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HEAVY METAL REMOVAL BY BACTERIAL ISOLATES FROM THE ANTARCTIC OCEANIC REGION

ANURADHA R MULIK[1] AND RAMA K BHADEKAR[1*]

[1]Department of Microbial Biotechnology, Rajiv Gandhi Institute of IT and Biotechnology, Bharati Vidyapeeth Deemed University, Katraj, Pune-411046, Maharashtra, India

ABSTRACT

Cost effectiveness, in situ or ex situ treatment, complete degradation of the contaminants are some of the advantages of bioremediation which is a natural process and perceived positively in the treatment of hazardous pollutants. In view of this, fourteen bacterial isolates from oceanic region were screened for their heavy metal tolerance. Among them, four isolates viz. *Kocuria sp* BRI 36, *Brevibacillus* sp 37, *Halomonas* sp 38 and *Oceanobacillus sp* 39 with maximum tolerance for Cd^{2+}, Cr^{3+}, Ni^{2+} and Pb^{2+} were selected for further study. Their maximum tolerable concentration (MTC) values were in the range of 300-600 ppm with cell viability in the range of 47.1 to 6.6 %. Maximum survival was observed in case of BRI 39 in presence of Ni^{2+}. Percentage of total metal removal in the range of 6 to 95.39 % was recorded using these isolates with i) 95.4 % removal of Cd^{2+} by BRI 36 ii) 86.7 % removal of Pb^{2+} by BRI 36 and iii) 88.2 % removal of Cd^{2+} by BRI 38 . Metal ion distribution studies indicated maximum intracellular accumulation of Cd^{2+} by BRI 36 (85 %) whereas Ni^{2+} was mainly depleted due to cell adsorption in case of BRI 38 (20.29 %). To summarize, *Kocuria sp* BRI 36 was observed to be the best candidate with significant potential for removal of Pb^{2+} and Cd^{2+}.

KEYWORDS: ***Antarctica, Adsorption, Bioaccumulation, Heavy metals***

RAMA K BHADEKAR*

Department of Microbial Biotechnology, Rajiv Gandhi Institute of IT and Biotechnology, Bharati Vidyapeeth Deemed University, Katraj, Pune-411046, Maharashtra, India

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INTRODUCTION

Heavy metals like Cu, Zn, Fe, As, Hg, Pb, Ni, Cd, Cr etc. are chemical elements which have a specific density which is five times greater than that of water and atomic mass greater than 20[1].Metals are present naturally in biological and geological resources and they play a vital role in metabolism of living organisms. Some metals are essential and are required as micronutrients in living organisms[2]. However, their high concentration in soil and water is threatening human health, food as well as biogeochemical cycles. It also adversely affects the flora and fauna of environmental niches[3].High concentration of heavy metals may cause acute or chronic toxicity in human beings[4].Inhalation of chromium leads to shortness of breath, coughing, wheezing, gastrointestinal and neurological disorders [5], cadmium is primarily toxic to the kidney and also causes bone demineralization[6]. Heavy metals also cause oxidative damage to cells directly by producing reactive oxygen species (ROS) and indirectly by inactivating cellular antioxidant species[7]. The two main contributors of heavy metal pollution are industrialization and urbanization. The problem of bioavailability and bioaccumulation of heavy metals in aquatic eco-systems is becoming increasingly severe all over the globe[8]. The severity of the problem is reflected by the reports stating their occurrence at toxic concentrations in relatively uninhabited areas of the globe like the Antarctic region. Antarctica (often considered as one of the last pristine regions), could be affected by pollution at global and local scale. Moreover anthropogenic activities cause increase in concentrations of Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Cd and Pb in Antarctic Peninsula[9, 10]. In this area maximum heavy metal pollution has been observed in bays and marine sediments near coastal stations. For instance, high concentration of Cu, Pb, Ag and Zn had been reported in McMurdo, Antarctica[11, 12].Thus, it is crucial to limit the problem of heavy metal pollution. Different traditional methods used to remove heavy metals include chemical precipitation, reverse osmosis, ion exchange, chemical oxidation and reduction, membrane filtration, electrochemical treatment etc.[13] However, they have certain limitations such as high cost, partial removal of metal ions, large amount of sludge containing chemical compounds, formation of metal hydroxides etc.[14] Hence, eco-friendly, renewable, effective and low cost strategies are being developed for remediation of heavy metal contamination. Biosorbents like bacteria, fungi algae and some agricultural waste have been found to play an important role in bioremediation. [15],Several microbial species have been successfully employed to remove heavy metals from contaminated soils and water bodies. However, very few reports are available on heavy metal tolerance in halotolerant microorganisms[16].In view of this, the present studies aim to evaluate heavy metal resistance in the Antarctic isolates and their potential in bioremediation.

