Resistance and Biosorption of Mercury by Bacteria Isolated from Industrial Effluents

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Abstract.- The present study is aimed at assessing the ability of two Hg^{2+} resistant bacterial strains, *Brevibacterium casei* and *Pseudomonas aeruginosa*, to uptake metal from the medium. For the bacterial isolates the minimum inhibitory concentration of Hg^{2+} ranged between 400-500 µg/mL. *Pseudomonas aeruginosa* could tolerate Pb^{2+} (600 µg/mL), Cu^{2+} (200 µg/mL), Cd^{2+} (50 µg/mL), Zn^{2+} (50 µg/mL), Ni^{2+} (550 µg/mL) and Cr^{6+} (50 µg/mL). *Brevibacterium casei,* on the other hand, showed resistance against Pb^{2+}, Cu^{2+}, Cr^{6+}, Ni^{2+}, Zn^{2+} and Cd^{2+} at a concentration of 650, 200, 150, 550, 50, and 50 µg/mL, respectively. The isolates showed typical growth curves but lag and log phases extended in the presence of mercury. Both isolates showed optimum growth at 37 ºC and pH varying from 7-7.5. Metal processing ability of the isolates was determined in a medium containing 100 µg/mL of Hg^{2+}. *Pseudomonas aeruginosa* could reduce 93% of mercury from the medium after 40 hours and was also capable to remove Hg^{2+} 35%, 55% 70% and 85% from the medium after 8, 16, 24 and 32 hours, respectively. *Brevibacterium casei* could also efficiently remove 80% mercury from the medium after 40 hours and was also able to remove Hg^{2+} 20%, 40%, 50%, and 65% from the medium after 8, 16, 24 and 32 hours, respectively. Both bacterial strains have shown remarkable ability to uptake metal ions from the culture medium. *Pseudomonas aeruginosa* was observed to uptake 80% and *Brevibacterium casei* 70% of Hg^{2+} from the medium after 24 hours of incubation at $37^{o}C$. The metal uptake ability suggests possibility of using these bacterial strains for removal of mercury from Hg^{2+} contaminated wastewater.

Keywords: Heavy metal accumulation, wastewater, bioremediation, heavy metal resistance.

INTRODUCTION

Heavy metal contamination due to natural and anthropogenic sources is a global environmental concern. Release of heavy metal without proper treatment poses a serious threat to public health because of its persistence, biomagnification and accumulation in food chain. Most of the heavy metals like chromium, cadmium, lead, mercury and copper are highly toxic for almost all the living organisms. The health of people living near the dumping grounds is also being constantly affected by the metal contamination of food and drinking water. A number of studies have elaborated the effects of heavy metals on animals, plants and human health (Chipasa, 2003; Chisti, 2004).

Mercury is one such metal which has been reported to produce metabolic disorders in variety of animals such as fish (Company *et al.,* 2004), rat (Reinhardt and Pelli, 1986), rabbit (Shakoori *et al.,*

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2002) and man (Miwa *et al.*, 1987). Various health problems such as pneumonitis, abnormal cramps, bloody diarrhea and suppression of urine, cancer, and hypersecretion of sweat glands are caused by mercurial and mercuric forms of mercury. Romero *et al.* (2004) studied the toxic effects of mercury chloride in two cell lines of renal origin. The most notable findings in treated cells were the presence of intracytoplasmic inclusion bodies and apoptotic bodies.

Recently, microbial bioremediation has emerged as an alternative technique to such traditional chemical treatments (Brierley, 1990). Mercury resistant bacteria have been reported by several authors (Chang *et al.*, 1998; Brown *et al.,* 2002; Mindlin *et al.*, 2005; Fortunato *et al.*, 2005). Mercury is also efficiently removed by algae (Davis *et al.,* 2003; Chojnacka *et al.,* 2004). The genus *Rhodosporidium* sp. was also isolated from metallurgical waste by Baldi and Pepi (1995). Mercury accumulation by plants is also reported by many researchers (Zeroual *et al.,* 2003; Bennicelli *et*

al., 2004).

One of the objectives of this study was to evaluate the minimum inhibitory concentration (MIC) of Hg^{2+} against the bacterial isolates and to determine their ability to uptake mercury.

