Journal of Environmental Science and Technology

ISSN 1994-7887

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Environmental Science and Technology 4 (1): 41-54, 2011
ISSN 1994-7887 / DOI: 10.3923/jest.2011.41.54

Novel Microbial Consortium for Laboratory Scale Lead Removal from City Effluent

[1]Sanhita Chowdhury, [2]Ashoke Ranjan Thakur and [1]Shaon Ray Chaudhuri

[1]Department of Biotechnology, West Bengal University of Technology, BF-142, Sector-1, Saltlake, Calcutta-700064, West Bengal, India

[2]Vice Chancellor's Office, West Bengal State University Barasat, Berunanpukuria, P.O. Malikapur, North 24 Parganas-700126, India

Corresponding Author: Shaon Ray Chaudhuri, Department of Biotechnology, West Bengal University of Technology, BF-142, Sector-1, Salt Lake, Calcutta-700064, India Tel: 00913323210731/108, 00913323341030 Fax: 00913323210730

ABSTRACT

In an earlier study, we had reported the isolation and characterization of eleven bacterial isolates from different sites of East Calcutta Wetland, the natural waste recycling site for the city of Calcutta, India. It receives high concentration of toxic heavy metals daily from different industries, tanneries, agricultural as well as health sectors along with domestic sewage. Considering the fact that bioremedial activity at East Calcutta Wetland depends to a large extent on the microbial activity, attempt was made to develop a metal removal package for treatment of waste water using bacterial consortium obtained from the above mentioned site. The nature of the extracellular proteases produced by the strains was analyzed using different extracellular protease specific primers during PCR. Energy Dispersive X-Ray Fluorescence (EDXRF) analysis showed variation in metal accumulation both within and among the strains. Transmission Electron Microscopy of the metal treated cells showed presence of nanoparticles within the cells. Scanning Electron Micrographs showed distinct cytoskeleton changes of the cell in presence of metals. Bioremedial package was developed under immobilized condition both with pure isolates as well as mixed consortia for treating heavy metal containing waste water. Efficiency of Lead removal was measured using atomic absorption spectroscopy. All the isolates were found to accumulate metals to different extent. Laboratory scale bioremediation study indicated effective lead removal by immobilized consortium as well as individual strains. The mixed consortium removed 83.68% metal from distilled water and 94.4% from Bheri water both supplemented with 5 mM lead nitrate salt, within 48 h.

Key words: East Calcutta Wetland, bioremedial package, energy dispersive x-ray fluorescence, atomic absorption spectroscopy, metal microbe interaction, protease, bioremediation of lead, bheri

INTRODUCTION

Though industrialization and urbanization in recent years brought economical boom to the world but at the same time it is the major cause of environmental pollution. Different heavy metals like lead, mercury, cadmium, arsenic, chromium are increasingly found in microbial habitats due to natural and industrial processes and as a result bacterial cells present in such an environment

are constantly exposed to stressful situations and an ability to resist those stresses is essential for their survival. Though some elements like calcium, cobalt, chromium, copper, iron, potassium, magnesium, manganese, sodium, nickel and zinc act as cofactors for essential enzymatic reactions in the bacterial cell; but beyond a certain level they become toxic to all living organisms (Bruins *et al*., 2000). There are different mechanisms adopted by microbes to tolerate the heavy metals including the efflux of metal ions outside the cell, accumulation of metal ions inside the cell, adsorption of metals to cell surface, precipitation and reduction of the heavy metal ions to a less toxic state (Shakoori and Muneer, 2002). Microbes are reported to take effective part in bioremediation of wastes (Chipasa, 2003; Lovley and Coatest, 1997; Ahluwalia and Goya, 2007). Few patents are available regarding microbial bioremediation (United States Patent 4898827, United States Patent 4293333). Microorganisms can neutralize the organic contaminants by oxidizing them to carbon dioxide, whereas they can remove heavy metals present in wastes by converting them to a less toxic form which in turn gets precipitated or volatilized from solution or by chelating the metal by siderophore or can change the redox state of the metal (Shakoori and Muneer, 2002; Lovley and Coatest, 1997). Doubler and Castin (2000) reported seven mercury-resistant *Pseudomonas* species that were able to remove 97% mercury within 10 h in a packed bed bioreactor from mercury contaminated effluent. Mercury concentrations of up to 10 mg L^{-1} were successfully treated by these microbes. *M. luteus* immobilized in 2% calcium alginate and 10% polyacrylamide gel beads was reported to uptake 61% copper (Leung and Wang, 2000).

Recently Lee *et al*. (2008) reported that an enriched consortium isolated from lake sediments was able to remove 99-100% of different heavy metals including copper, chromium, nickel, lead and zinc from heavy metal contaminated water. Living cell and nonliving biomass of *Saccharomyces cerevisiae* were reported to uptake uranium, zinc and copper by biosorption (Volesky and May-Philips *et al*., 1995). Microbes are also reported to generate metal nanoparticles within the cell (Salata, 2004). *Bacillus subtilis* was reported to produce octahedral gold nanoparticles (Mandal *et al*., 2006). A strain of *Bacillus* sp. isolated from atmosphere was reported to produce silver nanoparticles of size range 5-15 nm at their periplasmic space after 7 days incubation at room temperature in presence of silver nitrate solution (Pugazhenthiran *et al*., 2009). *Rhodococcus* sp. was reported to generate gold nanoparticles in the range of 5-15 nm on the cytoplasmic membrane and cell wall (Ahmad *et al*., 2003).

