Assessment of the bioremediation efficacy of the mercury resistant bacterium isolated from the Mithi River

Bhupendra Pushkar, Pooja Sevak and Suvarna Sounderajan

ABSTRACT

The contamination of natural resources with mercury has gained attention due to its high toxicity to all life forms. Bioremediation of mercury using bacteria is a useful technique to remediate mercury contamination. In the present study mercury resistant bacteria (MRB) were isolated from Mithi River water samples. Total heterotrophic bacteria (THB) and MRB present in the Mithi river water samples were enumerated. The count for THB was found to be 3.7×10^6 CFU/ml. MRB enumerated in the nutrient agar medium with mercury concentrations of 50, 100 and 150 ppm had counts of 2.8×10^6, 9.1×10^5 and 5.8×10^4 CFU/ml, respectively. The minimum inhibitory concentration (MIC) of the isolated bacterium was found to be around 500 ppm of mercury, and it was selected for further analysis. The bacterial isolate was found to tolerate a wide range of salt concentrations from 5 to 35 ppt of NaCl. The bacterial isolate was characterized by using standard biochemical tests and identified by using the 16S rDNA technique. Homology analysis of the 16S rDNA gene has confirmed the identity of the bacterium as *Bacillus thuringiensis* strain RGN1.2 with NCBI accession no. KX832953.1. It could remove 96.72%, 90.67% and 90.10% of mercury in 48 hours at 10, 25 and 50 ppm of mercury.

Key words | bacteria, bioremediation, mercury, minimum inhibitory concentration, Mithi River, salinity

Bhupendra Pushkar (corresponding author)
Pooja Sevak
Department of Biotechnology,
University of Mumbai,
Kalina, Mumbai 400098,
India
E-mail: *bhupendrapushkar@gmail.com*

Suvarna Sounderajan
Analytical Chemistry Biotechnology Division,
Bhabha Atomic Research Centre,
Trombay, Mumbai 400085,
India

INTRODUCTION

Increased mercury contamination due to anthropogenic activities has imposed negative impacts on the whole ecosystem. Mercury is a non-essential heavy metal, which is highly toxic to all forms of life (Velásquez-Riaño & Benavides-Otaya 2016). The elemental form of mercury is highly toxic to foetuses and young children as it can cross the placental and blood-brain barrier.One of the well-known examples of the effect of mercury pollution is Minamata disease, which was discovered in 1956 in Japan. This was the first incident to be recorded of mercury poisoning (Matsuyama *et al.* 2017). Other health issues in humans occurring due to mercury pollution are cardiovascular disease, hypertension, arteriosclerosis and coronary heart disease. Mercury also impairs physiological functions in the kidney, gastrointestinal system, respiratory system, hepatic, immune and integumentary systems (Ding *et al.* 2017).

The presence of mercury in natural resources like rivers, soil, ground water etc. allows contact between living beings and mercury (Jan *et al.* 2016). Mercury from various sources, which include natural and anthropogenic processes, enters the natural resources to cause mercury pollution (Xu *et al.* 2015). Activities such as urban discharges, agricultural materials, mining and combustion, and industrial discharges are the major anthropogenic events responsible for mercury pollution (Tay *et al.* 2017).

The intense level of mercury pollution has led many researchers to develop methods and techniques for its remediation. Physico-chemical methods developed for mercury remediation are not capable of tackling mercury pollution effectively (Sinha *et al.* 2012). Hence there is a need for alternative methods like bioremediation to address the issue of environmental pollution by mercury. The potentially

doi: 10.2166/ws.2018.064

low cost and environmentally safe nature of the process are attractive features of the bioremediation process (Sinha & Khare 2012; Chang *et al.* 2015). The bioremediation process makes use of any biological entity such as bacteria, algae, fungi, enzymes, or any biological material to mitigate the toxic impacts of pollutants on the environment (Dixit *et al.* 2015).

Bacteria are amongst the best bioremediation tools, being used for bioremediation of numerous pollutants. They can also serve in remediation of heavy metals from contaminated sites. Bacteria acclimatize to changing environmental conditions and develop mechanisms for heavy metal resistance (Banerjee *et al.* 2015). Various mechanisms involved in detoxification of heavy metals by bacteria include biosorption, reduction, alkylation, metal sequestration, metal binding peptides and precipitation (Gadd 2000; Park *et al.* 2011). These bacterial mechanisms could be employed for the remediation of environmental pollution caused by mercury.

