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Bioremediation of Mercury Compounds by using Immobilized Nitrogen-fixing Bacteria

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Abstract

The mercury (Hg) contamination is of great concern because of its toxicity and ubiquity. The environmental level of Hg is rising day by day because of anthropogenic activities. Bearing in mind the toxicity of Hg, in this study, we have isolated various strains of nitrogen-fixing bacteria (NFB) from nodules of different plants. The preliminary screening of NFB isolates resisting Hg was done by well plate assay. Further screening of selected isolates was done by quantitative assay for Hg remediation in liquid cultures by using dithizone method and hydrogen sulfide (H_2S) production by growing them on LA medium. Characterization of the selected strains for the Hg resistant and hydrogen sulfide producing NFB strains, identified as *Enterobacter, Cronobacter and Pseudomonas*, gave the most promising results in the detoxification of Hg. Finally these strains were immobilized in sodium alginate (synthetic beads) and their ability to detoxify Hg containing industrial effluents was determined as compared to free cells. It is concluded that immobilized NFB bacteria detoxified higher concentration of Hg as compared to free cell cultures. © 2014 Friends Science Publishers

Keywords: *Cronobacter*; Dithizone; *Enterobacter*; Nitrogen fixing bacteria; Sodium alginate; Immobilization

Introduction

Mercury (Hg) is potent cellular toxin and its compounds are considered health hazardous and cause many serious diseases in human. In the form of either ionic mercury (Hg^{2+}) or elemental Hg enters in the environment but undergoes numerous biotic and abiotic alterations. Methylmercury (MeHg), the most toxic compound formed from Hg^{2+} by the activity of anaerobic bacteria (Compeau and Bartha, 1985), is a human health and aquatic organisms concern because of its accumulation and biomagnifications in the food chain (Johnsson *et al*., 2005). Methylmercury interacts with soil constituents less strongly than Hg^{2+} and is therefore more mobile in the environment (Gabriel and Williamson, 2004). It is more problematic for living organisms as it causes serious neurological disorders (Brodkin *et al*., 2007; Holmes *et al.,* 2009).

Contamination due to Hg is of great concern because of its toxicity and ubiquity. Release of Hg from human activities and later atmospheric deposition into the air, aquatic and terrestrial ecosystems is creating global contamination problem. The environmental levels of Hg have risen due to anthropogenic activities, such as mining activities, petroleum and coal burning, and industrial wastes (Protano *et al.,* 2000; Barringer *et al*., 2005, 2006) as well as use of pesticide and fertilizers in agriculture. Metals contamination is a real threat not only to the human health but also to the aquatic organisms due to their persistent nature (Ubaid-ullah *et al*., 2004; Javed, 2013). Efforts are underway to reduce the level of Hg throughout the world by using different advance techniques such as ion exchange, reverse osmosis, precipitation, electrochemical treatment (Akpor and Muchie 2010), gold and manganese oxide nanoparticales etc. (Kamarudin and Mohamad 2010; Lisha *et al*., 2010). However, most of these techniques are expensive and generate hazardous by-products (Zhang *et al*., 2005).

In nature, the microorganisms have developed an array of resistant mechanisms to overcome Hg toxicity. These organisms can be used to detoxify Hg pollutants and prevent further metal contamination. Hg resistant bacteria including free living (Prantera *et al*., 2002), symbiotic (Radwan *et al*., 2007) and nitrogen-fixing bacteria have the potential for hydrocarbon utilization. Methylmercury is highly toxic and these bacteria have evolved genes that convert it to a much less toxic form. In this perception, microbial bioremediation seems to be most effective and potent approach to remove/detoxify Hg compounds due to its better efficiency and low cost. In the current study, Hg resistant plant growth promoting rhizobacteria (PGPR), especially N2-fixing bacteria (NFB), which have the ability to remediate the Hg and can survive at high levels of Hg as well were isolated. Conditions were established for the quantification of Hg by following dithizone method (Elly, 1973; Khan *et al*., 2007; Amin and Latif, 2011). By using this method, efficiency of Hg bioremediation by immobilized as well as free cells of selected bacterial species was checked. The same isolates

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were also used for the evaluation of their potential for the remediation of Hg from the industrial effluents.

