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Isolation, Characterization and Protein Profiling of Lead Resistant Bacteria

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Authors' contributions

This work was carried out in collaboration between all authors. Author SC designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors JD, SC and AS managed the analyses of the study. Author JD managed the literature searches. Author PC highlighted the toxicity of lead on human beings and helped in statistical analysis. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To isolate lead resistant bacteria from industrial effluent and characterize them by biochemical tests, effect of physical and chemicals factors on their growth and protein profiling of the isolates on with or without metal stress.
Study Design: Cross-sectional study of related research articles and papers.
Place and Duration of Study: Samples were collected from effluent water of Indian Iron and Steel Company (IISCO), Burnpur, West Bengal, India and studied at The Department of Biotechnology, The University of Burdwan. West Bengal, India, between September 2010 and June 2011.
Methodology: Isolation of the isolates was done by pour plating the diluted effluent water onto the metal (Lead acetate) containing agar plates. The basic characterization of the isolates was done on the basis of morphological, physical, biochemical and antibiogram tests. Quantification of metal absorption was determined by Atomic absorption Spectrophotometric analysis. Protein expressions of the isolates on metal stress were studied by SDS-PAGE.

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Results: Two bacterial isolates namely Pb^rB1 and Pb^rB2 were isolated; they showed resistance to lead up to 6mM Lead (Lead acetate) and also to different antibiotics. They are able to ferment different sugars even in presence of low concentration of metals. Their protein profiling by SDS-PAGE shows different expressions of proteins upon metal stress.
Conclusion: The isolates can be utilized to bioremediate lead from contaminated environment and further study of molecular mechanisms underlying lead accumulation process can be studied.

Keywords: Lead resistant bacteria; biochemical and antibiogram tests; Atomic absorption spectroscopy; SDS-PAGE.

1. INTRODUCTION

Microorganisms play an important role in the environmental fate of toxic heavy metals with a multiplicity of mechanisms effecting transformation between soluble and insoluble forms of the heavy metals [1]. Although some heavy metals are essential trace elements, most can be, at high concentrations, and some are in even in low concentration toxic to all branches of life, including microbes, by forming complex compounds within the cell. Because heavy metals are increasingly found in microbial habitats due to natural and environmental processes, microbes have evolved several mechanisms to tolerate the presence of heavy metals. These mechanisms include the efflux of metal ions outside the cell, accumulation and complexation of metal ions inside the cell and reduction of the heavy metal ions to a less toxic state. This is an important implication of microbial heavy metal tolerance because the oxidation state of a heavy metal relates to the solubility and toxicity of the metal itself [2]. Thus the heavy metal resistant microorganisms have significant role in treatment of the metal containing soil. The detoxifying ability of these resistant microorganisms can be manipulated for bioremediation of heavy metals in wastewater, metal contaminated soil etc. Effluents having heavy metals can be treated with these microorganisms by the processes like biosorption, bioaccumulation and bioprecipitation [3].

Among all the heavy metal Lead (Pb) is the second metal of concern especially because lead poisoning of children is common and leads to retardation and semi permanent brain damage [19]. Lead can affect almost every organ and system in the body. It inhibits porphobilinogen synthase and ferrochelatase, preventing both porphobilinogen formation and the incorporation of iron into protoporphyrin IX, the final step in heme synthesis causing subsequent microcytic anemia. Lead interferes with the release of neurotransmitters [4] and glutamate. The targeting of NMDA receptors is thought to be one of the main causes for lead's toxicity to neurons [5]. It can cause programmed cell death in brain cells [6]. Its exposure damages cells in the hippocampus, a part of the brain involved in memory. Hippocampi of lead exposed rats show structural damage such as irregular nuclei and denaturation of myelin compared to controls [5]. Lead also affects reproductive systems like reduce volume of sperm, miscarriage; prematurity, low birth weight can happen [7]. Not only to the human being, Lead and other heavy metals also have severe toxic effects on microbial cells.

Lead cause toxicity by interacting nucleic acid, by binding to essential respiratory proteins, to oxidative damage by producing reactive oxygen species and displacing Ca ion and Zn ion in proteins. Microorganisms generally use specific transport pathways to bring essential metals across the cell membrane into cytoplasm; unfortunately toxic metal can also cross

membrane, via diffusion or via pathways designed for other metals. Lead enters in microbial cells via transport systems for essential divalent cation such as Mn^{++}.