In the present study potential mercury resistant bacteria were isolated from the stretches of the Mithi River that are contaminated by mercury. Bacterial isolates resistant to mercury were characterised by standard biochemical tests used for the characterization of bacteria. The identity of the bacterial isolates was determined by analysing the 16S rDNA homology. Further, the bacterial isolates were tested for their mercury remediating capabilities by using cold vapour atomic absorption spectroscopy (AAS). Mercury resistant bacteria isolated from the Mithi River are well adapted to grow in the presence of various pollutants in the river, and so could be easily used to remediate the mercury pollution of the Mithi River without affecting their bio-remediating capacity.

MATERIAL AND METHODS

Water sampling

The water sample was collected from the Mithi River located in Mumbai, India, and was used to isolate the mercury resistant bacteria. The water sample was collected from the Mithi River stretch near Kalanagar (19°03′06.6″N 72°50′53.9″E).

Total heterotrophic bacteria (THB) and mercury resistant bacteria (MRB) enumeration

The total heterotrophic bacterial population in the Mithi River was enumerated by the spread-plate technique by using aliquots of 100 µl of 10^{-2}, 10^{-3} and 10^{-4} diluted water samples and spread-plating them on nutrient agar plates (5 g peptic digest of animal tissue, 5 g NaCl, 1.5 g beef extract, 1.5 g yeast extract and 13 g of agar). The pH of the medium was adjusted to 7.4. Mercury resistant bacteria were also enumerated in a similar way by using nutrient agar plates containing 50, 100 and 150 ppm of mercury. The nutrient agar plates were incubated at 37 °C for 24 h. The THB and MRB populations were counted as colony forming units (CFU) per ml of the water sample. The percentage of mercury resistance in the water sample was calculated using the formula: Percent MRB = (Total MRB × 100)/THB.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of mercury for the isolated bacteria was determined by the broth dilution method (Jorgensen & Turnidge 2015). In this method 10 ml of the nutrient broth was supplemented with different concentrations of Hg from the 5,000 ppm Hg stock. Then 1×10^6 cells/ml were inoculated in each tube and incubated at 37 °C for 24 h in an orbital shaker at 150 rpm with appropriate positive and negative controls. After overnight incubation the optical density (OD_{600}) was determined on a UV-Visible spectrophotometer (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific). The OD of the test was compared with the negative control and the MIC of the isolate was calculated.

Effect of salinity on the growth of the bacterial isolates

The salinity tolerance of the bacteria was studied over a range of 5 ppt to 35 ppt at intervals of 5 ppt. The salinity of the nutrient broth was adjusted to a defined salt concentration using NaCl. 1×10^6 cells/ml of the culture were inoculated in each tube and incubated at 37 °C for 24 h in

an orbital shaker at 150 rpm. Positive and negative controls were also maintained to compare the bacterial growth. The growth was determined by taking the OD at 600 nm with the UV-Visible spectrophotometer. The OD of the test was compared with the negative control.

Biochemical tests

Biochemical tests on the selected isolates were carried out to determine the characteristics of the bacterial isolates. Overnight cultures of the bacterial isolates were used for all the biochemical tests. The bacterial isolates were checked for their Gram status. The sugar fermenting capabilities of the bacterial isolates were determined by growing the cultures in different sugars (glucose and lactose). The isolates were tested for IMViC (indol, methyl red, Voges–Proskauer, citrate). The positive and negative controls were maintained for each test.

Sequencing of the 16S rDNA

The bacterial isolate showing high resistance to mercury was selected and identified using a molecular technique by amplifying 16S rDNA. The genomic DNA was extracted using HiPur^{TM}Bacteria genomic DNA Purification Kit (Himedia) and the amplification reaction was conducted using universal primers 347F-5′GGAGGCAGCAGTAAGGAAT-3′ and reverse primer 803R-5′CTACCGGGGTATCTAATCC-3′ (Wu *et al.* 2014). A polymerase chain reaction (PCR) was carried out in 50 µl of volume containing 25 µl ready-to-use Master Mix containing *Taq* DNA polymerase, dNTPs, $MgCl_2$ and 1 µl of 20 pmol/µl of each forward and reverse primer and 15 ng of template DNA. The final volume of PCR reaction mixture was made up to 50 µl using sterile DNAase free water. The PCR programme was used as an initial denaturation at 94 °C for 5 mins, followed by 30 cycles of denaturation at 94 °C for 30 seconds, primer annealing at 50 °C for 35 seconds, extension at 72 °C for 45 seconds and a final extension at 72 °C for 10 mins. The PCR was carried out in the Veriti Thermal Cycler. The sequence of the PCR amplified 16S rDNA was obtained from the Scigenom Laboratory, Cochin.

