Research Article

IMPLICATIONS OF BACTERIAL ISOLATES IN HEAVY METAL TOLERANCE TO THE POLLUTED ENVIRONMENT

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ABSTRACT

Two isolates namely, *Bacillus* sp. and *Leclercia adecarboxylata* were isolated from soil contaminated with wide range of heavy metals. Minimum inhibitory concentration (MIC) for both the isolates was at 2000 mg Pb L^{-1} and also exhibited high resistant to wide range of antibiotics.

Keywords: Heavy metal resistance, Antibiotics resistance, Bacteria, Bioremediation.

INTRODUCTION

Industries continuously pollute our environment and releases heavy metals in their effluent, which pose a great threat to all life forms[1]. Most of the metals such as K, Na, Mg, Ca, Mn, Fe, Cu, Ni, Zn, Mo have a direct role in microbial growth but the biological role of Al, Ag, Cd, Sn, Au, Sr, Hg and Pb was not yet known. But these elements interact with microbial cells and are accumulated as a result of physico-chemical mechanism and transport systems of varying specificity, independent on, or directly and indirectly dependent on metabolism[2]. Therefore, these processes are of biotechnological importance being relevant to metal removal and recovery from mineral deposits and industrial effluents for environmental bioremediation. The aim of this paper to isolate bacteria that have evolved mechanisms to detoxify heavy metals and to find out the correlation between tolerance to heavy metal and antibiotic resistance, which is a global problem currently threatening the environment.

MATERIALS AND METHODS

Experimental soil for isolation

The heavy metal contaminated soil for isolation of bacteria was collected from Narasimmanayakanpalayam, Coimbatore, Tamil Nadu, India. The basic properties of the soil such as pH and EC[3], Organic carbon[4] were analyzed. The total heavy metal contents using aqua-regia assisted digestion were analyzed using Atomic Absorption Spectrophotometer (Model - Perkin Elmer AAnalyst 200, USA) is given in table 1.

Isolation of Pb – resistant bacteria:

Soil samples were subjected to serial dilution in a Pb incorporated Luria Bertani's (LB) medium containing 1000 ppm of Pb as $Pb(NO_3)_2$ for the selective isolation of Pb resistant bacteria. Inoculated plates were incubated at 37°C for 24 h. After the incubation period, individual colonies on the selective media were sub cultured and obtained in the form of pure culture and identified on the basis of their morphology and biochemical characters using VITEX 2 Systems Version: 05.02. Two bacteria namely *Bacillus* sp. and *Leclercia adecarboxylata* were used for further studies. Pure cultures were routinely sub cultured in Nutrient agar medium and maintained at 4°C.

Determination of Minimum Inhibitory Concentration (MIC)

20,000, 10,000, 8000, 6000, 4000, 2000, 1000, and 0 mg L^{-1} of Pb solution was prepared in sterile distilled water. Disc was dipped in different concentration and sterilized in UV. Sterilized LB agar medium poured in 20 cm petriplate. Bacterial suspension 5 x 10^7 cells ml^{-1} was prepared in sterile distilled water and swabbed on the media. Disc containing different concentration of Pb was placed on the media. Plates were incubated at 37°C for 18 h. MIC was calculated to different concentration of Pb by diameter of inhibition zone. Experiment contains three replicates and repeated two times.

Determination of antibiotic sensitivity and resistance pattern

Antibiotic sensitivity and resistance of the isolated heavy metal resistant isolates were assayed according to the Kirby-Bauer disc diffusion method. Two isolates were tested against twenty different antibiotics. After incubation, the organisms were classified as sensitive or resistant to an antibiotic according to the diameter of inhibition zone given in standard antibiotic disc chart.

Statistical analysis

Data were statistically analysed for one-way ANOVA to find out the significant differences between the treatments.

RESULTS AND DISCUSSION

Isolation and characterization of bacteria

The heavy metal resistant bacteria could be a potential agent for bioremediation of heavy metals pollution. Therefore, Two Pb – resistant isolates namely *Bacillus* sp. and *Leclercia adecarboxylata* was isolated from the

contaminated soil indicating that these bacteria populations had a marked adaptation to heavy metal under constant metal stress for a long time [5].

Heavy metal and antibiotic resistance

Bacillus sp. and *Leclercia adecarboxylata* showed a very high degree of resistance to Pb at 2000 mg L^{-1} (table 2) and also exhibited multi antibiotic resistant characteristics (table 3). Singh (2010)[6] reported that *Pseudomonas* sp isolated from sewage of industrial effluent were resistant to heavy metals and antibiotics. Such high resistance towards Pb by the bacteria was attributed by variety of detoxifying mechanism developed by resistant microorganisms such as complexation by xopolysaccharides, binding with bacterial cell envelopes, metal reduction, metal efflux etc. These mechanisms are sometime encoded in plasmid genes facilitating the transfer of toxic metal resistance from one cell to another[7]. Many have speculated and have even shown that correlation exists between metal tolerance and antibiotic resistance in bacteria because of the likelihood that resistance genes of both (antibiotics and heavy metals) may be located closely together on the same plasmid in bacteria and are thus more likely to be transformed together in the environment[8,9].

Table 1: Basic chemical properties of the selected soil

pH	EC (mS cm^{-1})	OC (%)	Total Heavy metal contents (mg kg^{-1})					
			Cr	Ni	Cu	Zn	Cd	Pb
8.8	0.17	1.72	106	12	2000	348	0	1024

Table 2: Minimum Inhibitory Concentration (MIC) of bacteria at different concentration of Pb

S.No	Concentration of Pb (mg L^{-1})	*Bacillus* sp.	*Leclercia adecarboxylata*
1	20,000	1.5 a	1 a
2	10,000	1.1 a	0.75 b
3	8000	0.5 b	0.5 b
4	6000	0.5 b	0.25 c
5	4000	0.5 b	0.25 c
6	2000	0.5 b	0.25 c
7	1000	0 c	0 c
8	0	0 c	0 c

Data in the same column followed by the same letter are not significantly different, whereas with different letters data are significantly different ($p < 0.05$).

Table 3: Antibiotic sensitivity and resistant activity of heavy metal resistant bacteria

Bacteria	Sensitive to	Resistant to
Bacillus sp.	Clarithromycin, Gentamycin, Vancomycin, Cephalothin, Amikacin, Novobiocin, Erythromycin, Teicoplanin, Azithromycin, Ofloxacin, Linezolid, Clindamycin, Tetracycline and Chloramphenicol.	Oxacillin, Amoxyclav, Ampicillin, Penicillin, Methicillin and Co-Trimoxazole
Leclercia adecarboxylata	Streptomycin, Sparfloxacin, Nalidxic acid, Ceftriaxone, Ciprofloxacin, Co – Trimoxazole, Moxifloxacin Gentamicin, Gatifloxacin, Ofloxacin, Tobramycin, Norfloxacin, Amikacin, Levofloxacin, Kanamycin, Ticarcillin.	Cefpodoxime, Colistin, Imipenom, Augmentin,

CONCLUSION

These heavy metal resistant bacteria can be used for bioremediation. In order to use these heavy metal resistant bacteria for bioremediation purposes, these bacteria should be capable of oxidize or reduce heavy metals since the oxidation state of the heavy metal ion determines its solubility.

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