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Role of Pseudomonas species in Bioremediation of Mercury - contaminated soil

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Abstract

Mercury contamination is becoming more challenging because of the major amount of mercury entering the environment by natural activities.

It has been observed that major strains of *Pseudomonas species* have special clustered genes in an operon i.e. mer operon that easily detoxifies mercury contamination. Even some species of *Pseudomonas* such as *Pseudomonas aeruginosa,* and *P. putida* are involved in the remediation of mercury contamination in plants by phytoremediation phenomenon.

The present study is based on the role of mercury-resistant bacteria in mercury-contaminated soil and includes that bacterium in remediation design. To evaluate this assistance of mercury-resistant bacteria, this review focuses on determining the role of *Pseudomonas* species in the bioremediation of mercury-contaminated soil. Moreover, this review focuses on the mer operon and estimates the removal of mercury by *Pseudomonas* spp. and detects and describes the mercury reductase enzyme activity produced by a strain. Based on studies, *Pseudomonas* species were isolated from metal-contaminated and pesticide areas. And, among all P. species, two highly potent isolates that were resistant to high mercury concentration and able to remove mercury are *Pseudomonas aeruginosa* and *Pseudomonas putida.* Based on much research, the bacterial isolate *P. aeruginosa* showed the highest mercury bio-remediating capacity for Hg (II) i.e. 85% more than *Pseudomonas putida* under laboratory conditions. The results of this review article illustrate the occurrence of the diverse strain of *Pseudomonas*, bacteria capable of high tolerance to mercury. The mercury-reducing ability shown by *Pseudomonas* species signified their potential to develop bioremediation technologies and applications in the remediation of the environment and waste contaminated with mercury.

KEYWORDS: Bioremediation, Pseudomonas species, Mercury contaminated- soil, Heavy metal contamination, mer- operon, Heavy metal tolerance- *Pseudomonas spp., Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas fluorescens*, Mercury toxicity.

Introduction

A Bacterial species*, Pseudomonas* that is considered as potent species that remediate heavy metals contamination.

Pollution is one of the most serious issues in our World, posing numerous threats to humans, plants, and ecosystems.

Heavy Metals contaminate our environment as the result of activities of Industries, such as mining, refining, industrial effluent and electroplating etc.

Contamination by heavy metals in the environment is becoming more challenging in developing countries and this heavy metal pollution causes harmful effects on human health.

Therefore, researchers are searching for biotechnical methods which are eco-friendly and do not cause secondary pollution. Among all heavy metals mercury (Hg II) is considered the most toxic heavy metal in our environment. Acrodynia or pink discoloration disease is caused by mercury poisoning.

Mercury has the ability to;

1. Ability to travel long distances,
2. Ability to Bio-accumulate,
3. Ability to Bio-magnify.

Due to their ability to Bio-magnify and Bio-accumulate in the food chain, they adversely affect human and animal health.

Mercury is released into the environment as the result of various industries such as the pharmaceutical industry, paper industry, pulp preservative industry, agricultural industry, and other chemical drugs like chlorine and soda production industry.

Mercuric chloride and methyl mercury are highly carcinogenic, as declared by the Environmental Protection Agency (EPA). Contamination of mercury in the environment can cause harmful effects on human health like lung damage, vomiting, diarrhea, nausea, skin rashes, increased heart rate, headache, fatigue, tremors, depression, memory problems, fluctuation of blood rate, vision issues, etc.

Even also observed that the concentration of mercury in aboveground parts of plants appears to depend largely on the amount of HgO volatilized from the soil by uptake of foliar It has been noticed that uptake of mercury is plant-specific in bryophytes, lichens, wetlands, plants, Woody plants and crop plants.

Phytoremediation is a type of bioremediation by plants that has also emerged as an alternative technology for the management of toxic heavy metal contamination

Impacts of Mercury- contamination on the living system

Mercury contamination is a considerable public health and environmental issue because Methylmercury enters into the bloodstream easily and affects the brain.

The toxicity of mercury is based on its chemical form and the way of exposure like how the enters into the environment.

There are three different forms of mercury and all are toxic to human health. Their toxicity depends on the form to which people are exposed. The most toxic form of mercury is an organic form i.e., **Methylmercury.**

Methylmercury Poisoning; Methylmercury is the most toxic form of mercury contamination because this form of mercury can accumulate in the food chain and reach high concentrations.