MATERIALS AND METHODS

Organisms

Fourteen bacterial isolates were obtained from eight different samples of oceanic regions [17]. Out of eight, four [(i) Latitude S 64°59'50.7'' Longitude E 46°13'35.6'' (ii) Latitude S 60°13'29.1'' Longitude E 70°22'30.1'' (iii) Latitude S 70°45'37.7'' Longitude E 11°43'30.5'' (iv) Latitude S 70°45'53.1'' Longitude E 11°43'25.6''] belonged to the Antarctic region. The isolates were found to be the member of genera *Halomonas, Brevibacillus, Kocuria* and *Oceanobacillus* [17]. They were maintained on Marine Salt Medium (MSM) (composition per litre: 81.0 g NaCl, 10.0 g yeast extract, 9.6 g $MgSO_4$, 7.0 g $MgCl_2$, 5.0 g protease peptone, 2.0 g KCl, 1.0 g glucose, 0.36 g $CaCl_2$, 0.06 g $NaHCO_3$, 0.026 g NaBr and 15 g agar with pH adjusted to 7.0± 0.2). For all experiments 48 h grown isolates were used as inoculum at 10% (as per McFarland standard) concentration.

Chemicals

All chemicals used were of analytical grade. The media components were purchased from HiMedia Laboratories Pvt. Ltd (India). The stock solutions of cadmium, nickel, lead and chromium (prepared in 2% HNO_3) at concentration of 1000 ppm were purchased from Sigma-Aldrich.

Screening of isolates for heavy metal tolerance

Heavy metal tolerance of the bacterial isolates were examined individually for Cd^{2+}, Ni^{2+}, Pb^{2+} and Cr^{2+}. The MSM containing different concentrations (50 ppm to 200 ppm) of metal was inoculated with the BRI isolates individually followed by incubation at room temperature (RT) (3 30°C) with shaking at 120 rpm. In order to confirm the viability of microorganisms after incubation, each bacterial culture was serial diluted and used to determine total viable count (TVC) on MSM agar plates at RT for 48 h.

Determination of maximum tolerable concentration (MTC)

Kocuriasp BRI 36, *Brevibacillus*sp 37, *Halomonas*sp 38 and *Oceanobacillussp*39 were used to determine their maximum tolerable concentration (MTC) to Cd^{2+}, Ni^{2+}, Pb^{2+} and Cr^{3+} at concentrations ranging from 200-700 ppm as described above.

Growth curve studies

Toxic effect of heavy metals on bacterial growth was determined by growing each bacterial isolate at 10 ppm metal concentration individually in Luria Bertani **(**LB) (Composition per litre; Casein enzymichydrolysate 10 g, Yeast extract 5 g, Sodium chloride 10 g, pH 7.5±0.2) medium and growth was measured in terms of optical density at 620 nm at 8 h time interval upto 72 h using UV-Vis spectrophotometer (Thermo Fisher Scientific, Genesys 10UV). The medium inoculated in absence of any metal severed as biotic control for respective isolate. The uninoculated media amended with heavy metals individually served as abiotic controls. All the experiments were conducted in triplicate.

Measurements of heavy metals removal by bacterial isolates

The four isolates, BRI 36, 37, 38 and 39 were grown in LB medium supplemented with 1 ppm metal individually. At the end of 48 h incubation, cells were separated by centrifugation at 10,000 rpm for 10 minutes. The heavy

metal removal properties were estimated by measuring metal depletion in culture supernatants by inductively coupled plasma-optical emission spectroscopy (ICP-OES Agilent model 7700, G3281A). The ICP-OES system was calibrated by serial dilution of each metal standard within limits of detection ranging from 50 ppb to 50 ppm.