Material and Methods

Isolation and Purification of N_2-Fixing Bacteria from Root Nodules of Different Plants

Seven root samples of leguminous plants were collected from the Botanical garden of University of the Punjab, Lahore, Pakistan in zip-lock plastic bags. A selective medium, yeast extract mannitol (YEM) agar containing 1 g/L yeast extract, 10 g/L mannitol, 0.5 g/L dipotassium phosphate, 0.2 g/L magnesium sulfate, 0.1 g/L sodium chloride and 20 g/L agar (Cappuccino and Sherman, 2007) was prepared for the isolation of nitrogen-fixing bacteria. Each root nodule sample was washed thoroughly with water to avoid contamination from soil. Nodules were crushed using mortar and pestle to prepare 100% extract. After centrifugation of extract at 12,000 rpm speed for 5 min., 50 µL of each suspension was spread onto Petri dishes containing YEM medium and incubated at 37˚C for one to two days. Single colonies of NFB were picked up and checked for purity by repeated streaking and by microscopic examination of cellular morphology. The isolated colonies were then preserved as 30% glycerol stocks at -20^{o}C for further use.

Screening of Hg Resistant NFB

The isolated NFB were tested for their resistance against different concentrations of Hg by well-plate method. Each isolated strain was cultured in YEM broth at 37˚C overnight on continuous shaking. Optical density of each culture was set at 0.1 taken at 600 nm using YEM broth and 100 µL of each suspension culture was spread on the corresponding YEM plates. The YEM plates were left for 2 h to allow full embedment of the organisms. Under aseptic conditions, four wells were made in each plate by using sterile borer and 50 µL of four different concentrations (10, 20, 50 and 100 µg/mL) of mercuric chloride ($HgCl_2$) were added in each well. The plates were left at room temperature until $HgCl_2$ solution was absorbed in the medium. Plates were then incubated in the upright position at 37^{o}C for 24 h and results were monitored for the appearance of clear ones around wells. The diameters of the halo were taken as an index of the degree of sensitivity by measuring with a transparent plastic ruler at three different angles (Durairaj *et al*., 2009).

Biochemical Characterization of NFB

The strains showing resistance against Hg were characterized by subjecting to different biochemical tests, i.e., Grams staining, oxidase test, catalase test, triple sugar iron test, starch hydrolysis test, gelatin hydrolysis test, glucose peptone agar and lactose assay (Cappuccino and Sherman, 2007).

Qualitative Detection of Hydrogen Sulfide Production

For qualitative detection of hydrogen sulfide production, Petri plates containing lead acetate medium (Ono *et al*., 1991) were prepared. The bacterial strains were spotted in a grid pattern in replica. A plate of medium without lead acetate was also made and spotted the strains to use as negative control. The plates were incubated at 37˚C for one to two days and blackening of the colonies was observed which indicated the presence of hydrogen sulfide.

Screening of Hg-resistant NFB by Dithizone Method

The cultures were grown overnight at 37^{o}C and were spun down at 12,000 rpm speed for 5 min. The supernatant was used for the estimation of remainder Hg in the culture by dithizone method (Elly, 1973; Khan *et al*., 2007; Amin and Latif, 2011). Hg reacts with Dithizone solution and form orange color which was determined by spectrophotometer at 500 nm after acid digestion. The amount of Hg in supernatants was determined by using standard curve. The control culture medium with the same concentration of Hg was also run but without any bacterial inoculum.

Immobilization of Selected NFB

Supernatant from the overnight grown culture ($O.D_{600nm}$ 1.0) was discarded and cell pellet was mixed gently with the same volume of sterilized sodium alginate solution (2%) until it became homogeneous. The mixture was then extruded drop wise through a syringe into a solution of 75 mM $CaCl_2$ for bead formation. The beads were then allowed to harden in $CaCl_2$ solution at room temperature for 30 minutes. The beads were rinsed with 5 mM $CaCl_2$ solution and stored at room temperature for further use.

