International Journal of Geology, Earth & Environmental Sciences ISSN: 2277-2081 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jgee.htm
2014 Vol. 4 (3) September-December, pp. 51-58/Utkarsh and Tanaji
Research Article

LEAD BIOACCUMULATING BACTERIA FROM KARAD, (MS) INDIA

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ABSTRACT
Contamination of lead and other heavy metals is an environmental problem in worldwide because of their toxic effects on ecosystem and human. Removal of lead from the contaminated area is very difficult. Different chemical and physical are used for remove the metal from soil and water; but the use of biological method represents most cheap and efficient method. Eight lead bioaccumulating bacteria were isolated. The isolates had distinct morphological and biochemical features. Seven isolates grew on Luria agar with 10 mM lead. The isolates had ability to produce polysaccharides. The extent of lead accumulation was estimated using atomic absorption spectrometry (AAS), where the pbr 5 found to be most potent with accumulation of 2012 ppm of lead. The siderophore production and nitrogen fixation ability of isolates highlighted their possible role in plant growth promotion.

Keywords: *Lead Bioaccumulation, Bioremediation, Soil, AAS, Plant Growth Promotion*

INTRODUCTION
Lead is one of the most widely used elements in industries like petroleum, electronics, battery, paints, ceramic and ammunitions (Eisler, 1988). Biologically lead is nonessential element and its retention time in soil is like most of the heavy metal is 150-5000years (Lerda, 1992; Roane, 1999). Lead is known to cause the neurodegenerative diseases, reproductive impairment and renal failure in the humans (Fowler, 1998; Kamel *et al.,* 2003) DNA damage, oxidative damage to proteins and lipids; the binding ability of lead to essential proteins and enzymes leads to conformational changes and ultimately the loss of function (Asmub *et al.,* 2000; Hartwig *et al.,* 2002; Nies, 1999). Varieties of mechanism have been demonstrated within the bacteria for the survival in presence of heavy metals. Metal sorption, uptake and accumulation, mineralization, extracellular precipitation and enzymatic oxidation or reduction to a less toxic form, and efflux of heavy metals from the cell has been reported (Hughes *et al.,* 1991; Urrutia *et al.,* 1993). One of the defensive mechanisms elaborated by the resistant bacteria to keep alive in toxic metal environments is the production of extracellular polysaccharides (Gadd, 2004). In last few years alternative methods like biosorption has been developed (Volesky, 1990; Matheikal *et al.,* 1991; Williams *et al.,* 1998). In this method, accumulation of heavy metal is achieved by means of biological material; mediated by metabolic and physico – chemical pathways (Fourest and Roux, 1992). Heavy metal accumulation can be achieved by many processes dependent or independent of the metabolism. Living and nonliving biomass and cellular products like extracellular polysaccharide can be effectively applied for metal removal (Gadd, 1993). Some of the heavy metal resistant bacteria have ability to the produce siderophore and nitrogen fixation (Naik, 2010) e.g. the lead resistant *Pseudomonas aeroginosa*, it shows increased siderophore production upto the 0.5mM lead. The nitrogen fixing ability however of most of the strains seems decreased with increase in heavy metal concentration (Chen *et al.,* 2003).

MATERIALS AND METHODS
Collection of Samples
The samples were collected from the MIDC area Oglewadi, Karad. Four samples were collected from each point in sterile polythene bags, then immediately brought into the laboratory and processed.
Enrichment and Isolation of Lead Resistant Bacteria
Add 1 gm of sample in 50ml Luria-Bertani broth with containing 2mM lead acetate in flask. Four samples were added in four different flasks in 1 gm quantity and incubated for enrichment at ambient temperature for 48 hrs.

Determination of MIC

MIC of the isolates was determined by using lead concentrations in the range of 3mM to 10mM.

Siderophore Production

The siderophore production ability of isolates was determined on Chrome azurol –S (CAS) agar.
The plates were incubated at ambient temperature for 168 hrs. The intensity of siderophore production was determined in terms of selection ratio.

Nitrogen Fixing Ability

The ability of the isolates to fix the atmospheric nitrogen was determined by cultivating the isolates on Ashbey's nitrogen free mannitol agar (Ashby *et al.*, 1987) for 168 h.

Polysaccharide Test

The extent of polysaccharide production by the isolates was estimated and quantified using Dubois method (Dubois *et al.*, 1956).