Metal accumulation and distribution

The four isolates were grown in presence of four metals (10 ppm) individually in LB medium with shaking for 48 h at RT. Each culture broth was centrifuged at 10,000 rpm for 10 min to obtain the cell pellet and was analyzed for the distribution of metal ions within the cells. For this, intracellular accumulation and cell wall adsorption of metals were estimated using the method described by Al-Momani *et al*[18].Metal adsorbed on the cell wall were determined by washing the cell pellet of each isolate with 10 ml of 0.1 M sodium citrate for 10 min, 3 times to release the cations from the cell wall. Intracellular metal accumulation was estimated by digesting collected cell pellets by 10 ml of nitric acid (35% HNO_3). The samples were analyzed by ICP-OES. The metal distribution in each cellular part was calculated in terms of mg/g of biomass.

Antibiotic susceptibility testing

Isolates were studied for antibiotic susceptibility testing by disc diffusion assay[19]. The 48 h grown cultures were spread on LB agar plate and Hi media antibiotic discs containing Ampicillin (10mcg), ampoxyclav (30mcg), cefotaxin (30mcg), co-trimoxazole (25mcg), gentamicin (10mcg), tobramycin (10mcg), ceptazidine (30mcg), ciprofloyacin (5mcg), amikacin (30mcg), nitrofloxacin (300mcg), netillin (30mcg), tetracycline (30mcg), ofloxacin (5mcg), cefuroxacin (5mcg) for gram negative isolates and piperacillin (100mcg), linezolid (300mcg), teicoplanin (30mcg), vancomycin (30mcg), chloramphenicol (25mcg), penicillin G (1 unit), streptomycin (10mcg), sulphatriad (300mcg) for Gram positive were used for antibiotic susceptibility testing.

Statistical analysis

The experiments were performed in triplicates and standard deviation was calculated. Two-way ANOVA was applied to determine significant value.

RESULTS

Screening of isolates for heavy metal tolerance

All fourteen isolates exhibited their ability to grow in presence of Cd^{2+}, Cr^{3+}, Ni^{2+} and Pb^{2+}(Table 1). BRI 34, 35, 40, 41 and 43 showed growth upto 150 ppm metal concentration. BRI 36, 37, 38 and 39 were found to be the most promising isolates since they could tolerate all the metals upto 200 ppm concentration.

Maximum tolerable concentration

Based on the results of screening experiment, we used BRI 36, 37, 38 and 39 for further study. MTC determination experiments showed that, BRI 36 has highest MTC value for Ni^{2+} (600 ppm). It was 500 ppm for Pb^{2+}, Cd^{2+} and Cr^{3+} (Table 2). To summarize, the order of MTC values for Ni^{2+} was BRI 36>BRI37>BRI38>BRI39, for Cr^{3+} it was BRI36>BRI38>BRI39>BRI37, for Pb^{2+} it was BRI36>BRI37>BRI38>BRI39 and for Cd^{2+} it was BRI36>BRI37>BRI38>BRI39 (Table 2). The observations suggested BRI 36 as potential isolate for heavy metal removal. The viability of four isolates at their MTC was determined by estimating Total Viable Count (TVC). Maximum survival (47.1%) of BRI 39 was observed in presence of Ni^{2+}, followed by 30 % survival of BRI 38 in presence of Pb^{2+}, 29 % of BRI 36 in presence of Cd^{2+} and 16.6 % of BRI 38 in presence of Cr^{3+} (Table 2) .

Growth curve studies

Growth curve studies of four isolates (BRI 36, 37, 38 and 39) were performed in the presence of different heavy metals (Ni^{2+}, Cd^{2+}, Pb^{2+} and Cr^{3+}) individually. The growth responses by resistant isolates were entirely different in presence of metal in comparison with their respective controls grown under non-metal conditions (Fig. 1). Growth pattern of BRI 36 and BRI39 were comparatively less affected by heavy metals. Cd^{2+} appears to affect growth maximally among the metals selected. We noticed increasing lag phase by 8 h in BRI 37, 38 and 39 in presence of Cd^{2+}. Similar effect was also observed due to Pb^{2+} and Cr^{3+} on BRI 37. BRI 38 showed extending log phase in presence of all four metals. Whereas BRI 37 exhibited increase in log phase in presence of Ni^{2+} and Cr^{3+}.