Efficiency of Immobilized and Free Cells in Hg Remediation

Immobilized and free-cell culture of each bacterial strain was incubated at 37^{o}C overnight in YEM medium supplemented with 20 µg/mL $HgCl_2$. After 24 h, quantification of Hg detoxification by immobilized and free cell cultures was done by dithizone method (Elly, 1973; Khan *et al*., 2007).

Estimation of Hg in Effluents Collected from Different Industries

Effluent samples were collected from four industries: Nishat textile mills, Kamahan cold storage, Nishat chemical industry and Harvard rubber industry located in Lahore, Pakistan. Each sample was diluted 10-folds with autoclaved distilled water and the concentration of Hg in each sample was quantified by dithizone method (Elly, 1973; Khan *et al*., 2007), as done before.

Detoxification of Hg in Effluents by Immobilized and Free Cell Culture

Immobilized and free-cell culture of each strain was tested against four industrial effluents to remediate Hg (as done before in the case of $HgCl_2$). The flasks were incubated with continuous shaking at 37°C for 48 h and quantification of residual/detoxified Hg was done by dithizone method (Elly, 1973; Khan *et al*., 2007).

Statistical Analysis

All experiments with control for comparison were run in triplicates. For each treatment, three separate flasks were maintained. Each time three readings were taken and mean values and standard deviation were calculated. The recorded data were subjected to analysis of variance (ANOVA) using SPSS V.20 software. The separation of means was accomplished by Duncan's multiple range test (DMRT) at a probability level 0.05 to determine the significance of immobilized bacterial strains over free cells for the remediation of Hg.

Results

Isolation, Purification and Screening of N_2-fixing Bacteria

Eighteen bacterial strains were isolated from root nodules of different leguminous plants shown in Table 1. It was observed that all bacteria were highly sensitive against 50 and 100 µg/mL (Fig. 1) in well plate assay. Among all NFB strains (Fig. 2), only seven showed better resistance against $HgCl_2$ (10 and 20 µg/mL), which were isolated from *Sesbenia aegyptica* (Jantar), *Cyprus Rotundus* (purple nut sedge) and *Mellilotus indicus* (Sinji). It was concluded from the observations that the bacterial strains with minimum zone of inhibition exhibited greater resistance to $HgCl_2$.

Biochemical Characterization of NFB

Nine bacterial strains including seven resistant to 10 and 20 µg/mL $HgCl_2$ in well plate assay and two strains sensitive to $HgCl_2$ were characterized by different biochemical tests. Bacterial strains resistant to $HgCl_2$ were characterized as *Cronobacter* sp. (Z-A1, Z-A3, Z-A5 and Z-A11), *Enterobacter* sp. (Z-A2), *Klebsiella* sp. (Z-A6), and *Pseudomonas* sp. (Z-A8) and strains sensitive to $HgCl_2$ were characterized as *Bacillus* sp. (Z-A9) and *Micrococcus* sp. (Z-A12). From the results (Table 2), it is concluded that all *Cronobacter* sp. showed higher resistance to $HgCl_2$ as compared to other bacterial species (*Bacillus* and *Micrococcus* sp.).

Qualitative Assay for Hydrogen Sulfide Production

Seven bacterial strains resistant to Hg (Z-A1, Z-A2, Z-A3, Z-A5, Z-A6, Z-A8 and Z-A11) showed blackening in their

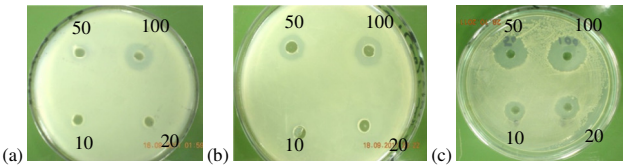

Fig. 1: Hg-resistant bacteria (a) Z-A2 (b) Z-A11, showing no inhibition zones on 10 µg/mL and 20 µg/mL concentration of $HgCl_2$; (c) Hg-sensitive bacteria Z-A12 showing inhibition zones on all concentrations

