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CO-RELATION BETWEEN HEAVY METAL RESISTANCE AND ANTIBIOTIC SUSCEPTIBILITY IN HALOTOLERANT BACTERIA ISOLATED FROM THE ANTARCTIC OCEANIC REGION

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ABSTRACT

Four bacterial isolates viz *Kocuria*sp *BRI 36, Brevibacillus* sp *BRI 37, Halomonas* sp *BRI 38* and *Oceanobacillu*s sp BRI*39* from Antarctic oceanic region exhibitinghigh tolerance to heavy metals like cadmium, chromium, nickel and lead were selected for this study. Their maximum tolerable concentration MTC values were found to be in the range of 300 to 600 ppm. Interestingly,the isolates exhibited growth in presence of multiple metals at various temperatures (15, 30 and 45°C). The characteristicof metal resistance is known to be linked to antibiotic resistance and mostly associated with plasmid DNA. In order to determine role of plasmid DNA, curing experiments were performed using ethidium bromide and Sodium dodecyl sulfate SDS. Cured BRI isolates were again examined for metal tolerance and antibiotic susceptibility pattern. MTC values for the selected four metals were almost similar to those prior to curing. However, antibiotic susceptibility experiments revealed increase in sensitivity to piperacillin and chloramphenicol in cured BRI 36 whereas, remaining isolates demonstrated similar response to the antibiotics tested after curing. The observations indicated possible role of plasmid in piperacillin and chloramphenicol resistance in BRI 36whereas, in other isolates it may or may not be plasmid linked. However, metal resistance characteristics of BRI isolates appear to be independent of extra chromosomal DNA, since the MTC results remained unaltered after curing.

KEYWORDS: ***Multi-metal, Maximum tolerable concentration (MTC), antibiotic resistance, plasmid isolation, curing***

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INTRODUCTION

Metals like Na, K, Mg, Ca, V, Mn, Fe, Co etc. play a vital role in metabolic processes of living organisms while some like Ag, Cd, Pb, Sn, Au, Hg etc. are toxic[1]. Element biotransformation and biogeochemical cycling, decomposition, bioweathering, and soil and sediment formation are some of the activities in which microorganisms are involved. However, increase in metal concentration beyond certain limit activates microbial resistance mechanism which enables them to survive under stress[2].Microorganisms exhibit various strategies to combat the metal stress. Various adaptation mechanisms include reduction of metals to less toxic form, sequestration, active efflux etc.[3]These mechanismsmay be attributed to the genetic elements present on chromosomes, transposons or plasmid.In majority of the cases, it had been shown to be associated withplasmid DNA. For example, cadmium resistance mechanism in *Enterobacter* sp and *Staphylococcus aureus*sp.,[4,5] *Bacillus* strain JMAK1[6], *Halomonas BVR 1*[7]etc..However, role of chromosomal DNA in metal resistance had been very rarely documented. Moreover, antibiotic resistance is known to be linked to metal resistance. The studies carried out on *Bacillus sp.* showed loss of antibiotic and heavy metal resistance in this organism after plasmid curing[8]. However, among halophilic and/or halotolerant microorganisms very few studies of such type have been published. Keeping this in mind we have selected four halotolerant isolates from Antarctic oceanic region with significant resistance to cadmium, nickel, lead and chromium[9] to establish the co-relation between heavy metal tolerance, antibiotic resistance and possible role of plasmid DNA.Recently we have reported effect of metals on pigment production by *Kocuria*sp. BRI 36[10].

MATERIALS AND METHODS

Organism

*Kocuri a*sp *BRI 36,Brevibacillus* sp BRI 37, *Halomonas* sp BRI 38 and *Oceanobacillus* sp *BRI* 39 were used in this work. The isolates were grown in Mineral Salt Medium (MSM) at 25±2°C for 48 h with shaking at 120 rpm[11]. They were further used for inoculation in all the experiments at 10% concentration.

Chemicals and Reagents

All chemicals used were of analytical grade. The media components were purchased from Hi Media Laboratories Pvt. Ltd. (Mumbai, India). The stock solutions of cadmium, nickel, lead and chromium (1000 ppm each) were purchased from Sigma-Aldrich.

Screening of BRI isolates for multi-metal resistance

All the four isolates were cultivated in MSM amended with combinations of different metals (each at 10 mg/l concentration) at 30°C with shaking at 120 rpm for 48 h. At the end of incubation the samples were used to determine total viable count (TVC).

Effect of temperature on growth in presence of multiple metals

All the four isolates were grown under different Conditions of temperature (15, 30 and 45°C) in presence of various combinations of metals each at 10 mg/l concentration with shaking at 120 rpm for 48 h.