Measurement of heavy metal removal by bacterial isolates

Heavy metal removal of BRI 36, 37, 38 and 39 was assessed by measuring metal depletion in culture supernatants by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Table 3). Highest removal (86.6-95.39 %) was observed in case of Cd^{2+} in all the isolates. This was followed by Pb^{2+} (72.9-86.7 %), Ni^{2+} (6.7-20.29 %) and Cr^{3+} (1.9-5.01 %). Among the four isolates tested, maximum removal of Cd^{2+} and Pb^{2+} was observed in BRI 36 and 38 while that of Ni^{2+} was detected in BRI 38.

Metal accumulation and distribution

The selected bacterial isolates were observed for intracellular metal accumulation and distribution on the cell wall after incubation with Cd^{2+}, Cr^{3+}, Ni^{2+} and Pb^{2+}. Results showed that accumulation of Pb^{2+} (8.11 mg/l) and Cd^{2+} (8.5 mg/l) in BRI 36 (Table 3). Whereas BRI 38 and BRI 39 exhibited their metal accumulation ability for Ni^{2+} (0.49 mg/l) and Cr^{3+} (0.39 mg/l) respectively. Observations of metal adsorption experiments revealed highest value (1.54 mg/l) for Ni^{2+} using BRI 38 followed by BRI 37 (0.8 mg/l), BRI 36 (0.7 mg/l) and BRI 39 (0.18 mg/l). It can be concluded that metal depletion from cell exterior is mainly due to accumulation in case of Pb^{2+} and Cd^{2+}.Whereas in case of Ni^{2+} cell wall adhesion appears to be the main reason.

Antibiotic susceptibility

We noticed high susceptibility to most of the antibiotics tested. BRI 36 showed resistance to 33 % antibiotics tested while BRI 39 showed resistance to only 16 % antibiotics tested (Table 4). Gram negative isolate BRI 37 (BRI 37 and 38) displayed resistance to only 6 % of antibiotics used and BRI 38 displayed resistance to 13 % of the antibiotics selected (Table 5) indicating absence of co-relation between heavy metal tolerance and antibiotic resistance.

Table 1
Screening of fourteen BRI isolates for heavy metal tolerance

	BRI isolates													
	32	33	34	35	**36**	**37**	**38**	**39**	40	41	42	43	44	45
	Lead (ppm)													
50	-	+	+	+	+	+	+	+	+	+	+	+	+	+
100	-	-	+	+	+	+	+	+	+	+	+	+	-	-
150	-	-	+	+	+	+	+	+	+	+	-	+	-	-
200	-	-	-	-	+	+	+	+	-	-	-	-	-	-
	Cadmium (ppm)													
50	-	-	-	+	+	+	+	+	+	+	+	-	+	-
100	-	-	-	+	+	+	+	+	-	-	-	-	-	-
150	-	-	-	-	+	+	+	+	-	-	-	-	-	-
200	+	-	-	-	+	+	+	+	-	-	-	-	-	-
	Nickel (ppm)													
50	+	+	+	+	+	+	+	+	-	-	-	-	-	-
100	+	+	-	-	+	+	+	+	-	-	-	-	-	-
150	+	+	-	-	+	+	+	+	-	-	-	-	-	-
200	-	-	-	-	+	+	+	+	-	-	-	-	-	-
	Chromium (ppm)													
50	-	-	+	+	+	+	+	+	+	+	-	-	+	-
100	-	-	-	-	+	+	+	+	-	-	-	-	-	-
150	-	-	-	-	+	+	+	+	-	-	-	-	-	-
200	-	-	-	-	+	+	+	+	-	-	-	-	-	-

+: growth; - : no growth

Table 2
Maximum tolerable concentration of four BRI isolates and their total viable count at MTC