MATERIALS AND METHODS

Sample collection

Wastewater samples were collected in screw capped sterilized bottles from five different ponds in industrial area of Sialkot (Pakistan). Some physicochemical parameters of wastewater *viz.,* temperature (ºC), pH and mercury (µg/mL) were measured (APH, 1992).

Isolation of mercury resistant bacteria

For isolation of mercury resistant bacteria, 100µL of the wastewater sample was spread on Luria-Bertani (LB) agar plates containing 50µg of Hg^{2+}/mL of the medium. LB agar plates were prepared by dissolving 1 g NaCl, 1 g tryptone and 0.5 g yeast extract in 100 mL distilled water, pH adjusted at 7.2 to 7.5 and then 1.5 g agar was added in the 250 mL flasks. The medium was autoclaved at 121ºC and 15 Lb pressure for 15 minutes. The growth of the bacterial colonies was observed after 24 hours of incubation at 37ºC. Isolated colonies were picked up with sterilized wire loop and streaked on LB agar medium plate containing 100µg Hg^{2+}/mL. It was again incubated at 37ºC for 24 hours. This process was repeated with successively higher concentrations of Hg^{2+} (150, 200, 250, 300, 350, 400, 450, 500µg Hg^{2+}/mL) until the minimum inhibitory concentration (MIC) of each isolate was obtained. The MIC is defined as the lowest concentration of Hg^{2+} at which a single colony-derived streak could not grow.

Physical, biochemical and molecular characterization of the bacterial isolates

For biochemical characterization the bacterial isolates were tested for oxidase activity, motility, citrate utilization, urease activity, triple sugar iron reaction, indole reaction, and MacConkey agar test. For physical and biochemical characterization of bacterial isolates the criteria adopted by Benson (1994) and those of Bergey's Manual of Determinative Bacteriology were followed. For further identification, genomic DNA was isolated and the 16S rRNA gene was amplified by PCR using two general bacterial 16S rRNA primers (RS-1; 5′-AAACTC-AAATGAATTGACGG-3′, RS-3; 5′-ACGGGCGGTGTGTAC-3′). The PCR product of 0.5 kb was removed from the gel and cloned in pTZ57R/T vector. The amplified 16S rRNA gene was purified with a Fermentas purification kit and the amplified products were electrophoresed on 1% agarose gel. Sequencing was carried out by Genetic analysis system model CEQ-800 (Beckman) Coulter Inc. Fullerton, CA, USA. The determined sequences obtained were compared with 16S rRNA gene sequences obtained from GenBank and ribosomal RNA databases.

Determination of optimum growth conditions

For optimum growth of the bacterial isolates, two parameters *i.e.* temperature and pH were considered. For determination of optimum temperature, 5 mL LB broth was added in 4 sets, each of three test tubes, for each isolate, autoclaved and inoculated with 20 µL of freshly prepared culture of isolates. The four sets of tubes were incubated at 25ºC, 30ºC, 37ºC and 42ºC. After an incubation of 12 hours, their absorbance was taken at 600 nm.

For determination of optimum pH, test tubes having 5 mL LB broth were prepared in 9 sets, each containing 3 test tubes, for each isolate and their pH was adjusted at 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 then autoclaved. These tubes were inoculated with 20 µL freshly prepared culture of the isolates. After incubation period of 12 hours, their absorbance was taken at 600 nm.

Growth curves of isolates

Growth curves of bacterial isolates were determined with (50µg Hg^{2+}/mL) and without mercury. For each isolate 50 mL LB broth was taken in one set consisting of 3 flasks, autoclaved and then inoculated with 50 µL of the freshly prepared inoculum. These cultures were incubated at their optimum temperature in a shaker at 60-80 rpm. An aliquot of culture was taken out in an oven sterilized tube, at regular intervals of 0, 4, 8, 12, 16, 20, 24, 36, and 48 hours. Absorbance was taken at

600 nm wavelength. Growth was plotted graphically.

Table I.- Physicochemical parameters of wastewater collected from Nullah Egg receiving effluents from industrial area of Sialkot, Pakistan.