Lead Bioaccumulation Ability

The isolates were cultivated in Luria-Bertani broth in presence of 2mM lead for 72 h. the cells were pelleted at 10000 rpm for 10 min. the pellet was retained and washed 3 times with double distilled water. The biomass was hydrolyzed with boiling 70 % HCL and analyzed for presence of lead by atomic adsorption spectrometry at M/s Nikhil analytical laboratory Pvt. Ltd., Sangli.

RESULTS AND DISCUSSION

Enrichment and Isolation of Lead Resistant Bacteria

The enrichment of lead resistant bacteria was achieved in LB broth amended with 2 mM of lead acetate at ambient temperature.

The isolation of bacteria from enriched samples was achieved on LB agar amended with 2 mM of lead acetate, where eight distinct isolates were obtained. The isolated showed distinct morphological and biochemical features (table 1-6).

Table 1: Morphology, Gram staining, Motility of isolate

Isolates	Morphology	Gram nature	Motility
Pbr1	Rods	Gram positive	Motile
Pbr2	Rods	Gram positive	Motile
Pbr3	Cocci	Gram negative	Actively Motile
Pbr4	Short rods	Gram negative	Motile
Pbr5	Short rods	Gram positive	Motile
Pbr6	Long rods	Gram negative	Motile
Pbr7	Short rods	Gram negative	Non Motile
Pbr8	Rods	Gram positive	Motile

Table 2: Endospores, Flagella, Capsule staining

Isolates	Endospores	Flagella	Capsule
Pbr1	Non spore former	Lophotrichous	capsulated
Pbr2	Non spore former	Monotrichous	capsulated
Pbr3	Non spore former	Peritrichous	capsulated
Pbr4	Non spore former	Lophotrichous	capsulated
Pbr5	Non spore former	Lophotrichous	capsulated
Pbr6	Non spore former	Peritrichous	capsulated
Pbr7	Non spore former	Non flagellated	capsulated
Pbr8	Non spore former	Amphitrichous	capsulated

Table 3: Carbohydrate utilization

Sugar	Isolates							
	Pbr1	**Pbr2**	**Pbr3**	**Pbr4**	**Pbr5**	**Pbr6**	**Pbr7**	**Pbr8**
Lactose	-	-	+	-	-	-	-	-
Galactose	-	-	+	⊕	-	⊕	-	+
Maltose	-	+	+	⊕	⊕	-	-	-
Mannitol	-	-	+	⊕	⊕	⊕	+	+
Sucrose	-	+	+	⊕	-	⊕	-	-
Xylose	-	-	⊕	⊕	⊕	⊕	⊕	-
Arabinose	-	-	-	-	⊕	+	-	-
Rhamnose	-	-	⊕	⊕	⊕	⊕	⊕	+
Glucose	-	-	⊕	-	-	⊕	-	+
Raffinose	-	-	+	⊕	-	⊕	-	+

(Note- + = only acid, ⊕ = acid and gas, - = No acid and gas)

Table 4: Hugh and Leifson test

Incubation Condition	Isolates							
	Pbr1	**Pbr2**	**Pbr3**	**Pbr4**	**Pbr5**	**Pbr6**	**Pbr7**	**Pbr8**
Aerobic	-	-	+	+	+	+	+	+
Anaerobic	-	-	+	+	+	+	+	+

(Note- + = positive, - = negative test)

Table 5: Enzymatic properties

Enzymes	Isolates							
	Pbr1	**Pbr2**	**Pbr3**	**Pbr4**	**Pbr5**	**Pbr6**	**Pbr7**	**Pbr8**
Catalase	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+
Amylase	-	+	+	+	+	+	+	+
Protease	-	+	-	-	+	-	+	-
Urease	-	-	+	+	+	-	+	+
Lipase	-	+	+	-	+	-	+	-
gelatinase	+	-	+	-	-	-	+	-
lecithinase	-	+	+	-	-	-	-	-

(Note- + = positive test, - = negative test)

Table 6: Biochemical characters of isolates

Biochemical Test	Isolate code							
	Pbr1	**Pbr2**	**Pbr3**	**Pbr4**	**Pbr5**	**Pbr6**	**Pbr7**	**Pbr8**
Nitrate reduction	+	+	+	+	+	+	+	+
H2S production	-	-	-	-	+	+	+	+
Phenyl alanine deaminase	+	-	-	+	+	-	-	+
Arginine hydrolysis	+	-	+	-	-	+	-	+

Minimum Inhibitory Concentration

The MIC was determined in order to acquire the knowledge about lead resistance capacity of isolates. All the isolate were capable of growing on 9mM lead conc. within 96 hrs at ambient temperature but Pbr8 was completely inhibited at 9mM lead conc.