In response to metals in the environment, micro-organisms have evolved ingenious mechanisms of Lead resistance and detoxification. Lead resistance in microbes is somewhat less studied but involvement of P-type ATPase and detoxification through sequestration is known as Lead inducible specific mechanism. P-type ATPases transports Lead from cell to periplasm using ATP [8,9], whereas CBA transporters are three-component transenvelope pumps of Gram negative bacteria that act as chemiosmotic antiporters [10,11,12] efflux Lead from periplasm to outside the cell and cation diffusion facilitator (CDF) family transporters act as chemiosmotic ion-proton exchangers are also involved in Lead efflux [13,14,12]. In general, P-type ATPases and CDF transporters are commonly found among different bacterial species, whereas the presence of a CBA transporter an RND (Resistance, Nodulation and cell Division) protein in Gram-positive bacteria is exceptional and indicates high-level resistance to heavy metal ions [15]. P-type ATPases and CDF transporters export metal ions from the cytoplasm to the periplasm; whereas CBA transporters mainly detoxify periplasmic metal (outer membrane efflux), i.e. CBA transporters further remove periplasmic ions transported there by ATPases or CDF transporters or before ions have entered the cytoplasm. While P-type ATPases and CDF transporters can functionally replace each other, they cannot replace CBA transporter and vice versa [15]. Probably all the Zn^{2+}/Cd^{2+} translocating P-type ATPases are also effective in Pb^{2+} export, however Pb^{2+} resistance is very seldom tested and there is no experimental proof for this hypothesis. One of the few characterized Pb^{2+} translocating P-type ATPases can be found from the plasmid-encoded lead resistance gene cluster *pbrTRABCD* from *Cupriavidus metallidurans* CH34. This gene cluster consists of six genes that code for the following proteins: PbrR, a transcription factor belonging to the MerR family; PbrT, a putative Pb^{2+} uptake protein; PbrA, a P-type ATPase; PbrB/PbrC, a predicted integral membrane protein and a putative signal peptidase; and PbrD, a putative intracellular Pb binding protein. The Pbr proteins are hypothesized to combine functions involved in uptake (PbrT), efflux (PbrABC) and accumulation (PbrD) of Pb^{2+} [16].

Sequestration is also a detoxification mechanism for Pb^{2+}. Several bacterial species use intra and extracellular binding of Pb^{2+} to avoid toxicity. *S. aureus*, *Citrobacter freundii* [17,18], *Vibrio harveyi* [19] and *Bacillus megaterium* [20] lower the concentration of free Lead ions by precipitating Lead as a phosphate salt. *Pseudomonas marginalis* avoids lead toxicity by precipitating it as an extracellular polymer [20]. However, the molecular mechanisms responsible for the formation of lead precipitates in these strains are not known. Many bacteria have a cell wall or envelope that is capable of passively adsorbing high levels of dissolved metals, usually via a charge-mediated attraction [21]. Binding of heavy metals by these organisms takes place mainly through exopolysaccharides (EPSs) [22]. Particular heavy metal binding capacity has been observed in uronic acid rich EPSs [21]. Although an EPS could act as a biosorbent of free metal ions, it cannot be considered to be an inducible resistance mechanism synthesized and developed in response to metals.

Thus, Lead resistance microorganisms can be used to remediate heavy metal containing soil, by a process known as bioremediation which is nothing but a pollution treatment technology that uses biological systems to catalyze the destruction or transformation of various chemicals to less harmful forms. Bioremediation is proven to be quite effective for the removal of metal ions from contaminated solution in a low cost and environment friendly manner. So, this study aimed to isolate and identify lead resistant bacteria from Indian Iron

and Steel Company (IISCO) effluent water and characterizes them, so that they can be useful for the bioremediation of Lead from lead contaminated area.

2. MATERIALS AND METHODS

2.1 Sample Collection

Samples were collected from effluent water of Indian Iron and Steel Company (IISCO) located in Burnpur of Burdwan district of West Bengal, India. Its different physical and chemical parameters are given in Table1.

Table 1. Characteristics of industrial effluent used for the isolation of heavy metal-resistant bacteria

Physical characteristics of the sample water	
Color:	Brownish black.
Odor:	Pungent greasy smell.
Turbidity:	Fully turbid.
Chemical characteristics of the sample water	
pH:	6.0
BOD:	345 mg/L
COD:	720 mg/L

2.2 Isolation of Lead Resistant Bacteria from the Collected Effluent

Bacteria were isolated from the effluent using sterilized nutrient agar (NA) medium (pH-7.0±2) and were prepared using peptic digest of animal tissue (5 g/L), yeast extract (1.5g/L), beef extract (1.5 g/L), NaCl (5 g/L) and agar (20 g/L) along with Lead [Lead acetate, $Pb(C_2H_30_2)_2$] in increasing concentrations in each plate from 1mM to 10mM with one control agar plate. 10^{-4} dilution was used and incubated at 37°C for 48 hrs.

2.3 Morphological Characterization

Morphological characteristics of the isolates were observed under bright field microscopy (Olympus (OIC), GB 89954) of simple stained preparation of the isolates at 100 X magnifications. The detailed surface features were observed by SEM.

2.4 Scanning Electron Microscopy

To determine the effect of metal on the surface feature of the cell, the metal treated as well as untreated cells were analyzed by Scanning Electron Microscopy (Hitachi Scanning Electron Microscope S-530). SEM images have a characteristic three-dimensional appearance and are useful for judging the surface structure of the sample. Both in control and treated cells, post growth, cells at a density of 10^8-10^9 cells mL^{-1} were harvested by centrifugation and analyzed using the software Quartz PCI version 8.0 image management systems.