GenBank accession number and phylogenetic analysis

The nucleotide sequences of the 16S rDNA gene of the isolated mercury resistant bacteria have been deposited in GenBank under the accession numbers KX832953.1.

Determination of mercury removal potential of the isolate by AAS

The mercury removal experimental set up was performed in a 250 ml Erlenmeyer flask containing 50 ml of the nutrient broth. Log phased bacterial culture (1×10^6 cells/ml) was inoculated into the experimental flask. The initial concentration of the mercury was set to 10 ppm, 25 ppm and 50 ppm in three different flasks. The flask without mercury was labelled as the control. The experiment was carried out by incubating the flask at 37 °C on a shaker condition of 120 rpm. Nutrient broth containing mercury was maintained as a negative control flask. The nutrient broth was further analysed for determining mercury removal from the medium. The growth of bacterial isolate and mercury removal from the nutrient broth were studied at different time intervals. Growth was determined by observing the OD_{600} of the culture medium. Mercury removal was determined by withdrawing aliquots of samples from the flask (culture with mercury) at various time intervals. The bacterial biomass was separated by centrifuging the culture medium at 8,000 rpm for 10 mins. The supernatant was transferred to fresh tubes and stored at 0 °C for Hg quantification. Mercury present in the supernatant of the culture medium was quantified using cold vapour atomic absorption spectroscopy (ContrAA 300, Analytikjena) at the Analytical Chemistry Division, BARC (Mumbai).

RESULTS

Total heterotrophic bacteria (THB) and mercury resistant bacteria (MRB) enumeration

Table 1 shows the THB and MRB populations of the water sample from the Mithi River. The MRB of the water sample was determined at different concentrations of mercury. The microbial load of the Mithi River water sample was 3.7×10^6 CFU/ml. The MRB population at 50, 100 and

Table 1 | Total heterotrophic bacteria and mercury resistant bacteria present in Mithi River water

Sr. no.	Hg (ppm)	CFU/ml	% MRB
THB	Without Hg	3.7×10^6	–
MRB	50	2.8×10^6	75.67
	100	9.1×10^5	24.59
	150	5.8×10^4	1.56

150 ppm of mercury was 2.8×10^6, 9.1×10^5 and 5.8×10^4 CFU/ml respectively. The percentage MRB population is also calculated as shown in Table 1.

Determination of minimum inhibitory concentration (MIC)

Five bacterial isolates were selected for further studies. Figure 1 shows the MIC for the bacterial isolates. The OD of the bacterial isolates at different concentrations of Hg was measured to determine the growth of the isolates in the presence of mercury. Bacterial isolate WHg3a could grow at 500 ppm of mercury and was selected for further study.

Effect of salinity on the growth of isolate

The bacterial isolate was grown at different salt concentrations (Figure 2). The salinity tolerance experiment was carried out in triplicate. The growth at different salinities was compared to the positive control. The selected isolate could grow in salinities up to 35 ppt without any major effect on its growth.

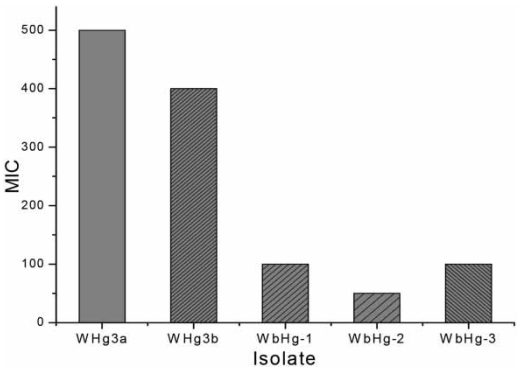

Figure 1 | MIC (ppm) of mercury for the isolated bacteria.

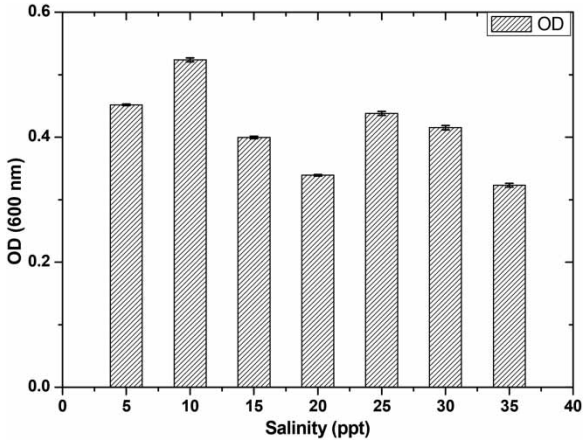

Figure 2 | Optical density of the WHg3a isolate at different salt concentrations at 600 nm.