Plasmid isolation

BRI isolates were grown in Luria-Bertani (LB) broth at 120 rpm for 48 h at room temperature. The cell pellets were harvested by centrifugation at 10000 rpm for 10 min at 4°C. The plasmid DNA was isolated by small scale boiling lysis method[12]. The samples were dissolved in Tris-EDTA ($T_{10}E_1$) buffer and analysed on 1% agarose gel.

Plasmid curing

BRI isolates were grown in sterile LB amended with ethidium bromide (100 mcg/ ml) and 2% SDS[13]. After incubation at 30°C for 48 h, the cultures were maintained on Luria agar plates. The cured BRI isolates were examined for i) presence of plasmid DNA ii) their MTC for Cd^{2+}, Ni^{2+}, Pb^{2+} and Cr^{3+} individually and iii)antibiotic resistance.

Maximum tolerable concentration (MTC)

Cured BRI isolates 36, 37, 38 and 39 were used to determine their maximum tolerable concentration (MTC) to Cd^{2+}, Ni^{2+}, Pb^{2+} and Cr^{3}+ individually in the range of 200-700 ppm as described previously[9.]

Antibiotic resistance

The antibiotic susceptibility of cured BRI isolates was examined as described previously[9] using 48 h grown cultures and antibiotic discs (Hi Media Pvt. Ltd. India).

RESULTS AND DISCUSSION

The four isolates viz. BRI 36, 37, 38 and 39 were examined to determine the multi-metal resistance andcorrelation between theirmetal resistance and antibiotic resistance characteristics. As reported previously, their MTC values for Cd^{2+}, Cr^{3+}, Ni^{2+} and Pb^{2+} were in the range of 300 to 600 ppm. Moreover, the isolates showed high susceptibility to most of the antibiotics tested[9].The resultsof multi- metal resistance experiment showed that all the BRI isolates have ability to resist all four heavy metals in different combinations at 10 mg/l concentration of each metal. Previously studies on multimetal tolerance had been reported in *Thiobacillus ferrooxidans by* Das et al[14]using Cu^{2+}, Zn^{2+} and Fe^{3+}.The authors had performed the experiments using the metals individually or in possible binary and ternary combinations. *Acinetobacter baumannii*strain HAF – 13 exhibited multi-metal resistance to Hg^{2+}, Pb^{2+}, Cd^{2+}, As^{5+} and Cr^{6+} at their MTC (75-250 mg/l)[15].The results of TVC experiments indicated maximum viability (23 %) of BRI 37 when exposed to all four metals followed by BRI 38 in presence of Ni^{2+}, Pb^{2+},Cd^{2+} and BRI 36 in presence of Cr^{3+}, Cd^{2+}, Ni^{2+}(Figure 1) at 30°C.We studied the effect of temperature (Table 1) on multi-metal tolerance of all the isolates. All BRI cultures exhibited normal growth at temperatures of 15, 30 and 45°C indicating their ability to survive and proliferate in presence of multiple metals. Kumar[16] had reported growth of *Pseudomonas aeruginosa*at different temperatures(25-45°) in presence of cadmium. He had observed maximumremoval at

35°C.In order to confirm the association between these characteristics and plasmid DNA, plasmid curing experiments were carried out (Figure 2 (a, b). Plasmid mediated resistance mechanism for cadmium, chromium and nickel in *E.coli* was studied by Lazar et al.[17]. Authors had discussed phenotypic data showing direct relation between multiple antibiotic resistance and heavy metal resistance. Cured BRI cultures (present work) were then reassessed for MTC values for Cd^{2+}, Ni^{2+}, Pb^{2+} and Cr^{3+} individually and also for antibiotic susceptibility. We observed MTC results almost similar to those obtained prior to curing[9]. However, antibiotic test results slightly differed from those prior to curing. Among the four BRI isolates,cured BRI 36 showed increased susceptibility to antibioticsincomparison to the previous sensitivity pattern[9]. Initially it had demonstrated resistance to piperacillin and chloramphenicol. However, same concentration of antibiotics wasfound to inhibit its growthafter curing.On the other hand BRI 37, 38 and 39 did not show any change in their antibiotic susceptibility pattern after curing.The results indicated plasmid mediated antibiotic resistance in halotolerant BRI 36 whereas it may or may not be plasmid derived in other BRI isolates. However, unalteredMTC values suggested absence of association with extra-chromosomal DNA. Thus, our results of heavy metal tolerance in BRI isolates showing susceptibility to majority of antibiotic tested indicate absence of direct correlation between these two characteristics. The present inference is currently under investigation. Similar resultsrepresenting absence of correlation between antibiotic resistance and metal tolerance were observed by Boga et al.[18]in Gram negative bacteria isolated from Lake Victoria wetlands.