Isolates	Metals	Maximum tolerable concentration (ppm)	% Survival
BRI 36	Ni^{2+}	*600*	40.3
	Pb^{2+}	*500*	16.1
	Cr^{3+}	*500*	14.8
	Cd^{2+}	*500*	29
BRI 37	Ni^{2+}	*500*	39
	Pb^{2+}	*400*	23.4
	Cr^{3+}	*300*	15.6
	Cd^{2+}	*300*	21.8
BRI 38	Ni^{2+}	*500*	30.3
	Pb^{2+}	*400*	30
	Cr^{3+}	*400*	16.6
	Cd^{2+}	*300*	22.7
BRI 39	Ni^{2+}	*500*	47.1
	Pb^{2+}	*400*	24.5
	Cr^{3+}	*400*	6.6
	Cd^{2+}	*300*	21.6

Table 3 A)
Cadmium removal by four BRI isolates

Isolates	Control mg/l	Intracellular accumulation mg/l	Adsorption mg/l	% Removal
BRI 36	10.0	8.5 $^{(0.026)*}$	1.04 $^{(0.02)*}$	95.39
BRI 37	10.0	7.56 $^{(0.015)}$	1.09 $^{(0.028)}$	86.6
BRI 38	10.02	7.87 $^{(0.02)}$	0.97 $^{(0.04)}$	88.2
BRI 39	10.02	7.76 $^{(0.045)}$	1.01 $^{(0.025)}$	87.7

Table 3 B)
Chromium removal by four BRI isolates

Isolates	Control mg/l	Intracellular accumulation mg/l	Adsorption mg/l	% Removal
BRI 36	6.03	0.19 $^{(0.025)*}$	0.09 $^{(0.026)}$	4.6
BRI 37	6.03	0.1 $^{(0.01)}$	0.2 $^{(0.015)}$	4.9
BRI 38	9.52	0	0.19 $^{(0.01)}$	1.9
BRI 39	9.52	0.39 $^{(0.026)}$	0.09 $^{(0.01)}$	5.01

Table 3 C)
Lead removal by four BRI isolates

Isolates	Control mg/l	Intracellular accumulation mg/l	Adsorption mg/l	% Removal
BRI 36	10.01	8.11 (0.01)	0.56 (0.011)	86.7
BRI 37	10.01	6.6 (0.011)	0.7 (0.005)	72.9
BRI 38	9.20	6.13 (0.01)	0.96 (0.01)	77.0
BRI 39	9.20	6.11 (0.01)	0.84 (0.04)	75.5

Table 3 D)
Nickel removal by four BRI isolates

Isolates	Control mg/l	Intracellular accumulation mg/l	Adsorption mg/l	% Removal
BRI 36	7.90	0.4 (0.005)	0.7 (0.026)	13.9
BRI 37	7.90	0.3 (0.01)	0.8 (0.01)	13.9
BRI 38	10.0	0.49 (0.01)	1.54 (0.04)	20.29
BRI 39	10.0	0.49 (0.02)	0.18 (0.015)	6.7

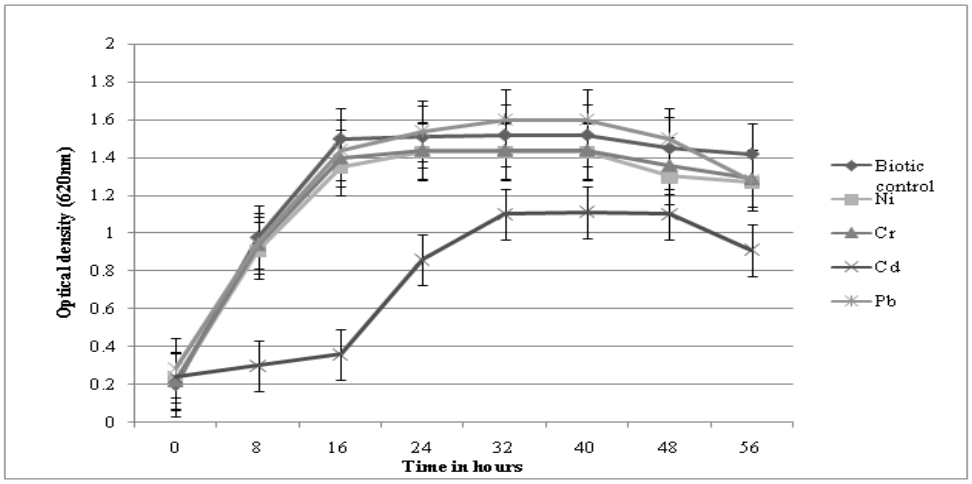
**** ± standard deviation (*p<0.05)**

Table 4
Antibiotic susceptibility of BRI 36 and 39 (Gram positive isolates)