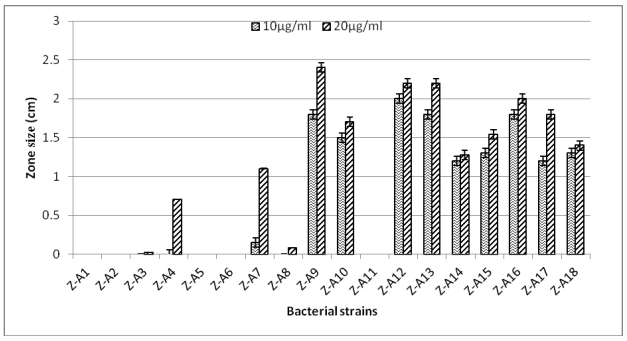

Fig. 2: Measurement of zones of inhibition in diameter (cm) at a final concentration 10 µg/mL and 20 µg/mL of $HgCl_2$. Bars represent mean ± standard deviation (S.D). Each was replicated three times (p<0.05)

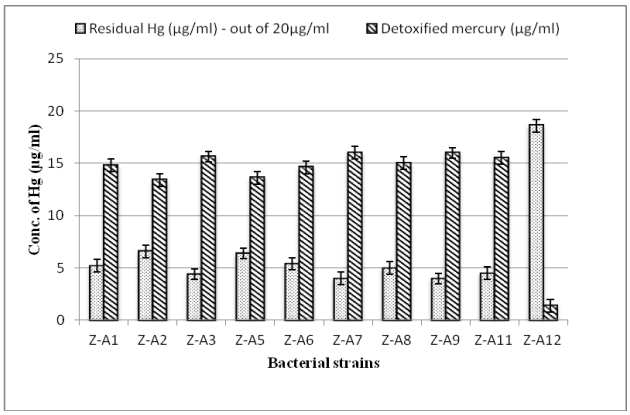

Fig. 3: Quantification of detoxified Hg by H_2S-producing bacterial strains. Bars represent mean ± S.D (p<0.05)

colonies on LA medium. Since it is known that hydrogen sulfide (H_2S) aids in the volatilization and detoxification of methylmercury (Ghosh, 1996). It is also reported that strains showing resistance to $HgCl_2$ are hydrogen sulfide-producing and methylmercury is converted from highly toxic state to a detoxified form. Our findings match the reports in literature (Ono *et al*., 1991). Hydrogen sulfide producing strains developed black (dark brown) color because of the formation of PbS (lead sulfide) and non-producing strains (Z-A9 and Z-A12) showed white colored colonies.

Table 1: Bacterial strains isolated and purified from root nodules of different plants

Source	No. of bacterial strains	Name of strains
Sesbania aegyptica (Jantar)	5	Z-A1, Z-A2, Z-A3, Z-A4, Z-A7
Cyprus rotundus (Purple fern)	1	Z-A5
Phasleolus radiates (Green gram)	4	Z-A8, Z-A13, Z-A14, Z-A15
Moringa oleifera (Sohanjna)	1	Z-A9
Medicago denticuleta (Maina)	4	Z-A12, Z-A16, Z-A17, Z-A18
Melitotus indicus (Sinji)	2	Z-A6, Z-A10
Red Fern (Lal jhari)	1	Z-A11

Fig. 4: Na-alginate beads of hydrogen sulfide producing NFB strains

Quantification of Hg by Dithizone Method

Dithizone is a reagent that changes its color from dark green to orange in the presence of Hg. The resultant dithizone product of the selected bacterial strains showed variations in green color that was indication of the Hg detoxification, whereas dithizone product of the non-H_2S producing strain gave orange color due to the presence of Hg (Fig. 3).

Selection of NFB Strains for Immobilization

On the basis of qualitative and quantitative analysis, three NFB strains were selected for Hg bioremediation. Two of them were H_2S-producing, *Cronobacter* sp. (Z-A1) and (Z-A3) and third as non-H_2S producing *Micrococcus* sp. (Z-A12), used as a control. Selected bacterial strains were encapsulated in sodium alginate as artificial beads (Carvalho *et al*., 2002; Latif *et al*., 2007; Amin and Latif, 2011). The immobilized beads (Fig. 4) were stored at 4˚C for further use.