The MIC of other isolates was 10mM (figure 1). The concentration less than 3mM were not used as the isolation itself was achieved using 2mM lead. Overall response of the isolates to the gradually increasing lead concentration indicated that the growth of the organism under study in delayed by higher concentration.

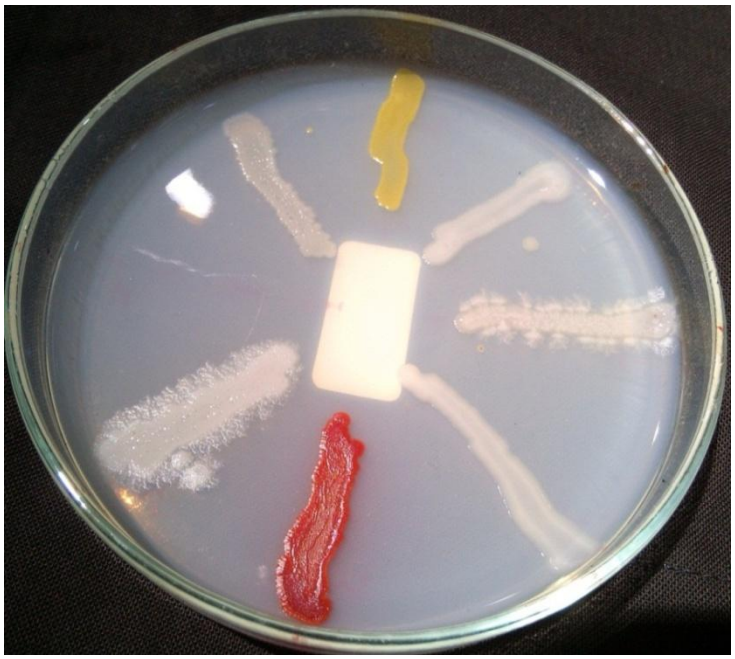

Figure 1: Growth of isolates on Luria agar plate with 10 mM lead acetate

Siderophore Production

On CAS agar, the growth was monitored for 168 h with periodic recording of colony and halo diameters with interval of 24 h (figure 2). Isolates pbr3 and pbr5 produced halo surrounding colony at 72 h indicating either late recognition of iron starved conditions by the isolates or delayed process of siderophoregenesis under the conditions. The isolates pbr6 and pbr7 excreted siderophore within 48 h indicating comparatively quicker response to iron starved conditions. The isolate pbr4 did not show any halo development; but the colony colour including medium turned orange leaving the possibility of presence of bound siderophore at the end of 96 h. All the isolates reached peak of siderophore production which remained constant thereafter. Most potent siderophore producer among the isolates was pbr6 with selection ratio 2. The siderophore elaborating property of isolates indicates the use of organisms in agriculture as well.

