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Introducing an Efficient Mercury-Resistant Bacteria (MRB) Which Can Be Used for Bioremediation Purposes

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ABSTRACT

Mercury is one of the most toxic metals which can lead to the irreversible damages on CNS and the other organs. As the various forms of this element are stable in the environment, many of the microorganisms have developed mercury resistance systems; therefore they can play a major role in the bioremediation of polluted sites. Thus the aims of this research are isolation and identification of the bacteria that are able to growth at the present of high concentrations of $HgCl_2$. In order to attain such biosorbent, all collected samples were cultured on modified Luria Bertani Agar medium (containing 10ppm $HgCl_2$). Then MIC, MBC and disc diffusion methods were used for selection of the most resistant isolate. Thereafter growth profile and biosorption mechanism were explored. Among the 87 screened isolates, the best isolate (CBS-H5) was selected. The obtained results determined that this isolate had the best quantity of MIC/MBC (400ppm). At the next stage, growth curve studies in the presence and absence of mercury stress (50ppm), didn't show any significant differences between two subset experiments. In addition, we investigated the biosorption mechanisms of this isolate and calculated the percent of removal efficiency (%RE). It was found that this isolate, was capable to biosorb 23.56 mg Hg/gdw from the medium with %RE equal to 94.2%. Compared to dead cells, living cells, were more effective. Based on the morphological, biochemical and molecular features, it was revealed that highly mercury resistant isolate (MRB) was belonged to the *Enterobacter* genus and deposited as accession JQ965667 in the Gene Bank database.

KEY WORDS: Heavy metal, mercury, microorganism, growth profile, biosorption

INTRODUCTION

Among the 90 natural elements, there are 21 non-metals, 16 light elements and 53 heavy metals [26]. Heavy metals are rare elements which have density at least 5 times more than water and introduce to the environment during the physicochemical erosion of soils and igneous rocks, volcanic actions and etc. [15, 34]. Some cations of heavy metals such as Hg^{2+}, Cd^{2+} and Ag^{2+} are capable to form strong toxic complexes which cause them to be dangerous for any physiological functions [24]. Some metals such as Pb^{2+}, Cd^{2+} and Hg^{2+} may be induce oxidative stress through replacement with metals which are naturally exist at cellular binding sites [24]. Heavy metals toxicity can cause decrease of mental actions or central nervous system disorder, energy loss, damage of blood composition, lungs, kidneys and other vital organs[34]. Therefore the intracellular concentration of heavy metals' ions must be controlled tightly [24].

Among these metals, mercury is a liquid metal in room temperature [37]. This element is one of the most toxic heavy metals along with cadmium and lead. Mercury exists in three important forms: pure, inorganic (such as mercury nitrate) and organic (such as phenyl mercury propionate) element [33]. Unlike other dangerous organic compounds, mercury can not change to harmless form and different forms of mercury are stable in natural environments [37]. Because of this ability of mercury, it has been proven that mercury can accumulate at different proportions in food chains; therefore make various problems for human [22].Uptake of mercury in humans occurs mainly through breathing or by eating contaminated foods and on a lower scale by skin [37]. Acute and chronic toxicity with mercury compounds can cause irreversible physical damage to kidneys, lungs, spinal cord and central nervous system [2]. Because this element can easily pass through blood-brain barrier, the most toxic effect of mercury is created on brain [31]. Therefore, this matter has transformed mercury to a neurotoxical metal which has diverse effects on the brain cellular functions [37]. The reason of these disorders is related to this fact that mercury can attach to sulfhydryl groups of enzymes and proteins and therefore inhibit vital functions of the cells [42]. Besides natural sources, mercury distribution is resulted from anthropogenic activities such as agriculture,

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mining and fossil fuels combustion [2, 42]. Conventional remediation processes using for remediation of polluted sites are expensive and frequently could be leading to remobilization of toxic mercury compounds[33]. Thus, nowadays, biological-based technologies which utilizing natural materials with biological origin such as bacteria, fungi, yeasts, algae and etc., have attracted the most attention in order to cleaning up the contaminated environments [39]. Consequently, finding resistant microorganisms as biological tools, introduce promising technologies because many of microorganisms (especially bacteria), have developed mercury-resistance systems and using them can play an important role in removing of environmental pollutions to mercury [37].