Biochemical tests and sequencing of the 16S rDNA

Table 2 shows biochemical characteristics of the bacterial isolate. The partial sequence of the 16S rDNA gene of the bacterial isolate WHg3a was checked for its sequence similarity in GenBank using BLAST. The bacterial isolate was identified as *Bacillus thuringiensis* strain RGN1.2.

GenBank accession number and phylogenetic analysis

The partial sequence of the 16S rDNA gene of bacterial isolate WHg3a isolate was submitted to NCBI GenBank and assigned the accession number KX832953.1. The phylogenetic tree was prepared in the CLUSTALW program by the neighbour-joining method. The tree has 15 nodes (Figure 3).

Determination of mercury removal potential of the isolate by AAS

Bacterial isolate WHg3a could remove the maximum mercury on the second day of growth which is at the end of the exponential phase and where bacteria just enter the stationary phase of growth. It could remove 94.87% of the mercury within a period of 6 hours at 10 ppm initial mercury concentration. The appropriate controls confirmed that the bacteria was responsible for the removal of mercury from the solution. No reduction in the mercury was observed in

Table 2 | Biochemical characteristics of the WHg3a bacterial isolate

Morphological test	
Shape	Circular
Colour	Creamish
Margin	Even
Opacity	Translucent
Elevation	Slightly elevated
Consistency	Mucoid
Gram staining	Gram +ve rods
Motility	Non motile
Size	2.5 mm
Biochemical test	
D-Glucose:	
Acid production	+
Gas production	−
Lactose:	
Acid production	−
Gas production	−
Indole Test	−
MR Test	−
VP Test	−
Catalase Test	−
Citrate Test	+

the flask without any bacteria (negative control). The growth of the isolate at varying concentrations of mercury at different time intervals is presented in Figure 4(a)–(c). A sharp increase in the growth of the bacteria is positively correlated with the mercury removal. The growth of the bacteria was assessed by observing the growth at OD_{600} which confirmed that the increased concentration of mercury in the medium has increased the time of the lag phase (Table 3). The isolate could remove 96.72% of mercury at 10 ppm of mercury, 90.67% of mercury at 25 ppm of mercury and 90.10% at 50 ppm of mercury (Figure 5).

DISCUSSION

The Mithi River is polluted with high concentrations of mercury. The concentration of mercury in the Mithi River was reported to be 32.06 ppm by Singare *et al.* (2015) which is higher than the permissible limit as per the standards of the Water Quality Assessment Authority, Government of India (Drinking Water Standards 1991). In these standards, the permissible limit of mercury in drinking water is 0.05 ppm and in industrial effluent the permissible limit is 0.1 ppm. The total bacterial count of the Mithi River water sample as determined in the present study was 3.7×10^6 CFU/ml. This is similar to the bacterial load of other polluted rivers reported in previous studies (Mirzaei *et al.* 2008; Nagarsekar & Kakde 2014). However the percentage of mercury resistance bacterial (MRB) population in the Mithi River is quite high as compared to previous reports. The MRB population of the Mithi River that could tolerate 50 ppm of mercury as determined in the current study was 75.67%. A higher percentage of the bacteria population showing resistance to mercury may be attributed to the environmental stress generated by the mercury present in the river. Increasing metal contamination present in the environment leads to the development of plasmid mediated resistance in microbes (Sandaa *et al.* 2001; Chihomvu *et al.* 2015).

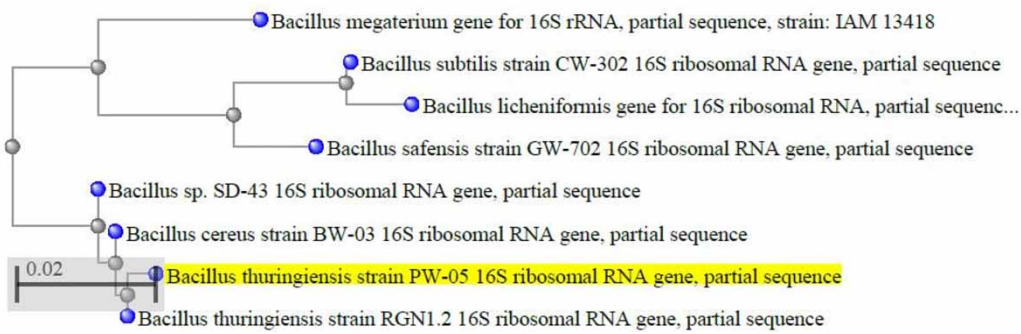

Figure 3 | Phylogenetic tree of the mercury resistant isolate prepared using the neighbour-joining method.