East Calcutta Wetland (ECW) located at the eastern edge of Calcutta (RayChaudhuri and Thakur, 2006) is acting as the natural sewage treatment plant to the city and it is an excellent example of integrated resource recovery where, city sewage is used for fisheries and agriculture for more than 100 years now (RayChaudhuri and Thakur, 2006; Chowdhury *et al*., 2008; Pradhan *et al*., 2008; RayChaudhuri *et al*., 2007, 2008). ECW receives about 600 million litres of liquid sewage and 2500 tonnes of garbage daily (Chowdhury *et al*., 2008). In addition to domestic wastes, it receives effluents containing heavy metals like chromium, copper, lead, zinc from industries, tanneries, battery manufacturing units, etc. located in and around Calcutta. The purification of the waste is mainly based on microbial activity which is facilitated by the hot and humid climate all throughout the year. Thus ECW is recognized as a source of biotechnologically important microbes (RayChaudhuri and Thakur, 2006). The early study on biodiversity screening of this area based on culture independent (300 clones analysed) approach revealed the existence of wide variety of microbial populations distributed in 12 different phyla within the domain bacteria. These phyla included β-γ-Proteobacteria, α-Proteobacteria, ϵ-Proteobacteria, Bacteroidetes, Cyanobacteria, δ-Proteobacteria, Brevinema, Fermicutes, Actinobacteria, Chloroflexi and two uncultured groups of microbes at different ratio (Fig. 1) (RayChaudhuri and Thakur, 2006).

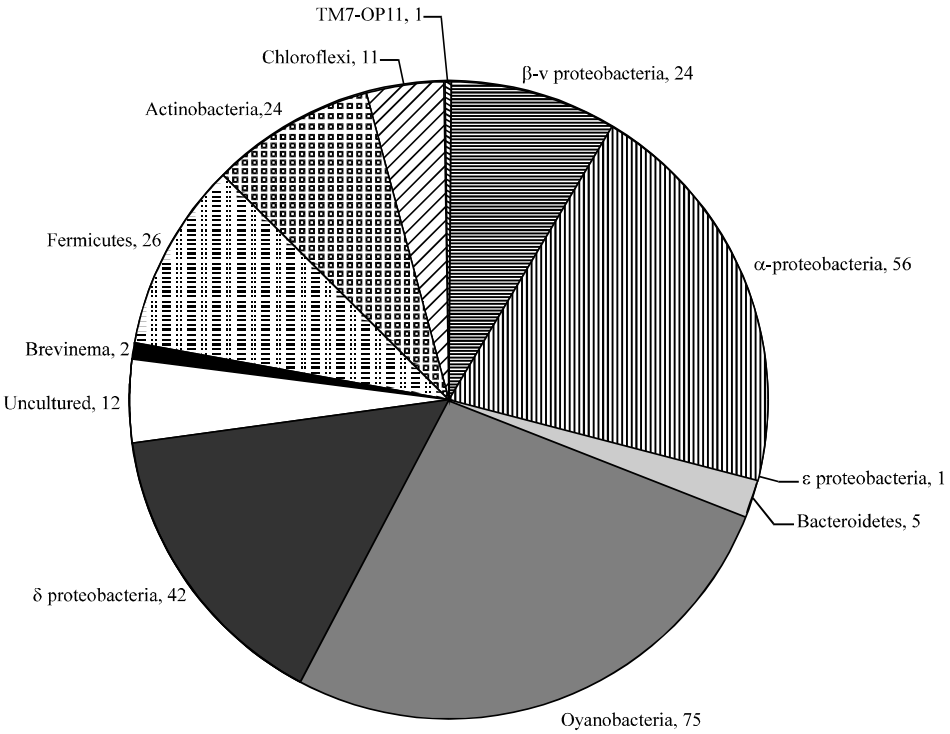

Fig. 1: Distribution of clones at ECW. Pie chart representing distribution of clones from different sites of ECW among the 12 bacterial phyla. The name and the numerical values represent the phyla and number of clones from ECW in each phylum

Presence of microbes from different phyla indicated the diverse bioremedial processes operating at ECW. The study revealed certain clones to be closest to *Streptococcus macedonicus*. *Streptoccous macedonicus* is a Gram-positive, non spore forming lactic acid bacteria that lives in chains of varying length belonging to family firmicutes. It is a strain producing extracellular protease and food grade bacteriocine (Georgalaki *et al.*, 2002). The growth condition of *Streptococcus macedonicus* (Georgalaki *et al.*, 2002) was thus used to cultivate different extracellular protease producing microbes from various sites of ECW (Chowdhury *et al.*, 2008).

Based on this background, eleven metal resistant bacteria were isolated from various sites of East Calcutta Wetland. Keeping in mind their source of origin, the objective of this study was to characterize the molecular nature of the protease that would be responsible for organic waste degradation and to check the role of these microbes in heavy metal removal using them either as pure isolates or as mixed consortium under immobilized condition for liquid waste treatment. These cells showed potential of removing lead from environmental samples as well as distilled water supplemented with lead nitrate salts at the laboratory scale.

MATERIALS AND METHODS

Microbes and culture conditions: Isolation of the strains was done on solid milk medium plates containing 10% double toned milk, 0.3% yeast extract and 1.5% agar (Chowdhury *et al.*, 2008). Extracellular protease secreting bacteria were identified by observing the clear zone around the colony due to the degradation of casein present in milk. Luria Bertani (LB) broth containing 1% tryptone, 0.5% yeast extract and 0.5% NaCl in distilled water (pH-7.5) was used for the

maintenance of the isolate and their further characterization. The pure isolates were preserved at -80°C as 70% (v/v) glycerol stock. All cultivations were carried out at 150 rpm and 37°C. The morphological, biochemical and physiological characterization of the strains have been reported earlier (Chowdhury *et al.*, 2008; Adarsh *et al.*, 2007).

Molecular nature of the protease: Among the eleven isolates, nine were found to produce extracellular protease and thus potential candidate for organic waste degradation. So attempt was made to assess the molecular nature of the extracellular proteases from these nine strains. Protease gene amplification using different extracellular protease specific primers were performed. Genomic DNA isolated from the strains as described by Adarsh *et al.* (2007) were used for PCR amplification of the *Pseudomonas aeruginosa* specific *aprA* gene (Kim *et al.*, 2006); *lasB* gene (Lin *et al.*, 2009) and protease *IV* gene (Caballero *et al.*, 2004) fragments as per reported protocols. After PCR the amplicons of *aprA* and protease *IV* gene were checked on a 2% agarose gel while that of *lasB* gene was run on 1% agarose gel with a 100 bp DNA ladder (Cat. No. SM0623, Fermentas) to determine the tentative length of the amplicon.

