

Mercury Resistant Bacteria: Eco-Friendly Way for Remediation

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ABSTRACT

Mercury is a heavy metal which is found naturally in the ecosystem in many different forms. Due to its highly toxic nature, its presence and increasing accumulation in the environment is of great concern. Methylmercury, an organic form of mercury, of all other forms act as global and serious problem for human as well as ecological health. Although all forms can be accumulated in the living organisms, but it has been observed that methylmercury can be taken up at a much faster rate as compared to others. This methylmercury has ill-effects on the human nervous system. It is also known to cause cancer, although it has not been proven yet. Mercury contamination has also been observed in aquatic ecosystem and it has disastrous results because its accumulation increases many fold in increasing level of the food chain. The adverse effect of mercury on the health holds in its ability of bioaccumulation and biomagnifications. The objective of this study was to isolate mercury-resistant bacteria and characterize them. These bacteria were characterized based on their morphological characters and biochemical tests conducted on them. Minimal Inhibitory Concentrations (MIC) was used to determine its bacterial susceptibility to Hg compounds.

Keywords: Heavy metals, Mercury contamination, Bacteria, mer operon

Mathematics Subject Classification: 92C70

JEL Classification : Q59

1. INTRODUCTION

The ongoing and thereby increasing development or urbanization has lead to the deterioration of the environment. The rate at which unorganized industrialization is increasing has also increased the amount of effluent waste. These effluents waste contains various toxic metals like mercury, chromium, arsenic, etc (Adelaja and Keenan, 2012). Heavy metals can thus enter water bodies through industrial discharge or consumer waste or even through acid rain which leads to heavy metal contamination in soil as well as water streams, rivers, lakes and groundwater. Heavy metals affect the diversity, number and activities of microbes present in the soil and water. Their level of toxicity depends upon various aspects like temperature, pH, organic matter, chemical forms of the metals, etc (Giller et al., 1998), example: copper toxicity increases by 7% when there is 4^o rise in temperature

from 20°C to 24°C (Khan et al., 2006). The negative influence of heavy metals is on the growth and activities of microorganisms which can directly affect their growth and development (Valentina et al., 2016). They are known to cause some serious ailments to the plant diversity also. (Table 1)

Table 1: Adverse affects of heavy metals on plants

Heavy Metal	Plant Name	Effect on Plant
Arsenic (As)	Rice (<i>Oryza sativa</i>)	Reduce seed germination and seedling height; leaf area & dry matter production also decreases (Abedin et al., 2002)
	Tomato (<i>Lycopersico esculentum</i>)	Fruit yield decreases (Barrachina et al., 1995)
Cadmium (Cd)	Wheat (<i>Triticum aestivum</i>)	Reduced plant nutrient content; decreased root and shoot length (Yourtchi and Bayat, 2013)
	Maize (<i>Zea mays</i>)	Inhibited root growth (Wang et al., 2007)
Mercury (Hg)	Rice (<i>Oryza sativa</i>)	Decreased plant height; reduced yield; bioaccumulation in root and shoot of seedling (Kibra, 2008)
	Tomato (<i>Lycopersicon esculentum</i>)	Reduced germination; reduction in flowering and fruit weight; chlorosis (Shekhar et al., 2011)
Lead (Pb)	Maize (<i>Zea mays</i>)	Suppressed growth; reduction in plant protein content (Hussai et al., 2013)
	Oat (<i>Avena sativa</i>)	Inhibited enzyme activity affecting carbon dioxide fixation ability (Moustakas et al., 1994)

Mercury is one of the most toxic metals and it's this toxicity is a serious matter of concern as it possess serious health issues to human as well as plants, fishes and animals (Müller et al., 2001a). Bacteria, due to its ability to survive in extreme conditions, can develop resistance against these heavy metals like mercury. These mercury resistant bacteria can be beneficial for the nature as they can help in bioremediation of heavy metals (mercury) and hence can help in its removal from the environment (Chatzieftimiou et al., 2007). Therefore, they can be isolated from the waste effluent water and can be characterized to analyze their biochemical properties. Thus, the main objective of the study is to isolate and characterize mercury-resistant bacteria which can be used for the betterment of nature.

