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Biosorption and Characterization of Metal Tolerant Bacteria Isolated from Palar River Basin Vellore

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Abstract

Metal pollution is a growing problem and microbes have adapted to tolerate the presence of metals and even use them. The investigation was carried out to screen for bisorption property of metals by bacteria and check for correlation between tolerance to heavy metals and antibiotic resistance. Soil samples were collected from Palar River basin site of Vellore and five distinct bacteria were isolated. Antibiotic resistance (bacitracin, chloramphenicol, streptomycin, rifampicin, penicillin and ampicillin) was checked and tolerance to heavy metals was screened (Cd, Pb, Cu and Zn). It was found that most of the bacterial isolates had multiple antibiotic resistances which might be due to the stress caused by heavy metals released into the Palar river basin, Vellore. The multiple antibiotics resistance of this bacterial species was found to be associated with tolerance to metals. Biosorption studies revealed that *Alcaligenes faecalis* could tolerate 59% Cd, 61% Pb, 40% Cu, 39% Zn and *Staphylococcus aureus* removed 60% Cd, 63% Pb, 42% Cu, 41% Zn and *Streptococcus lactis* absorbed 61% Cd, 57% Pb, 37% Cu, 38% Zn and *Micrococcus luteus* reduced 56% Cd, 61% Pb, 39% Cu, 41% Zn and *Enterobacter aerogenes* removed 60% Cd, 55% Pb, 62% Cu, 67% Zn.

Keywords: Antibiotic resistant; Heavy metal tolerance; Biosorption; Metal polluted soils.

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1. Introduction

Today heavy metal pollution is one of the major problems of our environment which can be from natural or anthropogenic sources with anthropogenic inputs of metals exceed natural inputs. Various industries produce and discharge wastes containing different heavy metals into the environment, such as mining and smelting of metalliferous, surface finishing industry, energy and fuel production, fertilizer and pesticide industry and metallurgy, iron and steel, electroplating, electrolysis, electro osmosis, leather working, photography, electric appliance manufacturing, metal surface treating, aerospace and

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atomic energy installation etc. Growing attention is being given to health hazards presented by the existence of heavy metals in the environment. Three categories of heavy metals which are of concern, are toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (such as Pd, Pt, Ag, Au, Ru etc.) and radionuclides (such as U, Th, Ra, Am, etc.) [1]. The introduction and progressive accumulation of antimicrobial agents, detergents, disinfectants and residues from industrial pollution, such as heavy metals contributes to the evolution and spread of resistant organisms in the receiving waters [2].

The study of interactions of heavy metals with microorganisms has focused in particular on the selection of metal resistant microorganisms from polluted environments [3, 4] and the possibility of using these bacteria for detoxifying polluted environments [5-7]. Microorganisms have developed resistance mechanisms that lead to the selection of resistant variants that can tolerate metal toxicity [8-10]. Microorganisms resistant to antibiotics and tolerant to metals appear to be the result of exposure to metal contaminated environments that cause co-incidental selection for resistance factors for heavy metals and antibiotics [11, 12]. Biosorption can be defined as the removal of metal or metalloid species, compounds and particulates from solution by biological material [13]. In recent years, applying biotechnology in controlling and removing metal pollution has been received much attention, and gradually for the past 15 years has shown outstanding application in the field of metal pollution control. Alternative process is biosorption, which utilizes certain natural materials of biological origin, including bacteria, fungi, yeast, algae, etc. These biosorbents possess metal sequestering property and can be used to decrease the concentration of heavy metal ions in solution from ppm to ppb level. It can effectively sequester dissolved metal ions out of complex solutions with high efficiency and rapidly, therefore it is an ideal candidate for the treatment of high volume and low concentration complex wastewaters [1]. We need a better understanding of the microbial tolerance mechanism in order to reduce the overall effect of toxic metals in the environment. An attempt has been made in this study to investigate antibiotic resistance and metal tolerance in isolates from metal polluted soils and to determine their biosorption capacity of Cd, Pb, Cu and Zn.