This organic form in food such as fish, is a certain human and animal health hazard because methylmercury is easily taken up into the body through food and enters the bloodstream, which further causes severe health – hazardous.

This organic mercury affects the immune system, alters enzyme systems, and genetics, and damages the nervous system which includes coordination and all five senses. Methylmercury also damages developing embryos, causing chromosomal aberration and a genetic disorder.

Methylmercury is the most poisonous form of mercury that can weaken the human body and cause death. In both freshwater and ocean sediments, Methylmercury is produced by the methylation of inorganic mercury in aquatic biota.

Then further they accumulate in micro-organisms and fish that bio-magnify into the find chain by concentrating up the food chain reaching high levels in edible fish. And Consuming these contaminated fish is the major cause of mercury poisoning.

Symptoms arise progressively from modest to moderate to advanced and can be lethal after one month of crucial exposure.

The first signs are a decrease in senses like senses of vision, taste, touch, and hearing which further leads to difficulties in coordination and finally breakdown nervous system, kidney failure, lung damage, and tremors.

High quantities of the contamination may result in death in about four to five weeks after the initial set of signs.

Reference can be made to the Minamata tragedy in Japan (1950's) where thousands of persons died from methylmercury poisoning by eating contaminated fish due to mercury explosions being dumped into the surrounding sea.

Even thousands of people also died in Iraq in the 1970s by eating bread made with mercury-contaminated grain.

Exposure to the organic form of mercury is usually by ingestion, and it is consumed more readily and excreted more slowly than other forms of mercury contamination.

Elemental Mercury Hg (0), causes tremors, gingivitis, and skin disorder. Although elemental mercury is slightly toxic than methylmercury, elemental mercury may be found in high quantities in environments such as gold mine sites, laboratories, and chemical industries.

Inorganic Mercury ($HgCl_2$); Ingestion of inorganic forms of mercury, such as the salt $HgCl_2$, which damages the gastrointestinal tract causes kidney failure, and harms the respiratory system.

People are exposed to organic Mercury such as methylmercury almost entirely by consuming contaminated fish that are at the top of aquatic food chains.

The National Research Council, in its 2000 report on the toxicological impacts of methylmercury, marked that the population at highest risk are developing embryos and pregnant women who consume large amounts of contaminated fish and seafood.

The report took place to estimate that more than 60,000 children are born each year in danger of adverse neurodevelopmental effects and genetic disorders due to in utero exposure to methylmercury.

Mercury Study report in its 1997, to Congress, the U.S. Environmental Protection Agency (EPA) presumed that mercury also may pose a hazard to some grown-ups and wildlife populations that consume large amounts of mercury-contaminated fish.

Sources of mercury

Both natural and anthropogenic origin are sources of mercury.

Mercury is released into the environment by natural activities such as volcanoes, soil erosion, volatilization from the oceans, geological deposits of Mercury, and biological formation of elemental and methylmercury.

Whereas, anthropogenic sources are industrial effluents, mining, refining, agricultural activities, domestic garbage, medical waste, urban runoff, Fossil fuel combustion, and electroplating, etc.

Thermal treatment of gold and mercury ores are major sources of Mercury pollution.

In the atmosphere, most of the Mercury pollutants released as gaseous elementals (Hg^0) which are able to travel long distances and further deposit into aquatic or terrestrial environments cause Mercury pollution in that area.

They all participate in the cycle of biogeochemical cycle through which mercury enters the environment through human and natural activities.