Table 1
Effect of temperature on multi-metal tolerance

Sr.no	Temperature (˚C)	15˚C				30˚C				45˚C			
	Metal	BRI 36	BRI 37	BRI 38	BRI 39	BRI 36	BRI 37	BRI 38	BRI 39	BRI 36	BRI 37	BRI 38	BRI 39
1	Ni^{2+},Pb^{2+}, Cr^{3+},Cd^{2+}	+	+	+	+	+	+	+	+	+	+	+	+
2	Ni^{2+},Pb^{2+}, Cr^{3+}	+	+	+	+	+	+	+	+	+	+	+	+
3	Ni^{2+},Pb^{2+}, Cd^{2+}	+	+	+	+	+	+	+	+	+	+	+	+
4	Pb^{2+}, Cr^{3+},Cd^{2+}	+	+	+	+	+	+	+	+	+	+	+	+
5	Cr^{3+},Cd^{2+},Ni^{2+}	+	+	+	+	+	+	+	+	+	+	+	+

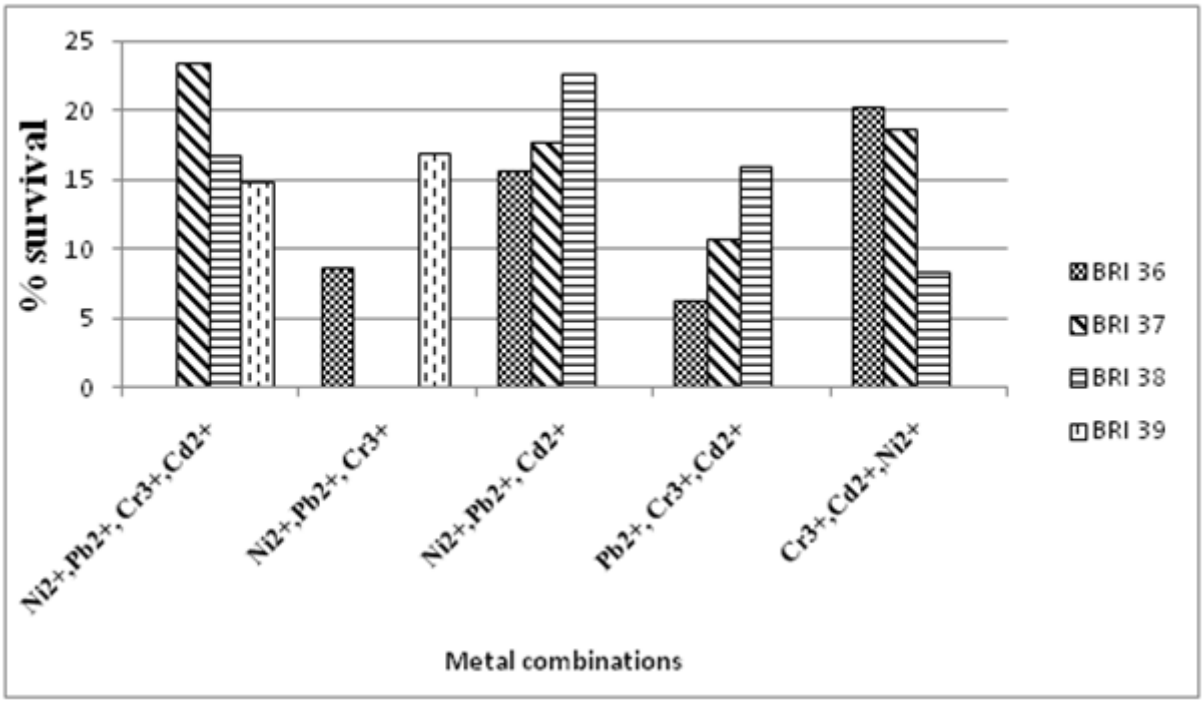

Figure 1
Percent survival of BRI isolates in presence of different combinations of metals.