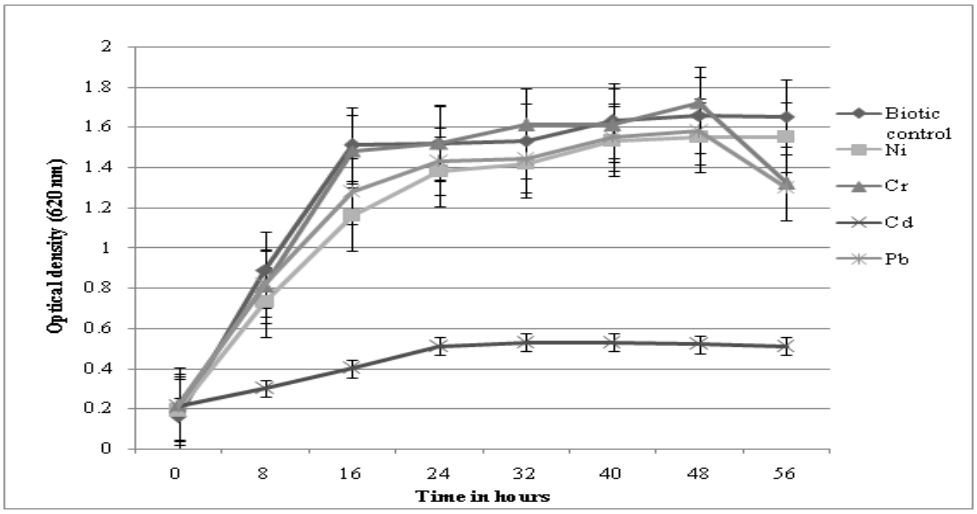
Name of Antibiotic	Concentration of antibiotic (µg)	BRI 36	BRI 39
Piperacillin	100	R	S
Linezolid	300	S	S
Ciprofloxacin	5	S	S
Teicoplanin	30	S	S
Vancomycin	30	S	S
Gentamicin	10	S	R
Ampicillin	10	S	R
Chloramphenicol	25	R	S
Penicillin G	1 unit	R	S
Streptomycin	10	S	S
Sulphatriad	300	R	S
Tetrocycline	25	S	S

Table 5
Antibiotic susceptibility of BRI 37 and 38 (Gram negative isolates)

Name of Antibiotic	Concentration of antibiotic (µg)	BRI 37	BRI 38
Ampicillin	10	S	S
Amoxyclav	30	R	R
Cefotaxin	30	S	S
Co-trimoxazole	25	S	R
Gentamicin	10	S	S
Tobramycin	10	S	S
Ceftazidine	30	S	S
ciprofloyacin	5	S	S
Amikacin	30	S	S
Nitrofloxacin	30	S	S
Netillin	30	S	S
Nalidixic acid	30	S	S
Tetracycline	30	S	S
Ofloxacin	5	S	S
Cefuroxime	30	S	S