2. MICROBIAL DIVERSITY

Our ecosystem is enormously diversified ranging from large varieties of macroscopic as well as microscopic organisms. This varying biodiversity is based on different levels: genetic, species and ecosystem level. Among these, microbial diversity is of great prevalence. It is the diversification present among microscopic organisms like bacteria, viruses, some protozoans and fungi also. The microbes present have been classified into three categories being Bacteria, Archaea, Eukarya. The organisms present under each one of them have different properties and behavioral changes according to their environment. All these microbes play a very vital role in maintaining a steady and

balanced cycle of our ecosystem. The importance of microbes is due to their metabolic activities occurring in the environment, their relationship and interaction with the plants and animals and moreover their use in biotechnological practices. Because of the inability to see microbes through naked eyes, their daily activities and activities carried out in their domains cannot be easily perceived and understood until or unless some special analyses are not carried out. All the microorganisms carry out immense tasks for sustaining in their ecosystem. The necessities performed by the microbes, though for their survival, like their growth and reproduction, photosynthesis, catabolic processes, etc, helps to attain and maintain a dynamic equilibrium of our ecosystem. Hence, it can be said that the environmental functioning and balance are encouraged and maintained by microorganism's actions.

Bacterial community is one of the most essential parts of ecosystem. It is not only important functionally but also provides stability to the environment through its physiological (Chatziefthimiou et al., 2007) and biochemical activities. Bacteria have a very unique but highly appreciable property of being able to adapt themselves to adverse or extreme conditions very rapidly. Due to this property, many researchers are using these bacteria to investigate or check the effluent discharges in the marine environment as these discharges are known to have heavy metal contamination. Presence of high amount of heavy metals like zinc, copper, nickel and mercury (Müller et al., 2001a) is altering the microbial community. Therefore, methods are being considered to preserve the microbial diversity not only to support human health (Srivastava et al., 2012) but also to augment its condition through appropriate utilization of genetic resources of microbial community.

3. TOXIC METALS IN THE ENVIRONMENT

Heavy metals are specified by their high atomic number and increased density. They are known to be present on the crust of our planet in trace amounts. They are mainly present in chemically bounded form like that in carbonate, sulfate, and oxide or can also be present in their elemental metallic forms (Hussain et al., 2010). Some of the heavy metals like copper or zinc are highly essential for the ecosystem and can possibly also help in human metabolism. Example: Cobalt, which is the central atom in vitamin B₁₂ complex, plays a crucial role in propionic acid fermentation pathway (Stowers et al, 2014). Although, these heavy metals are released into the environment through erosion and weathering but it can enter the environment through human activities also during their physical or chemical release basically from their ores. The agro-industrial, electrical, medical, domestic, and other uses are resulting in their excessive release into the terrestrial and marine ecosystem.

Heavy metals, when present in trace amounts, have beneficiary effects, but when its concentration exceeds a certain limit or present in high amounts can be toxic to all the life forms due to its ability to produce complex compounds in the cells. Some heavy metals like iron, nickel, copper are beneficial when present in trace amounts as they form a part of metabolic reactions. On the other hand, metals like mercury play no role in the nature and are very harmful for the organisms even when present in

small amounts (Alkorta et al., 2004). They cannot be degraded once enter the environment and hence causes various types of pollution viz, soil pollution, water pollution and air pollution. (Figure 1)

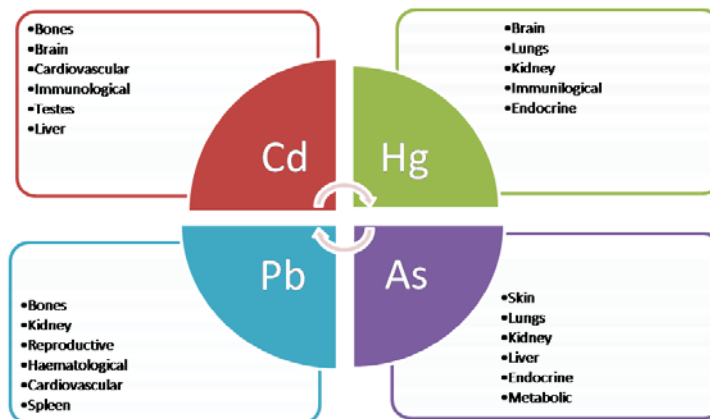


Figure 1. Impacts of heavy metals on environment (García-Niño and Pedraza-Chaverri, 2014)

Soil pollution, through heavy metals takes places due to various reasons particularly due to emissions from industries, mine tailing, leaded gasoline and paints, sewage disposal, wastewater irrigation, spillage of petrochemicals, etc. Thus, presence of heavy metals in the soil is of serious concern due to its indulgence in the food chain and hence can be destructive for the ecosystem. Various ways through which they can cause risks to animals, plants and humans are direct ingestion, utilization of contaminated water, variation in soil porosity, pH and its natural chemistry, thereby, affecting soil quality (Lee et al., 2002).