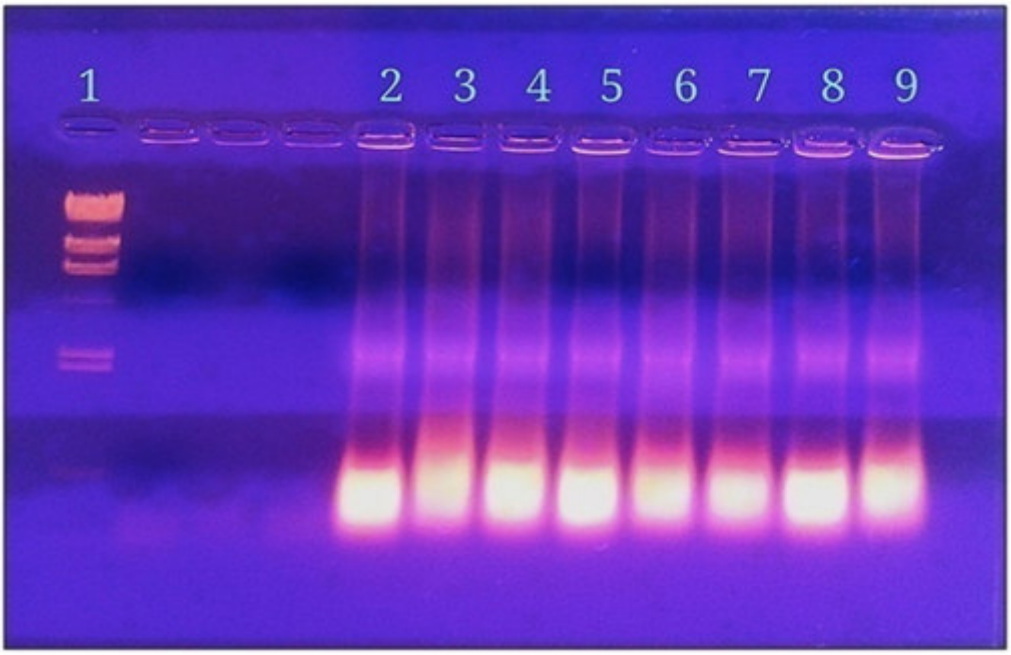

Figure2 (a)

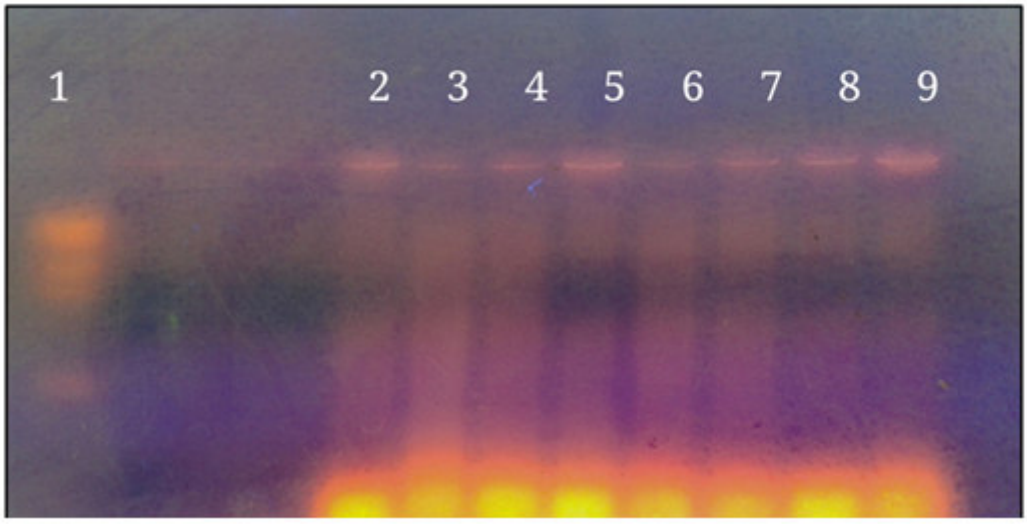

Figure2 (b)

Figure2

Agarose gel (1%) electrophoresis showing a) plasmid DNA from BRI isolates before curing and b) after curing. Lane 1- lambda DNA Hind III digest, lane 2 - BRI 36, lane 3- BRI 37, lane 4- BRI 38 and lane 5- BRI 39

CONCLUSION

Four bacterial isolates *Kocuria* sp BRI 36,*Brevibacillus sp BRI 37, Halomonas* sp *BRI 38 and Oceanobacillu*s sp BRI *39* from the Antarctic oceanic region exhibiting high tolerance to heavy metals like cadmium, chromium, nickel and lead were selected for this study. They exhibited an ability to tolerate multiple heavy metals in different combinations.MTC values for the selected four metals were almost similar before and after curing of BRI isolates. However, antibiotic susceptibility experiments indicated possible role of plasmid in piperacillin and chloramphenicol resistance in BRI 36 whereas, in other isolates it may not be plasmid linked.However, metal resistance characteristics of BRI isolates appear to be independent of extra chromosomal DNA, since the MTC results remained unaltered after curing.Their antibiotic susceptibility test results and unaltered MTC values before and after curing suggest lack of co-relation between heavy metal tolerance and antibiotic resistance in BRI isolates.

CONFLICTS OF INTERESTS

Conflict if interest declared none.

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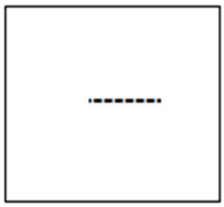

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