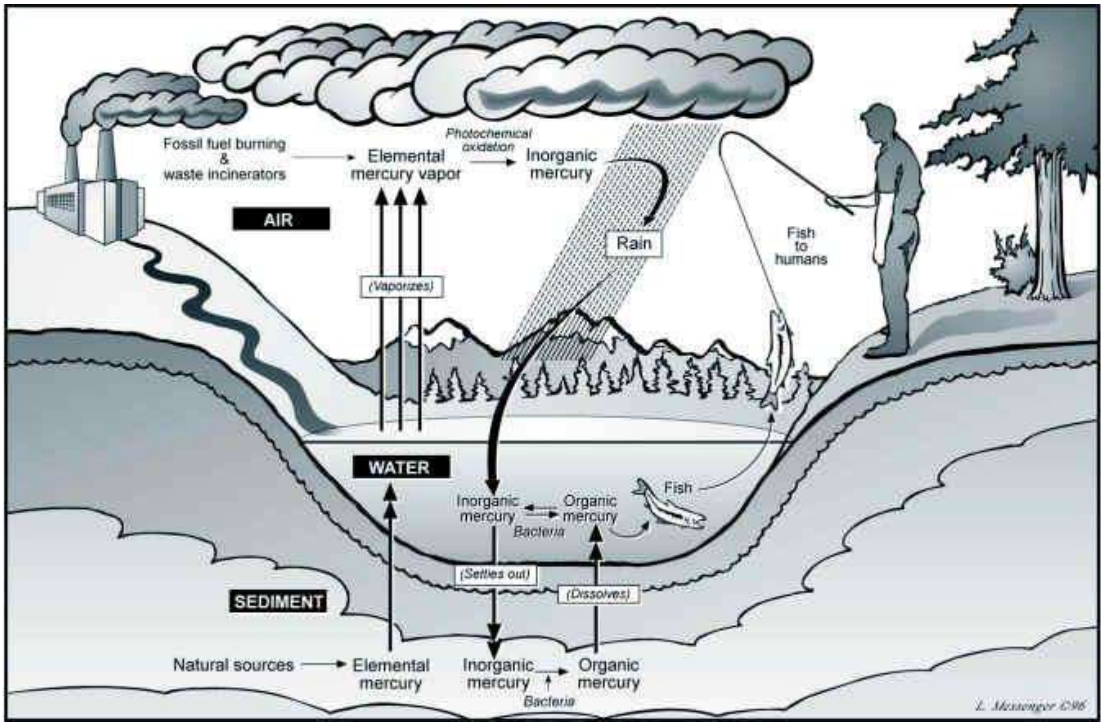

FIGURE 1: BIOGEOCHEMICAL CYCLE OF MERCURY [1]

Bacterial resistance to mercury

As a response to mercury pollutants globally distributed by geological and human activities, microbes have developed a surprising array of resistance mechanisms to resist Hg toxicity.

However, some bacterial populations residing in the mercury-contaminated regions can exchange mercury resistance genes with each other, because of continuous susceptibility to the toxic levels of mercury. After the acquisition of resistance genes, those bacteria will be resistant to.

A considerably studied resistance system based on clustered genes in an operon (i.e. mer), that allows bacteria to detoxify Hg2+ into volatile mercury by enzymatic deduction.

It seems that bacterial mercury resistance is an ancient mechanism, possibly acquired even before the anthropogenic usage of mercury.

Since the same biotransformation that includes the Hg biogeochemical cycle can take place inside the human body, interpreting its external transformations and transport processes will help in concluding which of these processes can exacerbate or reduce Hg toxicity in humans.

The genes involved in the mer operon are shown in Fig 2:

a) mer T, mer P for Transport.

b) mer A for Mercury reduction.

c) mer B for Cleavage of mercury from organic residue.

d) mer R and mer D for regulation.

e) mer C and mer E for Membrane proteins, conferring transport functions.

f) mer G for resistance to phenyl mercury.

