).

Impact of Heavy Metal Stress on Medicinal Plants

Humans need plants as food and medicine, so it's important to examine how heavy metals affect plants and their secondary metabolites (Rai et.al., 2005). Heavy metal poisoning of therapeutic plant products has had a variety of negative health effects, including liver and kidney failure and even death (Street, 2012). Reduced levels of protein, chlorophyll, and carotenoid compounds were produced by the cadmium stress in *Phyllanthus amaraus* (Rai et.al., 2005). The production of total phenolics, flavonoids, and saponin was decreased in *Gynura procumbens,* under cadmium and copper stress (Ibrahim et.al., 2017). The concentration of medicinally significant secondary metabolites Pseudo-hypericin and Hypericin was reduced in *Hypericin perforatum,* (the plant used to treat neurological disorders), grown in areas with nickel contamination (Murch et.al., 2003). Superoxide dismutase (SOD) activity was elevated in the leaf and root tissues of *Acalypha indica* regardless of the duration of lead exposure (Venkatachalam et.al., 2017). Chromium's toxic effects on *Ocimum tenuiflorum* were manifested in decreased levels of cysteine, ascorbic acid, non-protein thiols, and decreased nitrate reductase activity. However, *O.tenuiflorum* leaves produced more eugenol, a key ingredient in *Ocimum* essential oil, and showed greater proline concentration (Rai et.al., 2004).

Plant Growth Promoting Endophytic Bacteria

The internal tissues of plants are colonized by endophytic bacteria (beneficial or symbiotic), which are present in almost every plant on earth and have no detrimental impact on them (Ryan et.al., 2008). Endophytic bacteria were once believed to be weakly virulent plant pathogens, but more recent research has shown that they actually have a number of positive impacts on their host plants, including promoting plant development and boosting resistance to parasites and plant infections (Hallman et.al., 1997). Bacterial endophytes have been found to support the growth and development of plants in a way analogous to that of rhizospheric bacteria. Similar to rhizospheric plant growth-promoting bacteria, endophytic plant growth-promoting bacteria can aid in the growth of plants in horticulture, silviculture and agriculture, and phytoremediation. They can also help reduce chemical input in traditional agricultural practices and boost plant species nutrient uptake and stress resistance (Santoyo et.al., 2016, ALKahtani et.al., 2020). The production of diverse plant growth-promoting phytohormones by endophytic plant growth-promoting bacteria can increase plant growth while reducing the environmental toxicity of heavy metals. It is possible to isolate, alter or recombine the endophytic bacteria of a specific plant that aid in plant growth so that they can be injected into another plant to help detoxify heavy metals. Functional endophytic bacteria inoculation may lower phytotoxic effects and boost pollutant uptake and removal rates (Glick & Stearns, 2011). Numerous research has examined the interactions between plants and the bacteria they are linked with, to remove or stabilize metals from contaminated soils (Chen et.al., 2014).

Phytoremediation by Endophytic Bacteria

Arsenic

Arsenic is the widely distributed element in the earth's crust. It is primarily found in arsenopyrite and other sulphide minerals and is a constituent of more than 200 minerals. Numerous (bio) geochemical processes, including oxidation of arsenic-bearing sulphides, reductive dissolution, desorption from oxides and hydroxides, evaporative concentration, leaching from sulphides by carbonate, and microbial mobilization, are responsible for various mechanisms by which arsenic is released from the minerals (Garelick et.al., 2009). Bacterial arsenic tolerance is influenced by their capacity for As (III) oxidation and As (V) reduction as well as their ability to retain arsenic in the biomass to varied degrees. Endophytes from *Pteris vittate* were more resistant to arsenate (AsV), and those from *Pteris multifida* were more resistant to arsenite (AsIII) (Zhu et.al., 2014). *Arthrobacter, Bacillus, Brevibacterium, Kocuria, Micrococcus, Microbacterium, Pseudomonas, and Staphylococcus* were among the genera of arsenic-resistant endophytic bacteria that were found in the roots of *Prosopis laevigata* and *Spharealsea angustifolia* (Roman-Ponce et.al., 2018). The arsenic accumulation in rice grains might be reduced by the rice endophytic bacteria *Bacillus pumilus, Pseudomonas* sp., and *Bacillus thrungenesis* (Dolphen and Thiravetyan, 2019).