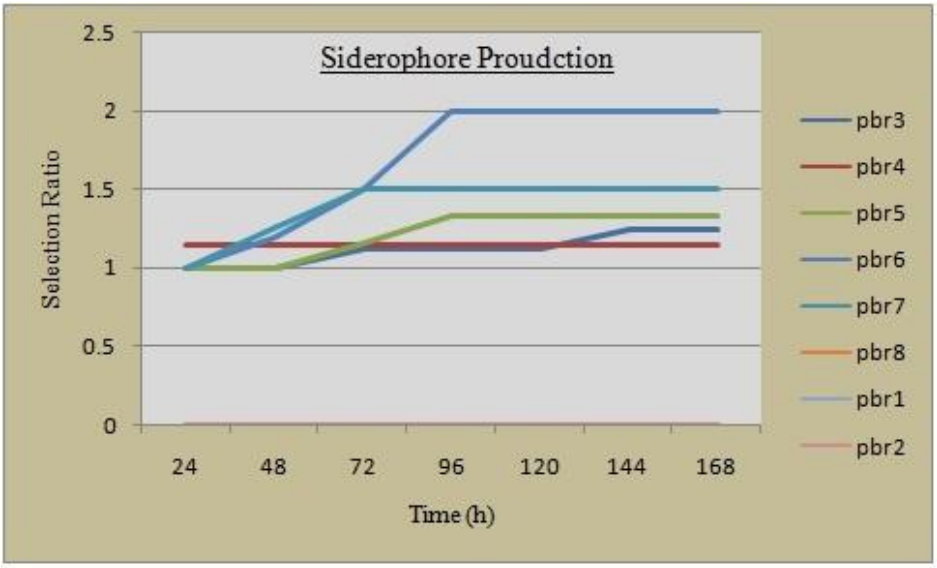

Figure 2: Siderophore production ability of the isolate

Nitrogen Fixation

After the 48 hrs incubation at ambient temperature the growth of pbr1, pbr3, pbr4, pbr5, pbr6, pbr7, pbr8 were growth observed on N2 free mannitol agar. When ability of isolates to assimilate atmospheric nitrogen under the normal oxygen pressure was determined, all the isolates except pbr2 were able to flourish on nitrogen free mannitol agar. The isolate pbr2 probably was incapable of growing on N2 free mannitol agar because of its inability to utilize the mannitol as carbon source hence the nitrogenase activity of pbr2 remains question.

Polysaccharide Production

Presence of capsule on the cells highlighted the polysaccharide synthesis by the cell. The involvement of extracellular polysaccharides in sequestration of metals has been demonstrated (Chen *et al.*, 1995b; Jensen-Spaulding *et al.*, 2004; Iyer *et al.*, 2005). Polysaccharides are known to sequester the toxicity imparting metals from the surrounding environment thereby decreasing the direct toxic impact imparted by the former on the cell (Chen *et al.*, 1995b; Maier *et al.*, 2000).

In this study, impact of lead on polysaccharide production by the isolates was assayed using Duboi's method. Two modes of metal exposure were used as – the cultivation of organisms in the medium containing lead and second one cultivating the organism first in medium without metal then adding the metal one hour prior to estimation. In case of cells cultivated in the medium containing lead, small amount of polysaccharide was detected in supernatant of all isolates. While some of the isolates showed greater amount of precipitated polysaccharide particularly the isolates pbr3, pbr4, pbr6, pbr7 and pbr8 indicating formation of insoluble precipitates of metal-polysaccharide complex (figure 3).