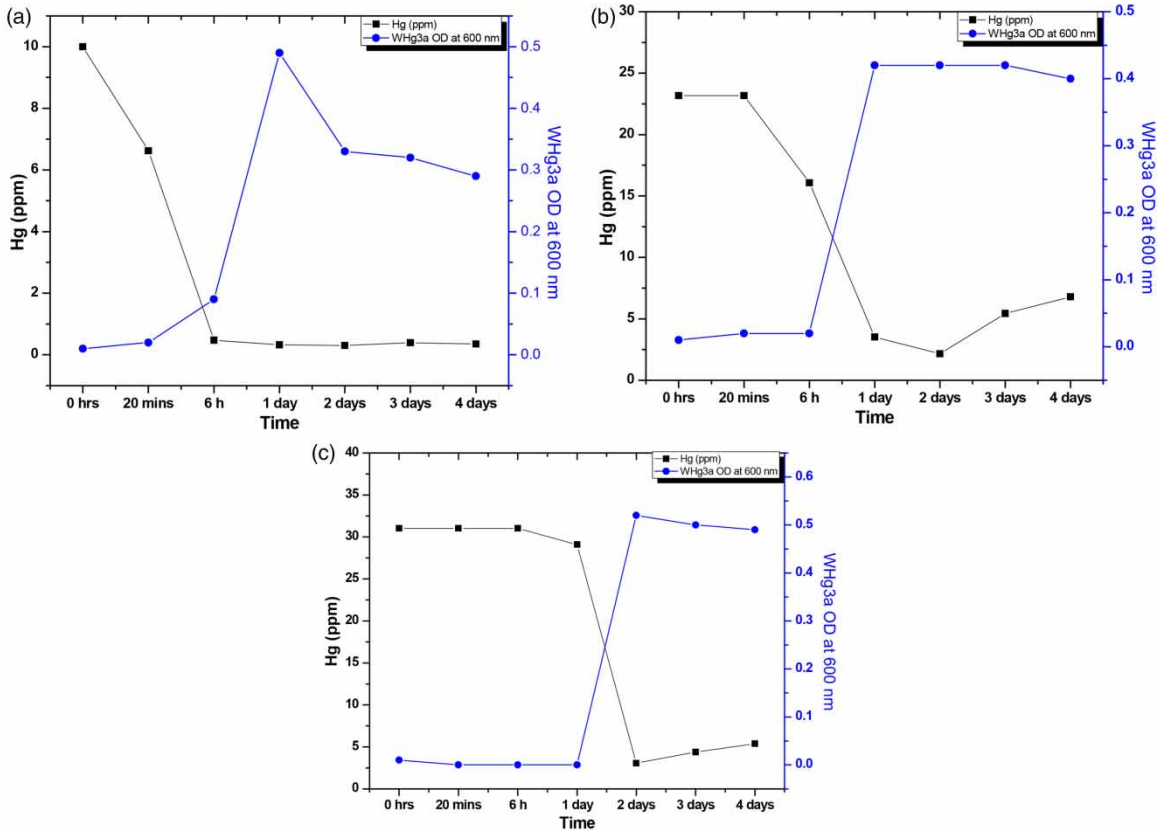

Figure 4 | The growth and mercury removed by bacteria at (a) 10 ppm of mercury, (b) 20 ppm of mercury and (c) 50 ppm of mercury.

Table 3 | Growth of the isolate in the presence and absence of mercury during mercury removal

Hg conc.	OD_{600}	20 mins	6 hrs	1 day	2 days	3 days	4 days
WHg3a	+ve control	0.02	0.21	0.49	0.3	0.3	0.28
10 ppm Hg	Test	0.01	0.09	0.49	0.33	0.32	0.29
25 ppm Hg	Test	0.02	0.02	0.42	0.42	0.42	0.4
50 ppm Hg	Test	0	0	0.3	0.52	0.5	0.49

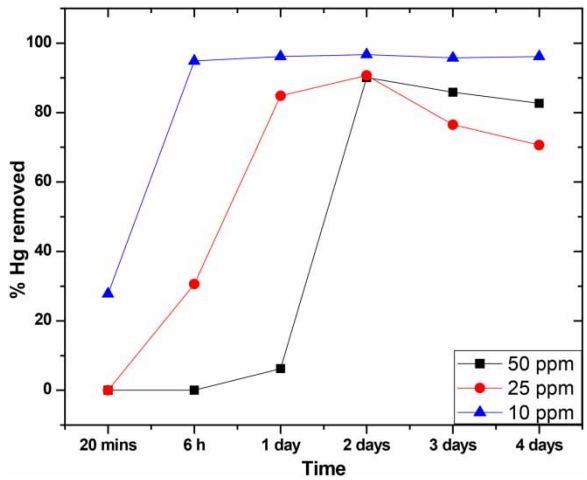

Figure 5 | Percentage removal of mercury by WHg3a isolate at varying concentrations of mercury.