2. Experimental

2.1. *Sample collection and isolation of microorganisms*

The metal polluted soil samples were collected from different sites in Palar River basin, Vellore, India (135 Km west of Chennai). 1 g of soil samples were transferred to a test tube containing 10 mL of sterile distilled water and mixed thoroughly. From this suspension serial dilution was prepared upto 10^{-6} and 0.1 mL of final dilution was transferred aseptically to nutrient agar plates (g/L: peptone 5, beef extract 3, agar 15 and pH 7), the diluted soil suspension was spread by using a sterile glass L-rod. The plates were incubated at 37 ºC for 24 to 48 h.

2.2. *Identification and characterization of the bacterial isolates*

The morphological and biochemical characterizations of bacterial isolates were performed according to the methods described in Bergey's manual of determinative bacteriology [14].

2.3. *Determination of antibiotic resistance*

Antibiotic resistances of bacterial isolates were determined by standard disc diffusion method [15].The antibiotic impregnated discs (Hi-Media) were placed on freshly prepared lawns of each isolate on Muller Hinton Agar plates and incubated at 37 °C for 24 h. After the incubation, all plates were examined for the presence or absence of zone surrounding each disc and the zones were measured. The antibiotic disc and their concentrations used were bacitracin (10 units/disc), chloramphenicol (30 mcg/disc), streptomycin (10 mcg/disc), rifampicin (5 mcg/disc), penicillin G (10 units/disc) and ampicillin (10 mcg/disc) respectively.

2.4. *Effect of metals on bacterial growth*

The bacterial isolates were grown in LB medium supplemented with Cd, Pb, Cu and Zn at the concentrations of 0.1 mM of the respective metals to check their effect on the organisms. The metals were added as $CdCl_2$, $Pb(NO_3)_2$, $CuSO_4$ and $ZnSO_4$ and culture was grown aerobically in 25 mL medium (pH 7.0) at 37 °C for 24 h. Culture grown in absence of metal was treated as control. Growth was monitored as a function of biomass by measuring the absorbance at 600 nm against blank [16].

2.5. *Estimation of heavy metals*

The biosorption of heavy metals was carried out using bacteria grown in 250 mL conical flasks containing 50 mL of LB medium supplemented with heavy metals at the concentration of 100 mg/L (Cd, Pb, Cu and Zn) and incubated at 37 °C for 48 h. At selected time intervals samples were harvested by centrifugation at 5000 rev/min, supernatant was collected and stored at –20 °C for heavy metal analysis. The heavy metals present in the solution were determined by a Varian Atomic Absorption Spectrophotometer. The amount of metals in samples was estimated by using known concentrations of metals in the medium as control [16].

3. Results and Discussions

Five different bacterial species were isolated from Palar River basin soil samples. The morphological and biochemical characteristics of bacterial isolates are presented in Table 1. The isolated bacterial species were *Alcaligenes faecalis, Staphylococcus aureus,*

Streptococcus lactis, *Micrococcus luteus and Enterobacter aerogenes*. Most of the bacterial isolates had multiple antibiotics resistance and is presented in Table 2. The *Alcaligenes faecalis*, *Streptococcus lactis* and *Micrococcus luteus* were resistant to all antibiotics and sensitive to one antibiotic each ampicillin, penicillin G and chloramphenicol respectively. *Staphylococcus aureus* was resistant to bacitracin, rifampicin, penicillin G and they were sensitive to three antibiotics whereas *Enterobacter aerogenes* was resistant to bacitracin, streptomycin, rifampicin, and ampicillin and sensitive to chloramphenicol and penicillin G.

Table 1. Morphological and biochemical characteristics of bacterial species isolated from metal polluted soil.

