IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 6, Issue 1 (Jan. – Feb. 2020), PP 06-14
www.iosrjournals.org

Bacteria With Heavy Metal Bioremediation Potential Isolated From The Polluted River Water Of Bangladesh.

Sanjeeda Haider Nupur[1], Abu Rayhan[1] and Sangita Ahmed*[2]
[1]*(Department of Microbiology, University of Dhaka. Dhaka-1000. Bangladesh).*
[2]*(Professor, Department of Microbiology, University of Dhaka. Dhaka-1000. Bangladesh).*
**Corresponding author: Sangita Ahmed*

Abstract
Background: *With the increased urbanization and industrial development, the rivers surrounding Dhaka city are getting highly polluted due to discharge of municipal and untreated industrial waste waters which are overloaded with different types of toxic heavy metals including chromium, cadmium, lead, copper, arsenic, nickel etc, which imposes great threat to public health. This study aimed to isolate bacteria with heavy metal bioremediation ability that could be used to remove the toxic heavy metals from water bodies.*
Method: *Bacteria were isolated from water sample collected from the Buriganga river and were identified by using morphological, cultural, biochemical tests and 16s rDNA sequencing. Chromium reduction was investigated by DPC method (Ilias et al., 2011).*
Results: *Two bacteria were isolated and were identified as Proteus mirabilis strain ALK428 and Pseudomonas aeruginosa strain Pse12. Both isolates could tolerateupto500ppm of lead, chromium, cadmium, aluminum, arsenic, zinc and copper. The whole cell of Proteus mirabilis strain ALK428 reduced 38.6%, 27.7%, 13.6% and 7.7% hexavalent chromium when Cr(VI) concentration (as* $K_2Cr_2O_7$*) was 5 mg/l, 10 mg/l, 20 mg/l and 30 mg/l respectively. This reduction by whole cell of Pseudomonas aeruginosa strain Pse12 was about 68.4%, 57.2%, 33.7% and 19.8%. None of the isolates were able to reduce 40 mg/l Cr (VI) concentration. Both Proteus mirabilis strain ALK428 and Pseudomonas aeruginosa strain Pse12 were found to possess several genes associated with heavy metal resistance, including chrA (chromate resistance determinant), czcC (cobalt, zinc and cadmium resistance determinant) and aioA (arsenic oxidase determinant).*
Conclusion: *The Proteus mirabilis strain ALK428 and Pseudomonas aeruginosa strain Pse12 characterized in this study possess high potential for heavy metal bioremediation and further investigations are necessary for applying these bacteria in practical field.*
Key words: *heavy metal, bioremediation, tolerance, chromium reduction*

Date of Submission: 10-02-2020 Date of Acceptance: 25-02-2020

I. Introduction

Dhaka, the capital city of Bangladesh depends on the major rivers surrounding the city including the Buriganga, the Turag and the Shitalakhya the Balu as the main sources of water supply. Unfortunately in recent years, water from most of these rivers have been polluted to such an extent that it is getting very difficult to cope up with the increasing demand of water (Islam *et al.,* 2018). One of the main reasons of this pollution is direct discharge of industrial wastes that have been built along these river banks. These industrial wastes carry huge load of different types of heavy metals. Analysis of the heavy metal concentrations of the Buriganga showed that in water concentration of Pb, Cd, Ni, Cu and Cr varied seasonally and spatially from 58.17 to 72.45µg/L, 7.08 to 12.33µg/L, 7.15 to 10.32µg/L, 107.38 to 201.29µg/L and 489.27 to 645.26µg/L, respectively (Ahmad *et al*., 2010). Recently, in a multi-industry district close to Dhaka city, assessment of concentrations of chromium, copper, zinc, arsenic, cadmium and lead in irrigation water contaminated with industrial wastes have shown that except for Cd and Pb, concentrations of all other heavy metals exceeded the permissible limits concentrations (Ahmed *et al*., 2018). These toxic metals enter the water bodies and soil through wastewater and finally human body via food chain (Sultana *et al*., 2017). In children, exposure to high concentration of these heavy metals can cause potentially permanent learning and behaviour disorders (Hsueh *et al.,* 2017). They also have harmful effects on heart, bones, intestines, endocrine system, reproductive and nervous system of adults and might cause death in extreme cases (Jaishankar *et al*., 2014). Therefore to ensure good public health it is very important to remove the heavy metal contamination of aquatic bodies in Bangladesh.

There are some physicochemical methods for removal of heavy metals from environmental samples (Barkat, 2011). However, most of these methods have several disadvantages, such as less efficiency, high costs and the problem of the safe disposal of the materials. As an alternative to the physicochemical removal methods,

use of microorganisms to reduce, eliminate or detoxify heavy metals has achieved growing attention for recent years. Bacteria possess several mechanisms for bioremediation of heavy metals, such as absorption of heavy metals into bacterial cell, transformation of toxic metal into less toxic form or degradation (Jin *et al*., 2018)

Although in Bangladesh heavy metal pollution of water has been a major environmental as well as health concern, the current water treatment method does not have any suitable technique for removal of these toxic metals. This work therefore aims to develop a sustainable bacterium based heavy metal bioremediation method.