Parameters	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5
Temperature (ºC)	23.66±0.47	24.66±0.47	23.00±0.47	22.00±0.81	20.53±0.47
pH	8.47 ±0.04	8.52 ±0.12	8.38 ±0.04	8.648±0.04	8.05.±0.12
Mercury (µg/mL)	1.10 ±0.08	1.50 ±0.08	0.70±0.08	1.30±0.04	1.41±0.04

*Means±standard deviation, n=3

Cross metal and antibiotic resistance

The cross heavy metal resistance of bacterial isolates was determined by using stock solutions of 10 mg/mL of different metal salts such as, lead nitrate, cadmium chloride, copper sulphate, potassium dichromate, zinc sulphate and nickel chloride. The cross metal resistance was checked by increasing the concentration of respective metal in a stepwise manner with 50 µg/mL of metal increased every time. Streaked plates containing metal ions, incubated at 37°C for 24 hours and growth was observed for four days. Antibiotic sensitivity against bacterial isolates was checked by measuring the zone of inhibition. The antibiotics used were ampicillin (10µg), chloramphenicol (30µg), gentamicin (10µg), carbenicillin (100µg), oxytetracyclin (30µg) and penicillin (10µg).

Estimation of Hg^{2+} processing ability of the isolates

The metal processing capability of bacterial isolates was checked by adding Hg^{2+} at a concentration of 100 µg/mL in the culture medium. The control culture medium was also run for mercury containing the same concentration as in treated one *i.e.* 100 µg/mL but was without the bacterial isolates. The cultures were incubated for 40 hours and from each medium (control and treated) 5 mL culture was taken out under sterilized conditions after 0, 8, 16, 24, 32 and 40 hours, respectively. The cultures were spun down at 3000 rpm for 5 minutes and the supernatants were used for the estimation of Hg^{2+} by Atomic Absorption Spectrophotometer (Varian, U.S.A) at wavelength 253.7nm. The amount of metal in the supernatants was determined by using standard curve. The percentage reduction in the amount of Hg^{2+} in the medium was calculated.

Bioaccumulation of mercury by bacterial isolates

The uptake of mercury by bacterial isolates in LB medium was carried out by acid digestion. Hg^{2+} (50 µg/mL) was added in the culture medium and cells were collected after 24 hours of incubation at 37°C, washed three times in saline solution and acid digested (H_2SO_4: HNO_3 1:1). Metal content of the digest was measured by Atomic Absorption Spectrophotometer (AAS) at 253.7nm wavelength. Amount of mercury uptake by bacterial cells was calculated in µg/mL by using standard curve.

Statistical analysis

Observations were made and all the experiments run in triplicate. At least three separate flasks were usually maintained for one treatment. Each time three readings were taken, their mean, and standard error of the mean were calculated.

RESULTS

Physicochemical characteristics of industrial wastewater

Table I shows physicochemical characteristics of industrial wastewater of five different samples, from where mercury tolerant bacteria were isolated. The temperature of different samples ranged between 20.5ºC to 24.6ºC, pH ranged between 8.05 and 8.6, and Hg^{2+} ranging between 0.70±0.08 and 1.50±0.08 µg/mL.

Identification of bacterial isolates

The morphological and biochemical characteristics of bacterial isolates have been shown

in Table II. The partially amplified (500bp) PCR product of 16S rRNA gene from local isolates (CBL-Hg1 and CBL-Hg2) was sequenced and blasted against similar sequences in the NCB1 databank. The blast query revealed that this gene was 90% homologous to already reported gene of *Brevibacterium casei* (CBLHg1) and 87% homology with *Pseudomonas aeruginosa* (CBL-Hg2). The nucleotide sequences coding for 16S rRNA gene of *Brevibacterium casei* and *Pseudomonas aeruginosa* have been submitted to the GenBank database under accession number AB262512 and AB262513, respectively (Figs. 1, 2).

Table II.- **Morphological and biochemical characteristics of bacterial isolates.**

Characters	*Pseudomonas aeruginosa*	*Brevibacterium casei*
Gram-reaction	-	-
Morphology	Rods	Rods
Colour	Off-white	Off-white
Urease production	-	-
Citrate utilization	-	-
Oxidase reaction	+	+
Triple sugar iron reaction	+	+
Indole reaction	-	-
Motility	+	+

+, positive; -, negative.