Hg Remediation by using Immobilized and Free Cells

Results showed that NFB strains Z-A1 and Z-A3 reduced 14.8 and 15.6 µg/mL of Hg by immobilization whereas 6.5 and 8.3 µg/mL of Hg by free cells respectively (Fig. 5a and 5b) The remediation of Hg by non-H_2S producing strains was different from NFB strains in immobilized bacteria and free cells ($p< 0.05$).

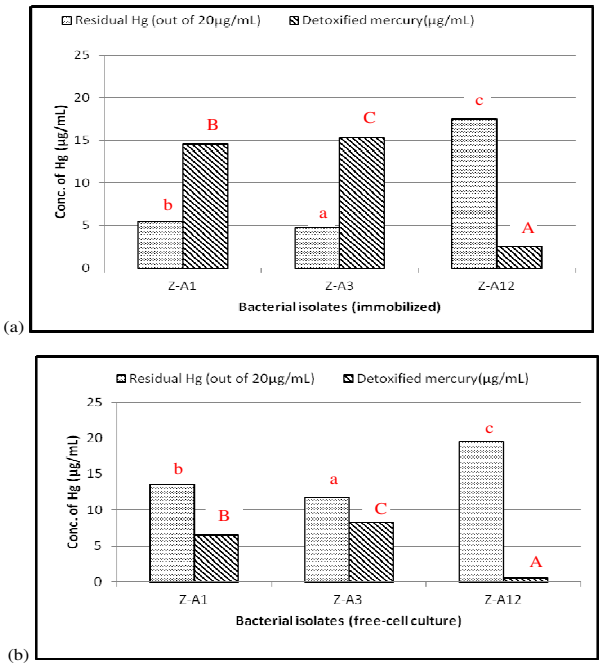

Fig. 5: Quantification of Hg detoxification from medium supplemented with 20 µg/mL $HgCl_2$ (a) immobilized bacterial strains (b) free cell, (Z-A1 and Z-A3, *Cronobacter* sp; Z-A12*Micrococcus* sp). Bars represent mean of replicates and different letters on bars indicate significant difference between treatments using DMRT (P=0.05)

Estimation of Hg in Effluents Collected from Different Industries

Among different effluent samples collected from four industries, maximum concentration of Hg was 7.5 µg/mL detected in the effluent of textile industry and minimum concentration was 3.5 µg/mL in cold storage (Fig. 6).

Hg Reduction in Effluents by Immobilized and Free Cell Culture

The efficiency of immobilized *Cronobacter* sp. (Z-A1 and Z-A3) was determined to remediate Hg from industrial effluents. *Micrococcus* sp. (Z-A12), sensitive to Hg was used as control. Data in Fig. 7 showed that strains Z-A1, Z-A3 and Z-A12 both in immobilized and free bacterial cell against chemical textile, rubber and cold storage were highly effective ($p< 0.05$). It was observed that after 48 h, the resultant products of *Cronobacter* sp. (Z-A1 and Z-A3) were green in color after dithizone assay, which was a clear indication of the detoxification of Hg. Non-H_2S producing strain, *Micrococcus* sp. (Z-A12) gave orange color indicating the presence of Hg. The behavior of both *Cronobacter* sp. was almost same to detoxify Hg,

Table 2: Different biochemical tests for the characterization of bacterial strains

Bacterial isolates	Gram's Reaction	Oxidase	Catalase	Gelatin Hydrolysis	Starch hydrolysis	TSI	Name of Characterized Species
Z-A1	-	-	-	-	+	+	*Cronobacter* sp.
Z-A2	-	-	-	-	+	-	*Enterobacter* sp.
Z-A3	-	-	-	-	+	+	*Cronobacter* sp.
Z-A5	-	-	-	-	+	+	*Cronobacter* sp.
Z-A6	-	-	-	-	+	+	*Klebsiella* sp.
Z-A8	+	-	-	-	+	+	*Pseudomonas* sp.
Z-A9	+	+	+	+	-	-	*Bacillus* sp.
Z-A11	-	-	-	-	+	+	*Cronobacter* sp.
Z-A12	+	+	+	+	+	-	*Micrococcus* sp.