II. Materials and Methods

Isolation and identification of bacteria

To isolate bacteria with heavy metal bioremediation capacity, the initial approach was to isolate bacteria which could grow in the presence of heavy metal. For this purpose, water sample collected from the Buriganga river was passed through membrane filter paper and the filter paper was placed into Luria-bertani (LB) agar medium supplemented with 50 ppm chromium followed by incubation at 37°C for 24 hrs. The morphologically dissimilar isolated colonies were picked up from the plates and sub-cultured on LB agar to obtain pure colonies. To identify, all isolates were screened for their morphological features by Gram staining and biochemical properties (Indole test, Methyl red test, Voges proskauer test, Citrate utilization test, Kligler iron agar test, Catalase test, Oxidase test, Urease test and motility test) as suggested by the Bergey's Manual of Systematic Bacteriology Volume 2 (2005).

To further confirm the identification of the bacterial isolates 16s rDNA sequencing was carried out, which is a common tool used for identification of bacteria. Partial 16S rDNA gene sequence of studied bacteria was analyzed with nucleotide BLAST search in GenBank (NCBI). Phylogenetic relationship of this species was analysed with other closely related bacterial species present in GenBank.

Analysis the tolerance pattern and growth condition in different concentrations of other heavy metals

Tolerance of isolates to increased concentrations of Cr (as $K_2Cr_2O_7$, Cd (as$CdCl_2.H_2O$), Cu (as $CuSO_4.5 H_2O$), Zn ($ZnSO_4.5H_2O$), Pb(as $Pb(NO_3)_2$)and As (as As_2O_3) was observed separately. Each isolate was grown on LB agar plate supplemented with 50ppm (as $K_2Cr_20_7$) at 37^0C for 24 hours. Isolated colony for each isolate was inoculated in 50 ml LB broth supplemented with different concentrations (100mg/L, 250mg/L, 500mg/L, 750mg/L) of chromium and incubated at 37^0C for 24 hours in shaker (150 rpm)**.** Growth of each isolate was monitored measuring OD at 600nm.Each experiment was done in triplicates. Tolerance to each metal was observed following this same protocol.

Chromium reduction by whole cell in buffer

Reduction of hexavalent chromium by whole cell in buffer was performed by following method described by Ilias *et al.,* (2011). Bacterial cells were grown in 50 ml LauriaBertani broth supplemented with 100 ppm Cr (as $K_2Cr_2O_7$) in a 250 ml Erlenmayer flask. After 24h of incubation at 37^0C and 150 rpm agitation, cells were harvested from 1.5 ml culture by centrifugation at 10,000 rpm for 5 min. The cell pellet was collected in an eppendorf tube and was washed twice with phosphate buffer followed by resuspension in 0.5 ml phosphate buffer (100 mM, pH 7.0). For Cr(VI) reduction, 0.5 ml of $K_2Cr_2O_7$ was added in different concentrations (2.5 mg/l; 5mg/l; 10 mg/l; 20 mg/l; 30 mg/l and 40 mg/l) into the cell suspension in phosphate buffer separately and incubated at 37°C in a water bath for 45 min. The reaction mixture was centrifuged at 10,000 rpm for 5 min and the supernatant was analysed for residual Cr (VI) determination by the following method.

Residual hexavalent chromium was determined colorimetrically with a spectrophotometer following the S-diphenylcarbazide (DPC) method (Ilias *et al*., 2011).The DPC reagent was prepared by adding 24ml of 85% H_3PO_4 to 56ml distilled water. This solution was mixed with 0.076g DPC previously dissolved in 20ml of 95% ethanol. The reagent was stored in dark at 4 °C. To determine the amount of Cr(VI) in the sample, 125 µl of the DPC reagent was added to 1ml of chromium samples, mixed gently and kept at room temperature for 20 minutes to complete the reaction. Presence of pink color indicates positive result. The absorbance was measured at 540 nm using a spectrophotometer. A reaction blank was prepared by adding 0.5 ml phosphate buffer to 0.5 ml of $K_2Cr_20_7$(2.5 mg/l, 5mg/l; 10 mg/l; 20 mg/l; 30 mg/l; 40 mg/l) and considered as 100% Cr (VI). Phosphate buffer was used to set the spectrophotometer to zero.

Detection of heavy metal tolerant genes

To detect specific heavy metal tolerant genes, *chrA* (chromate transport protein), and *czcC* (cobalt-zinc-cadmium outer membrane resistance protein), primers were designed (Table 3) using Primer3Plus software. Genomic DNA of isolates were collected and purified. Polymerase chain reaction was carried out using each primer with the genomic DNA of each isolate as template. The PCR program was as follows: one cycle at 95°C

for 4 minutes, 40 cycles at 94°C for 30seconds, 57°C for 1 minute and 72°C for 1 min; and a final extension of 10 minutes at 72°C.