Mercury tolerances of various bacteria such as *Pseudomonas putida*, *Escherichia coli* strains and *Sphingomonas* were found to be 180.53 ppm, 55 ppm and 48.48 ppm as reported in earlier studies (Zeyaullah *et al.* 2010; Giovanella *et al.* 2016; Mahbub *et al.* 2016). The *B. thuringiensis* strain RGN1.2 isolated from the Mithi River in the current study, could tolerate 500 ppm of mercury, which is very high as compared to previous studies. Long term exposure to high mercury concentrations present in the Mithi River could be one of the factors responsible for tolerance of high mercury concentrations of bacterial isolates from the Mithi River.

The salinity of the medium in which bacteria grow plays an important role in the survival and metabolism of bacteria. The difference in salt concentrations of the surrounding environment and the cytoplasm of bacteria exerts an osmotic pressure on the bacterial cell wall. The cell membrane of the bacteria is disrupted when this osmotic pressure is high. The change in salinity also affects the functioning of various enzymes present in bacteria (Díaz *et al.* 2002; Jiang *et al.* 2017). The salinity of the surrounding medium may affect the bio-remediating capability of bacteria. The bacterial isolate identified in the present study could tolerate a wide range of salt concentrations in the medium. It could grow in the presence of salt concentrations ranging from 5 ppt to 35 ppt. Earlier studies have also reported on the *Bacillus* sp. which could tolerate high salt concentrations without much effect on the growth (Giri *et al.* 2014). The wide range of salt tolerance of the bacterium identified in the current study will help in bioremediation of mercury from various stretches of the Mithi River having different salinities.

Earlier studies on bioremediation suggest the versatile nature and application of *Bacillus* sp. in bioremediation of various pollutants (Guo *et al.* 2010; Pandey *et al.* 2013). *Bacillus thuringiensis* strain RGN1.2 isolated from the Mithi River in the current study could tolerate 500 ppm of mercury and was capable of removing 96.72% of mercury in 48 hours. Also it could remove 94.87% of mercury within a short period of 6 hours. Earlier studies on bioremediation reported a *Bacillus* sp. which could remove 80% of mercury in a period of 24 hours (Chien *et al.* 2012). This suggests a high efficiency of the bacterial isolate of *Bacillus* sp. from the Mithi River for the removal of mercury. The bacteria resistant to mercury are generally capable of transforming organic and inorganic forms of mercury to less toxic or elemental forms with the help of genes present on the mer operon. The mer operon can be located on transposons, plasmid or bacterial chromosomes (Dash *et al.* 2014). The mer gene present in mercury resistant bacteria encodes for the mercury reductase enzyme which helps in volatilization of mercury by reducing Hg^{2+} to Hg^{0}. Mercury resistant bacteria have been reported to bioaccumulate mercury using a Hg^{2+} transport system and metallothionein (Ndu *et al.* 2015). Exopolysaccharide secreting bacteria use biosorption as one of the mechanisms for mercury removal (Dash & Das 2015). *Bacillus thuringiensis* strain RGN1.2 isolated from the Mithi River in the current study may be using one of the above mechanisms for the removal of mercury. The isolated bacterium can be further studied for its mechanism of mercury removal, which can help in increasing the efficiency of the bacterium. Bacteria isolated from the Mithi River can be used for the remediation of mercury from the Mithi River and other mercury contaminated sites.

CONCLUSION

The Mithi River harbours the mercury resistant bacterium due to mercury contamination of the river water. The mercury resistant bacterial strain was isolated from the Mithi River. The bacterial isolate was identified as *B. thuringiensis* strain RGN1.2, which could tolerate high concentrations of mercury. It could remove mercury in a very short period. The isolate could remove 94.87% of mercury within a short period of 6 hours and 96.72% of mercury in 48 hours at 10 ppm of mercury. Thus it can be concluded that the bacterial strain *B. thuringiensis* strain RGN1.2 isolated from the Mithi River in the current study has potential for bioremediation of mercury. Further the bioremediation efficiency of the isolate should be determined in the river water conditions before applying it to actual remediation.