Key: + = positive results, - = negative, TSI = Triple sugar iron test

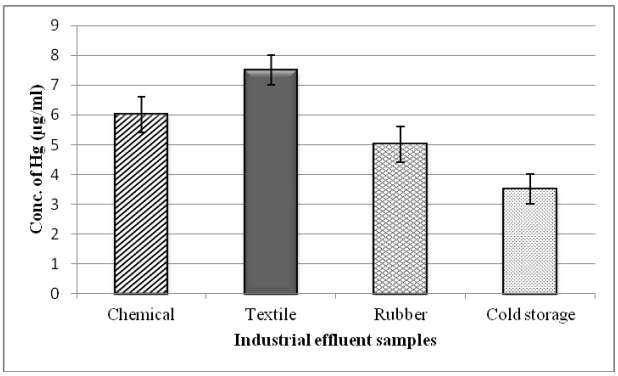

Fig. 6: Concentration of Hg (µg/mL) in effluents collected from different industries. Bars represent mean ± S.D

whereas bacterial strains sensitive to Hg are not significantly important for the remediation of Hg from any industrial effluent collected from different industries.

Discussion

Bioremediation technology uses microbial consortia or microbial processes to reduce, eliminate or transform hazardous substances present in soils, sediments, water, and air to benign products (Chishti and Arshad, 2013). Hg is the sixth most toxic element in a universe of 6 million substances on earth. Its levels have been raised due to environmental contamination from human activities, which can increase local Hg levels several thousand-fold above background (Tuovinen, 1984). So, there is a need to adopt an environment friendly technology for the remediation of Hg. Biological processes have been employed in bioremediation. The use of microorganisms for removing metal from contaminated environments is a promising technology. Microorganisms in contaminated environments have developed resistance to Hg and are playing a major role in natural decontamination.

The detoxification of Hg by different nitrogen-fixing bacteria isolated from root nodules of different plants was quantified (Elly, 1973; Khan *et al*., 2007; Amin and Latif 2011). The basic reason to adopt dithizone method was the limited availability of resources and cost-effectiveness. The method is based on the spectrophotometric measurement of colored complex extracted in inorganic solvent in which all the Hg in the sample has been converted to Hg^{2+} by acid digestion and made complex with dithizone.

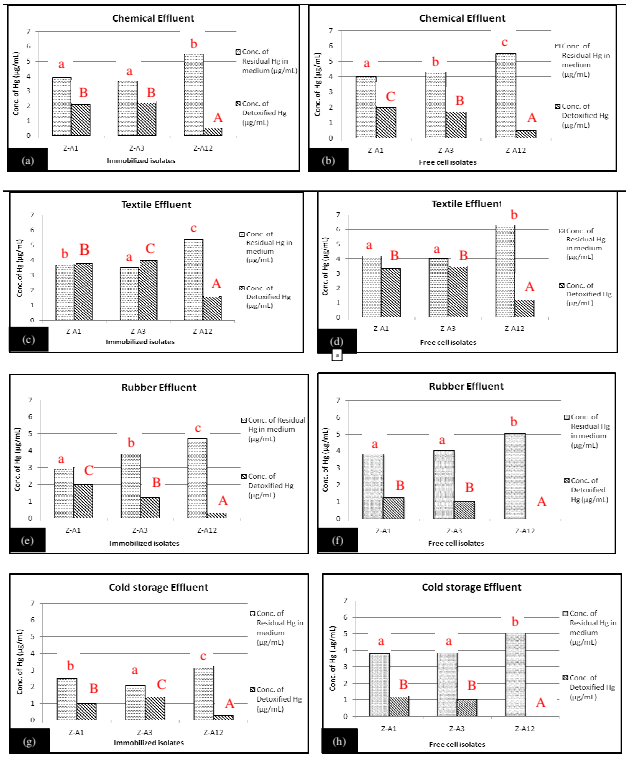

Fig. 7: Bioremediation of Hg from different effluents collected from different industries. In the left column (a, c, e and g), immobilized NFB isolates whereas in the right column (b, d, f and h), free cells were used to determine the efficiency of bacterial isolates to remediate Hg. Bars represent mean of nine replicates and different letters on bars indicate significant different between treatments using DMRT (P=0.05)