Optimum growth conditions

The most suitable temperature for both the mercury resistant bacterial isolates was found to be 37°C. Maximum growth for *P. aeruginosa* was observed at pH 7.5, while *B. casei* showed maximum growth at pH 7. The growth curve pattern was studied by growing the organisms in the presence of Hg^{2+} (50µg/mL) and comparing with the control culture in which no metal ions were added. Although the growth pattern of these isolates were not significantly different from those of control but the growth of both isolates was inhibited in the presence of Hg^{2+}. The growth of *P. aeruginosa* was more affected than *B. casei*. It is interesting to note that the lag phases of both isolates were extended from 4 hours to 12 hours in *P. aeruginosa*, and from 4 hours to about 8 hours in *B. casei*. The growth pattern has been shown in Figure 3.

Heavy metal resistance

B. casei was found to be resistant to mercury at a concentration of 500µg/mL, while *P. aeruginosa* showed maximum resistance at a concentration of 400µg Hg^{2+}/mL. The bacterial isolates were also checked for their resistance to various other heavy metals, *viz.*, chromium, cadmium, copper, lead, zinc and nickel (Table III). *P. aeruginosa* showed maximum resistance against Pb^{2+} at a concentration of 600 µg/mL and the order of resistance regarding the metal concentration was $Pb^{2+}>Ni^{2+}>Cu^{2+}>Cr^{6+}>Cd^{2+}>Zn^{2+}$. *B. casei* was found to be resistant to Ni^{2+} at a concentration of 550µg/mL. The order of resistance regarding the metal concentration was $Pb^{2+}>Ni^{2+}>Cu^{2+}>Cr^{6+}>Zn^{2+}>Cd^{2+}$.

Table III.- **Cross resistance of mercury resistant bacterial isolates from industrial wastewater against other heavy metals.**

Heavy metal	*P. aeruginosa*	*B. casei*
Cr^{6+} (µg/mL)	50	150
Cd^{2+} (µg/mL)	50	50
Cu^{2+} (µg/mL)	200	200
Pb^{2+} (µg/mL)	600	650
Zn^{2+} (µg/mL)	50	50
Ni^{2+} (µg/mL)	550	550
Hg^{2+} (µg/mL)	400	500

Table IV.- **Antimicrobial susceptibility test profile for mercury resistant bacterial isolates.**

Antibiotics	Diameter (mm) of Inhibition Zones	
	P. aeruginosa	*B. casei*
Chloramphinicol (30µg)	R	R
Penicillin (10µg)	R	R
Gentamycin (10µg)	S (16)	S (20)
Carbenicillin (100µg)	S (14)	S (14)
Oxytetracyclin (30µg)	S (18)	S (22)
Ampicillin (10µg)	S (16)	R

S, Sensitive; R, Resistant.

Antibiotic resistance

The mercury tolerant bacterial isolates were also tested for antimicrobial susceptibility profile (Table IV). Both bacterial isolates, *P. aeruginosa* and *B. casei,* were found resistant to penicillin and

chloramphenicol and sensitive to gentamycin, carbenicillin and oxytetracyclin. *B. casei* showed resistance against ampicillin, while *P. aeruginosa* was found sensitive to it.