Industrialization and urbanization are two main reasons behind heavy metal accumulation in water bodies. This occurs due to waste discharges or runoffs from the industries leading to its sedimentation in soil and water bodies (Lee et al., 2002). Although they are present in trace amounts in water, yet it is toxic for the living form due to its property of bioaccumulation. Its accumulation increases with every level in food chain. Thereby, being maximum threat to humans because of sea food consumption.

Besides releases from industries and factories, heavy metals are released into the air through volcanic eruptions, dust storms, rock weathering and other anthropogenic processes (Soleimani et al., 2018). Some of the heavy metals like copper, cadmium, nickel, aluminum, iron, etc arise from the industrial release as a part of particulate matter (Soleimani et al., 2018).

Presence of heavy metals is of great concern for living forms as it is known to affect the cell organelles like mitochondria, endoplasmic reticulum, cell membrane, nuclei and sometimes enzymes also which are a necessitate for metabolism, damage repair and various other practices (Wang and Shi., 2001). They cause some conformational changes in DNA and other nuclear proteins leading to carcinogenesis or sometimes apoptosis also (Beyersmann and Hartwig., 2008). Because of their high level of toxicity, metals like arsenic, cadmium, mercury, chromium and lead are considered the main

priority for health purposes. These can be the cause of multiple organ failure even when present in low amount. As claimed by United States Environmental Protection Agency (U.S.EPA) and International Agency for Research and Cancer (IARC), these metals can be categorized as 'probable' or 'known' human carcinogens (Tchounwou et al., 2012).

4. MERCURY TOXICITY IN THE ENVIRONMENT AND ITS HARMFUL EFFECTS

Mercury, a heavy metal, belongs to transition series element. It is known to be used in various dimensions namely in thermometers, some medications and in dental fillings. But its usage in thermometers was banned by European Union Directive due to its high level of toxicity. It exists in various forms in nature, mainly elemental, organic and inorganic, and all these forms have its own toxicity profile (Clarkson et al., 2003). It exists in liquid form at room temperature and releases in to the environment as mercury vapors. It can also subsist as cations with +1 and +2 oxidation states (Clarkson et al., 2003). The highly toxic nature of mercuric mercury (Hg^{+2}) is accredited due to its coherence with sulphydryl groups of the cysteines present in vital enzymes and proteins, thereby severely affecting the gut and renal systems. Hg^0 (elemental form) is toxic as it can easily be inhaled by the organisms due to its high vapor pressure. It spreads throughout the body including brain of the organism through blood and gets converted into Hg^{2+} in liver, RBCs, and central nervous system. In organic form, it usually occurs as mono or di-methylmercury or as phenylmercuric acetate, all of them being lipid soluble, hence, can easily be absorbed into the body. It can even penetrate blood-brain barrier, transforming into Hg^{2+} and causing serious neuromuscular malfunctions and other disorders (Barkay, 2000).

Methylmercury is known as one of the most poisonous form of mercury. It is particularly dangerous for humans because of its high power of accumulation in the body of animals including humans. It gets accumulated at a very profound rate in food chain especially among aquatic food chain (Keating, 1997). Its accumulation power increases tremendously amongst fish and by the time it is consumed by humans, its concentration is high enough to cause serious ailments in the body. In pregnant ladies, it can lead to still births of child or even babies with some deformities or nervous system disorders (Tsuchiya et al., 1984). Hence, it can be said that due to its accumulation in humans, it can cause some serious problems to nervous system, heart and kidney and other organs also. It has the ability to cross blood-brain barrier and even placenta, critically affecting the brain and fetus respectively. Minamata disease is one of the most common problem caused due to its accumulation resulting in symptoms like paresthesia, numbness in fingers, etc (UNEP, 2003). (Figure 2)