2.5 Biochemical Characterization

2.5.1 Amylase test

This test was performed by growing the pure bacterial culture in sterilized Starch Agar plate (containing both with Pb and without Pb) followed by 48 hours incubation and Gram's iodine was used to indicate the presence of starch.

2.5.2 Catalase test

This test was done by adding a loopful of appropriately grown (in medium containing Pb and without Pb) pure culture in the test tube containing substrate i.e. 3% H_2O_2. If Catalase is produced by the bacteria; for the chemical reaction, 2 H_2O_2 --> O_2 + 2 H_2O will liberate free O_2 gas. Bubbles of O_2 represent a positive Catalase test; the absence of bubble formation is a negative Catalase test.

2.5.3 Indole test

This test was done by growing pure bacterial culture in sterile tryptophan or peptone broth (both with Pb and without Pb) for 24-48 hours. Following incubation, 5 drops of Kovac's reagent (isoamyl alcohol, p-Dimethylaminobenzaldehyde and concentrated hydrochloric acid) was added to the culture broth. A positive result is shown by the presence of a red or red-violet color in the surface alcohol layer of the broth. A negative result appears yellow. A variable result showing an orange color can also occur.This is due to the presence of skatole, also known as methyl indole or methylated indole, another possible product of tryptophan degradation.

2.5.4 Protease test or gelatin hydrolysis test

This test was performed by using Gelatin liquefaction (the formation of a liquid) and tested by stabbing the pure culture of the isolates in nutrient gelatin deep tubes (both with Pb and without Pb) and incubated at 4^0C until the bottom of the medium resolidifies. If gelatin has been hydrolyzed, the medium will remain liquid after refrigeration. If gelatin has not been hydrolyzed, the medium will resolidify during the time it is in the refrigerator.

2.6 Effect of Temperature on the Growth of the Isolates

Each microbial species requires a temperature growth range that is determined by the heat sensitivity of its particular enzymes, membranes, ribosome, and other components. So in order to get the optimum growth temperature (i.e. the temperature at which the rate of cellular reproduction is most rapid) of the isolates, test tubes containing Nutrient both with Pb and without Pb were inoculated with the pure cultures and incubated at different temperatures 10°C, 20°C, 30°C, 37°C, 45°C, 60°C for 24 hours. Optical density was measured to observe growth in terms of turbidity using a spectrophotometer at 660 nm.

2.7 Effect of pH on the Growth of the Isolates

Acidic and basic environment or pH of an environment dramatically affects bacterial growth. The pH affects the activity of enzymes especially those that are involved in biosynthesis and growth. Each microbial species possesses a definite pH growth range and a distinct pH

growth optimum. Different microbial groups have characteristic pH optima. Thus to get the pH optima of the isolates this experiment was done. For this experiment separate test tubes containing Nutrient broth with Pb and without Pb were adjusted to different pH using 1(N) Hydrochloric acid (HCl) and 1(N) Sodium hydroxide (NaOH). The different pH taken for the experiment was pH 2, pH 4, pH 6, pH 7, pH 8 and pH10 and after inoculation, incubated at 37°C. After 48 hours growth of the isolates were observed using a spectrophotometer at 660 nm.

2.8 Effect of Osmotic Pressure on the Growth of the Isolates

Since, bacteria were separated from their environment by a selectively permeable plasma membrane; they can be affected by changes in the osmotic pressure or water availability of their surroundings. The experiment of effect of osmotic pressure was done using Nutrient Broth containing Sodium chloride (NaCl) in different test tubes in varying concentration like 2M, 4M, 6M, 8M, 10M for both the cases with Pb and without Pb in the media and inoculations were done. The tubes were incubated for 48 hours at 37°C and optical density was taken using spectrophotometer at 660nm.

2.9 Determination of Growth Profile

The four phases (lag, logarithmic, stationary, and death or decline) of growth of bacterial population were determined by measuring the turbidity in a broth culture. However, turbidity is not a direct measure of bacterial numbers but an indirect measure of biomass, which can be correlated with cell density during the log growth phase. The construction of a complete bacterial growth curve (increase and decrease in cell numbers versus time) was done by transferring aliquots of a 24 hrs incubated pure active culture in freshly prepared nutrient broth medium containing both with Pb and without Pb in side arm flasks and kept in shaker incubator (at 120 rpm) for proper aeration at 30°C. Optical density of the cultures was measured after each 1 hour at 660nm (UV-1700 Pharma Spectrophotometer, Shimadzu).

2.10 Multiple Sugar Fermentation Tests

Fermentative degradation of various carbohydrates was detected by acid, gas production utilizing that particular carbohydrate. For this test fermentation medium (1% sugar solution with indicator Bromothymol Blue) were prepared, sterilized and poured in culture tubes with Durham tube (with Pb and without Pb). After inoculation the tubes were incubated at 30°C for 48hrs. Utilization of sugars was observed by means of changed color of medium from blue to green (acid production) and bubble production in the Durham's tube (gas production).