Characteristics	Bacterial isolates				
	A2	M3	P1	P2	P5
Gram staining	-ve	+ve	+ve	+ve	-ve
Shape	Rod	Cocci	Cocci	Cocci	Rod
Motility	M	NM	NM	NM	M
Indole test	-ve	-ve	-ve	-ve	-ve
Methyl red test	-ve	+ve	+ve	-ve	-ve
Voges proskauer test	-ve	-ve	-ve	-ve	+ve
Citrate utilization test	-ve	-ve	-ve	-ve	+ve
Catalase test	+ve	+ve	-ve	+ve	+ve
Oxidase test	+ve	-ve	-ve	-ve	-ve
TSI test	KS/NCB	–	–	–	AS/AB
Urease test	-ve	-ve	-ve	+ve	-ve
Nitrate reduction test	+ve	+ve	-ve	-ve	+ve
H_2S test	-ve	-ve	-ve	-ve	-ve
Starch test	-ve	-ve	-ve	-ve	-ve
Gelatin Liquefaction	-ve	+ve	-ve	+ve slow	-ve
Sugar fermentation					
(a) Glucose	-ve	+ve	+ve	-ve	+ve
(b) Sucrose	-ve	+ve	+ve	-ve	+ve
(c) Lactose	-ve	+ve	+ve	-ve	+ve

Note: A2 = *Alcaligenes faecalis*, M3 = *Staphylococcus aureus*, P1 = *Streptococcus lactis*, P2 = *Micrococcus luteus*, P5 = *Enterobacter aerogenes*, +ve = Positive, -ve = Negative, KS = Alkaline slant, NCB = No change butt, AS = Acid slant, AB = Acid butt, M = Motile, NM = Non motile.

The bacterial Isolates showed growth in heavy metals such as Cd, Pb, Cu and Zn were measured by UV-vis spectrophotometer and growth rate is represented in Table 3. Bacterial growth in LB medium supplemented with heavy metals was observed due to efficient tolerance of heavy metals. Metal uptake capacity of bacterial isolates was determined using LB medium supplemented with heavy metals at the concentration of 100 mg/L (Cd, Pb, Cu and Zn). After 48 h of incubation decrease of metal concentration in the solution was observed with increase in growth due to efficient uptake of metals. The uptake capacity was measured by AAS (Atomic Absorption Spectrophotometer) analysis.

The highest amount of biosorption of heavy metals was observed with Pb, Cd while the lowest was seen with Zn and Cu, respectively.

Table 2. Antibiotic resistance of bacterial isolates from metal polluted soil.

Antibiotics	Conc.	Diameter of inhibition zone (mm) of bacterial isolates				
		A2	M3	P1	P2	P5
Bacitracin	10 units	NZ (R)	NZ (R)	NZ (R)	NZ (R)	NZ (R)
Chloramphenicol	30 mcg	NZ (R)	19 (S)	17 (R)	32 (S)	19 (S)
Streptomycin	10 mcg	11 (R)	18 (S)	10 (R)	11 (R)	11 (R)
Rifampicin	5 mcg	16 (R)	16 (R)	NZ (R)	16 (R)	NZ (R)
Penicillin G	10 units	8 (R)	NZ (R)	28 (S)	NZ (R)	25 (S)
Ampicillin	10 mcg	26 (S)	19 (S)	NZ (R)	NZ (R)	NZ (R)

Note: NZ = No zone, R = Resistant, S = Sensitive.

Table 3. Heavy metal tolerance profiles of bacterial isolates.

Organisms	OD value at 600 nm				
	Control	Cd	Pb	Cu	Zn
Alcaligenesfaecalis	0.728	0.648	0.713	0.522	0.507
Staphylococcus aureus	0.768	0.719	0.757	0.651	0.633
Streptococcus lactis	0.784	0.753	0.769	0.462	0.523
Micrococcus luteus	0.676	0.608	0.674	0.538	0.516
Enterobacteraerogenes	0.799	0.625	0.589	0.718	0.675

The isolates identified as *Alcaligenes faecalis, Staphylococcus aureus, Streptococcus lactis, Micrococcus luteus* and *Enterobacter aerogenes* are similar to the results of previous works of bacterial isolates in metal contaminated environments [17]. Microorganisms resistant to antibiotics and tolerant to metals appear as the result of exposure to metal contaminated environments which cause co-incidental selection for resistance factors for antibiotics and heavy metals. Microbial resistance to antibiotics and metal ions is a potential health hazard because these traits are generally associated with transmissible plasmids. Association between resistance to antibiotics and heavy metals has been reported by several workers that the mechanism of tolerance is generally efflux pumping and enzymatic detoxification [11, 12, 17-19]. Here we find similar results, *Alcaligenes faecalis, Streptococcus lactis* and *Micrococcus luteus* were resistant to all antibiotics except they were sensitive to ampicillin, penicillin G and chloramphenicol respectively. *Staphylococcus aureus* was resistant to bacitracin, rifampicin, penicillin G whereas *Enterobacter aerogenes* was resistant to bacitracin, streptomycin, rifampicin, ampicillin. All bacterial isolates were found to be tolerant to Cd, Pb, Cu and Zn. Metal tolerance and antibiotic tolerance behavior of all the stains have revealed a very