III. Results

Isolation and identification of heavy metal tolerant isolates

After inoculation of water sample on Luria-bertani agar medium supplemented with 50 ppm chromium, two types of bacterial colonies were isolated. Gram staining of each isolate revealed that each was Gram negative rod. Therefore, for identification each isolate was subjected to a series of biochemical tests commonly applied for identification of Gram negative bacteria. From the results of biochemical tests isolate 1 was presumptively identified as *Proteus* sp while isolate 2 as *Pseudomonas* sp (Table 1). For further confirmation of the identification, genomic DNA of each isolate was subjected to PCR using universal primers specific for 16s rDNA sequence (Table 3). Purified PCR products were sequenced by Bioneer, Malaysia. The sequenced data were aligned in Chromas software and blasted in NCBI GeneBank. Blast result shows 97% similarity with *Proteus mirabilis* strain ALK428 for isolate 1 and 96% similarity with *Pseudomonas aeruginosa* strain Pse12 for isolate 2 (Table 2.0).

Heavy metal tolerance pattern of Proteus mirabilis strain ALK428 and Pseudomonas aeruginosa strain Pse12

To investigate whether the *Proteus* sp., and *Pseudomonas* sp. have potential for heavy metal bioremediation, each was tested initially for their ability to grow on increased concentration of different heavy metals such as chromium (Cr), lead (Pb), zinc (Zn), arsenic (As), copper (Cu),and cadmium (Cd). Both isolate could tolerate upto 500ppm of each metal in a similar way (Figure 1). However, the *Proteus mirabilis* strain ALK428 could grow at 750ppm of all heavy metals, while the *Pseudomonas aeruginosa* strain Pse12 strain failed to grow at 750ppm of arsenic (Figure 1b) and was able to tolerate 750ppm of other heavy metals tested.

Tolerance of Proteus mirabilis strain ALK428 and Pseudomonas aeruginosa strain Pse12 to heavy metals as Co-culture

To investigate how *Proteus mirabilis* strain ALK428 and *Pseudomonas aeruginosa*strain Pse12respond when they are grown together in increased concentration of heavy metals, both were inoculated in LB broth supplemented with different concentration of either cadmium and chromium and growth was monitored as mentioned previously. Results showed that both isolates could tolerate upto 750ppm of chromium as well as cadmium (Figure 3a and b).

Tolerance of Proteus mirabilis and Pseudomonas aeruginosa to combination of multiple heavy metals (As, Cr, Cd, Cu, Zn, Pb)

In the environment, different heavy metals co-exist. Therefore to investigate how *Proteus mirabilis* and *Pseudomonas aeruginosa*might respond when they would be exposed to combinations of heavy metals, each was grown in LB broth supplemented with different heavy metals including Chromium, Lead, Copper, Zinc, Cadmium, and Arsenic.Result shows that *Pseudomonas aeruginosa* could tolerate upto 400ppm of combination of heavy metals, while *Proteus mirabilis* could tolerate only upto 250ppm.

Reduction of hexavalent chromium reduction by Proteus mirabilis strain ALK428 and Pseudomonas aeruginosastrain Pse12

Chromate reduction activities of whole cells of both *Proteus mirabilis* strain ALK428 and *Pseudomonas aeruginosa* strain Pse12 were investigated at 37°C. Residual Cr(VI) for *Proteus mirabilis* strain ALK428 and *Pseudomonas aeruginosa* strain Pse12 was 52.3% and 20.6% respectively, which suggests that whole cell of *Proteus mirabilis* strain ALK428 reduced 47.7% of the initial Cr(VI) concentration (2.5 mg/L as $K_2Cr_2O_7$), while that of *Pseudomonas aeruginosa* strain Pse12 was 79.4% (Figure 4).At Cr (VI) concentration of 5 mg/L as $K_2Cr_2O_7$, *Proteus mirabilis* strain ALK428 and *Pseudomonas aeruginosa* strain Pse12 reduced 38.6% and 68.4% Cr(VI), respectively (Figure 4). When concentration of Cr (VI) was increased to 10 mg/L, 20 and 30 mg/L($K_2Cr_2O_7$), reduction by *Proteus mirabilis* strain ALK428 was 27.7%; 13.6% and 7.7% respectively, while that of *Pseudomonas aeruginosa* strain Pse12 was 57.2% ; 33.7% and 19.8% respectively (Figure 4). At concentration of 40 mg/L (as $K_2Cr_2O_7$) none of the isolate managed to reduce hexavalent chromium. Comparison of the chromium reduction ability of the two isolates at different chromium concentration showed that at each concentration of chromium tested, reduction of hexavalent chromium was more by *Pseudomonas aeruginosa* strain Pse12 than *Proteus mirabilis* strain ALK428.

Detection of heavy metal tolerant genes

To investigate whether *Proteus mirabilis* strain ALK428 and *Pseudomonas aeruginosa* strain Pse12possess the heavy metal tolerant genes, genomic DNA from *Proteus mirabilis* and *Pseudomonas aeruginosa* strain Pse12was subjected to PCR using primers specific for *chrA*(chromate transport protein), *czcC*(cobalt-zinc-cadmium outer membrane resistance protein) and *aioA* (Arsenite oxidase subunit). Both isolates found to possess all the heavy metal tolerant genes (Figure 5).