ACKNOWLEDGEMENT

The authors acknowledge the financial support provided for the study by Mumbai Metropolitan Region – Environment Improvement Society (MMR-EIS). The authors are also thankful to Yogini Ghare, Deputy Registrar, T.A.A.S, University of Mumbai for her support in executing the administrative tasks related to the research project.

REFERENCES

Banerjee, G., Pandey, S., Ray, A. K. & Kumar, R. 2015 Bioremediation of heavy metals by a novel bacterial strain *Enterobacter cloacae* and its antioxidant enzyme activity, flocculant production, and protein expression in presence of lead, cadmium, and nickel. *Water, Air, & Soil Pollution* **226** (4), 91.

Chang, S., Wei, F., Yang, Y., Wang, A., Jin, Z., Li, J., He, Y. & Shu, H. 2015 Engineering tobacco to remove mercury from polluted soil. *Applied Biochemistry and Biotechnology* **175** (8), 3813–3827.

Chien, M., Nakahata, R., Ono, T., Miyauchi, K. & Endo, G. 2012 Mercury removal and recovery by immobilized *Bacillus megaterium* MB1. *Frontiers of Chemical Science and Engineering* **6** (2), 192–197.

Chihomvu, P., Stegmann, P. & Pillay, M. 2015 Characterization and structure prediction of partial length protein sequences of pcoA, pcoR and chrB genes from heavy metal resistant bacteria from the Klip River, South Africa. *International Journal of Molecular Sciences* **16** (4), 7352–7374.

Dash, H. R. & Das, S. 2015 Bioremediation of inorganic mercury through volatilization and biosorption by transgenic *Bacillus cereus* BW-03 (p PW-05). *International Biodeterioration & Biodegradation* **103**, 179–185.

Dash, H. R., Mangwani, N. & Das, S. 2014 Characterization and potential application in mercury bioremediation of highly mercury-resistant marine bacterium *Bacillus thuringiensis* PW-05. *Environmental Science and Pollution Research* **21** (4), 2642–2653.

Díaz, M. P., Boyd, K. G., Grigson, S. J. & Burgess, J. G. 2002 Biodegradation of crude oil across a wide range of salinities by an extremely halotolerant bacterial consortium MPD-M, immobilized onto polypropylene fibers. *Biotechnology and Bioengineering* **79** (2), 145–153.

Ding, E., Guo, J., Bai, Y., Zhang, H., Liu, X., Cai, W., Zhong, L. & Zhu, B. 2017 MiR-92a and miR-486 are potential diagnostic biomarkers for mercury poisoning and jointly sustain NF-κB activity in mercury toxicity. *Scientific Reports* **7** (1), 15980.

Dixit, R., Malaviya, D., Pandiyan, K., Singh, U. B., Sahu, A., Shukla, R., Singh, B. P., Rai, J. P., Sharma, P. K., Lade, H. & Paul, D. 2015 Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. *Sustainability* **7** (2), 2189–2212.

Drinking Water Standards 1991. *Bureau of Indian Standards, Drinking Water Standard IS: 10500: 1991*. Water Quality Assessment Authority, Government of India.

Gadd, G. M. 2000 Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. *Current Opinion in Biotechnology* **11** (3), 271–279.

Giovanella, P., Cabral, L., Bento, F. M., Gianello, C. & Camargo, F. A. O. 2016 Mercury (II) removal by resistant bacterial isolates and mercuric (II) reductase activity in a new strain of *Pseudomonas* sp. b50a. *New Biotechnology* **33** (1), 216–223.

Giri, S., Dash, H. R. & Das, S. 2014 Mercury resistant bacterial population and characterization of *Bacillus* sp., isolated from sediment of solid waste discharged point of steel industry. *National Academy Science Letters* **37** (3), 237–243.

Guo, H., Luo, S., Chen, L., Xiao, X., Xi, Q., Wei, W., Zeng, G., Liu, C., Wan, Y., Chen, J. & He, Y. 2010 Bioremediation of heavy metals by growing hyperaccumulator endophytic bacterium *Bacillus* sp. L14. *Bioresource Technology* **101** (22), 8599–8605.

Jan, A. T., Azam, M., Choi, I., Ali, A. & Haq, Q. M. R. 2016 Analysis for the presence of determinants involved in the transport of mercury across bacterial membrane from polluted water bodies of India. *Brazilian Journal of Microbiology* **47** (1), 55–62.

Jiang, J., Pan, C., Xiao, A., Yang, X. & Zhang, G. 2017 Isolation, identification, and environmental adaptability of heavy-metal-resistant bacteria from ramie rhizosphere soil around mine refinery. *3 Biotech* **7** (1), 5.