MATERIALS AND METHODS

Primary screening of the most Hg^{2+}-resistant isolates:

In order to isolation of the most resistant bacteria to mercuric ion, wastewater and sludge samples were collected from Amir Kabir, Farsit and Carbon black plants of Khouzestan province, Iran, and immediately transferred to the laboratory on ice without freezing [23]. 0.1ml of each serial dilution(10^{-1}-10^{-5}) from collected samples, were spread on mLBA medium [containing (per liter): 5.0g yeast extract, 10.0g bacto-tryptone and 15.0g agar; Merck, Germany], pH 7.0, containing 10ppm $HgCl_2$(Merck, Germany). Incubation was performed aerobically at 30°C for 24-48hr and discrete colonies were selected for further studies after purification on nutrient agar (NA) medium (Merck, Germany) [10, 19].

Screening of resistant isolates to high concentrations of $HgCl_2$:

All gathered isolates in previous stage, were introduced to mLBA medium with high concentrations of Hg^{2+} ranging from 20-140ppm in separately levels. Incubation was done aerobically in 30°C for 24hr. Representing colonies on medium containing high Hg^{2+} concentration were selected for next studies [1].

Qualitative assessment of Hg^{2+} resistant levels for selection the best isolate:

Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and disc diffusion methods used for selection of the most resistant isolate.

MIC and MBC determination:

The MIC was defined as the lowest concentration of metals, metalloids or antibacterial agents that inhibit growth of a microorganism. In this study, for determination of MIC, 100µl of bacterial suspension of each isolate (set to McFarland Standard No.0.5) was inoculated in mLB broth medium containing various concentrations of Hg^{2+} ranging from 25-800ppm. These tubes incubated in 30°C for 24-48hr and 150rpm and the amount of Optical density (OD) of them was measured at 600nm. Ultimately, tubes which have no sign of growth considered as MIC. In the next stage, MBC was determined by culturing of 50µl inoculum from tubes without any growth on mLBA medium. After incubation time, those plates that didn't show any colony, considered as MBC [13, 41].

Disc diffusion method:

In order to analysis of toxicity effect of any antibacterial agent (e.g. toxic concentration of Hg^{2+} in this study), this test was done on selected isolates. For this mean, after introduction of blank disc in 25-800ppm of $HgCl_2$ at a given time, 50µl of bacterial suspension were spread on mLBA medium (without metal). After incubation at 30°C, the inhibition zone was measured. All tests were done in three times [23, 28].

Growth kinetic of test isolate:

In this stage, flasks which having 50ml mLB broth medium were utilized for two sub-set experiments, in triplicates:1. challenging with 50ppm Hg^{2+}; 2. without any metal

The inoculum was consisted of 1ml of bacterial suspension that growth overnight, aerobically. Over the period of incubation time, growth was monitored by absorbance measuring at 600nm using spectrophotometer (Analytikjena, Germany) until stationary phase was reached [1, 4, 8, 9].

Evaluation of biosorption capacity:

Measurement of bio-removal ability of resting bacterial cells:

This stage was performed for determination of quantitative ability of Hg^{2+}-resistant isolate. At first, the best strain was cultured aerobically in mLB broth and incubation was continued at 30°C at 150rpm until mid-exponential phase was reached. Then, centrifugation was performed at 4°C, 6000rpm and 15min and harvesting cells washed twice with double-distilled water. The biosorption capacity of resting cells was investigated by re-suspension of about 0.4g (dry weight) from obtained cells in 40ml of Hg^{2+} solution(50ppm). At this stage, incubation was also done at 30°C, 150rpm until equilibration was reached [5, 17,36].

Study of metal removal mechanism by selected isolate:

Determination of bioremoval mechanisms and comparison of these mechanisms, were performed by resting cells in both metabolically active and inactive biomasses. The process of inactivation was done by:

1. Heating at 100°C, overnight 2.Autoclaving at 121°C for 15min.

These biomasses were challenging with Hg^{2+} solution, separately, in triplicates as described above [3, 38].

Computing the amount of biosorbed metal:

In order to achieve this object, residual amount of Hg^{2+} in the supernatant, was measured using atomic absorption spectrophotometer (SAVANtAA A7104, Australia) at 253.70 nm. The fraction of biosorbed metal on the cells was calculated by the following equation (1):

$$\text{Metal uptake} = V(C_i - C_f)/S \quad (1)$$

In the given formula C_i, C_f, V and S considered as: initial metal concentration (mg/l), final metal concentration (mg/l), volume of reaction (l) and total biomass (g), respectively. Bacterial pellets laid overnight at 100°Cto measuring the dried biomass weight. It should be noted that before starting each stage of the test samples measurement, standard curve of Hg^{2+} sorption was drawn by metal solution containing 10, 20, 50, 70 and 100ppm of Hg^{2+} [25, 40].