A.   Partial sequence of 16S rRNA gene of CBL-Hg1.

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GAAAAAAAGGGCCCCCGCCGGGAAAAAAAGCCGCGTTTCTGCACTGG
TDCCAATAACTTATGCGGGTCGTGAAAGAAACATGGACCGGGAAACT
ATTAAACCCCGCAGACGTTGCGATTTTGCGATTACTAGACGACTCCGA
CTTCACGTAGTCGAATTGCAGACCTACGATCCGAAACTGAGACTGGAC
TTAAGGGATTCCGGCTTGCCCCTCACGGGTTTCGCCTCTCTCTGTACCA
AGACCATTGTAGCATGACGTGAAGACCCAAGANATAAAGGGCATGAT
GATTTGACGTCATCCCCACCTTCCTACCGAGTTGAACCCGGCAGTTCT
ACCTATGAGTTTCCCACCATNACGTGACTGGCAACATAGAACGAGGGT
TTGAGACTCGTTGACGGGAACTTAAACCCATTATNTCACGANAACGAG
ACTGACGACAAACCATGCACCACCTGTACACCAGACTTCAAAGAAGA
AGACCTGTTTCCAGGACGGTCCGGTGTATGTCAAGACCTTGGTAAGGT
TTCTTCGACGTTGCATTCGAATTTAATTTCCGACATTGACTACCGGCCC
GTCCTTTGGTGGACGGGGGACCCCCCCGGTTACAAATTTTCCACCTTT
TTGAAAGGTTTTTAAAATTTCGGGAAATTCACCCGGGGGACCCCGGTA
CGAACTGNAAGAGGGACCTGCATTGCAAGACTTTACCTATAAGTGAGT
CGTATTAGAGCT ]GGCGTAATCATGGGTCATAGACTGTTTTACTGTGGT
GAAATTGGGTAANTACCGCCCTCACCAAATTTCCCACCACCAAAACAN
TAACCG
```

B.   90% homology with *Brevibacterium casei* (AY468375.1)

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Query     121   TGCGATTACTAGACGACTCCGACTTCACGTAGTCGAATTGCAGACCTACGATCCGAAACT   180
                |||||||||||||| |||||||||||||||||||||||||||||||| |||||||||| ||
Subject  1331   TGCGATTACTAG-CGACTCCGACTTCACGTAGTCGAATTGCAGAC-TACGATCCGAA-CT   1275

Query     181   GAGACTGGACTTAAGGGATTCCGGCTTGCCCCTCACGGGTTTCGCCTCTCTCTGTACCAA   240
                |||||||||  ||||||||||||  |||||||| |||||||| ||||||||||||||||||
Subject  1274   GAGACTGGCTTTAAGGGATTC--GCTTGCCC-TCACGGGTT-CGCCTCTCTCTGTACCAG   1219

Query     241   GACCATTGTAGCATGACGTGAAGACCCAAGANATAAAGGGCATGATGATTTGACGTCATC   300
                  ||||||||||||| |||||| ||||||| |||||||||||||||||||||||||||||
Subject  1218   --CCATTGTAGCATG-CGTGAAG-CCCAAGACATAAAGGGCATGATGATTTGACGTCATC   1163

Query     301   CCCACCTTCCTACCGAGTTGAACCCGGCAGTTCTACCTATGAGTTTCCCACCATNACGTG   360
                |||||||||| |||||||||| |||||||||| || |||||||||| |||||||| |||||