Chromium

Chromium is a well-known non-essential hazardous metal for microorganisms and plants and is a severe environmental pollutant. Chromium occurs in nature as two primary species: the trivalent form, Cr(III), which is comparatively harmless, and the hexavalent form, Cr(VI), which is thought to be more hazardous because it prevents sulphate membrane transit and causes oxidative damage to biomolecules. The most well-known bacterial resistance mechanism to chromate involves chromate ion outflow from the cell cytoplasm and reduction of Cr(VI) to Cr(III). By interacting with the carboxyl and thiol groups of enzymes, Cr(III) interferes with DNA replication, resulting in mutagenesis, and changes in the structure and function of enzymes. Chromate efflux by the ChrA transporter, which is an energy-dependent mechanism driven by membrane potential, has been demonstrated in *Pseudomonas aeruginosa* and *Cupriavidus metallidurans* (formerly *Alcaligenes eutrophus*).

One of the biggest issues with environmental protection is the contamination of hexavalent chromium [Cr(VI)]. Since the Cr(VI) is mobile and highly toxic, mutagenic, carcinogenic, and spreads considerably beyond the location of original contamination, it poses a risk to human health (Ramierz-Daiz et.al., 2008, Viti et.al., 2014). The *Pantoea stewartii*, *Microbacterium arborescens*, and *Enterobacter* are the endophytic bacteria of *Prosopis juliflora*, have the potential to promote plant growth (ACC deaminase activity, siderophore, and IAA production, Phosphate solubilization) and tolerance to higher concentrations of heavy metals and salt (Khan et.al., 2015). Endophytic bacterium *Kocuria rhizophila* of *Oxalis corniculate* (hyperaccumulator plant), is capable of accumulating metal ions and exhibited greater resistance Cr (Haq et.al., 2016).

Mercury

Mercury is a non-essential metal that is persistent and quite poisonous (Selin, 2014). Manufacturing of paints, disinfectants, pharmaceuticals, pulp and paper, fungicides, and bactericidal agents are few anthropogenic sources of mercury. In addition, mercury is released into the environment through mercury mining, gold refining, fuel burning, and instrument manufacturing (Moreno et.al., 2008). Impacts on society, the environment, human and animal health result from the bioaccumulation and biomagnification of mercury in the trophic chain (Matulik et.al., 2017). *Bacillus amyloliquefaciens* of *Elusine indica* and *Jeotgalicoccus huakuii* of *Cynodon dactylon,* are two endophytic bacteria used for phytoremediation of Hg-contaminated soil, for its high siderophore production and lack of haemolysis (Ustiatik et.al., 2021). *Acinetobacter baumannii*, *Serratia marcescens*, *Pseudomonas* sp., *Klebsiella pneumoniae,* and other mercury-resistant endophytic bacteria were inoculated into the maize plants to enhance growth on mercury-contaminated substances and reduce mercury phytotoxicity (Mello et.al., 2020). The growth of corn (*Zea mays*) on mercury-supplemented substrates was facilitated by the bacteria *Bacillus* sp., *Burkholderia* sp., *Enterobacter* sp., *Klebsiella pneumoniae, Lysobacter soli, Pantoea* sp. (Mello et.al., 2019).

Zinc

Increased zinc (Zn) concentration at dangerous levels in agricultural land due to various human activities, such as the application of metal-contaminated sewage sludge or mining operations, may pose a risk to sustainable and high-quality food production (Li & Christie, 2001). The genus *Cupriavidus, Klebsiella, Serratia, Micrococcus, Pseudomonas, Streptomyces, Proteus,* etc., are notable Zn tolerant PGPR strains (Ortiz-Ojeda et.al., 2017, Bhojiya & Joshi et.al., 2016, Chen et.al., 2014 (a), Afzal et.al., 2017). *Paenibacillus* sp. RM (Host – *Tridax procumbens*), is a viable candidate for the bioremediation of zinc due to its potential function in promoting plant development, formation of secondary metabolites, and heavy metal bioremediation (Govarthanan et.al., 2016). *Mesorhizobium loti* and *Agrobacterium rediobacter*, two symbiotic isolates, have the highest potential for HM resistance and PGP characteristics (Fan et.al., 2018). With their inherent ability to promote plant growth, *Sedum alfredii's* bacterial endophytes VI8L2, II8L4, and VI8R2 may be one of the best options for enhancing phytoremediation of Zn-contaminated soil (Long et.al., 2013).