Quantification of metal accumulation: Complex metal salts $Al(NO_3)_3.9H_2O$; $CuSO_4.5H_2O$; $AgNO_3$; $Pb(NO_3)_2$; $NiCl_2.6H_2O$; $HgCl_2$; $CoCl.6H_2O$; AgCl and $CdCl_3$ were used for metal tolerance study. Minimum inhibitory concentration (MIC) of each metal for each strain was determined as per the protocol of Adarsh *et al.* (2007). Post MIC determination, the fate of the metal within the cell was checked. The concentrations of the metals within the cell were measured using Energy Dispersive X Ray Fluorescence Analysis (EDXRF) in Jordan Valley EX 3600 EDXRF system. The sample preparation and analysis was as reported by Adarsh *et al.* (2007).

Transmission electron microscopy for nanoparticle detection: For confirmation of the intracellular localization of metals by the isolates, Transmission Electron Microscopy (TEM) of the unstained metal treated as well as control cells were done. The detailed procedure for Transmission electron microscopy was as reported by Chowdhury *et al.* (2008).

Development of heavy metal removal package using immobilized cells

Generation of mixed consortium: For generation of bioremedial package, a mixed consortium was first developed. All the eleven isolates were grown separately for overnight in LB medium at their optimum growth condition. Next day, 0.1% inoculum from each strain was added to a 3 mL LB broth. The tube was incubated at 37°C for overnight with continuous shaking at 150 rpm and mixed consortium was developed. The mixed consortium was grown for 4-5 generation using same growth conditions.

Immobilization of the cell: The cells of mixed consortium were immobilized by the method reported by Malathu *et al.* (2008). The 1% inoculum from the consortium was added to LB medium and incubated at 37°C for overnight with continuous shaking at 150 rpm. The culture was then mixed with equal volume of 8% Na-alginate solution. Beads were made using the slurry by pouring the later drop wise into a sterile chilled solution of 1 M $CaCl_2$, kept on an ice bath aseptically using a fine micro pipette. The beads were kept in $CaCl_2$ solution for 4 h and subsequently stored in sterile water at 4°C.

Kinetics of lead removal from distilled water as well as environmental water sample: In order to test the kinetics of lead removal two distinct set of experiments were performed. In the control set 2 mM lead nitrate was added to sterile distilled water as well as Bheri (flat bottomed sewage fed fish pond) water and kept for 0 (immediately after addition), 24 and 48 h before the samples were prepared for Atomic Absorption Spectroscopy (AAS). In the experimental set three separate combinations were tried out. In the first combination Bheri water was inoculated with Immobilized Mixed Consortium (IMC), in the second combination Bheri water supplemented with 2 mM lead nitrate was incubated with IMC while in the third combination sterile distilled water containing 2 mM lead nitrate was incubated with IMC. The samples for the experimental set were collected after 0, 1, 6, 12, 24 and 48 h of incubation.

Among the 11 isolates of the mixed consortium, 2 strains could not survive above 5 mM concentration of lead nitrate. The experimental set was again repeated in presence of 5 mM lead nitrate with samples collected after 0, 24 and 48 h of incubation.

The method for AAS analysis as reported earlier (Roy *et al.*, 2008) was modified further mostly in the step of digestion. The supernatant (10 mL) was incubated overnight at room temperature with 6 ml of concentrated nitric acid. It was heated at 95°C until the cessation of red fume. Then it was cooled down and 2 mL perchloric acid was added to it and again heated at 95°C until the volume reduced to 2 mL. It was cooled down. The acid digested solution was then filtered through Whatman filter paper and finally the volume was made up to 10 mL using distilled water. The lead concentration of the sample was measured using PERKIN- ELMER 5100 PC Atomic Absorption Spectrophotometer at 283.3 wavelength and 10 mA current.

To understand the role of each individual isolate present in the consortium in metal removal, individual strains were grown in LB medium. Calcium alginate beads were made from each strain separately as mentioned earlier. One percent inoculum was added to distilled water and Bheri water both supplemented with 5 mM lead nitrate. The concentration of lead remaining in the solution after 0, 24 and 48 h of incubation at 37°C was measured through atomic absorption spectroscopy.

Amplification of SOD gene to understand the molecular mechanisms: Superoxide dismutases are a class of enzymes that break superoxide into oxygen and hydrogen peroxide and are present in most of the aerobic organisms. To understand the mechanism of metal tolerance of the isolates, superoxide dismutase gene fragment were amplified from bacterial genomic DNA (isolated as per the method of Adarsh *et al.* (2007)) using the method of Zolg *et al.* (1994), Amplicon from one of the samples was sent for sequencing to Chromas Biotech Pvt. Ltd.

Scanning electron microscopy for metal induced cytoskeletal changes: To determine the effect of metal stress on the cell surface structure and morphology Scanning Electron Microscopy (SEM) of the metal treated as well as untreated cell were done. The sample preparation and analysis procedure was as reported by Chowdhury *et al.* (2008).

RESULTS

Isolation of microbes: Eleven bacterial strains namely SRC-001, SRC-002, SRC-003, SRC-004, SRC-005, SRC-006, SRC-007, SRC-008, SRC-009, SRC-010, SRC-011 were isolated from different sites of ECW. Out of these eleven isolates nine produced extracellular protease as evident from casein clearing assay of milk media plates. The Isolate SRC-003 and SRC-009 were non caseinase producing but able to grow on milk media plates.