Optical density (620nm)
Time in hours
Biotic control
Ni
Cr
Cd
Pb

a)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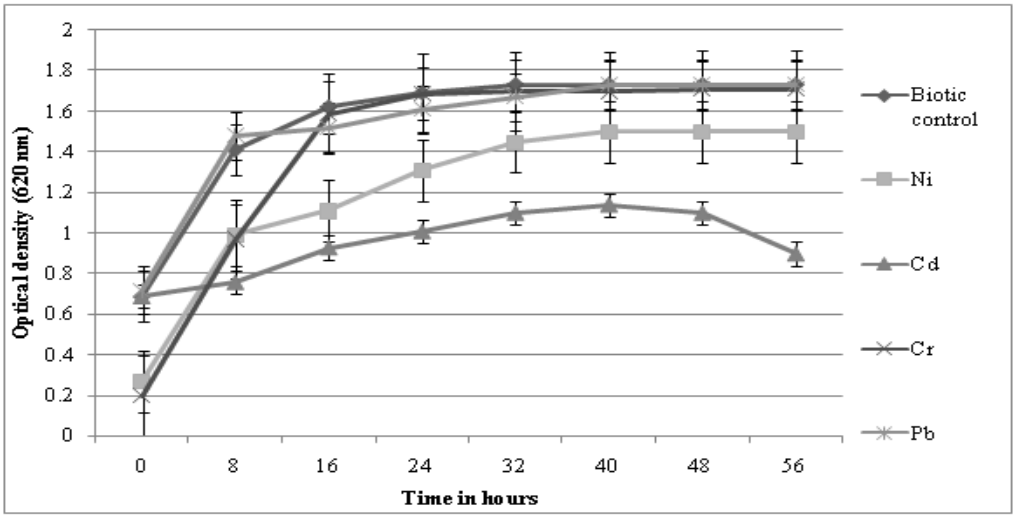
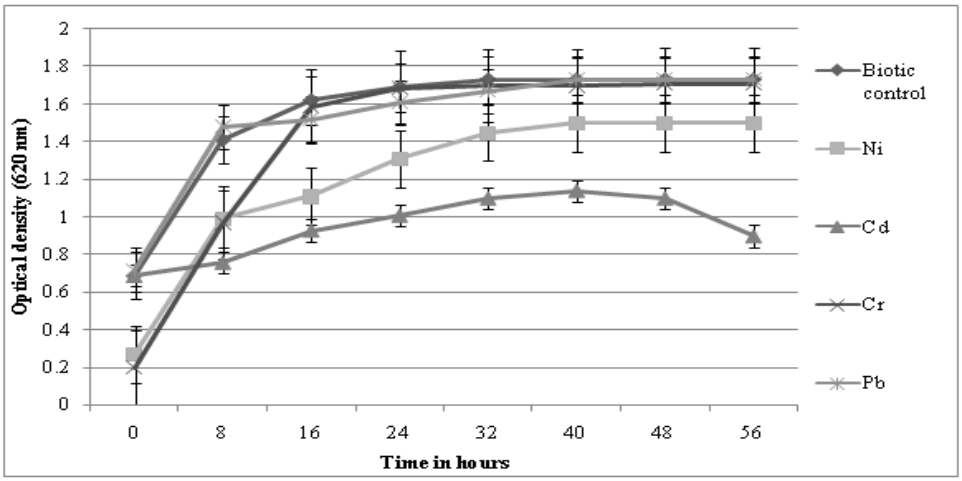
Time in hours
Biotic control
Ni
Cr
Cd
Pb

b)


Optical density (620 nm)
Time in hours
Biotic control
Ni
Cd
Cr
Pb

c)



d)

Figure 1

Growth curve pattern of a) BRI 36, b) BRI 37 c) BRI 38 and d) BRI 39 in presence of Ni^{2+}, Cr^{3+}, Cd^{2+} and Pb^{2+}. Data were analyzed by two –way ANOVA ($p<0.05$) and vertical bars represent standard error.