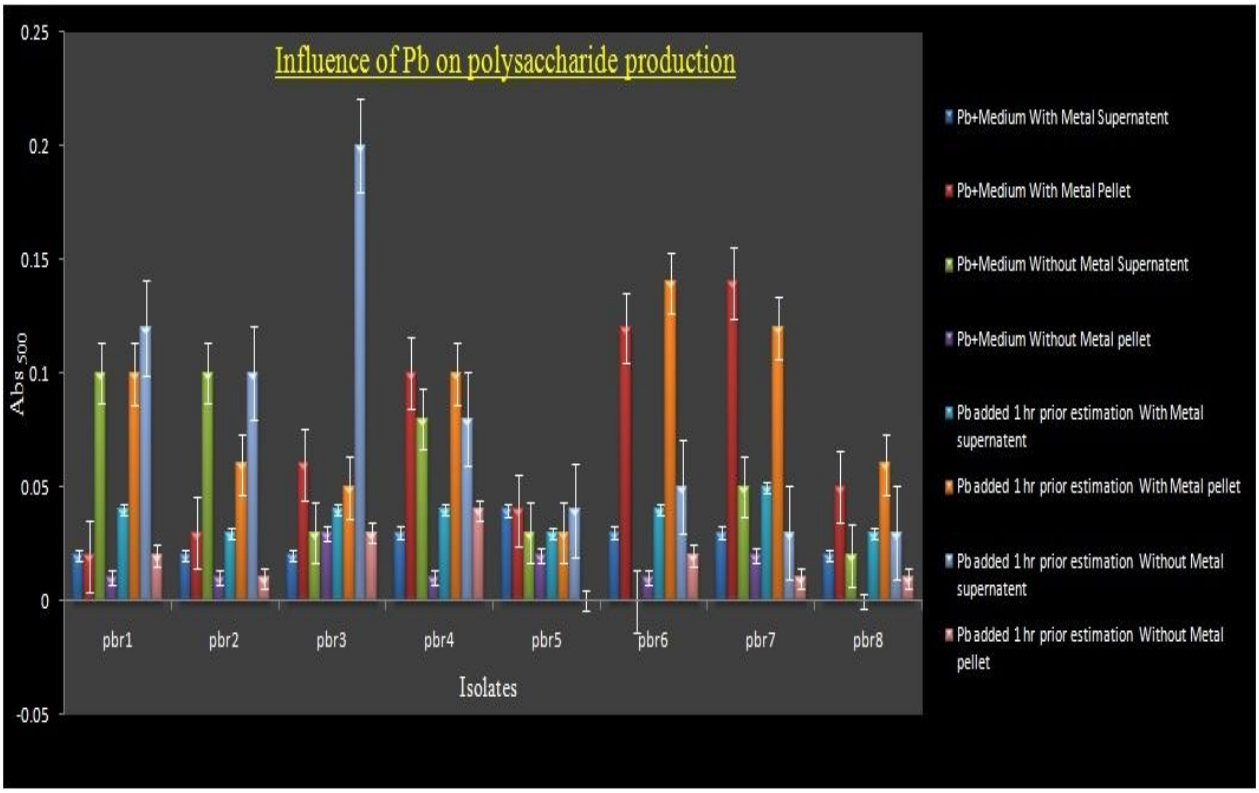

Figure 3: Effect of lead on the production of polysaccharide

In case of isolates cultivated in the medium without lead, with lead added one hour prior estimation it was observed that polysaccharide content in culture extracts was noted corresponding to the first case. While in case of isolates, pbr6, pbr7 and pbr8 the polysaccharide content in precipitate found markedly raised indicating higher affinity of polysaccharides towards the metal added.

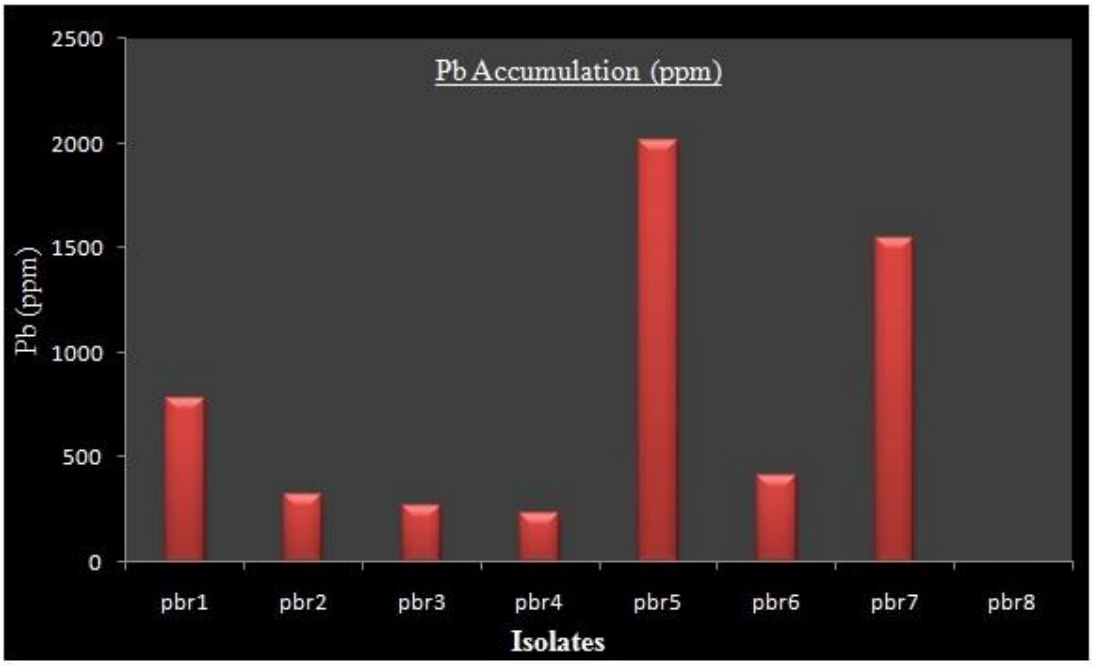

Figure 4: Lead accumulating ability of isolates

Lead Bioaccumulation Potential of the Isolates

Lead content was analyzed by atomic adsorption spectroscopy, Pbr5 has most potential lead accumulator they accumulate 2012 ppm lead (figure 4). Then Pbr7 is also accumulated1544 ppm lead. All other isolates were relatively low lead accumulator.

Conclusion

It was confirmed that all isolates successfully accumulated lead. Pbr5 and Pbr7 were more potent lead accumulators. The isolates can be employed for bioremediation of lead contaminated environments. The siderophore production and nitrogen fixation by the isolates highlights their use in agriculture.

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