Jorgensen, J. H. & Turnidge, J. D., 2015 Susceptibility test methods: dilution and disk diffusion methods. In: *Manual of Clinical Microbiology* (J. H. Jorgensen, M. A. Pfaller, K. C. Carroll, G. Funke, M. L. Landry, S. S. Richter & D. W. Warnock, eds), 11th edn. American Society of Microbiology, Washington DC, USA, pp. 1253–1273.

Mahbub, K. R., Krishnan, K., Megharaj, M. & Naidu, R. 2016 Bioremediation potential of a highly mercury resistant bacterial strain *Sphingobium* SA2 isolated from contaminated soil. *Chemosphere* **144**, 330–337.

Matsuyama, A., Yano, S., Taninaka, T., Kindaichi, M., Sonoda, I., Tada, A. & Akagi, H. 2017 Chemical characteristics of dissolved mercury in the pore water of Minamata Bay sediments. *Marine Pollution Bulletin*. DOI: 10.1016/j.marpolbul.2017.10.021.

Mirzaei, N., Kafilzadeh, F. & Kargar, M. 2008 Isolation and identification of mercury resistant bacteria from Kor River, Iran. *The Journal of Biological Sciences* **8** (5), 935–939.

Nagarsekar, A. S. & Kakde, U. B. 2014 A bacteriological study of Mithi River water in Mumbai metropolis. *International Journal of Researches in Biosciences, Agriculture & Technology*. **2**, 362–374.

Ndu, U., Barkay, T., Mason, R. P., Schartup, A. T., Al-Farawati, R., Liu, J. & Reinfelder, J. R. 2015 The use of a mercury biosensor to evaluate the bioavailability of mercury-thiol complexes and mechanisms of mercury uptake in bacteria. *PLoS One* **10** (9), e0138333.

Pandey, S., Ghosh, P. K., Ghosh, S., De, T. K. & Maiti, T. K. 2013 Role of heavy metal resistant *Ochrobactrum* sp. and *Bacillus* spp. strains in bioremediation of a rice cultivar and their PGPR like activities. *The Journal of Microbiology* **51** (1), 11.

Park, J. H., Lamb, D., Paneerselvam, P., Choppala, G., Bolan, N. & Chung, J. W. 2011 Role of organic amendments on enhanced bioremediation of heavy metal (loid) contaminated soils. *Journal of Hazardous Materials* **185** (2), 549–574.

Sandaa, R. A., Torsvik, V. & Enger, Ø. 2001 Influence of long-term heavy-metal contamination on microbial communities in soil. *Soil Biology and Biochemistry* **33** (3), 287–295.

Singare, P. U., Ferns, S. E. L. & Agharia, E. R. 2015 Studies on toxic heavy metals in sediment ecosystem of Mahim Creek near Mumbai, India. *International Letters of Chemistry, Physics and Astronomy* **43**, 62–70.

Sinha, A. & Khare, S. K. 2012 Mercury bioremediation by mercury accumulating *Enterobacter* sp. cells and its alginate immobilized application. *Biodegradation* **23** (1), 25–34.

Sinha, A., Pant, K. K. & Khare, S. K. 2012 Studies on mercury bioremediation by alginate immobilized mercury tolerant *Bacillus cereus* cells. *International Biodeterioration & Biodegradation* **71**, 1–8.

Tay, P. K. R., Nguyen, P. Q. & Joshi, N. S. 2017 A synthetic circuit for mercury bioremediation using self-assembling functional amyloids. *ACS Synthetic Biology* **6** (10), 1841–1850.

Velásquez-Riaño, M. & Benavides-Otaya, H. D. 2016 Bioremediation techniques applied to aqueous media contaminated with mercury. *Critical Reviews in Biotechnology* **36** (6), 1124–1130.

Wu, J., Lin, I., Hayes, R. B. & Ahn, J. 2014 Comparison of DNA extraction methods for human oral microbiome research. *British Journal of Medicine & Medical Research* **4** (10), 1980–1991.

Xu, J., Bravo, A. G., Lagerkvist, A., Bertilsson, S., Sjöblom, R. & Kumpiene, J. 2015 Sources and remediation techniques for mercury contaminated soil. *Environment International* **74**, 42–53.

Zeyaullah, M., Islam, B. & Ali, A. 2010 Isolation, identification and PCR amplification of merA gene from highly mercury polluted Yamuna river. *African Journal of Biotechnology* **9** (24), 3510–3514.

First received 7 July 2017; accepted in revised form 9 March 2018. Available online 22 March 2018