Subject  1162   CCCACCTTCCT-CCGAGTTGACCCCGGCAGT-CT-CCTATGAGTT-CCCACCATCACGTG   1107

Query     361   ACTGGCAACATAGAACGAGGGTTTGAGACTCGTTGACGGGAACTTAAACCCATTATNTCA   420
                ||||||||||||||||||||| || | |||||| ||||| |||| ||||| |||| || |||
Subject  1106   -CTGGCAACATAGAACGAGGGT-TGCG-CTCGTTG-CGGGA-CTTAA-CCCAACATCTCA   1053

Query     421   CGANAACGAGACTGACGACAAACCATGCACCACCTGTACACCAGACTTCAAAGAAGAAGA   480
                ||| | ||||| ||||||||| ||||||||| |||||||||||||||| |||||||||| ||
Subject  1052   CGACA-CGAG-CTGACGACAA-CCATGCACAACCTGTACACCAG-CTTCAAAGAAGAGGA   997

Query     481   CCTGTTTCCAGGACGGTCCGGTGTATGTCAAGACCTTGGTAAGGTT   526
                ||||||||||||||||||||||||||||||||| ||||||||||||
Subject  996   CCTGTTTCCAGGACGGTCCGGTGTATGTCAAG-CCTTGGTAAGGTT   952
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Fig. 1. Partial sequence of 16S rRNA gene of CBL-Hg1 (A), which showed 90% homology with *Brevibacterium casei* (AY468375.1) (B).

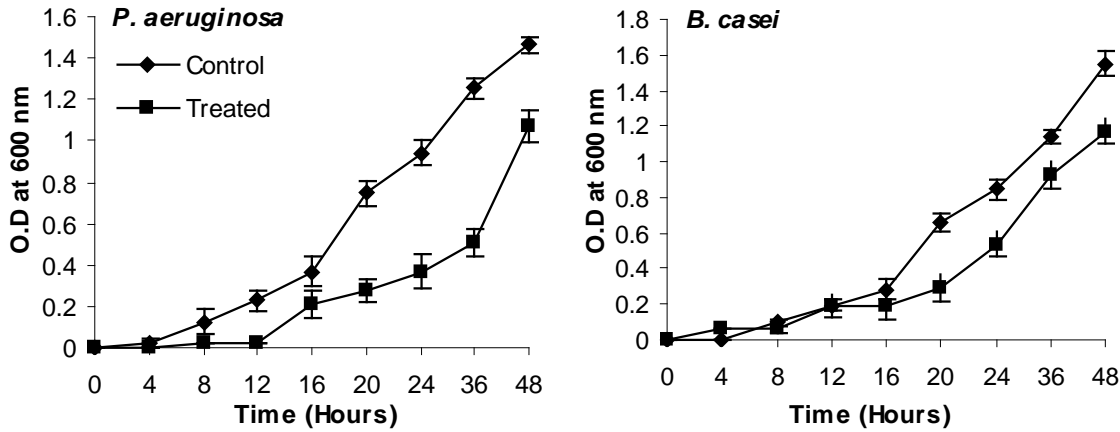A.   Partial sequence of l6S rRNA gene of CBL-Hg2

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CAGTGAATGCGAGACTACGGGTACCTACGACTAATGCTGCTAGATTAA
AACTACAAATGAATGTGAACGGGGGACCCGACAAACAANGACGGTGG
AAGAACATGTGGTTTAAATTACGAAAAGACAAACGAACAAAGAAACC
TTAAACCCTGGACCTTGAAACATGACTGAAGAAAACTTTAAACACAAG
AAGAATGGATTGGTTGCCCTTACGGGAAACTCAGAAAACAGGTGACT
GAATGGACTGTCGTAAGACTAGTTGTACGTGAGATGTTTGGGTTAAAG
TCCCCGTAAACGNAGACAAAAAACCCTTGTTACCTTAAGNTTAACAAA
GACAACCTACGGGTTGGGACAACTACTAAGGGAGAANCTGACCGGGT
GAAACAAAACCGGAAGGAAAGGGTGGGGAATGAACGTCAAGTCATCA
TGGACCCTTAACGGACAGGGACTANAAAACGTGACTAACAAATGGGT
ACGGGTAAAAAAGGGGGTTGGACCAAAGACCGACGAAGGGGTGGAA
GAACTAAATACACATTAAAAAAACCGAATACGTAGGTCACGGANACG
AAGGTCTGCAACTAGAACTGAGTGAAGTCGGAATCGATAGTAATCGT
GAATCAGAATGTCACGGTGAATACGTTACCGGGACCTGAACACACCG
CACGTAATCGGATCCCGGGACACGTCGACTGCAGAGGACTGCATGAC
AGACTTACCTATAGNGAGTCGTATTAGAGACTGGACGTAATAATGGTA
TAGCTGTTTACTGTGTGAAATGGTATACGCTACAATCCACACAACTAC
AGCCGAAGCTAAAGGTAAGCCGGGTGCTATAGGAACACCAACTAATG
CGTGCCCACG
```