H_2S pathway is the major pathway that is utilized by the microorganisms to resist Hg and to convert it from highly toxic form to a less toxic form. We have provided evidence that the nitrogen-fixing bacteria detoxify methylmercury by using H_2S but the chemical reaction in this phenomenon is not known yet. Since it is known that the H_2S aids the volatilization of methylmercury, removal of methylmercury from the cell via such a mechanism is possible. However, it is also possible that methylmercury is converted to Hg^{2+} in the cell and then reacts with H_2S to yield highly insoluble and non-toxic HgS (Ono *et al*., 1991). Our results match with the above described pathway. We have given stress of 20

µg/mL of mercuric chloride in the medium with the inoculation of hydrogen sulfide producing strains and the removal of Hg was upto 15.6µg/mL. These resistant NFB will be of great agronomic value as they can be used at heavy metal contaminated site, where they have been shown to play two vital roles simultaneously, (1) conversion of toxic form of Hg to a non-toxic form and; (2) increase the process of nitrogen fixation thereby enhancing the soil fertility.

It is also clear from this study that effluents from different industries have a major contribution in the introduction of toxic Hg compounds in the environment. Detection of Hg in effluent samples from 4 different industries was done by dithizone method and it is shown by the results that each sample contained surprisingly high amounts of Hg ranging from 3.5 to 7.5µg/mL. Effluent from textile industry contained highest concentration of Hg and effluent of cold storage contained the lowest concentration.

In the current study, hydrogen sulfide producing and non-hydrogen sulfide producing bacterial cells were immobilized with sodium alginate. By using dithizone method, the effect of immobilization on their ability to detoxify Hg was checked. It was concluded that immobilization with sodium alginate increased the ability of bacterial strains to bioremediate Hg. Moreover, the effect of immobilized and free cell culture of hydrogen sulfide producing bacterial strains to detoxify Hg from different industrial effluents was also checked by the same method and comparison was made. It was finally concluded that the immobilization in sodium alginate provides favorable micro-environmental conditions for the bacterial cells, protects against shear damage, improves genetic stability and most importantly, increase the shelf-life. Another important reason is that there are more chances for the immobilized bacterial cells to adsorb materials (i.e., Hg) onto their surfaces then the free cell culture. That's why, the non-hydrogen sulfide producing strains that were immobilized with sodium alginate also showed a little amount of Hg reduction from the industrial effluents.

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References

Akpor, O.B. and M. Muchie, 2010. Remediation of heavy metals in drinking water and wastewater treatment systems: processes and applications. *Int. J. Phys. Sci.,* 5: 1807–1817

Amin, A. and Z. Latif, 2011. Isolation and characterization of H_2S producing yeast to detoxify mercury containing compounds. *Int. Res. J. Microbiol*., 2: 517–525

Barringer, J.L., Z. Szabo, L.J. Kauffman, T.H. Barringer, P.E. Stackelberg, T. Ivahnenko, S. Rajagopalan and D.P. Krabbenhoft, 2005. Mercury concentrations in water from an unconfined aquifer system, New Jersey coastal plain. *Sci. Total Environ.,* 346: 169–183

Barringer, J.L., Z. Szabo, D. Schneider, W.D. Atkinson and R.A. Gallagher, 2006. Mercury in ground water, septage, leach-field effluent, and soils in residential areas, New Jersey coastal plain. *Sci. Total Environ*., 361: 144–162

Brodkin, E., R. Copes, A. Mattman, J. Kennedy, R. Kling and A. Yassi, 2007. Lead and mercury exposures. Interpretation and action. *Can. Med. Assoc. J*., 176: 59–63

Cappuccino, J. and N. Sherman, 2007. *Microbiology-A Laboratory Manual*, 7th edition. Dorling Kindersley Pvt. Ltd., New Delhi, India

Carvalho, W., S.S. Silva, A. Converti and M. Vitolo, 2002. Metabolic behavior of immobilized *Candida guilliermondii* cells during batch xylitol production from sugarcane bagasse acid hydrolyzate. *Biotechnol. Bioeng*., 79: 165–169