IV. Discussion

This study aimed to investigate the heavy metal bioremediation potential of two bacteria isolated from polluted river water of the Buriganga and identified as *Proteus mirabilis* strain ALK428 and *Pseudomonas aeruginosa* strain Pse12.

Investigation of heavy metal tolerance of *Proteus mirabilis* strain ALK428 and *Pseudomonas aeruginosa*strain Pse12 showed that both isolates could tolerate 500ppm of each metal, although as the concentration of heavy metal increased, the growth of the two isolates decreased. In a number of studies, *Proteus* and *Pseudomonas* have been found to be associated with heavy metal bioremediation (Abimbola *et al*., 2019, Oaikhena *et al,* 2016, Kalayu and Ahemad, 2014). Oaikhena *et al,* (2016) isolated heavy metal tolerant *Proteus vulgaris* and *Pseudomonas aeruginosa* from petroleum refinery effluent, while Kalayu and Ahemad (2014) isolated *Proteus* sp and *Pseudomonas* sp along with several other heavy metal tolerant bacteria from the Nile river water in Ethiopia which showed minimum inhibitory concentration to heavy metals ranging from 200 to 2300ppm.

Heavy metal contaminated water bodies contain a combination of various heavy metals and bacteria capable of bioremediation must tolerate these multiple heavy metals. Therefore, *Proteus mirabilis* strain ALK428 and *Pseudomonas aeruginosa* strain Pse12 were examined for their tolerance to a combination of heavy metals. *Pseudomonas aeruginosa* could tolerate upto 400ppm of combination of heavy metals, while *Proteus mirabilis* could tolerate upto 250ppm. Again, in nature bacteria often work in mutualism or synergism with each other to survive against the odd. Therefore *Proteus mirabilis* strain ALK428 and *Pseudomonas aeruginosa* strain Pse12 were grown together in increasing concentration of different heavy metals to investigate whether they show better tolerance when they are grown together. In co-culture both isolates could tolerate upto 750ppm of cadmium and chromium. However, *Proteus mirabilis* strain ALK428, when grown alone, showed better tolerance to heavy metals compared to its growth together with *Pseudomonas aeruginosa* strain Pse12. In a similar study, mixed culture consortium of *Pseudomonas aeruginosa, Staphylococcus aureus, E. coli, Proteus vulgaris* and *Klebsiella pneumonia* remediated higher percentage of cadmium (100%), chromium (33.4%), nickel (73.9%), and zinc (90.1%) from petroleum refinery effluent than pure culture isolates (Oaikhena*et al,* 2016).

Investigation of Cr (VI) reduction capability of *Proteus mirabilis* strain ALK428 and *Pseudomonas aeruginosa* strain Pse12 showed that they reduced 47.7% and 79.4% of the Cr(VI) at a concentration of 2.5mg/l in phosphate buffer, respectively. When the Cr(VI) concentration was increased, both isolates were able to reduce up to 30 mg/l Cr(VI) in phosphate buffer at 37°C, pH 7.0, although reduction by whole cell of each isolate decreased with increased Cr(VI) concentration. None of the isolates managed to reduce Cr (VI) when concentration was 40 mg/l. In a previous study Ilias *et al.,* (2011) reported that whole cell of *Staphylococcus aureus* subsp and *Pediococcus pentosaceus* ATCC 25745 reduced 24% and 30% of the initial Cr (VI) concentration (1 mg/l) in 45 min, respectively. In another study, *Pseudomonas* sp. isolated from mining areas of Orissa, India was shown to reduce more than 80% of 2 mM chromium (Dey and Paul, 2013). Compared to these bacterial isolates the *Proteus mirabilis* strain ALK428 and *Pseudomonas aeruginosa*strain Pse12 seem to be more efficient to reduce Cr(VI). Data from the current study also suggests that *Pseudomonas aeruginosa* strain Pse12 is more efficient than *Proteus mirabilis* strain ALK428.

Identification of Chromium and cadmium resistant genes in *Proteus mirabilis* strain ALK428and *Pseudomonas aeruginosa* strain Pse12 was carried out to further understand the molecular basis of chromium and cadmium reduction. Both isolates possessed several genes associated with chromium, cadmium and arsenic tolerance which included *chrA, czcC* and *aioA*. In a similar study in Ibadan, Nigeria, Abimbola *et al*., detected metal resistance genes such as *chrA, chrB, pbrA,* cusCBA, PWAP3 etc in several bacteria including *Pseudomonas aeruginosa, Proteus, Bacillus, Klebsiella, Citrobacter, Providencia* isolated from wastewater generated from printing operations.

This study has successfully isolated two bacterial isolates which can tolerate high concentrations of various heavy metals and reduce toxic hexavalent chromium to non-toxic form. They also contain genes associated with heavy metal bioremediation. Further investigations are necessary to understand their mechanism of tolerance to other heavy metals. They could also be investigated for the presence of other heavy metal tolerant genes. Strain development using genetic engineering could also be applied to improve their heavy metal bioremediation potential.

V. Declarations

Ethics approval and consent to participate: Not applicable
Consent for publication: Not applicable
Availability of data and material: Not applicable
Competing interests: The authors declare that they have no competing interests
Funding: This work has been funded by the Ministry of Education, Government of the People's Republic of Bangladesh.