DISCUSSION

We have screened fourteen bacterial isolates from Antarctic oceanic region for heavy metal tolerance and metal removal ability.Their MTC values were in the range of 300 ppm to 600 ppm. In 2015, Vashishth and Khanna[20] had worked on the isolates from battery manufactured polluted soil and shown MTC values of 500 ppm ,750 ppm and 2000 ppm for Cd and Ni and Pb respectively. Bacterial isolates from Indian Ocean Equatorial region were studied by Devika et al[21]. They had recorded MTC of 150 ppm for Cd in case of the isolate CD- 10 while it was 800 ppm for another isolate LD 5- 3. Ivanova *et al* [22] and De salva[23] had published MTC value of 100 ppm for cadmium in marine bacteria. Studies by Samanta *et al* [24] in *Bacillus sp.* revealed MTC values of 1000 ppm, 750 ppm, 500 ppm for Cd, Cr and Ni respectively. Comparatively lower MTC values in the range of 80 ppb to 100 ppm were documented by Tripathy *et al* [25] and Priyalaxmi *et al*[26] Considering this. Our isolates exhibit moderate tolerance to toxic metals. Moreover, their viability is also significant in presence of toxic metals. Our experiments on change in growth behaviour in presence of metals indicated maximum effect of Cd^{2+}.Similar results of increase in adaptation time causing prolonged lag phase due to metal stress were published by Deb *et al*[27] based on their studies using *Pseudomonas stutzeri.* We observed maximum removal of Pb^{2+} and Cd^{2+} from cell exterior in case of BRI 36 and 38 respectively which can be accounted for intracellular metal accumulation. Moderately halophilic bacteria isolated from saline environments of Iran could uptake more than 90% and 50% of lead and cadmium, respectively[28].Massadeh *et al*[29]have reported bacterial isolates from Dead Sea shore showed Pb and Cd absorption 86.8 to 96.0 % and 82.60 % to 93.2 %, respectively in 3 weeks. Bacteria isolated from soil by Hrynkiewicz *et al* [30] had shown accumulation of Cd^{2+}in the range of 0.87 to 1.32 weight %. The halotolerant isolate from shrimp pond, (Thailand) was recorded to reduce Pb upto 39 % and Cd upto 5 % [31]. Among the genus *Kocuria*, *K.carniphila* MY and *K.polaris* MO isolated from Nile river, Egypt showed absorption of Pb and Cd in the range of 32.2 to 74.6 %[32]. However the genera *Brevibacillus* and *Oceanobacillus* are very less studied for heavy metal removal. In contrast to this Ni^{2+} was majorly depleted from cell exterior due to cell adsorption in case of BRI 38 We had come across very few reports on heavy metal adsorption in bacteria using live cells. Biosorption of chromium was studied by Velásquez and Dussan[33] in *Bacillus sp*. They observed 25 % and 32 % biosorption of Cr^{3+} in case of *B. sphaericus* OT4b31 and *B. sphaericus* IV(4)10 respectively. The authors proposed role of S-layer proteins in metal ions entrapment. Role of Carboxylate and phosphate groups of peptidoglycan and teichoic acids as major metal binding sites had been proposed by Rho and Kim[34].Some of the earlier studies showed correlation between metal tolerance and antibiotic resistance in bacteria which may be due to close association of respective loci on extra chromosomal genetic material and thus suggesting their simultaneous transfer in environment[35]. In order to investigate our isolates for this characteristic feature, we carried out antibiotic susceptibility test. Our results suggested lack of co-relation between metal tolerance and antibiotic resistance. Similar results were also reported by Boga *et al* [36] during their work on Gram negative bacteria.BRI isolates also have ability to grow in presence of high salt (15 %), low temperature (10°C) and at acidic pH (4.0)[17]. Very few reports are available on potential of microorganisms in removing heavy metals under saline conditions[37]. Significant metal removal ability of these psychrotolerant and halotolerant isolates suggest their possible application in bioremoval of heavy metals from extreme environment such as industrial wastes containing high salt concentration[38] and cold habitats in polar region.

CONCLUSION

All the BRI isolates have potential to tolerate high concentration of Cd^{2+}, Cr^{3+}, Ni^{2+} and Pb^{2+} (300 -600 ppm). Among the four isolates tested, maximum removal

of Cd^{2+} and Pb^{2+} was observed in BRI 36 and 38 while that of Ni^{2+} was detected in BRI 38. Maximum accumulation of Pb^{2+} and Cd^{2+} was observed in BRI 36 while BRI 38 and BRI 39 exhibited considerably less metal accumulation ability for Ni^{2+} and Cr^{3+}. These observations indicate BRI 36 as a potential candidate for heavy metal removal. Further experimentation is underway to affirm its applicability in bioremediation.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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Reviewers of this article

Dr.Pradnya Kanekar M.Sc.Ph.D.Microbiology

CSIR Emeritus Scientist
Department of biotechnology
Modern college of arts,Science and commerce
Shivajinagar,Pune- 411005

Asso.Prof.Dr. R. Usha, MSc, M.Phil, Ph.D.

Associate Professor, Department of Microbiology,
Karpagam University, Eachanari (PO),
Coimbatore - 641 021, Tamil Nadu, India.

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

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