Chishti, Z. and M. Arshad, 2013. Growth linked biodegradation of chlorpyrifos by *Agrobacterium* and *Enterobacter* spp. *Int. J. Agric. Biol*., 15: 19–26

Compeau, G.C. and R. Bartha, 1985. Sulfate-reducing bacteria: principle methylators of mercury in anoxic estuarine sediment. *Appl. Environ. Microbiol.*, 50: 498–502

Durairaj, S., S. Srinivasan and P. Lakshmanaperumalsamy, 2009. *In vitro* antibacterial activity and stability of garlic extract at different pH and temperature. *Elect. J. Biol*., 5: 5–10

Elly, C.T., 1973. Dithizone procedure for mercury analysis. *J. Wat. Pollu. Cont. Fed*., 45: 940–945

Gabriel, M.C. and D.G. Williamson, 2004. Principal biogeochemical factors affecting the speciation and transport of mercury through the terrestrial environment. *Environ. Geochem. Health,* 26: 421–434

Ghosh, S., P.C. Sadhukhan, D.K. Ghosh, J. Chaudhuri and A. Mandal, 1996. Volatilization of mercury by resting mercury-resistant bacterial cells. *Bull. Environ. Contam. Toxicol*., 56: 259–264

Holmes, P., K.A.F. James and L.S. Levy, 2009. Is low level environmental mercury exposure of concern to human health? *Sci. Total Environ*., 408: 171–182

Javed, M., 2013. Chronic effects of nickel and cobalt on fish growth. *Int. J. Agric. Biol.,* 15: 575–579

Johnsson, C., A. Schutz and G. Sallsten, 2005. Impact of consumption of freshwater fish on mercury levels in hair, blood, urine, and alveolar air. *J. Toxicol. Environ. Health, Part A.*, 68: 129–140

Kamarudin, K.S.N. and M.F. Mohamad, 2010. Synthesis of gold (Au) nanoparticles for mercury adsorption. *Amer. J. Appl. Sci.,* 7: 835–839

Khan, M.S., A. Zaidi and P.A. Wani, 2007. Role of phosphate solubilizing microorganisms in sustainable agriculture: a review. *Agron. Sustain Dev*., 27: 29–43

Latif, Z., A. Idrees and S. Riazuddin, 2007. Indigenous production of synthetic seeds in *Daucus carota. Pak. J. Bot.*, 39: 849–855

Lisha, K.P., S.M. Maliyekkal and T. Pradeep, 2010. Manganese dioxide nanowhiskers: a potential adsorbent for the removal of Hg(II) from water. *Chem. Eng. J.,* 160: 432–439

Ono, B.I., N. Ishi, F.I. Shizu and I. Aoyama, 1991. Role of hydrogen sulfide Ions in methylmercury resistance in *Saccaromyces cerevisiae*. *Yeast*., 12: 1153–1162

Prantera, M.T., A. Drozdowick, S. Gomes-Leite and A. Soares-Rosado, 2002. Degradation of gasoline aromatic hydrocarbons by two N_2-fixing soil bacteria. *Biotech. Lett.*, 24: 85–89

Protano, G., F. Riccobono and G. Sabatini, 2000. Does salt water intrusion constitute a mercury contamination risk for coastal fresh water aquifers? *Environ. Pollut.*, 110: 451–458

Radwan, S.S., R.H. Al-Hasan, H. Mahmoud and M. Eliyas, 2007. Oil-utilizing bacteria associated with fish in the Arabian Gulf. *J. Appl. Microbiol.*, 103: 2160–2167

Tuovinen, O.H., 1984. Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds: physiological, biochemical and genetic analyses. *Microbiol. Rev.*, 48: 95–124

Ubaid-ullah, M., M. Javed and S. Abdullah, 2004. Impact of waste disposal on the uptake and accumulation of heavy metals in the planktonic biomass of the river Ravi. *Int. J. Agric. Biol.,* 6: 629–632

Zhang, F.S., J.O. Nriagu and H. Itoh, 2005. Mercury removal from water using activated carbons derived from organic sewage sludge. *Water Res.,* 39: 389–395

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