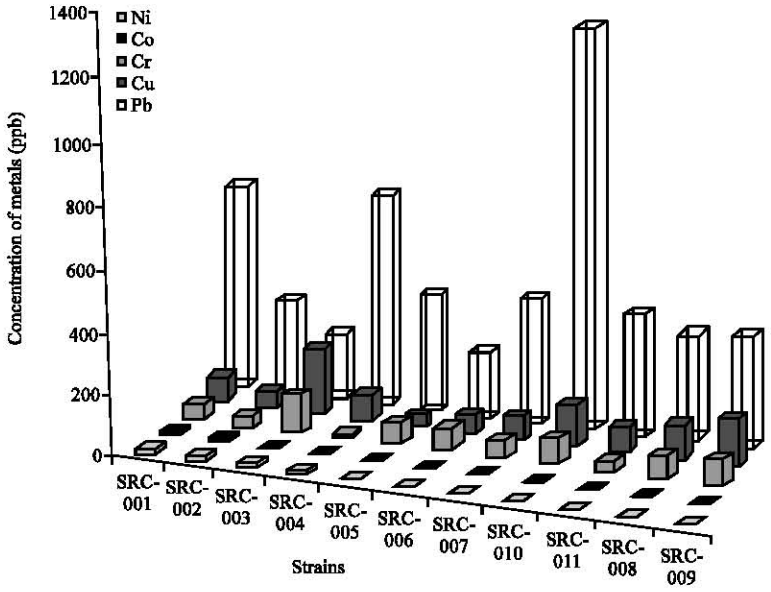

Fig. 2: Relative accumulation of metals. Graph representing relative accumulation of metals inside the cells as determined from EDXRF analysis. After growth in highest metal concentration, cells were washed with 0.1 N HCl and PBS thrice for removal of adsorbed metals and media components; resuspended in PBS; vacuum filtered through 0.45 μ Whatmann filter; dried and analysed using EDXRF. Y axis represented the concentration of metal in ppb, while X axis represented the strains. Different metals are designated by different patterns as shown in legend

Molecular nature of the protease: The aprA gene was found to be amplified in case of SRC-001, SRC-002, SRC-004, SRC-006, SRC-007, SRC-008, SRC-010 and SRC-011. The size of the amplicon was around 400 bp as compared to 100 bp DNA ladder. The genes were sequenced. All of them were found to be novel and they were submitted to Genbank under the accession number GQ202009-GQ202016. From phylogenetic analysis it was observed that all our strains were closely related. That might be due to the common isolation procedure applied during isolation of the strains. The lasB gene was amplified in isolate SRC-007. Protease IV Set A primers amplified the gene from strains SRC-005, SRC-007, SRC-008, SRC-010 and SRC-011. The size of the amplicon was around 1000 bp. Set B primers amplified the gene from strains SRC-008, SRC-010 and SRC-011. The size of the amplicon was around 700 bp. Set C primers amplified the gene from strains SRC-001, SRC-005, SRC-006, SRC-007 and SRC-008. The size of the amplicon was around 400 bp as evident from 100 bp DNA ladder.

Quantification of metal accumulation: All the eleven isolates were found to grow in presence of most of the metals used for the study to different extent, only strain SRC-005 could not grow in presence of cadmium salt. The strains were found to tolerate aluminium, lead and iron more in comparison to other metals, while the MIC for mercury was very low for all the strain. EDXRF analysis indicated that all the strains accumulated lead in maximum amount followed by copper, chromium, nickel and cobalt (Fig. 2). Isolate SRC-010 accumulated lead (1306 ppb) in maximum amounts (Fig. 2), while strain SRC-001 accumulated nickel (13 ppb) and cobalt (9.9 ppb) and isolate

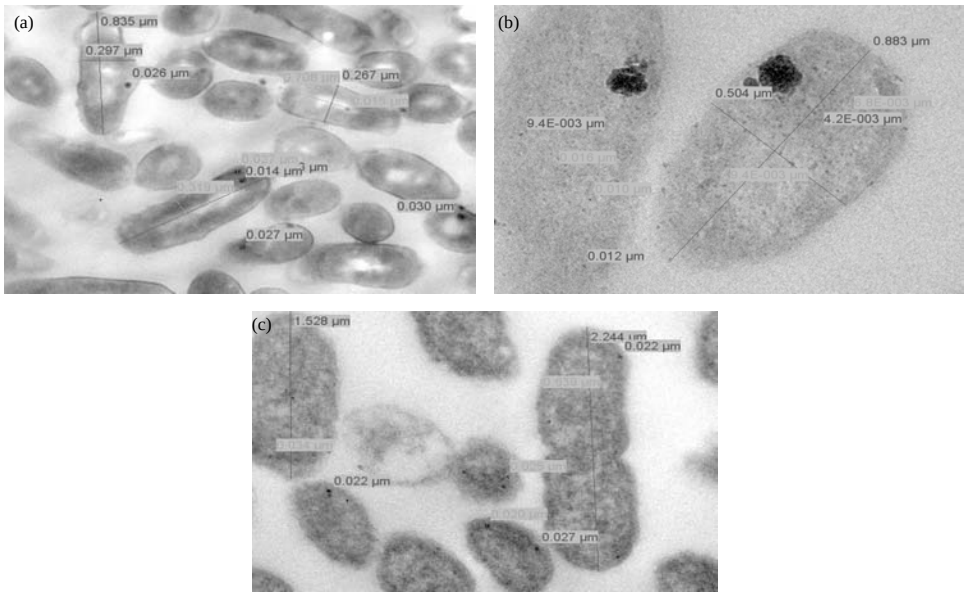

Fig. 3: Transmission electron micrographs. Transmission electron micrographs of metal treated cells showing presence of nanoparticles within cell. The cells were fixed with 2.5% gluteraldehyde and 2% para formaldehyde in sodium phosphate buffer (pH-7.4) for 4 h. Staining was avoided since, the intracellular metal deposit would themselves act as scattering centre for electrons and thereby develop the contrast for visualization. Cells were visualized as cross sections in a transmission electron microscope (model number FEI Philips Morgagni 268D) at 100kV transmission mode. (a) Isolate SRC-010 in presence of mercury (magnification-11000X), (b) Isolate SRC-002 in presence of lead (magnification-8900X) and (c) Isolate SRC-009 in presence of chromium (magnification-5600X)

SRC-003 accumulated copper (222.712 ppb) and chromium (125 ppb) most (Fig. 2). This is an important finding from environmental point of view. These bacteria could have a role in removal of those metals from contaminated sites.

Nanoparticle generation by the isolates-transmission electron microscopy: Almost all the isolates were found to generate nanoparticles in presence of lead and mercury (Fig. 3). Most of the isolates were found to produce more nanoparticles in presence of lead in comparison to other metals. The size and number of nanoparticles per cell varies from isolate to isolate and metal to metal. The size of the particles was relatively larger in presence of lead and chromium. These results supported the finding of EDXRF analysis for detection of total metal accumulation within cell.