2.11 Antibiotic Resistance Test

The agar cup assay method was used to determine antibiotic sensitivity of the isolates. Ampicillin, Chloramphenicol, Kanamycin, Rifampcin, Streptomycin and Tetracycline are the antibiotics used for the experiment with the concentration of 5mg/ml. Isolates were spread plated on the Mueller Hinton Agar plates (HiMedia). 0.5ml antibiotic solutions were added into the cup/well made by borer (0.5mm in diameter) on the inoculated plates and incubated at 37°C for 48 hours. Zones of inhibition were measured and were classified as resistance or sensitive strains.

2.12 Quantification of Lead Accumulation

To quantify Pb accumulation Atomic Absorption Spectroscopy (AAS) was used. For AAS, metal is determined by aspirating a metal solution into an air-acetylene flame to atomize the metal. A metal specific lamp is placed into the AAS and used to determine the difference in light absorbance between a reference source and the metal solution. For this purpose isolates are grown in media with 4mM lead [Pb $(C_2H_30_2)_2$] concentration and a control were kept only with lead and media, with no inoculums. After 72 hour the supernatant were collected by pelleting out cells by centrifugation and were analyzed by Pollution Control Board (Govt. of West Bengal, India).

2.13 Protein Profiling of the Isolates by SDS-PAGE

To compare the protein expression in the stressed i.e. with lead and normal condition, insoluble and soluble bacterial proteins were isolated and SDS-PAGE was done. For protein isolation 48 hrs incubated pure culture (at 37^0C) was used to harvest the cells at 5,000 rpm for 10 mins. The pellet was then resuspended in 150 µl of B-PER reagent [0.5 gm Sodium chloride (NaCl) dissolved in 100 ml 20mM Tris HCl (pH- 7.5)] and again centrifuged at 13,000 rpm for 10 mins at 4°C. Supernatant contains soluble proteins and pellet which was dissolved in Phosphate buffer saline (PBS) contains the insoluble proteins. Both type of proteins were then separated by Sodium Dodecyl Sulphate-Poly-Acryl amide gel electrophoresis (SDS-PAGE).

3. RESULTS AND DISCUSSION

3.1 Isolation of the Lead Resistant Organism

Two bacterial isolates namely Pb^rB1 and Pb^rB2 were isolated and selected from the industrial effluent of IISCO which are Pb resistant. These bacterial strains were found to be resistance up to 6mM concentration of Pb and selected by depending upon their predominant growth over others.

3.2 Colony and Morphological Characterization of the Isolates

The preliminary morphological characterizations of these isolates were done on the basis of simple staining which implies the short rod structure with spore forming nature of the isolates. The scanning electron micrograph confirms the rod shape of the bacteria and demonstrated the effect of Pb [Lead acetate, Pb $(C_2H_30_2)_2$] on cell morphology and a slimy appearance surrounding the metal treated cells might be the extra polymeric substances produced by the cells [(Fig. 1.) & (Table 2)].

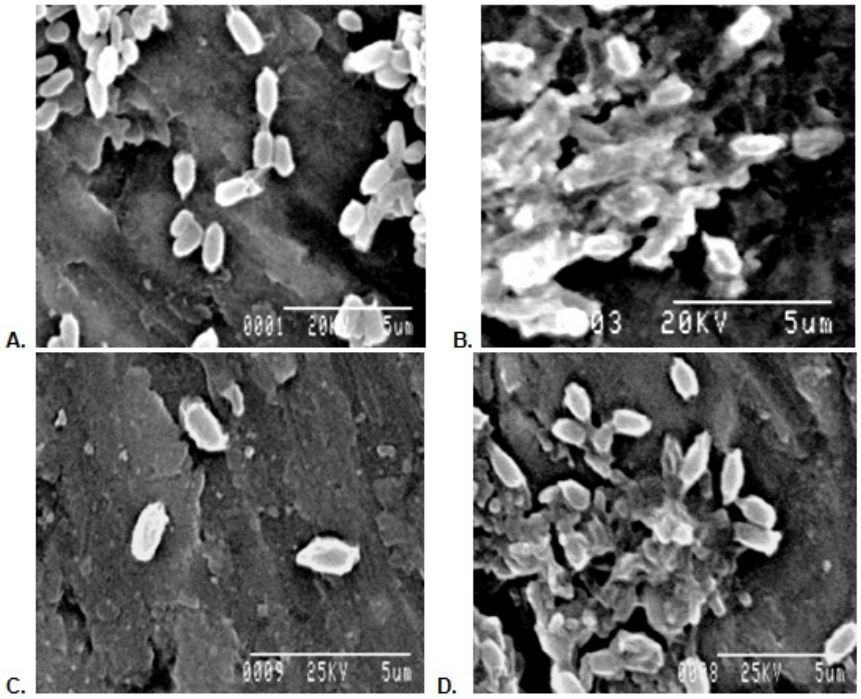


Fig. 1. SEM images of the isolates at different magnifications.
(A.) Pb^{r}B1 at 6000X without Pb; (B.) Pb^{r}B1 at 6000X with Pb. (C.) Pb^{r}B2 at 8000X without Pb; (D.) Pb^{r}B2 at 10000X with Pb.

