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Bioremediation of lead and chromium by bacteria isolated from coal mining areas

Rini Roy [a1] and Aditi Nag Chaudhuri [b2]

[a]*Post Graduate Department of Microbiology, Bidhannagar College, Kolkata*
[b]*Post Graduate Department of Microbiology, Lady Brabourne College, Kolkata*

Abstract: The microorganisms present in the soil of coal mining areas are naturally exposed to various heavy metals. Bacterial strain was isolated from soil of the coal mining areas of Raniganj. Metallothioneins (MTs) are a group of low molecular mass, cysteine-rich proteins used in metal homeostasis. The main objective of this study was to isolate and extract MT from the isolated bacterial culture. Induction of MT having molecular weight 14 kD occurs in isolated bacteria upon treatment with heavy metals like lead (Pb) and chromium (Cr) as evidenced from SDS PAGE. The thiol content increased in metal treated cultures when compared to the control sample. FTIR study shows the interaction of Pb (II) and Cr (VI) with cell wall components. The expression of MT was confirmed by Western blot technique. Heavy metal tolerating mechanisms within the isolated bacterial cells may be exhibited in the form of metallothioneins for their survival.

Key words: lead; chromium; bacteria; metallothionein; thiol

1. Introduction

Excessive heavy-metal accumulation and circulation in the biosphere are important environmental and health concerns, due to the toxicity both in essential (Cu, Cr, Zn, Mn, Fe, Ni and Mo) and xenobiotic metals (Cd, Pb and Hg) at increased levels of bioavailability [1]. Heavy metals have an important role in different biochemical reactions and are poisonous for cells in high concentrations [2]. Unlike organic contaminants which can be converted into non-toxic derivatives, metals are intrinsically persistent in nature [3]. As chromium compounds were used in dyes and paints and the tanning of leather, these compounds are often found in soil and groundwater at abandoned industrial sites. Toxic Cr (VI) is reduced to less toxic Cr (III) by bacterial system found in the industrial belt. Bioreduction of Cr (VI) has been demonstrated in several bacterial species including *Pseudomonas* sp., *Escherichia coli*, *Bacillus* sp., *Desulfovibrio* sp., *Microbacterium* sp., *Shewanella* sp., *Achromobacter* sp. and *Arthrobacter* sp. Direct bacterial reduction of Cr (VI) to Cr (III) is the most promising practice with proved expediency in bioremediation.

Because heavy metals are increasingly found in microbial habitats due to natural and industrial processes, microbes have evolved several mechanisms to tolerate the presence of heavy metals [4]. Although some heavy metals are essential trace elements, most can be, at high concentrations, toxic to all branches of life, including microbes, by forming complex compounds within the cell. Metallothioneins (MTs) are a group of low molecular mass, cysteine-rich proteins with a variety of functions including involvement in metal homeostasis, free radical scavenging, protection against heavy metal damage, and metabolic regulation *via* Zn donation [5, 6]. Owing to their rich thiol content, metallothioneins bind a number of trace elements including lead, zinc, cadmium, mercury, platinum & silver & also protects cells & tissues against heavy metal toxicity. Besides, levels of MTs in invertebrates and aquatic vertebrates well correlate with heavy metal pollution of an environment and, thus, serve as bio-environmental marker. The main objective of this study was to isolate and

[1] Email: *for_rini_roy@yahoo.co.in*

[2] Email: *odditybiom@gmail.com*

extract MT from the isolated bacterial culture. Induction of MT was observed in isolated bacteria upon treatment with heavy metals like lead (Pb) and chromium (Cr) at different concentrations. The thiol content was also estimated from the cell extract of the treated and untreated isolated bacteria. Probable cell metal interaction was predicted through FTIR study. Finally, MT was confirmed by SDS PAGE and Western Blotting.

2. Methodology

Among the toxic heavy metals lead (lead nitrate) and chromium (Potassium dichromate and potassium chromate) were used for studying the induction of metallothionein protein on the isolated bacteria. The concentrations used for each of the metals were 1g/L and 1.75 g/L.

2.1 ***Isolation of the bacteria***

Bacterial cultures were isolated from a pristine soil sampled from Raniganj coal mining area, West Bengal, India using the standard dilution plate technique. A 10-fold dilutions of fresh soil (1 g) were made in phosphate buffered saline (pH7.5) and 0.1 ml from each of these dilutions were spread on minimal media agar plates supplemented with active charcoal as the sole carbon source. Plates were incubated at 37°C for 2-3 days. Colonies with different morphological appearance were selected from these culture plates and purified by further sub culturing in the same minimal media. The isolates were further grown in minimal media broths which were supplemented with Pb (II) and Chromium (VI) having concentrations 1g/L and 1.75 g/L each. 4 ml of the isolated bacterial culture was inoculated in 100 ml of Nutrient Broth since this media proved to be the growth promoting media for the successful extraction of metallothionein.