Development of bioremedial package to treat heavy metal contaminated water: Immobilized mixed consortium were found to remove lead from distilled water supplemented with lead, Bheri water supplemented with lead and only Bheri water. The reduction started within 1 h of inoculation. After 48 h of incubation the consortium reduced 77% of lead in case of distilled water containing lead, more than 99% lead in case of Bheri water and 92% lead for Bheri water supplemented with 2 mM lead nitrate (Fig. 4a). It was found that the immobilized consortium was

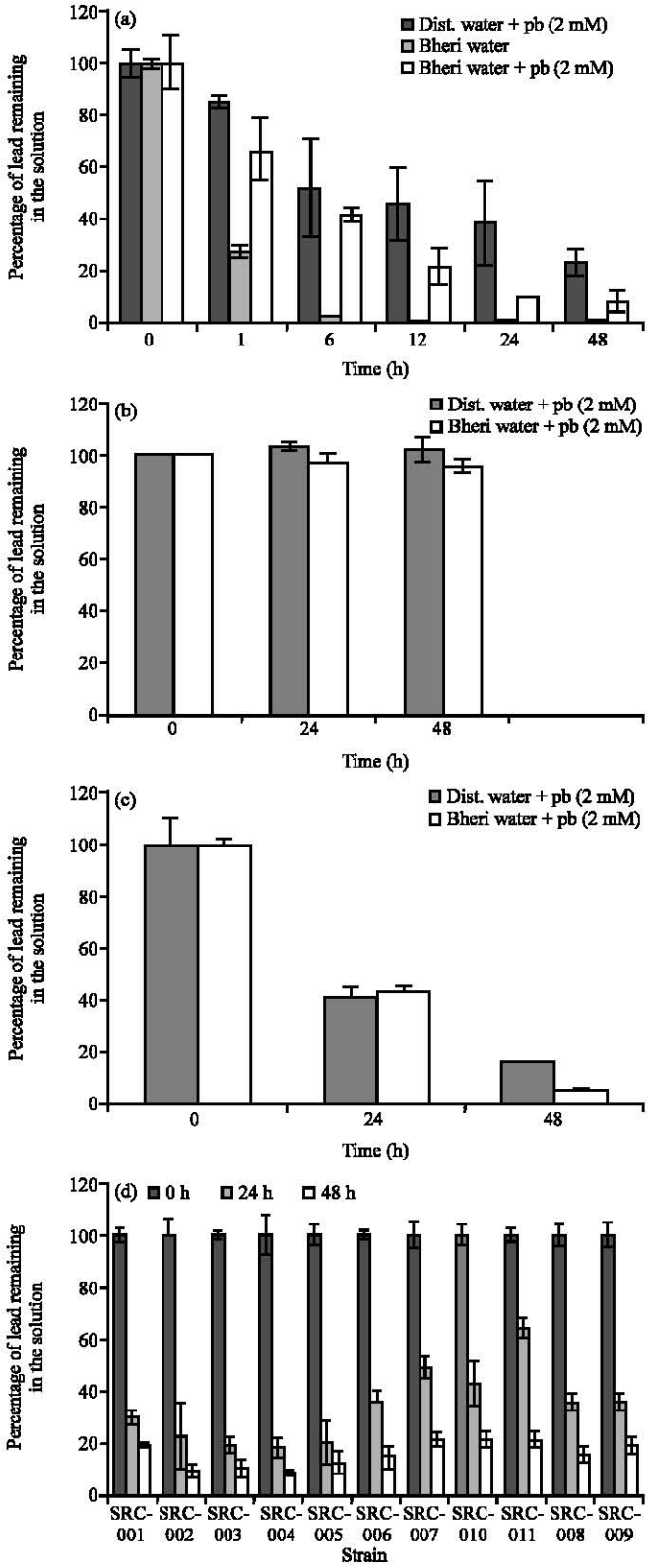

Fig. 4: Continued

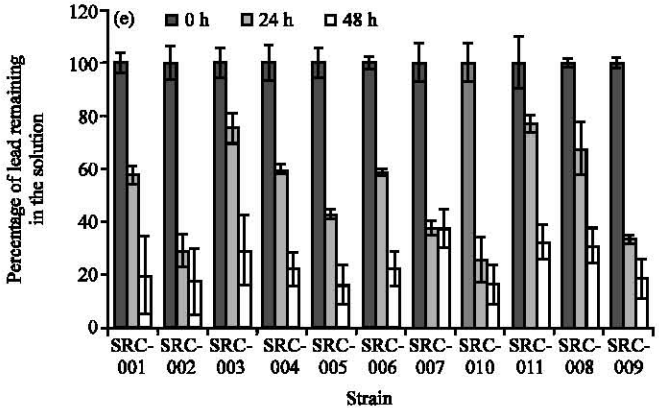

Fig. 4: Metal removal study. Bar diagram representing the extent of lead removal by immobilized mixed consortium and individual strains. The procedure is described in materials and methods section. Time was plotted along X axis and residual lead after microbial treatment along Y axis. (a) The removal efficiency was checked in distilled water and Bheri water containing 2 mM of Pb as well as Bheri water without any supplement. The reduction started within 1 h. After 48 h the mixed consortium reduced 99% lead from Bheri water, 77% lead from lead supplemented distilled water, 92% lead from lead supplemented Bheri water, (b) Bar diagram representing the extent of lead removal from distilled water as well as Bheri water containing 2 mM Pb, without any additions of the microbe (control). The results indicated that there was 3.5 and 5% reduction of lead concentration after 24 h and 48 h, respectively. in case of Bheri water by the natural microflora present in Bheri while there was no reduction of lead in case of sterile distilled water supplemented with lead nitrate solution, (c) Bar diagram representing the removal of lead by the mixed consortium from distilled water and Bheri water containing 5 mM lead nitrate. Result indicated that the consortium removed 83.64% lead from distilled water and 94.4% from Bheri water after 48 h of incubation, (d) The graph representing efficiency of lead removal by individual isolates from distilled water supplemented with 5 mM lead nitrate. Result indicated that all the isolates were able to reduce the lead content in distilled water with maximum (90%) reduction in case of strain SRC-003 and (e) Bar diagram representing removal of lead by individual isolates from Bheri water supplemented with 5mM lead nitrate. Result indicated that all the strains were able to reduce lead but none of the isolate could remove lead from water as efficiently as mixed consortium