Table 2. Colony characteristics of isolated bacterial strains

Characteristics	Pb^{r}B1	Pb^{r}B2
Color	Brown	Off white
Shape	Round, Uniform edged	Round, Uniform edged
Nature	Mucoid, Slimy, Sticky	Slimy but not sticky
Size	Medium	Small

3.3 Biochemical Characterization of the Isolates

From the biochemical characterization [Table3] it can be confirmed that both the isolates being strong catalase producer were obligate aerobes. The presence of catalase provides a defense mechanism against the reactive oxygen species and thus ability to exist in aerobic conditions and thereby facilitates the metabolic efficiency. Both the bacterial isolates lacks tyrptophanase enzyme which confirms through negative result for the Indole test. Pb^{r}B2 produced a significant amount of amylase and protease both in presence and absence of lead, where as Pb^{r}B1 produced significant amount of amylase and protease without metal, but metal stress inhibited production of the enzymes.

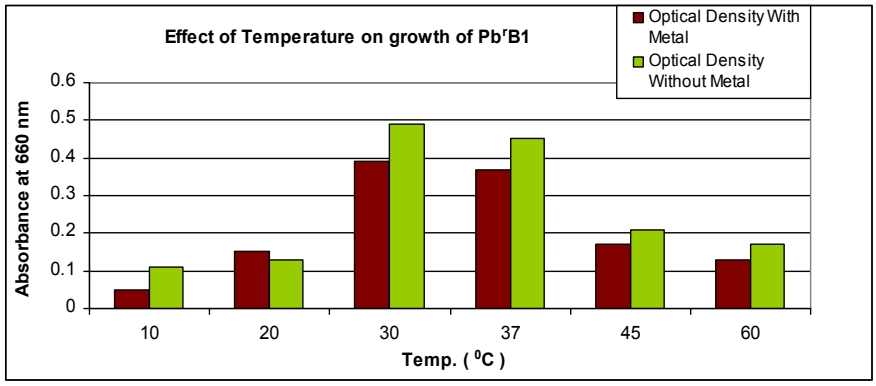
Table 3. Biochemical characteristics of the isolates with and without lead [Pb $(C_2H_30_2)_2$]

Tests	Pb^{r}B1 (With Metal)	Pb^{r}B1 (Without Metal)	Pb^{r}B2 (With Metal)	Pb^{r}B2 (Without Metal)
Amylase	-	+	+	+
Catalase	+	+	+	+
Indole	-	-	-	-
Protease	-	+	+	+

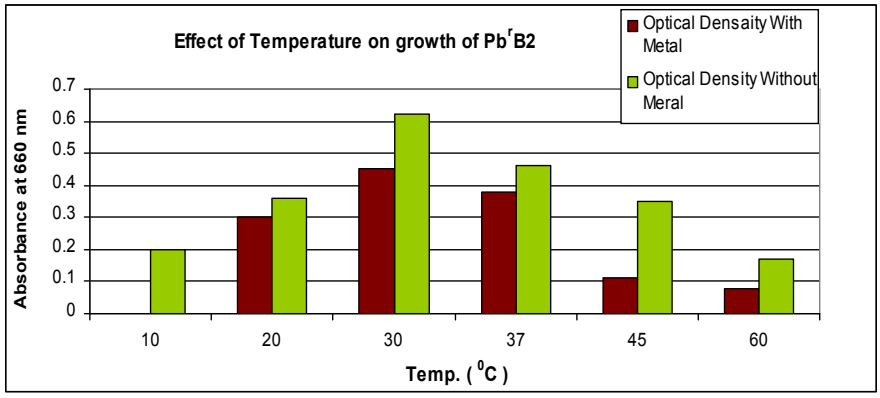
(+) indicate positive result and (-) indicate negative result.

3.4 Effect of Temperature, pH, Salt Concentration on the Growth of the Isolates

Optimum temperature and pH for the growth of Pb^{r}B1 was found to be 30°C and pH 7 and it can grow well on 37°C and pH 6, as showed near about same growth for both the temperature (30^{0}C and 37°C) and pH (pH 6 and pH 7) [Fig2- (A) (C)]. But metal stress suppressed growth in both the cases. In case of Pb^{r}B2 growth was optimum at 30°C and pH 7, [Fig. 2.- (B) (D)] and growth was also affected by the metal stress. Both the isolates found to tolerate 2mM salt (sodium chloride, NaCl) concentration however Pb^{r}B2 showed tolerance up to 4mM salt concentration [Fig. 2.- (E) (F)].