interesting pattern, which indicate that the strains which showed tolerance against metal have also shown tolerance against antibiotics. The prevalence of metal tolerant and antibiotic resistant microorganisms is ecologically very important as both characters are plasmid borne. Under environmental conditions of metal stress, these organisms will adopt faster multiplication and spread through R-factor than by mutation and natural selection thus leading to a very rapid increase in their numbers [20]. Resistance to antibiotics acquired by a chance in the genetic makeup of a bacterium, which can occur by either a genetic mutation or by transfer of antibiotic resistance genes between bacteria in the environment. Thus a pathogenic organism having multiple stresses and the capacity to adapt itself to the changing environment will ultimately result in difficulty while treating the clinical infections. When the organisms are exposed to stress they are found to evolve different mechanism whichever is required for their survival.

In the present study the isolated bacterial species were capable of removing significant amount of Pb, Cd, Cu and Zn during growth within 48 h even though it has been reported in previous studies *P. aeruginosa* BC15 removed 93% Ni, 65% Pb, 50% Cd and 30% Cr within 48 h through biosorption process [16]. *B. circulans* EB1 was found to remove as much as 65% of Zn and 60% of the Cu [21]. *Alcaligenes faecalis removed* 59% Cd, 61% Pb, 40% Cu, 39% Zn and *Staphylococcus aureus* utilized 60% Cd, 63% Pb, 42% Cu, 41% Zn and *Streptococcus lactis* removed 61% Cd, 57% Pb, 37% Cu, 38% Zn and *Micrococcus luteus* absorbed 56% Cd, 61% Pb, 39% Cu, 41% Zn and *Enterobacter aerogenes* removed 60% Cd, 55% Pb, 62% Cu, 67% Zn within 48 h through biosorption process (Fig. 1). Puyen *et al.* [22] reported, *Micrococcus luteus* DE2008 has the ability to absorb Pb and Cu. *M. luteus* DE2008 exhibited a specific removal capacity of 408 mg/g for Cu and 1965 mg/g for Pb.

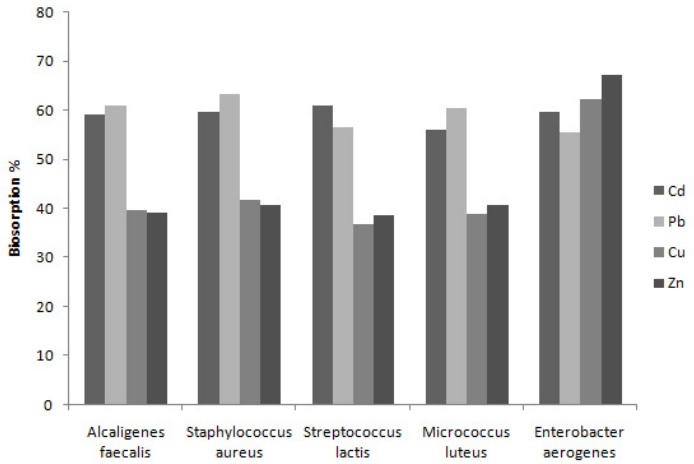

Fig. 1. Biosorption of metals in LB broth containing Cd, Pb, Cu and Zn (each at 100 mg/L concentration).

4. Conclusion

Our findings confirmed that metal polluted soils from Palar River basin site, Vellore, contribute to the antibiotic and metal resistance among microbes found in the environment. The waste water thus discarded in the environment not only changes the microbial community but can have serious effects on man. Biosorption studies have shown that the bacterial strains are efficient in removing heavy metals and have the ability to grow over a wide range of metal concentrations under aerobic conditions with clear indications of the advantages that may offer to employ these organisms for metal remediation process.

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