more effective in natural Bheri water than distilled water. This might be due to the participation of natural miocroflora present in Bheri water in reduction of lead concentration. This was supported by the control experiment. There was about 5% reduction of lead concentration after 48 h in case of Bheri water by the natural microflora present there while there was no reduction of lead concentration in case of sterile distilled water supplemented with lead nitrate solution without any inoculation (Fig. 4b). When the lead nitrate concentration was increased to 5 mM, the consortium efficiently removed 83.68% lead from distilled water and 94.4% from Bheri water after 48 h of incubation (Fig. 4c). Since, the mixed bacterial consortium was found to remove the metal from water, the role of individual strain in reduction of lead from water was checked. All of the eleven bacterial isolates were able to reduce lead from water. In case of distilled water mixed consortium

was able to reduce 83.68% lead from water while in case of individual strains there was 80.67% reduction for SRC-001, about 90% for SRC-002 and SRC-003 and SRC-004, 87% for SRC-005, 85% for SRC-006, 78% for SRC-007, 77% for SRC-010, 79% for SRC-011, 85% for SRC-008 and 80% reduction by strain SRC-009 within 48 h (Fig. 4d). But in case of Bheri water no strain could remove lead from water as efficiently as mixed consortium (Fig. 4e). Here there was 94.4% reduction of lead in case of mixed consortia while in case of individual strain SRC-001 could reduce 78%, SRC-002 reduced 82%, SRC-003 reduced 77%, SRC-004 reduced 78%, SRC-005 reduced 85%, SRC-006 reduced 78%, SRC-007 reduced 63%, SRC-010 reduced 83%, SRC-011 reduced 68%, SRC-008 reduced 69% and strain SRC-009 reduced 80% of initial lead concentration of water after 48 h of incubation.

Amplification of SOD gene to understand the molecular mechanisms: Gene amplification using Superoxide dismutase gene specific primer was observed for three strains namely, SRC-008, SRC-005 and SRC-011, while isolate SRC-004 gave a smear (Fig. 5). The sizes of the amlpicons were around 500 bp when compared with 100 bp ladder. The expected size of the amplicon was 489 bp (Zolg *et al*., 1994). The amplicon of SRC-008 strain was sequenced and found to be novel with 79% similarity with SOD gene of *Mycobacterium* sp. The sequence was submitted to GenBank with accession number FJ-930099.

Scanning electron microscopy for metal induced cytoskeletal changes: Scanning electron micrographs of metal treated cells revealed three different types of changes in the cell size and surface morphology in compare to control cells. First kind of change was shortening and thickening of the cells in presence of metals. Second type of change was appearance of a woolly coat around

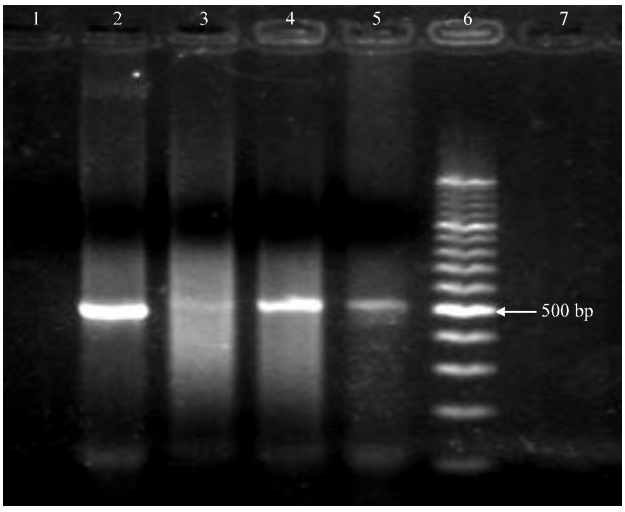

Fig. 5: Molecular mechanism of metal tolerance. Photograph of ethidium bromide stained 2% agarose gel run at 100 V cm^{-2} for 1 h showing the amplification of SOD gene using universal SOD specific primer sets. Lane 1- negative control, lane 2- SRC-008, lane 3- SRC-004, lane 4-SRC-005, lane 5- SRC-011, lane 6-100 bp DNA ladder (Cat. No. SM0643, Fermentas). Photograph of the gel showed amplification of SOD gene (500 bp amplicon) in isolate SRC-008, SRC-005 and SRC-011

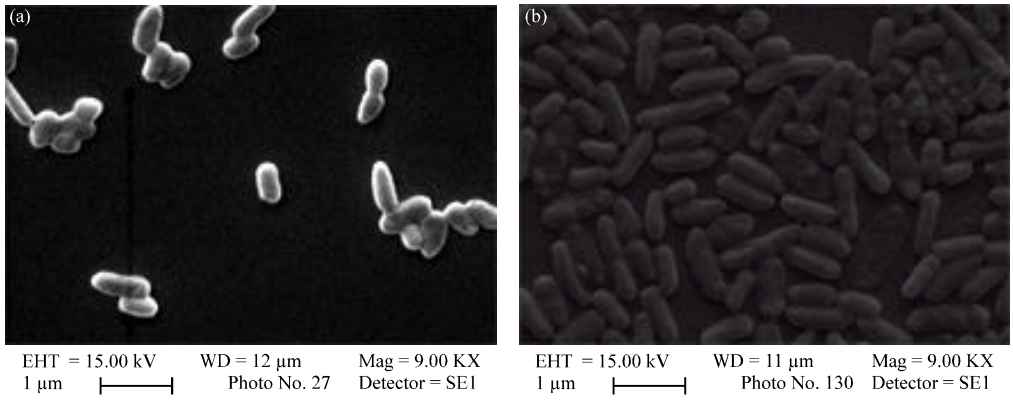

Fig. 6: Scanning electron micrographs. Scanning electron micrographs of the isolate SRC-004 showing the effect of metal stress on the cell morphology and dimensions in absence and presence of Cr treatment. Both control and metal treated cells were fixed with 2.5% gluteraldehyde and 2% para formaldehyde in sodium phosphate buffer (pH-7.4) and the smear was prepared on a coverslip which was then fixed onto a stub. Post gold coating, the cells were visualized under Scanning Electron Microscope model No.- LEO 435 VP. (a) control untreated cells and (b) cells treated with Cr. Magnifications of both the images were 9000X

the outer surface which might be due to an extra polysaccharide secretion by the cell that can reduce the surface area of contact between the cell and metal and thereby prevent further uptake. The third mechanism is the elongation of the cells which might be due to the inability to divide the cell under metal accumulated condition (Fig. 6a, b).