2.2 Characterization of bacteria

The organisms were characterized on the basis of detailed colony morphology, cellular arrangements, Gram nature by Bright Field Microscope, and cell motility by Phase Contrast Microscopy.

2.3 Extraction of metallothionein from bacteria

Equivalent mixtures of dithiolthreitol (DTT), phenyl methane sulfonyl fluoride (PMSF), and glycine buffer, pH=8.5 were added to 0.5 g of wet weight of above samples (harvested at the end of log phases and centrifuged at 10000 rpm for 10 min at 4 ºC).Then the bacterial samples (metal treated and metal untreated) were lysed in sonicator for 3 min and cooled on ice. Then the sonicated samples were centrifuged at 10000 rpm for 10 minutes [7]. The supernatant was then further used for SDS PAGE. A minute amount of sample was kept aside for estimation of protein amount by Bradford method [8].

SDS PAGE: 12% SDS PAGE was performed using the standard protocol followed by Coomassie brilliant blue staining.

2.4 Estimation of total thiol content

For total thiol, total cell extract prepared (by taking the supernatant after centrifuging the bacterial culture at 10,000 rpm for 10 minutes) was used to measure total thiol content [9] of protein using Dithionitrobenzoic acid (DTNB), Sodium bicarbonate, Phosphate buffer – 0.1M (pH 7.4). Protein was measured by Bradford method.

FTIR study: After growth of isolated bacteria in NB, the bacterial culture was centrifuged at 10,000 rpm for 5 mins. The pellet obtained was washed with buffer and distilled water for thrice. Lyophilisation of the bacterial

culture treated with or without metals was then performed. A thin uniform film of the lyophilised bacterial culture was drawn on a cover slip and FTIR was performed [10]. The IR spectra of dried whole cell were recorded with instrument having model number L1600300 spectrum Two FTIR Sl. No. 94372 (Perkin Elmer, U.S.). The sample was scanned between 600-4000 wave number in cm^{-1} at transmittance mode taking air as reference.

Western blot: Western blot technique was carried out following standard method using primary Anti Metallothionein UC1MT (ab 12228), protein marker, BCIP (5-bromo-4-chloro-3-indolyl phosphate), NBT (nitro blue tetrazolium), NBT-BCIP buffer, Secondary AP tagged antibody.

3. Discussion

Isolation of the bacteria: From the isolated bacteria grown in Nutrient Broth, further study was carried out.

Characterization of bacteria: Gram staining was done and found that the isolated culture was of gram-negative character, coccus in shape, non-motile in nature as confirmed by Phase Contrast Microscopy.

Extraction of metallothionein from bacteria: Protein was estimated from the supernatant after sonication in the extraction procedure and were then used for SDS PAGE.

SDS PAGE: 12% SDSPAGE was run using 14.3-97.4 kD protein markers. The gels were stained overnight in Coomassie Brilliant Blue R-250, fixed in 0.5% acetic acid and destained in destaining solution prior to scanning for documentation. Prominent bands could be seen in the gel, with respect to protein marker at 14 kD in control as well as in the different concentrations of Pb and Cr.

Estimation of total thiol content: From the data obtained by calculating the thiol content /mg of protein present in the cell, it could be seen that the thiol content is increasing in metal treated cultures in comparison with the control sample for both Pb and Cr. Comparing the two different metal treated cultures, it was observed that chromium treated bacteria showed higher thiol content than that of lead treated bacteria.

FTIR study: Existence of carbonyl group is high in the membrane of the organism which is supposed to interact with the metal. The shifting of peak in case of Pb and Cr shows binding of metal with the functional group on the membrane of bacteria.

Western blot: Since the protein reacted with anti-metallothionein antibody, so bands were observed, hence it was concluded that the sample contained metallothionein confirming MT induction with metal ion treatment of isolated bacteria.

4. Conclusion

From the band patterns observed in SDS PAGE with respect to the protein marker, it could be concluded that metallothionein activity is induced in lead treated and the chromium treated cultures. Thiol content was increased due to heavy metal challenge in all the cases for Pb and Cr. It can be concluded that isolated bacteria possess metal tolerance capacity by induction of metallothionein protein. One of the useful aspects could be the use of the isolated bacteria to clean up metal contaminated sites, viz; lead and also for Chromium (VI) contaminated site such as in industrial wastes.

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