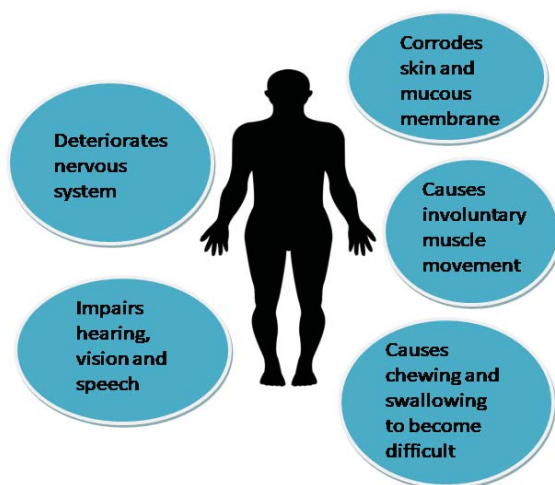


Figure 2. Effects of mercury on human body (Krabbenhoft and Rickert, 1995)

Therefore, mercury, because of its high toxicological properties, affects not only flora but also wildlife and human health. Because of its high accumulating power, it has detrimental effects on both high predators and microbial community. Due to its passive uptake, it moves up towards higher hierarchy leading to bio-magnification and resulting in some acute or chronic ailments in humans.

5. WHY MERCURY-RESISTANT BACTERIA?

Bacteria have advanced and progressed itself to tolerate high concentration of metal ions in extreme areas like volcanic zone, hydrothermal vents ore deposit regions, etc. Therefore, to live in that area, these microbes developed some survival strategies against the heavy metal ions. The resistant factors are generally found on plasmids or transposons (Bruins et al., 2000). The resistance mechanisms usually work by expelling out the toxic metals ions from the cell cytoplasm. There are five chief resistance mechanisms adopted by the bacteria against heavy metals (Bruins et al., 2000; Choudhury and Srivastava, 2001):

Extracellular barrier: It prevents the entry of metal ions into the cell. Bacteria usually do this by adsorbing the metal ions by using the ionizable groups (like carboxyl, amino, phosphate groups) present on cell wall or the capsule. Even dead bacterial cells are known to have the ability to bind the metal ions. Example: non-viable cells of *Pseudomona putida* (Pardo et al., 2003), *Bacillus sp* (Green-Ruiz, 2006) have been observed to adapt this mechanism.

Active efflux of metal ions: Most of the bacteria exhibit resistance mechanism through efflux or active mechanism. This method is used to export metal ions from the cell. These metal ions are exported

from the cells through ATP hydrolysis (Rensing et al., 1999) or through electrochemical gradient (Nies, 1995).

Extracellular sequestration: It is defines as the accumulation of accretion of metal ions cellular components in the periplasm or mixing up of metal ions as insoluble compounds. This resistance mechanism is generally seen to be followed by copper-resistant bacteria. Example: *Pseudomonas syringae* (Cha and Cooksey, 1991).

Intracellular sequestration: It is the complexation of metal ions in the cytoplasm of the cell. This resistant mechanisms are observed in cadmium, silver, etc tolerant bacteria. Example: *P. putida* (cadmium tolerant) possess intracellular sequestration of copper and zinc ions along with the help of cysteine rich proteins of low –molecular weight (Higham et al., 1986).

Reduction in metal ions: Bacteria, with the help of metals and metalloids as electron donor or acceptor as energy generators, have the ability to reduce a large spectrum of heavy metals. Example: (Table 2)

Table 2: Reduction processes shown by different microbes

Reduction process	Microorganism
Hg^{2+}/Hg^0	<i>Bacillus cereus</i> (Gupta et al., 1999) <i>Klebsiella pneumonia</i> (Zeroual et al., 2001)
Fe^{3+}/Fe^{2+}	<i>B. thermoamylovorans</i> (Slobodkina et al., 2007) <i>Geobacter sp</i> (Snoeyenbos et al., 2000)
As^{5+}/As^{3+}	<i>S. aureus</i> (Ji and Silver, 1992)

Heavy metal resistant bacteria can be isolated from industrial waste water (Mergeay et al., 2003), mines (Saxena et al., 2002), soils contaminated with heavy metals (Viti et al., 2003), etc. Although plasmids are basically known as the genetic determinants of resistant mechanisms, but similar system is found in some chromosomes also. Microbes like *E. coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* has Ars operon which are structurally similar to plasmid genetic determinants (Mukhopadhyay et al., 2002). There still can be difference between chromosomal and plasmid resistance, for instance, vital metal ion homeostasis genes are present on chromosomes whereas toxic metal resistant genes are located on plasmid (Bruins et al., 2000; Cervantes et al., 2001). However, horizontal gene transfer (HGT) has been observed to be a part of evolution of genetic determinants for mercury tolerance (Osborn et al., 1997).