DISCUSSION

All the eleven isolates were found to tolerate heavy metals as evident from EDXRF analysis and Transmission Electron Microscopy. Many bacteria are reported to accumulate different metals like silver, copper, chromium, cobalt, nickel (Nakajima and Sakaguchi, 1986; Pumpel and Schinner, 1986; Goddard and Bull, 1989; Mengoni *et al*., 2001). *Pseudomonas* (strain CRB5) was reported to reduce chromate [Cr(VI)] to an insoluble Cr(III) form and could tolerateup to 520 mg of Cr(VI) L^{-1} (McLean and Beveridge, 2001). Strain SRC-003 was found to accumulate 125 ppb chromium which was maximum among our 11 isolates. A strain of *Klebsiella planticola* Cd-1 isolated from salt marsh sediments has been reported to tolerate up to 15 mM $CdCl_2$ (Sharma *et al*., 2000) while our isolates were able to tolerate upto 4mM $CdCl_2$ (isolate SRC-001, SRC-007 and SRC-008). Different species of *Pseudomonas, Xanthomonas, E. coli* (Yang *et al*., 1993) were reported to tolerate copper. *E. coli* K-12 strain was reported to tolerate upto 18mM $CuSO_4$ added to their growth media (Williams *et al*., 1993). *Pseudomonas fluorescens* 09906 strain was reported to tolerate 1.6 mM Cu (Yang *et al*., 1993). In this study, the MIC for copper was maximum in case of isolate SRC-003, SRC-009 and SRC-011 (5 mM). A strain of *Pseudomonas fluorescens* isolated from uranium mine were found to uptake 1048 nmol Ni^{2+}/mg of dry wt., 845 nmol Co^{2+}/mg of dry wt., 828 nmol Cu^{2+}/mg of dry wt. and 700 nmol Cd^{2+}/mg of dry wt. (Choudhary and Sar, 2009). Metal accumulating bacteria could be used as source for production of metal nanostructured particles (Klaus-Joerger *et al*., 2001; Salata, 2004). The strains were able to produce nanoparticle in

presence of metals like mercury, lead, chromium, copper, cadmium, cobalt, nickel. These metal accumulating properties could also be applied for development of bioremedial package. The mixed consortium under immobilized condition efficiently removed 83.68% lead from distilled water and 94.4% from Bheri water after 48 h of incubation. A novel *Alicyclobacillus* sp. isolated from lead contaminated sea spot was reported to remove lead upto 64 and 65.3% from distilled water supplemented with 0.5 and 0.9 ppm of pb^{2+} respectively after 2 h of incubation (Mohamed and El-Sersy, 2009). An enriched consortium from lake sediments was reported to remove 99-100% of different heavy metals including Cu, Cr, Ni, Pb and Zn from heavy metal contaminated water (Lee *et al*., 2008). It was found that the immobilized mixed consortium was more effective in natural Bheri water than distilled water. Possible explanation for this is that natural microflora present in Bheri water was actively involved in removal of heavy metal. In case of Bheri water mixed consortium was found to reduce more metal than any individual strain. This might be because of synergistic effect of the isolates with the natural microflora present in Bheri water. The microbes under immobilized condition could be used in retention tanks for treating the liquid wastes containing heavy metals from different industries. The final effluent coming out will either be free of heavy metals or contain them in lesser amounts, fit to be released into the environmental source.

ACKNOWLEDGMENT

The authors would like to thank West Bengal University of Technology for the computational facility. Authors acknowledge the financial assistance of IUC-DAE, under the CRS scheme (CRS-088 of 2005) for sponsoring the fellowship for the student. The authors thank Department of Atomic Energy under the BRNS scheme (grant No. 2005/36/31/BRNS/2574 dated 2nd March 2006) for financial support.

REFERENCES

Adarsh, V.K., M. Mishra, S. Chowdhury, M. Sudarshan, A.R. Thakur and S. Ray-Chaudhuri, 2007. Studies on metal microbe interaction of three bacterial isolates from East Calcutta Wetland. Online J. Biol. Sci., 7: 80-88.

Ahluwalia, S.S. and D. Goyal, 2007. Microbial and plant derived biomass for removal of heavy metals from wastewater. Biores. Technol., 98: 2243-2257.

Ahmad, A., S. Senapati, M.I. Khan, R. Kumar, R. Ramani, V. Srinivas and M. Sastry, 2003. Intracellular synthesis of gold nanoparticles by a novel alkalotolerent actinomycete, *Rhodococcous* species. Nanotechnology, 14: 824-828.

Bruins, M.R., S. Kapil and F.W. Oehme, 2000. Microbial resistance to metals in the environment. Ecotoxicol. Environ. Safety, 45: 198-207.

Caballero, A., B. Thibodeaux, M. Marquart, M. Traidej and R. O'Callaghan, 2004. *Pseudomonas Keratitis*: Protease IV gene conservation, distribution and production relative to virulence and other *Pseudomonas* proteases. Invest. Ophthalmol. Visual Sci., 45: 522-530.

Chipasa, K.B., 2003. Accumulation and fate of selected heavy metals in a biological wastewater treatment system. Waste Manag., 23: 135-143.

Choudhary, S. and P. Sar, 2009. Characterization of a metal resistant *Pseudomonas* sp. isolated from uranium mine for its potential in heavy metal (Ni^{2+}, Co^{2+}, Cu^{2+} and Cd^{2+}) sequestration. Bioresour. Technol., 100: 2482-2492.

Chowdhury, S., M. Mishra, V.K. Adarsh, A. Mukherjee, A.R. Thakur and S.R. Chaudhuri, 2008. Novel metal accumulator and protease secretor microbes from East Calcutta Wetland. Am. J. Biochem. Biotechnol., 4: 255-264.

Doubler, J.W. and H.V. Castin, 2000. Removal of mercuric from chemical wastewater by microorganisms in technical scale. Environ. Sci. Technol., 34: 4628-4634.