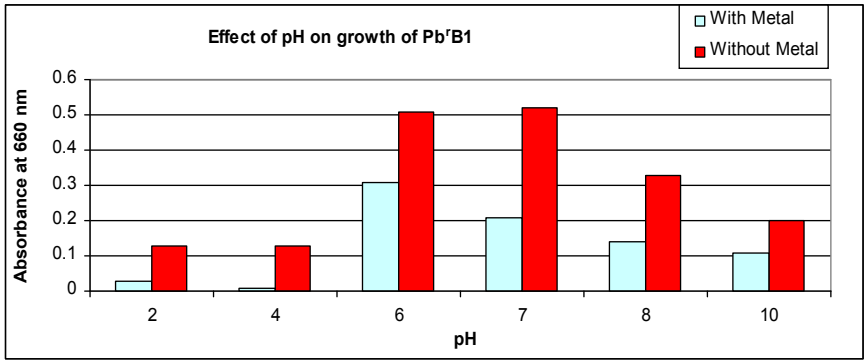


A.

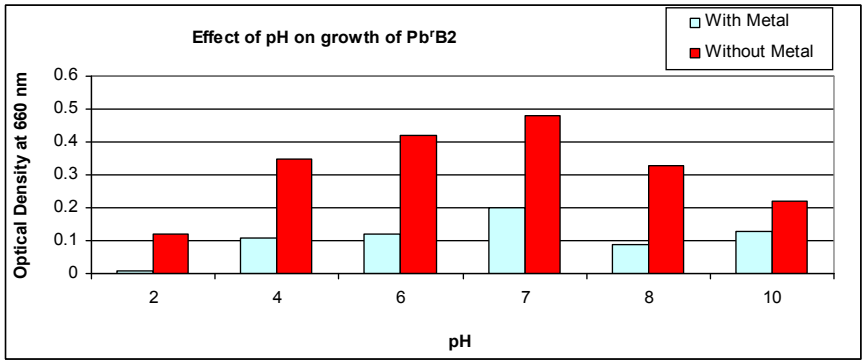


B.

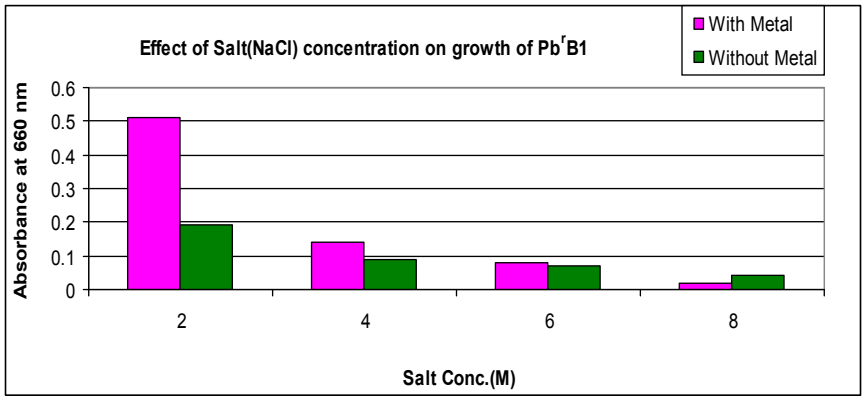
C.


Effect of pH on growth of Pb^{r}B1
With Metal
Without Metal
Absorbance at 660 nm
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
7
8
10
pH

D.


Effect of pH on growth of Pb^{r}B2
With Metal
Without Metal
Optical Density at 660 nm
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
7
8
10
pH

E.


Effect of Salt(NaCl) concentration on growth of Pb^{r}B1
With Metal
Without Metal
Absorbance at 660 nm
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
8
Salt Conc.(M)

F.

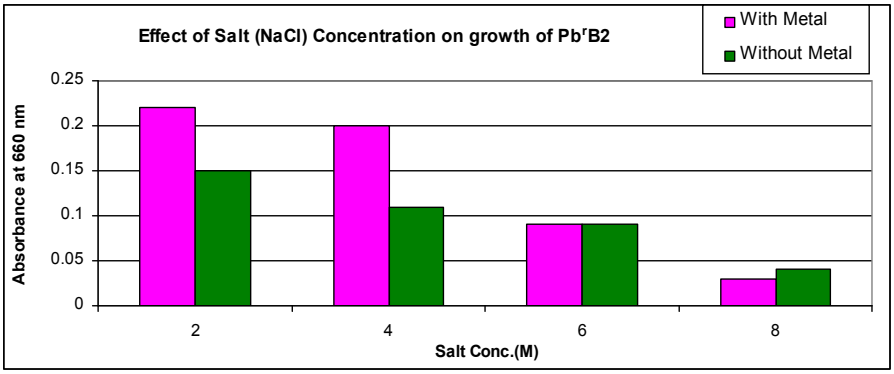
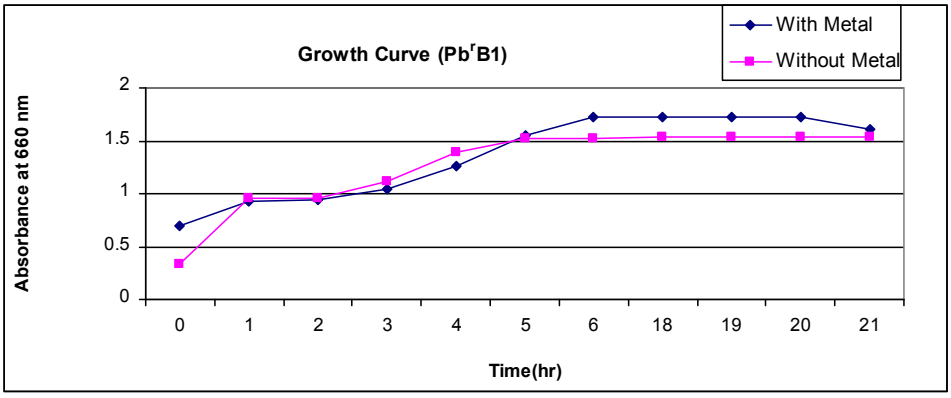