6. ISOLATION OF MERCURY- RESISTANT BACTERIA

As mercury contamination is a big problem, many conventional methods were employed for it but because of its costliness and further release of harmful effluents, they could not be carried further. As a result, mercury-resistance has been observed to be linked to natural flora (Müller et al., 2001b).

Hence, microorganism mediated mechanism of mercury bioremediation has come into light. According to previous studies, bacteria have different genetic mechanisms to convert toxic form of mercury into its lesser toxic forms (Pepi et al., 2013). Mercury- resistant bacteria are ubiquitous in nature and consider approximately 5-6% of aerobic heterotrophic bacteria (Müller et al., 2001a). Half of the strains of bacteria can easily be isolated on basal media like nutrient agar media and Luria Bertani agar media enriched with as high as 10 ppm of Hg^{2+} . On the other hand, some sensitive strains can tolerate only 1 μM of Hg^{2+} in growth media (Barkay, 2000). Even though, mercury resistant bacteria are known to be found in areas with mercury contamination, but still, they have been isolated from uncontaminated and healthy environment also.

Resistant mechanisms have been observed in both gram positive bacteria (eg: *S. aureus*, *Bacillus* sp.) and gram negative bacteria (eg: *E.coli*, *P. aeruginosa*, *Thiobacillus ferrooxidans*) (Misra, 1992). Mercury resistant bacteria, in cold regions, have been found to belong to the *Alpha*-, *Beta* and *Gammaproteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacterioidetes* (Annette et al., 2011). It has also been observed that among them approximately 31% are only culturable (Annette et al., 2011). However, their resistant levels were not found to be temperature dependent (Møller et al., 2011). They work with the help of mercuric reductase enzyme which reduces active Hg^{2+} to elemental, volatile and inert $\text{Hg}(0)$, a relatively less toxic form.

7. MODE OF ACTION OF MRB

There are various bacteria which act as a MRB (mercury resistant bacteria). Some of them belong to *Pseudomonas*, *Proteus*, *Xanthomonas*, *Aeromonas* and *Enterobacteriaceae* (De et al., 2003). Organisms like *Citrobacteria*, *Klebsiella*, *Rhodococcus* are generally seen to be preferred for bioremediation mechanisms (Keramati et al., 2011). Examples: Tributyltin resistant isolate of *Enterobacter cloacae* help in bioremediation of high amount of methylmercury (by using carbon-source in the pollutants to mineralize the organic compound) (Adelaja and Keenan, 2012); *Pseudomonas putida* SP-1 is capable to volatilize 89% of mercury enabling to be very efficient for marine ecosystem (Zhang et al., 2012). Anaerobic bacteria can also be used for bioremediation as they can precipitate Hg^{2+} to insoluble cinnabar (HgS). But they are a bit different from aerobes in their mechanism as they methylate mercury in anoxic environment (Kerin et al., 2006). Example: *Geobacter* species can volatilize mercury but on the other hand can also produce methylmercury (Kerin et al., 2006). Hence, there is a danger involved during mercury transformation under this anoxic condition and need more proper and careful surveillance during bioremediation.

Mer operon is the most known and studied resistance mechanism against mercury. It was discovered in 1970s, and since then it has been focused on in detail on every aspect being the genes involved, their structure, functions and even their regulation mechanisms (Liebert et al., 1999). This operon allows bacteria to detoxify Hg^{2+} into lesser toxic volatile form by enzymatic reduction (Silver and Phung, 1996). Mer operon is located on plasmid (Griffin et al., 1987), chromosome (Wang et al., 1987), transposons (Misra et al., 1984) and integrons (Liebert et al., 1999). The mer operon performs three major functions:

- Movement of Hg^{2+} into the cell.
- Convert ionic mercury into elemental mercury (Hg^0) (less toxic form) through enzymatic NADPH- dependent mechanism
- Regulate functional genes

The operon has been well sequenced in both gram-positive and negative bacteria. These are conferred by genes (Misra, 1992):