Georgalaki, M.D., E.V. Berghe, D. Kritikos, B. Devreese and J.V. Beeumen *et al.*, 2002. Macedocin, a food-grade lantibiotic produced by *Streptococcus macedonicus* ACA-DC 1998. Applied Environ. Microbiol., 68: 5891-5903.

Goddard, P.A. and A.T. Bull, 1989. The isolation and characterisation of bacteria capable of accumulating silver. Applied Microbiol. Biotechnol., 31: 308-313.

Kim, S.J., R.Y. Park, S.M. Kang, M.H. Choi, C.M. Kim and S.H. Shin, 2006. *Pseudomonas aeruginosa* alkaline protease can facilitate siderophore-mediated iron-uptake via the proteolytic cleavage of transferrins. Biol. Pharm. Bull., 29: 2295-2300.

Klaus-Joerger, T., R. Joerger, E. Olsson and C. Granqvist, 2001. Bacteria as workers in the living factory: Metal-accumulating bacteria and their potential for materials science. Trends. Biotechnol., 19: 15-20.

Lee, E.Y., J.S. Lim, K.H. Oh, J.Y. Lee, S.K. Kim, Y.K. Lee and K. Kim, 2008. Removal of heavy metals by an enriched consortium. J. Microbiol., 46: 23-28.

Leung, W.C. and M.F. Wong, 2000. Removal and recovery of heavy metals by bacteria isolated from activated sludge. Water Sci. Technol., 41: 233-240.

Lin, X., W. Xu, K. Huang, X. Mei and Z. Liang *et al.*, 2009. Cloning, expression and characterization of recombinant elastase from *Pseudomonas aeruginosa* in *Picha pastoris*. Protein Expression Purification, 63: 69-74.

Lovley, D.R. and J.D. Coates, 1997. Bioremediation of metal contamination. Curr. Opin. Biotechnol., 8: 285-289.

Malathu, R., S. Chowdhury, M. Mishra, S. Das and P. Moharana *et al.*, 2008. Characterization and wash performance analysis of microbial extracellular enzymes from East Calcutta Wetland of India. Am. J. Applied Sci., 5: 1650-1661.

Mandal, D., M.E. Bolander, D. Mukhopadhyay, G. Sarkar and P. Mukherjee, 2006. The use of microorganisms for the formation of metal nanoparticles and their application. Applied Microbiol. Biotechnol., 69: 485-492.

McLean, J. and T.J. Beveridge, 2001. Chromate reduction by a Pseudomonad isolated from a site contaminated with chromated copper arsenate. Applied Environ. Microbiol., 67: 1076-1084.

Mengoni, A., R. Barzanti, C. Gonnelli, R. Gabbrielli and M. Bazzicalopo, 2001. Characterization of nickel-resistant bacteria isolated from serpentine soil. Environ. Microbiol., 3: 691-698.

Mohamed, E.A.H. and N.A. ElSersy, 2009. Molecular characterization of some novel marine alicyclobacillus strains, capable of removing lead from a heavy metal contaminated sea spot. Biotechnology, 8: 100-106.

Nakajima, A. and T. Sakaguchi, 1986. Selective accumulation of heavy metals by microorganisms. Applied Microbiol. Biotechnol., 24: 59-64.

Pradhan, A., P. Bhaumik, S. Das, M. Mishra and S. Khanam *et al.*, 2008. Phytoplankton diversity as indicator of water quality for fish cultivation. Am. J. Environ. Sci., 4: 271-276.

Pugazhenthiran, N., S. Anandan, G. Kathiravan, N.K. Udaya-Prakash, S. Crawford and M. Ashokkumar, 2009. Microbial synthesis of silver nanoparticles by *Bacillus* sp. J. Nanoparticle. Res., 11: 1811-1815.

Pumpel, T. and F. Schinner, 1986. Silver tolerance and silver accumulation of microorganisms from soil materials of a silver mine. Applied Microbiol. Biotechnol., 24: 244-247.

RayChaudhuri, S. and A.R. Thakur, 2006. Microbial genetic resource mapping of East Calcutta Wetland. Curr. Sci., 91: 212-217.

RayChaudhuri, S., S. Salodkar, M. Sudarshan and A.R. Thakur, 2007. Integrated resource recovery at East Calcutta Wetland: How safe is these. Am. J. Agric. Biol. Sci., 2: 75-80.

RayChaudhuri, S., M. Mishra, P. Nandy and A.R. Thakur, 2008. Waste management: A case study of ongoing traditional practices at East Calcutta Wetland. Am. J. Agric. Biol. Sci., 3: 315-320.

Roy, S., A.N. Ghosh and A.R. Thakur, 2008. Uptake of Pb^{2+} by a cyanobacterium belonging to the genus *Synechocystis*, isolated from East Kolkata Wetlands. Biometals, 21: 515-524.

Salata, O.V., 2004. Applications of nanoparticles in biology and medicines. J. Nanobiotechnol., 2: 3-3.

Shakoori, A.R. and B. Muneer, 2002. Copper-resistant bacteria from industrial effluents and their role in remediation of heavy metals in wastewater. Folia Microbiol. (Praha), 47: 43-50.

Sharma, P.K., D.L. Balkwill, A. Frenkel and M.A. Vairavamurthy, 2000. A new *Klebsiella planticola* strain (Cd-1) grows anaerobically at high cadmium concentrations and precipitates cadmium sulfide. Applied Environ. Microbiol., 66: 3083-3087.

Volesky, B. and H.A. May-Philips, 1995. Biosorption of heavy metals by *Saccharomyces cervisae*. Applied Microbiol. Biotechnol., 42: 797-806.

Williams, J.R., A.G. Morgan, D.A. Rouch, N.L. Brown and B.T. Lee, 1993. Copper-resistant enteric bacteria from United Kingdom and Australian piggeries. Applied Environ. Microbiol., 59: 2531-2537.

Yang, C.H., J.A. Menge and D.A. Cooksey, 1993. Role of copper resistance in competitive survival of *Pseudomonas fluorescens* in soil. Applied Environ. Microbiol., 59: 580-584.

Zolg, J.W. and S. Philippi-Schulz, 1994. The Superoxide dismutase gene, a target for detection and identification of *Mycobacteria* by PCR. J. Clin. Microbiol., 32: 2801-2812.