Fig. 2. Figure showing (A) & (B) Effect of temperature on the isolates, (C) & (D) Effect of pH on the isolates, (E) & (F) Effect of salt concentration on the growth of the isolates with and without lead [Pb $(C_2H_30_2)_2$].

3.5 Bacterial Growth Curve Determination:

The growth profiles (Optical density Vs Time graph) of both the isolates followed a sigmoidal pattern of growth in presence as well as in absence of Pb. The growth pattern of Pb^{r}B1 showed difference in the lag and exponential phase of its growth, as with metal it showed a short lag phase, and slight long exponential phase with metal condition [Fig3- (A)]. It might be due to the presence of metal which slowed down the time for duplication. Whereas in case of Pb^{r}B2, the cell entered into stationary phase more rapidly in metal stressed condition i.e. have a short exponential phase than that of without metal condition [Fig3- (B)]. It might be due to the more rapid utilization of nutrients to duplicate in metal stress condition than in the normal condition.

A.



B.

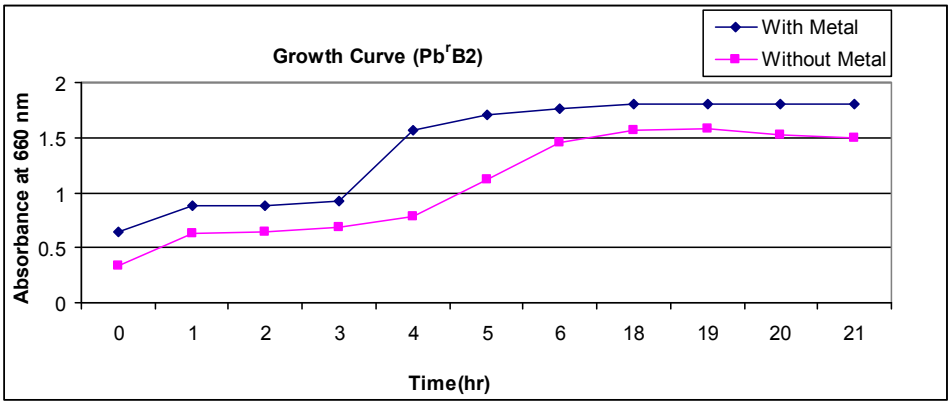


Fig. 3. Growth profile of the isolates, with and without lead [Pb $(C_2H_30_2)_2$]. (A) Pb^{r}B1, (B) Pb^{r}B2

3.6 Determination of Antibiotic Resistance

Antibiotic sensitivity pattern [Table 4] showed that the Pb^{r}B1 was resistant only to Streptomycin, whereas Pb^{r}B2 showed the resistance to Ampicillin, Chloramphenicol, Rifampcin and Streptomycin. Resistance to multiple drugs might involved some multidrug resistance genes.

Table 4. Antibiotic sensitivity pattern of the isolates.

Antibiotic	Class	Spectrum of activity	Zone of inhibition (cm)		Effect on Isolates	
			Pb^{r}B1	Pb^{r}B2	Pb^{r}B1	Pb^{r}B2
Ampicillin	β-lactam	Broad	1.6	0	Sensitive	Resistant
Chloramphenicol	Chloramphenicol	Broad	2.8	0	Sensitive	Resistant
Kanamycin	Aminoglycoside	Broad	4	2.8	Sensitive	Sensitive
Tetracycline	Tetracycline	Broad	3.7	1.	Sensitive	Sensitive
Rifampcin	Semisynthetic	Broad	3.2	0	Sensitive	Resistant
Streptomycin	Aminoglycoside	Broad	0	0	Resistant	Resistant

3.7 Multiple Sugar Fermentation Tests

Multiple sugar fermentation test were done with the isolates with respect to the sugars Dextrose, Galactose, Lactose, Maltose, Sucrose, Xylose and the acid-gas production by the isolates utilizing these sugars were observed which was given in the tabulated form [Table5]. From the observations it can be concluded that both the isolates can utilize the available sugars without presence of metal (2mM), but as the metal concentration increases up to 4mM they lose the ability, it might be due to the reason that in the presence of metal the cell stops the expression of the proteins responsible for utilization of carbon sources to save the energy for expression for the essential proteins. These proteins helped to exist in the metal stress environment.