Nickel

About 0.008% of the earth's crust is made up of Ni, the 24th most abundant element (twice as abundant as Cu), which occurs in igneous rocks either as a free metal or in combination with iron (Hedfi et.al., 2007). Though it is a necessary heavy metal that organisms need in very small amounts, high Ni concentrations in polluted soils can seriously harm ecosystems and endanger human health (Luo et.al., 2017). Low molecular weight proteins like glutathione were depleted by Ni (Kukkola et.al., 2000). Interesting advantages for endophytic bacteria and their host plants could result from the removal of nickel through sequestration or bio-precipitation processes and a resulting decrease in the free nickel concentration (Lodewyckx et.al., 2001). Three new NiEB of *Tamarix chinensis*, identified as *Stenotrophomonas* sp., *Pseudomonas* sp., and *Sphingobium* sp., exhibit various plant growth-promoting features Indole Acetic Acid (ACC), siderophores, and 1-aminocyclopropane (Chen et.al., 2020).

Copper

Copper (Cu) is a crucial element, which takes part in many physiological processes and serves as a vital cofactor for many metalloproteins. Copper has the potential to be poisonous at high doses, generating signs including chlorosis and necrosis, stunting, discolored leaves, and restriction of root growth (Van Assche & Clijsters, 1990, Marschner, 1996). It hinders vital cellular functions including photosynthetic electron transport and prevents plants from growing (Yruela, 2005). Numerous enzymes, including cytochrome C oxidase, amino oxidase, laccase, plastocyanin, and polyphenol oxidase, use copper ions as cofactors. At the cellular level, Cu is vital for signaling the transcription and protein trafficking machinery, oxidative phosphorylation, and iron mobilization, whereas Cu poisoning leads to i) binding to sulfhydryl groups in proteins, hence limiting enzyme activity or protein function, ii) induction of lack of other critical ions, iii) defective cell transport mechanism, and iv) oxidative damage at the cellular level (Halliwell and Gutteridge, 1984, Van Assche & Clijsters, 1990, Meharg, 1994).

The endophytic *Bacillus amyloliquefaciens* from rice seeds had the highest tolerance to Cu stress and its inoculation significantly boosted seedling biomass, and growth characteristics, and promoted physiochemical response by lowering Cu uptake, whereas the levels of stress-responsive phytohormones such as abscisic acid (ABA) and jasmonic acid were dramatically decreased (Shahzad et.al., 2019). By accumulating large amounts of copper and encouraging rice development, *Bacillus amyloliquefaciens* of *Boswellia sacra* showed much stronger bioremediation potential (Khan et.al., 2017). *Elsholtzia splendens*, a host plant, and *Brassica napus*, a non-host plant on inoculation with metal-resistant plant growth-promoting endophytic bacteria showed increased absorption of Cu by exhibiting high levels of ACC deaminase activity (Sun et.al., 2016).

Cadmium

Cadmium is a heavy metal that is widely utilized in industries, primarily in galvanizing and electroplating, in batteries, in electrical conductors, and in the production of plastics and alloys. and in the stabilization of phosphate fertilizers (Byrne et.al., 2009). The total protein pool is impacted by Cd, because it binds to the thiol groups of proteins, which can cause an inhibition of their function or structural breakdown (Van Assche and Clijsters, 1990). It was shown by the NMR technique that *Suaeda salsa* was affected by Cd (50 gL1) in ways that increased protein breakdown, disrupted osmotic regulation, and altered energy metabolism (Liu et.al., 2011). The hybrid *Pennisetum* endophyte *Bacillus megaterium* showed a strong potential for removing cadmium and was more tolerant to cadmium (Wu et.al., 2019). Endophytic *Klebsiella pneumoniae* of *Vigna mungo* can survive Cd (II) concentrations up to 100 g/mL and can solubilize phosphate, produce indole acetic acid, hydrogen cyanide, and siderophore (Dutta et.al., 2018a).

Conclusion

In this work, the possibility of endophytic bacteria that promote plant growth, being able to lessen the toxicity of heavy metals is investigated. Various Species of bacteria belonging to *Bacillus, Enterobacter,* and *Pseudomonas* genus pose multi-metal tolerance and can be exploited as a viable candidates for the bioremediation of heavy metals. By lowering the toxicity of heavy metals, they aid in boosting physiological processes, oxidative stress reduction, and nutrient uptake, all of which contribute to the growth of plants. Additionally, research was needed on the inoculation of effective bacterial strains on various plants to identify the better plant for a larger percentage of bioremediation. The efficiency of individual strains in detoxification and the plant-bacteria relationship of the plants growing in metalliferous soils should be studied. Despite the degradation techniques, the application of genetically engineered microbes may have significant potential in bioremediation, but their influence on the ecosystem has to be elucidated before commercialization.

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