Authors' contributions: We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all authors. The research work has been conceptualized and designed by Sangita Ahmed, while Ms Sanjeeda Haider Nupur and Mr Abu Rayhan conducted the research work as well as analysed and interpreted data with equal contribution. Corresponding author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

Acknowledgements: The authors acknowledge Professor Dr Anowara Begum for providing technical support to conduct the research work.

References

[1]. Abimbola OA, Olawale OA, Anthony IO, Obasola EF (2019) Metal-resistance encoding gene-fingerprints in some bacteria isolated from wastewaters of selected printeries in Ibadan, South-western Nigeria. J Taibah Univ Sci. 13(1):266–273.

[2]. Ahmad MK, Islam S, Rahman S, Haque MR, Islam MM (2010). Heavy metals in water, sediment and some fishes of buriganga river, Bangladesh. Int J Environ Res 4:321-332.

[3]. Ahmed M, Matsumoto M, Kurosawa K (2018) Heavy Metal Contamination of Irrigation Water, Soil, and Vegetables in a Multi-industry District of Bangladesh. Int J Environ Res12:531–542.

[4]. Barakat MA (2011) New trends in removing heavy metals from industrial wastewater. Arab J Chem 4: 361–377.

[5]. Dey S, Paul AK (2013) Hexavalent chromium reduction by aerobic heterotrophic bacteria indigenous to chromite mine overburden. Braz J Microbiol 44(1):307-315.

[6]. Garrity GM, Brenner DJ, Krieg NR, Staley JT (2005) Bergey's Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore, USA.

[7]. Hsueh YM, Lee CY, Chien SN et al (2017) Association of blood heavy metals with developmental delays and health status in children. Sci Rep 7:43608.

[8]. Ilias M, Rafiqullah IF, Debnath BC, Mannan KSB, Hoq MM (2011) Isolation and characterization of chromium(VI)-reducing bacteria from tannery effluents. Ind J Microbiol 51(1): 76-78.

[9]. Islam MM, Karim MR, Zheng X, Xiaofang L (2018) Heavy Metal and Metalloid Pollution of Soil, Water and Foods in Bangladesh: A Critical Review. Int J Environ Res Public Health 15(12): 2825.

[10]. Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN et al (2014) Toxicity, mechanism and health effects of some heavy metals. InterdiscipToxicol7(2):60–72.

[11]. Jin Y, Luan Y, Ning Y, Lingyan W (2018) Effects and Mechanisms of Microbial Bioremediation of heavy metals in soil: a critical review. App Sci 8:1336-52.

[12]. Kalayu G, M Ahemad (2014) Incidence of heavy metal and antibiotic resistance in bacterial isolates from Blue Nile river water in Ethiopian region. Int J Environ Stud 71(4):546-569.

[13]. Oaikhena EE, Makaije DB, Denwe SD, Namadi MM, Haroun AA (2016) Bioremediation Potentials of Heavy Metal Tolerant Bacteria Isolated from Petroleum Refinery Effluent. Am J Environ Prot 5(2): 29-34.

[14]. Sultana MS,RanaS, Yamazaki S, Aono T, Yoshida S (2017) Health risk assessment for carcinogenic and non-carcinogenic heavy metal exposures from vegetables and fruits of Bangladesh. Cogent Environ Sci 3:1–17.

Figure legends

Figure 1: Tolerance of *Proteus mirabilis* strain ALK428 (blue line) and *Pseudomonas aeruginosa*strain Pse12 (red line) to different concentrations of heavy metal.

Figure 2: Tolerance of *Proteus mirabilis* strain ALK428, *Pseudomonas aeruginosa*strain Pse12 and co-culture of the isolates to increased concentrations of chromium and cadmium.

Figure 3: Tolerance of *Proteus mirabilis* strain ALK428 (blue line), *Pseudomonas aeruginosa*strain Pse12 (red line) to multiple heavy metals

Figure 4: Reduction of different concentrations of heaxavalent chromium by whole cell of *Proteus mirabilis* strain ALK428 and *Pseudomonas aeruginosa*strain Pse12

Figure 5: Figure 3: Detection of *chrA* (a), *czcC* (b) and *aioA* (c) genes of *Proteus mirabilis* strain ALK428 and *Pseudomonas aeruginosa* strain Pse12:

(a) Lane 1 =DNA marker, Lane 2 = Negative control, Lane 3= *Proteus mirabilis* strain ALK428 and Lane 4= *Pseudomonas aeruginosa* strain Pse12

(b) Lane 1 =DNA marker, Lane 2 = *Proteus mirabilis* strain ALK428 and Lane 3= *Pseudomonas aeruginosa* strain Pse12

(c) Lane 1 =DNA marker, Lane 2 = Negative control, Lane 3= *Proteus mirabilis* strain ALK428 and Lane 4= *Pseudomonas aeruginosa* strain Pse12