B.   87% homology with *Pseudomonas aeruginosa* (AB 262513.1)

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Query    571   GTCTGCAACTAGAACTGAGTGAAGTCGGAATCGATAGTAATCGTGAATCAGAATGTCACG   630
               |||||||||| || ||| ||||||||| |||||| ||||||||||||||||||||||||||
Subject  407   GTCTGCAACT-CGACTGCGTGAAGTCAGAATCGCTAGTAATCGTGAATCAGAATGTCACG   465

Query    631   GTGAATACGTTACCGGGACCTGAACACACCGCACGTAATCGGATCCCGGGACACGTCGAC   690
               |||||||||| ||||| | |   | ||||||||| |||||||||||||||||| | |||||||
Subject  466   GTGAATACGTGCCCGGGCCTGGCACACACCGCCCGTAATCGGATCCCGGG-CCCGTCGAC   524

Query    691   TGCAGAGGACTGCATGACAGACTTACCTATAGNGAGTCGTATTAGAGACTGGACGTAATA   750
               ||||||||| ||||||| || || |||||||| ||||||||||||||| || | |||||||
Subject  525   TGCAGAGGCCTGCATGCAAGCTTTCCCTATAGTGAGTCGTATTAGAG-CTTGGCGTAATC   583

Query    751   ATGGT-ATAGCTGTTTACTGTGTGAAAT   777
               ||||| |||||||||| |||||||||||
Subject  584   ATGGTCATAGCTGTTTTCTGTGTGAAAT   611
```

Fig. 2. Partial sequence of 16S rRNA gene of CBL-Hg2 (A), which showed 87% homology with *Pseudomonas aeruginosa* (AB 262513.1) (B).

Metal processing ability

Mercury processing capability of both the bacterial isolates was checked by adding Hg^{2+} at 100µg/mL in the culture medium (Fig. 4). *P. aeruginosa* could reduce 93% of mercury from the medium after 40 hours. The *P. aeruginosa* was also capable to remove Hg^{2+} (100 µg/mL) 35%, 55% 70% and 85% from the medium after 8, 16, 24 and 32 hours, respectively. *B. casei* could also efficiently process mercury from the medium, 80% mercury was removed from the medium after 40 hours. The organism removed 20%, 40%, 50%, and

Fig. 3. Growth curves of mercury resistance *Brevibacterium casei* and *Pseudomonas aeruginosa* in LB medium containing 50µg Hg^{2+}/mL after incubation at 37°C.
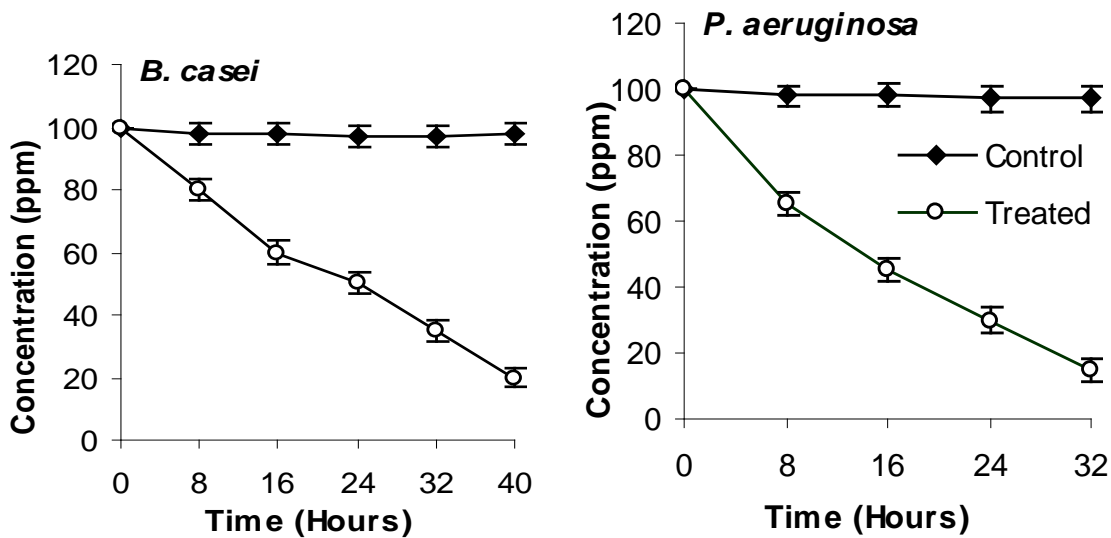

Fig. 4. Uptake of Hg^{2+} by mercury resistant bacterial isolates growing in Hg^{2+} containing medium. The control did not contain cells of the isolates.

65% Hg^{2+} from the medium after 8, 16, 24 and 32 hours, respectively.

Mercury uptake by bacterial isolates

The biosorption term has been used in the present study to indicate that the metal was removed by one or more of these processes. In general, there are two main mechanisms of mercury resistance. The first mechanism is enzymatic mercury detoxification, in which organic mercury compounds are degraded to form inorganic mercury (*e.g.*, mercuric ion). The mercuric ions are further reduced enzymatically to form metallic mercury, which subsequently volatilizes from the microorganisms to the atmosphere. The second mechanism is biosorption, in which metal ions adsorb onto cell surface or accumulate inside the cells. During the present investigation the

bioaccumulation of mercury for *B. casei* was observed 70% and for *P. aeruginosa* was 80% (Table V).

Table V.- **Percentage bioaccumulation of Hg^{2+} by bacterial isolates with initial concentration of 50.0 µg/mL of Hg^{2+} in the LB broth medium at their optimum temperatures.**

Percent bioaccumulation of Hg^{2+} after 24 hrs	Bacterial isolates	
	P. aeruginosa	*B. casei*
Supernatant (µg/mL)	10	15
Pellet (µg/mL)	40	35
% bioaccumulation	80	70

DISCUSSION

A number of microorganisms have evolved resistance mechanisms to deal with mercury compounds. Mercury resistance was first reported in *Streptomyces aureus* (Moore, 1960) and since then has been described in a number of bacterial species. One of the best defined mercury resistance determinants is the *mer* operon encoded by transposon Tn*501*, found in Gram-negative bacteria. The functions of the minimal number of proteins required to confer full resistance are as follows (Hobman and Brown, 1997): MerR is the *mer* regulatory protein which controls the expression of all the other proteins in the operon in response to the presence of Hg^{2+}. MerP, the periplasmic Hg binding protein, transfers Hg^{2+} to the MerT transport protein located in the cytoplasmic membrane. This passes mercuric ions to the cytoplasmic mercuric reductase, which reduces Hg^{2+} to Hg(0) using as NADPH as the reductant. Hg(0) is then lost from the cell in the gas phase.