- merT (transport)
- merP (transport)
- merA (Hg reduction)
- merB (Hg cleavage from organic residue)
- merR (regulation)
- merD (regulation)
- merC and merF (membrane proteins for transportation)
- merG (provide resistance to phenylmercury)

All the genes have different functions to perform. Mer R suppresses the operon in the absence of inducible whereas turn on the transcription in its presence. Mer D, on the other hand, coregulates the expression of operon (Silver and Phung, 1996; Misra, 1992). Hence, it can be said that mer operon is regulated by merR and merD, which are further inducible by $\text{Hg}(\text{II})$. MerT and merP are present in the periplasm and inner membrane of the plasmid respectively. They help in transportation of metal ions across the cell membrane. Mercury transportation is handled by the products of genes merC and merF, which acts as membrane proteins (Morby et al., 1995). MerB plays an important role by cleaving the carbon-mercury bond of the organomercurials. The result of the cleavage, $\text{Hg}(\text{II})$, can further be reduced by the mercuric reductase (Silver and Misra, 1988). Mercuric reductase, a flavin adenine dinucleotide consisting of disulfide oxidoreductase, is the result of merA. It is the most essential enzyme used for detoxification of $\text{Hg}(\text{II})$. Its ability to shift electrons from NADPH or NADH to $\text{Hg}(\text{II})$, leads to the formation of elemental $\text{Hg}(0)$, which is harmless and gradually diffuses out of the cell (Misra, 1992; Siver and Phung, 1996; Osborn et al., 1997). Thus, metallic mercury volatilizes and as a result, the cell detoxifies its microenvironment. Sometimes, the operon might contain some special genes also like merG. The product of this gene helps to provide resistance against phenylmercury by decreasing the cell permeability towards it (Kiyono and Pan-Hou, 1999).

It is an inducible operon. Hence, presence of Hg^{2+} is compulsory in order to activate resistance expression or transcription will be suppressed in its absence (Misra et al., 1984). Bacteria with “narrow spectrum resistance” are resistant to only $\text{Hg}(\text{II})$, whereas, the ones resistant to both $\text{Hg}(\text{II})$ and organomercurials are known to be with “broad spectrum resistance”. Broad Spectrum ie, cleavage of mercury from organic residue, is also important for detoxification.

$\text{Hg}(\text{II})$ is known as the environmental sensor as it controls the switch of mercury resistance proteins components. MerT and merC carry two pairs of cysteine and merP holds one pair of cysteine. It is

presumed Hg (II) are sequenced and transferred to the thiols of cytoplasmic mercuric reductase because of these cysteine pairs (Misra, 1992). Transmembrane protein merT plays the role in this transportation process. Therefore, Hg (II) is never on the loose for the interaction with the cell components and thus only Hg (0), the harmless form, enters cytoplasm.

It is clearly evident that increased mercury level in the environment creates selection pressure on the microbes. Those which can adapt to this environment can live through the heavy metal contaminated environment. These bacteria adapt various strategies to cope up with the toxicity: demethylation of methylmercury which is further converted to mercuric sulphide compounds, conversion of mercuric mercury to volatile methylmercury, enzymatically reduce Hg^{2+} to Hg^0 , etc (Kiyono and Pan-Hou, 1999).

Despite the variation in physical positioning to mer operon, essential genes are present in all but unexpectedly, there are very few studies to mercury resistance in marine isolates at molecular level. It was found that only 12% of culturable MRB from aquatic habitat hybridized to mer, indicating that MRB from marine habitat encode either some new mer genes or use some other mechanism for providing resistance against Hg (Reyes et al., 1999).