Figure 1

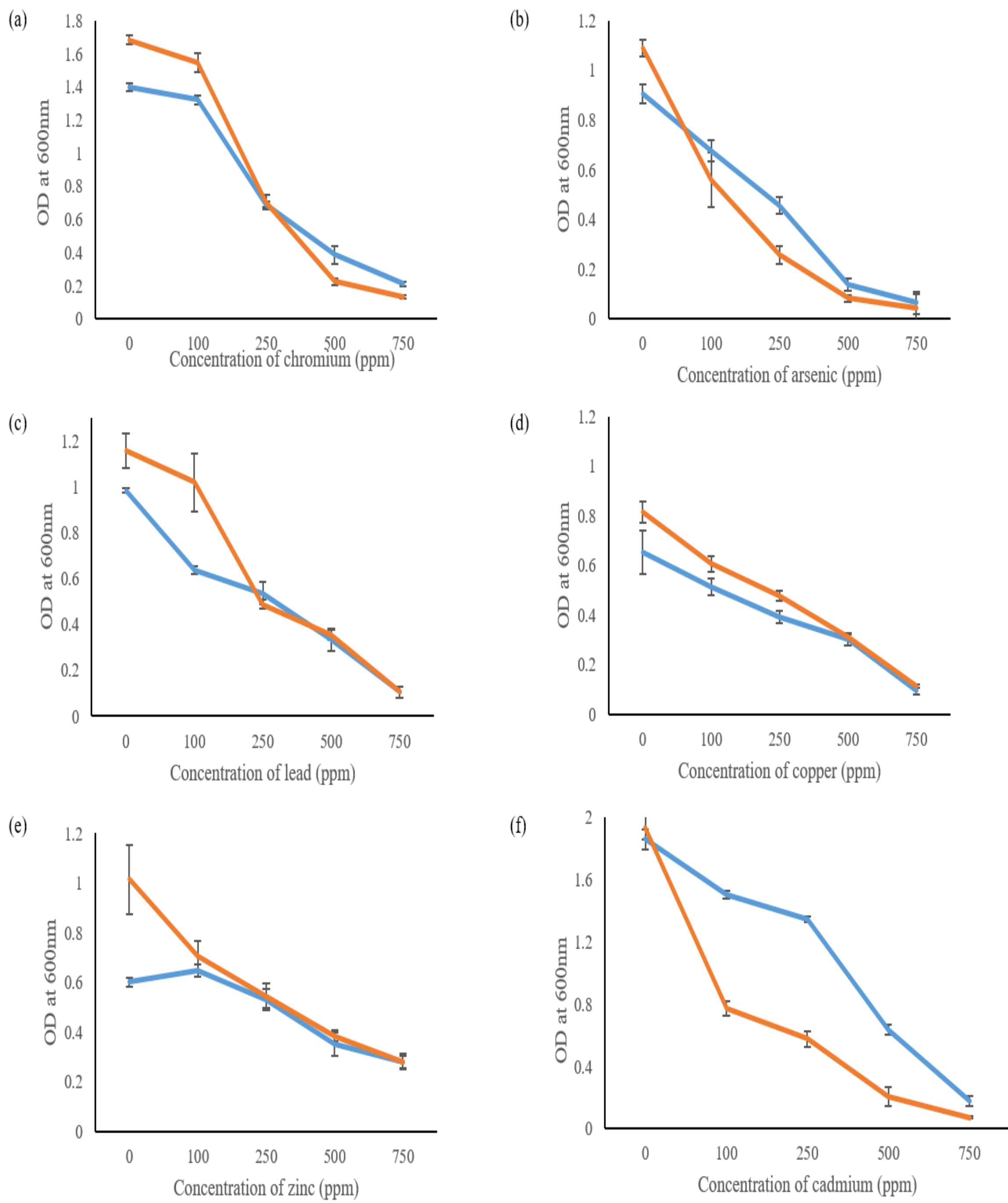


Figure 2

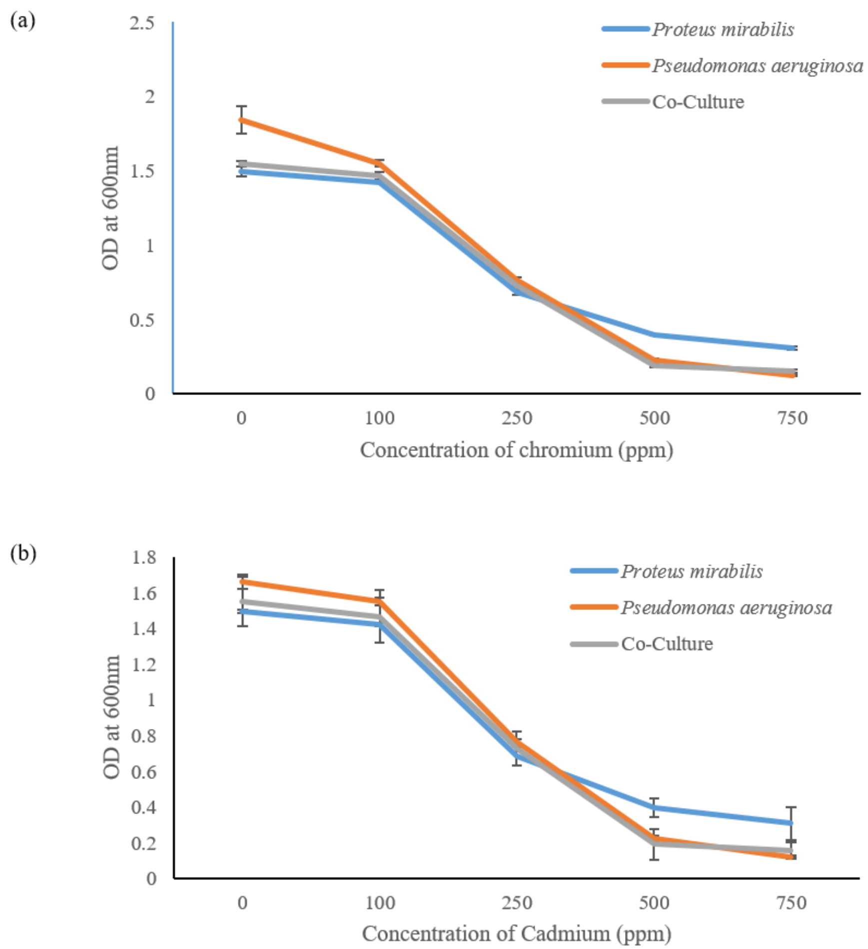


Figure 3

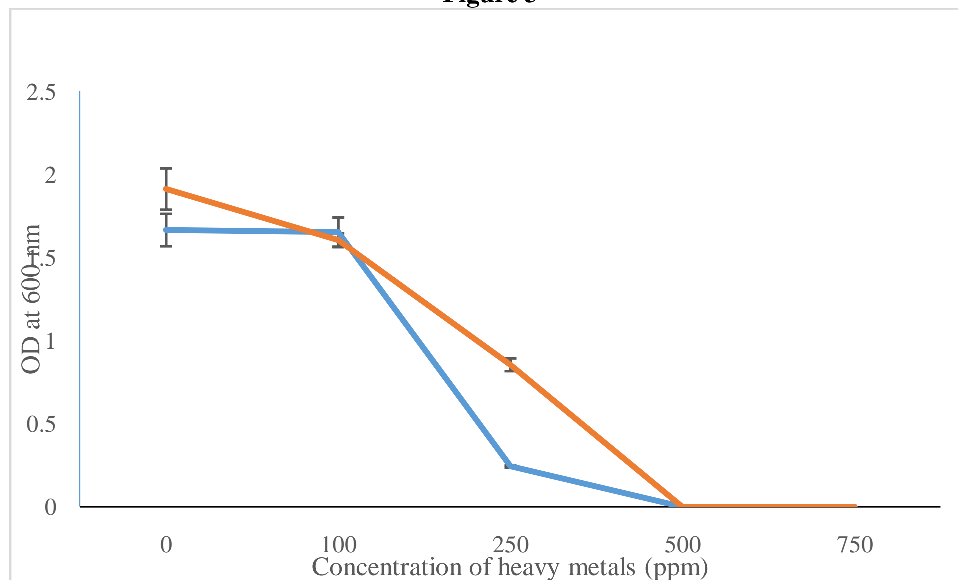


Figure 4

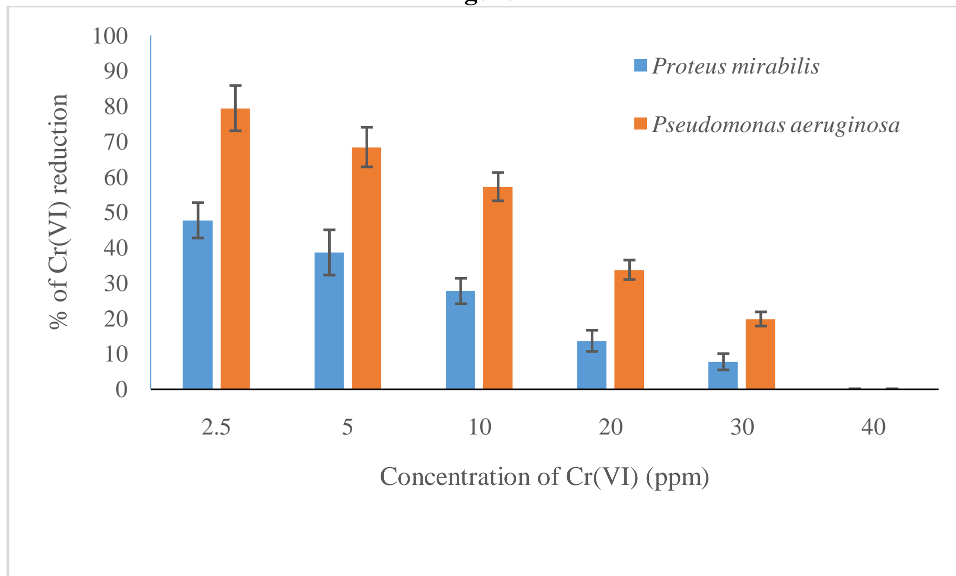
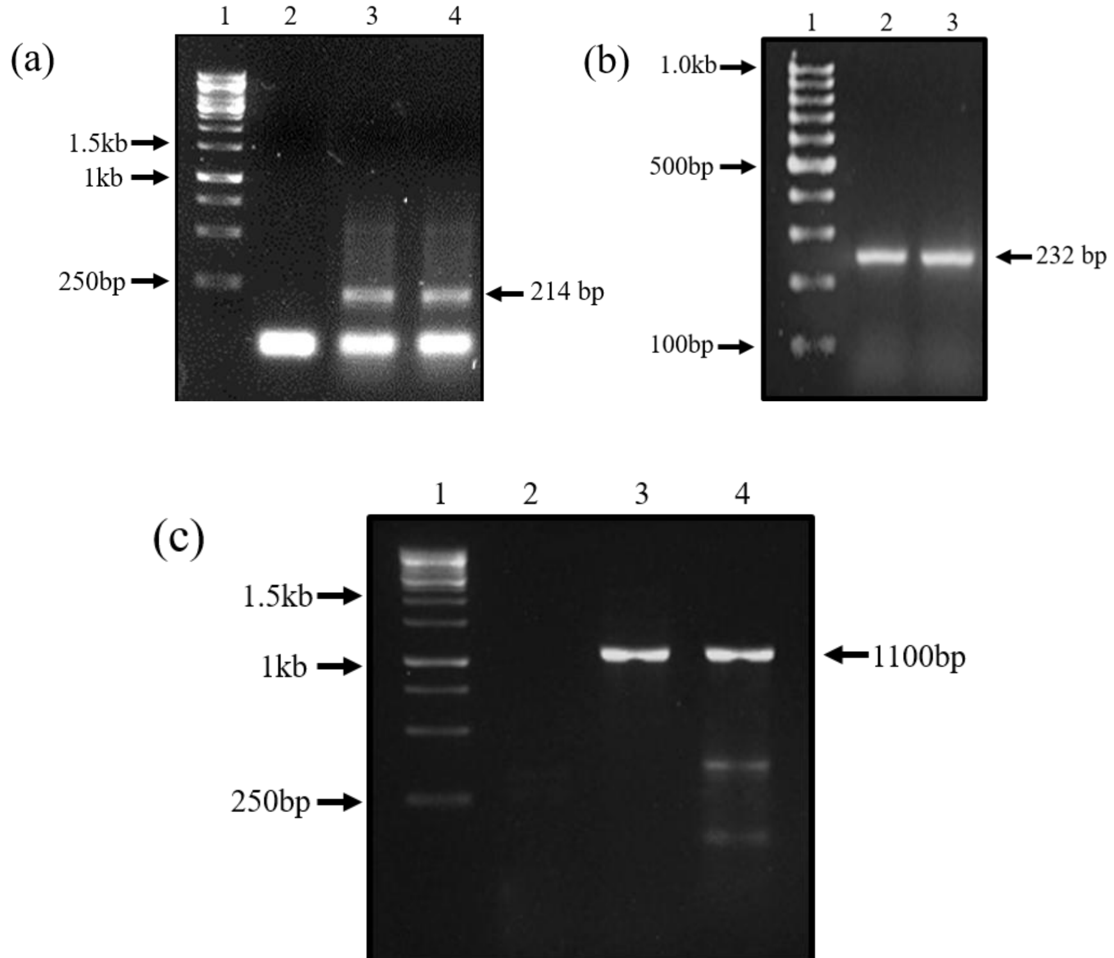


Figure 5



Tables

Table 1: Identification of Gram negative short rod isolates

Isolate	Oxidase	Catalase	Motility	Indole	Urease	Gas production	KIA	Citrate	MR	VP	H_2S	Presumptive identification