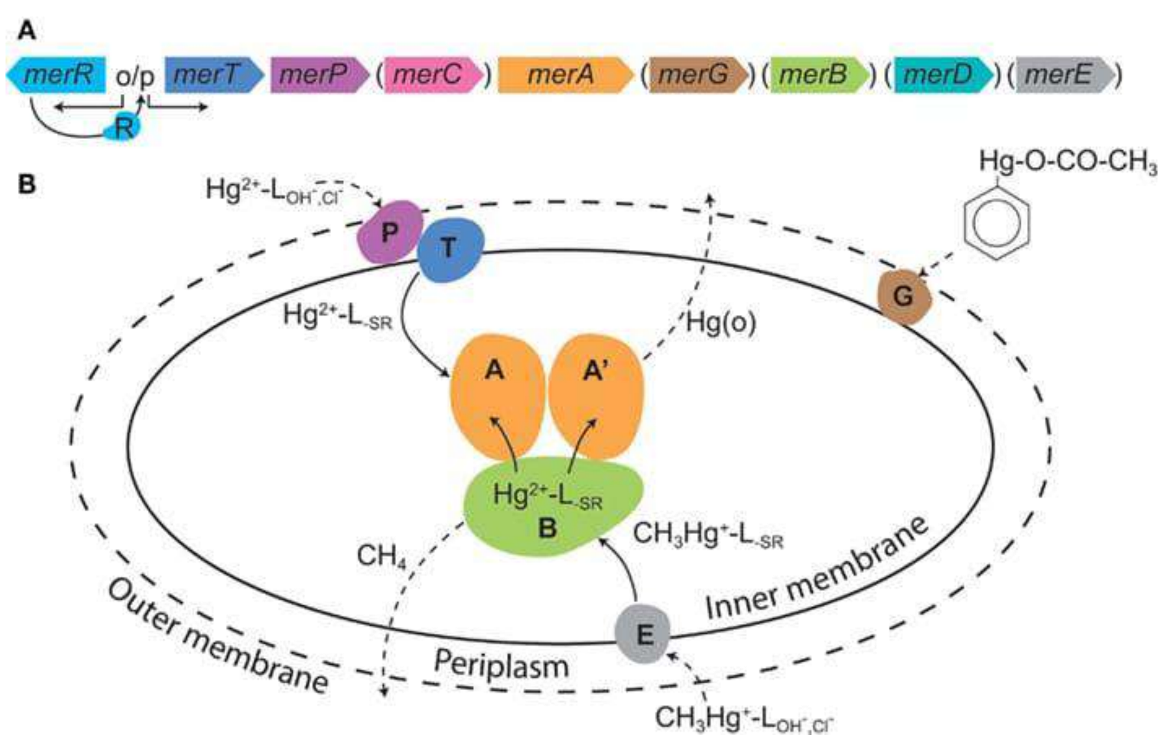

Figure 2 Mer operon [2]

Pseudomonas Species in bioremediation of mercury contamination

Pseudomonas is characterized as a Gram-negative rod bacterial genus.

Pseudomonas species are ubiquitous in waters and soils, being one of the most flexible bacteria in distinct environments.

Pseudomonas spp. is a tremendous microorganism for use in bioremediation because of the flexibility and plasticity of its metabolic ways.

Pseudomonas and the genus have enormous heterogeneity.

Pseudomonas species includes several scientifically and medically studied bacteria that are used in many chemical tools for remediation of contamination of heavy metals, such as P. aeruginosa, and there is an intensifying availability of *Pseudomonas strain* genome sequences.

Pseudomonas is well known for its metabolic versatility, because of its ability to utilize a barely wide range of organic compounds.

Major species of this genus are able to metabolize pollutants, including *P. fluorescens, P. putida*, *P. cepacia, P. vesicularis, and P. paucimobilis.*

As a result, these bacterial species are often isolated and studied for their bioremediation capabilities.

1: Pseudomonas putida

Pseudomonas putida belongs to the wide group of *fluorescens Pseudomonas species* and P. *putida* is a ubiquitous rhizosphere, soil colonizer, and saprophytic bacteria.

Strain KT2440 of P. putida is the best-characterized member of the group, came to be a model laboratory species that attracted significant attention as a cell host for metabolic engineering and synthetic biology due to its remarkable and versatile metabolism, which has grown up to withstand drastic environmental conditions and physicochemical stress, etc.

P. putida has also maintained the proficiency to survive and thrive in natural soil environments.

P. putida mt-2 and other isolates have been identified and used as tools for bioremediation due to their ability to grow on complex substrates, including aromatic compounds, organic compounds, and heavy metals.

According to many research, it has been observed that among all *Pseudomonas spp*., a strain *Pseudomonas putida* removed approximately 78% mercury contamination at 10 μg/ml (MIC), of mercury concentration, and by strain KT2440 removed approximately 62%.

2: Pseudomonas aeruginosa

Pseudomonas aeruginosa is a bacterial strain that is considered a potent strain that remediate heavy metal contamination.

Pollution is one of the most serious issues in our world, posing numerous threats to humans, animals, plants, and ecosystems.

P. aeruginosa is the cheapest and most eco-friendly tool for biotechnology to remediate environmental pollution.

P. aeruginosa expressed the highest mercury bio-remediating capacity for Hg (II). *Pseudomonas aeruginosa* is one of the most important bacteria present in almost all contaminated sites.