8. APPLICATION OF MRB FOR BIOREMEDIATION PROCESS

Bioremediation is, thus, need of the hour. Bioremediation is the term used to describe the process which helps to detoxify the chemicals and harmful materials preset in soil, water bodies (or waste water from industries) or ground water. It can also be defined as use of living creatures to eliminate the harmful and toxic chemicals and metal ions present in the environment. Microbial bioremediation is now-a-days the most used and preferred form. Utilization of microorganisms for degrading and detoxifying the amount of toxic particles present in the water, soil surface, waste water, etc is called microbial bioremediation. Example: *Pseudomonas putida* (Pushkar et al., 2019). It works best along with the presence of surfactants, pH stabilizers, oxygen and some nutrients. The main purpose of usage of microbes is that they convert the harmful chemicals into a form which is not hazardous for the environment and human life. A bioremediation process depends on various factors like the contaminant, hydrogeological factors, microbial ecology and other environmental factors. Diversified aspects are kept in mind during microbial assay, viz, microbial growth, degrading population amount, enzymes and their mechanisms which can possible destroy the harmful contaminants and controlled conditions during the process (Dua et al., 2002). If conducted properly, under controlled conditions and proper management, it can be very cost-effective and can easily destroy all the contamination without causing any affect on the environment (Dua et al., 2002; Wagner-Döbler., 2003).

Mercury bioremediation using mercury- resistant bacteria (MRB) was first carried out in 1984 and since then it has been in practice to find new ways to perform it. The best method was found, by reducing Hg through MRB from chloralkali waste (Wagner-Döbler, 2003). Another, an inexpensive and promising method, was also reported which involved adsorption of metal into algal or bacterial biomass (Mulligan et al., 2001). Various researches have been conducted to for removal of mercury

contamination. Methods like bioprecipitation of metal ions on the surface of cell (Diels et al., 1995); using recombinant microbial biomass for biosorption (Pazirandeh et al., 1995); utilization of genetically engineered *E. coli* (Chen and Wilson, 1997b); etc have been reported against mercury decontamination.

It is, hence, preferred because of its less cost, effectiveness and more efficient as compared to the other physiochemical processes. It only requires presence of bacteria along with a feeding source such as sucrose, glucose, fructose or others which act as an energy source for the bacteria to carry out the reduction of mercury. Some researchers (Gupta and Ali, 2004) were able to remove mercury from chloralkali electrolysis wastewater using a mercury resistant bacteria *Pseudomonas putida*. It was able to transform about 97% - 98% wastewater with about 8 ppm of Hg^{2+} (Wagner-Döbler, 2003). These kind of studies lead to the development of technical scale bioreactor which could decontaminate mercury polluted chloralkali wastewater effluents in situ (Wagner-Döbler, 2003).

Nevertheless, it also has its limitations, for example, production of intermediate products, which may be more harmful than the original substrate; some products produced can be non-biodegradable. These disadvantages can be overcome by being more specific during the process and handling all the substrates with utmost care and precautions.

In spite of large initial interest using sorption as removal technology, no initiative has been taken for commercial application (Gadd, 2000). They have only been restricted to laboratories. Mercury in radioactive wastes was examined for microbial bioremediation by the use of *Deinococcus geothermalis* (Brim et al., 2006). Therefore, still studies are being carried out to use this microbial bioremediation technique commercially and on a larger scale for healing of environment (Sinha et al., 2012).

All things considered, these mercury resistant bacteria can be used for bioremediation purposes of many kinds of mercury contamination (Shazia et al., 2002) for the betterment of the environment. It is a very efficient, environment friendly and moreover an economic way to eliminate mercury from pollutes wastewater and also contaminated soil enabling it as a reusable land (Iyer et al., 2005).

9. CONCLUSIONS

Due to the increasing status of mercury in the environment, it has become important to find out solutions which are ecological as well as effective. Hence, utilization of mercury-resistant bacteria is coming into consideration and is being followed up by many researchers as in how to use these bacteria into our lives for the betterment of the environment. MRB is not only an eco-friendly way to solve this problem but is also a reliable and practical method. To degrade the mercury in the waste, MRB must possess some mechanism to handle its highly toxic nature and hence its characterization and biochemical analysis helps us study all the factors for dealing with the toxic metal. The genes present on the *mer* operon helps in transforming the toxic organic and inorganic form of mercury into less toxic or elemental form. Bacteria like *Pseudomonas putida*, *Sphingomonas*, *Bacillus thuringiensis* have been known to show high level of mercury tolerance. These bacteria can be used commercially

by industries and production plants in their waste treatment processes to remove the presence of mercury before releasing the wastes into the environment. They can remove almost 95% of contamination in very short period of time. Further, these isolated bacteria can be studied for their bioremediation mechanisms to help increase its efficiency and put to better use.

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11. CONFLICT OF INTEREST

Authors declare no conflict of interest.

12. AUTHORS' CONTRIBUTION

All authors have direct and intellectual contribution to the work, and approved it for publication.

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