In the present study growth rate of both bacterial isolates in the presence of Hg^{2+} was slightly slower as compared with that of non-treated (control) bacterial culture. Chang and Law (1998) described that specific mercury detoxification rate was dependent on cell growth phases as well as the initial mercury concentrations. This happened because of higher concentration of metals that probably poisoned essential biochemical reactions (Perego and Howell, 1997). Growth period was delayed when concentration of heavy metal was increased (Brady *et al.,* 1994).

In the present investigation Gram-negative bacteria, *B. casei* and *P. aeruginosa,* were found to be resistant to mercury at a concentration of 500 and 400µg Hg^{2+}/mL, respectively. Glendinning *et al.* (2005) reported that *Bacillus* sp. and *Bacillus cereus* RC607 were resistant to Hg^{2+} at 60µg/mL. Thiomersal biodegrading mercury resistant *P. putida* (Fortunato *et al.,* 2005) and growth promoting *P. fluorescens* (Gupta *et al.,* 2005) strain have also been isolated and characterized from other laboratories.

Silver and Phung (2005) reported that in addition to the MerA-mediated mechanism of Hg reduction other enzymes are also able to reduce Hg^{2+}. Essa *et al.* (2000) demonstrated three different mechanisms *viz.* enzymatic reduction to Hg$^{\circ}$ and volatilization, formation of insoluble HgS and biomineralization of Hg^{2+} as an insoluble mercury-sulphur complex other than HgS, for mercury detoxification in *Klebsiella pneumoniae* M426, which may increase the capture efficiency of mercury. Mercuric reductase reduces Hg^{2+} into Hg$^{\circ}$ in the presence of NADPH and a sulfhydryl compound. Hg$^{\circ}$ volatilizes out of the system due to its high vapour pressure (Ghosh *et al.,* 1996). Many bacteria belonging to the genera *Pseudomonas, Bacillus* and *Staphylococcus* have been reported to reduce Hg^{2+} to Hg$^{\circ}$ (Silver and Phung, 2005; Saha *et al*., 2006).

Bacterial plasmids encode resistance system for toxic metals including Ag^{2+}, As^{2+}, Cd^{2+}, Cr^{6+}, Hg^{2+}, Ni^{2+} and Pb^{2+} (Silver, 1998). With in various Gram-positive and Gram-negative bacteria Cd^{2+}, Cr^{6+}, Cu^{2+} and Hg^{2+} resistant genes have frequently been found on plasmids or transposable elements (Yureiva *et al*., 1997). Saha *et al*. (2006) reported that *Pseudomonas* strains UR2, PS4 and UR5 contained plasmid DNA and were highly resistant to mercury. Mercury resistance in these *Pseudomonas* strains may be plasmid determined. Nakahara *et al*. (1997) reported that in some cases resistance to certain antibiotics and metals is mediated by the same plasmid.

Microorganisms have a high surface area-to-volume ratio because of their small size and therefore provide a large contact area that can interact with metals in the surrounding environment

(Ledin, 2000). Clean-up of mercury containing wastewater by mercury resistant microbes is a simple, environmental friendly and cost effective alternative to current treatment technologies (Wanger, 2003).

During the present study both *B. casei* and *P. aeruginosa* showed good biosorption ability to uptake mercury from the medium *i.e.* 70% (*B. casei*) and 80% (*P. aeruginosa*). Further work is needed to know that what mechanism is employed by these bacteria after up-taking the mercury from the environment. The mercury resistant bacteria isolated during the present study showed high level of mercury resistance and accumulated substantial amount of Hg^{2+} from the medium and therefore may be applicable for the treatment of the wastewater.

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