Isolate1	-ve	+ve	+ve	-ve	+ve	+ve	Glu (-ve) Lac (-ve)	-ve	+ve	-ve	+ve	*Proteus sp*
Isolate2	+ve	+ve	+ve	-ve	-ve	-ve	Glu (-ve) Lac(-ve)	+ve	-ve	- ve	-ve	*Pseudomonas sp*

Table 2 Identification of the isolates by 16s rDNA sequencing

Samples	Sequence length	Query	E value	Organisms
Isolate - 1	1441	98%	0.00	*Proteus mirabilis* strain ALK428
Isolate -2	1444	97%	0.00	*Pseudomonas aeruginosa* strain Pse12

Table 3: Primers for detection of heavy metal tolerant genes

Gene	Direction	Primer sequence	Amplicon size
chrA	Forward Reverse	5'-GCT ATT TCC GCG ACG AGT T-3' 5'-CCG AGA GCG AAG AGT ACC AG-3'	214bp
czcC	Forward Reverse	5'-GCA ACC AGG GCA ACA TCT AC- 3' 5'-AGC ACG TCG AGG AAG TTG AA- 3'	232bp
aox	Forward Reverse	5'-CAC TTC TGC ATC GTG GGN TGY GGN TA-3' 5'-TGT CGT TGC CCC AGA TGA DNC CYT TYT C-3'	1100bp

Sangita Ahmed. "Bacteria With Heavy Metal Bioremediation Potential Isolated From The Polluted River Water Of Bangladesh." *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB), 6(*1), (2020): pp. 06-14.