Table 5. Production of acid and gas by the isolates utilizing given sugars with and without lead [Pb $(C_2H_30_2)_2$]

Sugar	Without metal		With metal (2 mM)		With metal (4mM)	
	Pb^rB1	Pb^rB2	Pb^rB1	Pb^rB2	Pb^rB1	Pb^rB2
Dextrose	+	+	+	+	-	-
Galactose	+	+	-	-	-	-
Lactose	+	+	-	+	-	-
Maltose	+	+	-	-	-	-
Sucrose	+	+	-	+	-	-
Xylose	+	+	+	+	-	-

(+) indicate positive result and (-) indicate negative result.

3.8 Quantification of Pb accumulation:

Quantification of lead accumulation was done using Atomic absorption spectroscopy. It showed a decline in metal concentration after 72 hrs from the initial amount of metal present in the solution [Table 6].

Table 6. Table showing the results of atomic absorption spectroscopy

Isolates	Lead concentration in supernatant at 0 hr (mg/L)	Lead in supernatant after 72 hr (mg/L)	Amount of Lead absorbed (mg/L)	Percentage of lead abosorption (%)
Pb^rB1	1517.32	896.5	620.82	40.92
Pb^rB2	1517.32	618.3	899.02	59.25

3.9 Protein Profiling of the Isolates by SDS-PAGE

Soluble and insoluble protein of the isolates grown with and without metal were isolated and SDS-PAGE was done. The band pattern in SDS-PAGE showed [Fig.4] the expression of proteins in presence and absence of lead [Pb $(C_2H_30_2)_2$]. As for the insoluble protein the thick band showed the high expression of proteins in presence of metals, whereas protein expression was high without metal in case of soluble proteins. From this it can be concluded that there might be an involvement of metal stress for the expression of these proteins. More specifically, in case of insoluble proteins with metal stress H protein (Approximately of 29 kDa) expressed well whereas in normal condition G protein (Approximately of 35 kDa) expressed well, so it might be possible that these proteins have some important role on stress conditions.

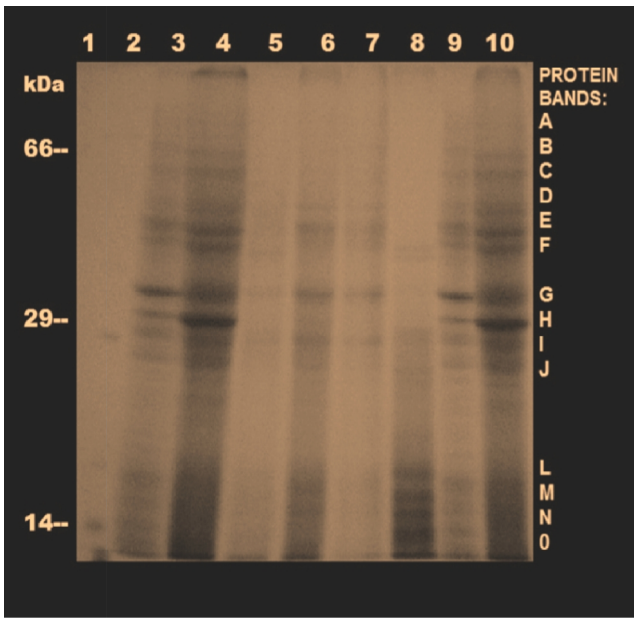


Fig. 4. The SDS-PAGE of the proteins of the isolates showing protein bands (A,B,C,D,E,F,G,H,I,J,L,M,N,O).

Lane3 and lane 9 is the insoluble protein of Pb^rB1 and Pb^rB2 repectively without lead [Pb $(C_2H_30_2)_2$], Lane 4 and lane10 is the isoluble protein of Pb^rB1 and Pb^rB2 with Pb [Pb $(C_2H_30_2)_2$], lane 5 an lane 7 is the soluble protein of Pb^rB1 and Pb^rB2 without Pb [Pb $(C_2H_30_2)_2$],lane 6 and lane 8 is the soluble protein of Pb^rB1 and Pb^rB2 with Pb [Pb $(C_2H_30_2)_2$].

4. CONCLUSION

The growing industry, Oil refineries introduce lots of heavy metal in the environment, so in order to remediate these heavy metal pollutants by biological means is more eco-friendly, as it does not need introduction of any chemical in the environment for the process. The isolated bacteria can be used in Lead bioremediation as they show the capacity to tolerate high concentrations of Lead.

Further study can proceed towards designing of a bioremedial package constituting either of single pure culture or as a consortium with microbes in varying proportion after observing the field application. One of the main applications can be in economical recovery of metals like Lead from ores and minerals. The further studies from other lead contaminated area can proved the use of isolated strains as bioindicator i.e. in the presence of Lead in environment can be detected by the presence of the bacteria. On the other hand the lead accumulation process can be further investigated for the study of molecular mechanisms underlying the bioremediation phenomenon and the study of differential expression of genes in response to the metal stress can explore an interesting area of research.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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