It has been observed that *P. aeruginosa* is resistant to antibiotics, detergents, heavy metals, and organic solvents.

Many studies have proved that *P. aeruginosa* is one of the versatile and high-tolerance mercury-resistant bacteria isolated from different habitats.

P. aeruginosa can be used as a suitable bio-sorbent for the removal of mercury and other heavy metals from contaminated water and soil.

According to many research, it has been observed that among all *Pseudomonas spp*., a strain of *Pseudomonas aeruginosa* removed approximately 85% mercury contamination at 10 µg/ml (MIC), of mercury concentration.

3: Pseudomonas fluorescens

Pseudomonas fluorescens is a gram-negative bacterial strain that has been seen to be a good bacterial strain for bio-remediation of mercury-contaminated soil.

Pseudomonas fluorescens is not only resistant to mercury contamination however this strain is resistant to other heavy metal contamination such as chromium, cadmium, nickel, and lead.

According to many research, it has been observed that among all *Pseudomonas spp*., a strain of *Pseudomonas fluorescens* removed approximately 34.30% mercury contamination at 10 µg/ml MIC (minimum inhibitory concentration) of mercury concentration.

P. fluorescens not only detoxifies mercury or heavy metal contamination but also degrades hydrocarbon contamination in soil.

Conclusion

Bacteria are a profitable tool to remediate Mercury because they have crucial reactive interfaces for the absorption of minerals, nutrients, and foreign contaminants on their cell surface.

The bacterial membrane acts as an area of exudation and uptake and gives a lot of enzymatic action.

In the case of metal contaminants, some bacterial cells reduce or oxidize them by a special enzymatic system.

According to many research, it has been observed that bacteria have a resistance system based on clustered genes in an operon (i.e. mer), that allows bacteria to detoxify Hg2+ into volatile mercury by enzymatic deduction.

Pseudomonas species are the most potent bacterial species involved in bioremediation of mercury contamination because it has been observed that major strains *of Pseudomonas species* have special clustered genes in an operon i.e. mer operon that easily detoxifies mercury contamination.

Even some species of *Pseudomonas* such as *Pseudomonas aeruginosa*, *P. putida* are involved in the remediation of mercury contamination in plants by phytoremediation phenomenon.

According to many research, it has been concluded that at a mercury concentration of 10 µg/ml, MIC (minimum inhibitory concentration), 65% of the mercury removed by a *Pseudomonas species* and mercury removing rate of other species or strains are; *Pseudomonas aeruginosa* approximately 85%, *Pseudomonas putida* approximately 78% and by strain KT2440 of P. putida approx. 60-62%, *Pseudomonas fluorescens* approx. 32.30%, *Vibrio fluviailis* approx. 26.05% and other bacterial species are also involved in bioremediation of mercury-contaminated soil or water as follows: *Bacillus sp*. Approximately removed mercury, 68.1%, and*,* *Bacillus thuringiensis* approx. 42.7%.

Human activities such as domestic wastewater, urban usage, agricultural activities, mining, smelting operations, and industries that use heavy metals and metal-containing compounds are the major sources of heavy metals that outcome in environmental heavy metal contamination and subsequent human exposure.

Many physical, chemical, and biological methods have been largely used for their effectiveness in the removal of heavy metals from different environmental media. Among these techniques, bioremediation is deemed an innovative technology and remedial strategy to remove heavy metal contaminants provided its cost-effectiveness and eco-friendly nature. Bacterial systems, described by high surface-to-volume ratios are considered superior bioremediation agents.

Moreover, the bacteria have an enzymatic system that functionally and structurally different proteins that aid redox reaction in the bioremediation process.

The effectiveness of bacterial bioremediation also depends on various biotic and abiotic factors. Bacterial enzymes like reductases, oxygenases, etc., also influence the process of bioremediation. Phytoremediation is a type of bioremediation by plants that has also emerged as an alternative technology for the management of toxic heavy metal contamination. Cell immobilization is a well-known technique, which increases the performance of heavy metal uptake from the contaminated environment.

The comprehensive review of the literature available in bioremediation implies that genetic engineering of the bio-sorbents to enhance their heavy metal sobbing properties, the utilization of biomaterials left out after commercially significant production processes for bio-sorption and the fate of the absorbed substances as growth-enhancing factors are still largely unexplored and offer scope for future research.

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