Metal Removal Efficiency:

This parameter considers as a comparison between absorbed metal and initial metal concentration and was calculated by following equation (2):

$$Hg^{2+} \text{ removal efficiency } (\%) = (C_i - C_f) \times 100 / C_i \quad (2)$$

Where, C_i and C_f represented initial and final metal concentration (mg/l), respectively [38].

Statistical analysis:

The differences amount and the meaningful level of samples were analyzed for the measurement of OD_{600} and inhibition zone assay stages, using one-way anova test (ANOVA), SPSS software, 19 version. Gathered data from MIC, MBC, %RE and the Study of removal mechanism studied by Chi-Square test (0.95 confidence level).

Characterization of selected isolate:

Morphological characterization:

The cellular morphology was determined by light microscopy on an OLYMPUS BX51 microscope (Japan).

Biochemical identification:

This isolate was checked out and characterized by several physiological key conventional tests for basic differentiation of bacteria according to Bergey's manual of bacteriology [39].

16SrRNA amplification:

Using a pure culture from a single colony of the test bacterial strain,genomic DNA was prepared according to the DNA extraction Kit (CinnaGen, Iran) [10, 30]. Bacterial 16SrRNA was amplified using universal 16SrRNA primers, F and R. Sequence of each primer are:

F-primer sequence: 5'- CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG- 3'

R-primer sequence: 5'- CCCGGGATCCAAGCTTACGGTTACCTTGTTACGACTT- 3'

The PCR mixture (25µl) contained: 1µl template, 2.5µl of 10 × Taq DNA polymerase buffer (CinnaGen, Iran), 1µl of $MgCl_2$ (50mM) (CinnaGen, Iran), 1µl of dNTP at 10mM (CinnaGen, Iran), 0.3µl of 1.5unite Taq polymerase (CinnaGen, Iran), 0.5µl of each primer (25µmol). PCR was performed according to Jiang et al. [1]. PCR products were analyzed by agarose (CinnaGen, Iran) gel electrophoresis[30]. The obtained sequence was subjected to nucleotide BLAST and the novel sequence was deposited to GenBank database. Phylogenetic analysis was done by neighbor joining method by MEGA 4.0 software [34].

RESULTS

Screening of the best Hg^{2+}-resistant isolates:

The results of the first screening stage showed that 87 isolates could growth at the presence of 10ppm $HgCl_2$. These isolates were used to next screening stage in which the levels of Hg^{2+} concentration increased to 20-140ppm, gradually. Therefore the number of Hg^{2+}-resistant isolates decreased to 7 isolates that utilized for the next experiments.

Determination of Hg^{2+} toxicity effects on bacterial isolates:

The effects of Hg^{2+} cations on bacterial isolates were concluded from the results of MIC, MBC and disc diffusion methods.

According to Table 1, CBS-H5 has the highest MIC which this competency was confirmed using chi-square statistical analysis: CBS-H5 isolate has meaningful difference along with other isolates ($X^2_{0.05,2}$>5.991). But MBC results didn't show any meaningful differences between all isolates ($X^2_{0.05,2}$<5.991).

Table 1. MIC and MBC of 7 bacterial isolates

Test / Isolate name	MIC (ppm)	MBC (ppm)
CBW-H1	50	50
CBW-H2	25	25
CBS-H3	50	50
CBS-H4	200	400
CBS-H5	400	400
CBS-H6	200	400
AMW2-H7	50	50

All data was taken as the average of three experimental results

As we noted above, investigation of Hg^{2+}inhibitory effect on the seven top isolates, was studied by inhibition zone assay (table 2). At this stage, it was not observed any meaningful differences among the three repetition of growth inhibition zone assay results of all isolates using statistical study (P>0.05) (Fig. 1). Therefore, the best isolate was selected here according to diameter of created inhibition zone.

Table 2. Inhibition zone assay of selected bacterial isolates[1]

	Inhibition zone (mm)						
Concentration (ppm) / Isolate name	800	400	200	100	50	25	0
CBW-H1	11.3	9.7	9.3	9	-	-	-
CBW-H2	15.3	14	12	10.3	10	8.7	-
CBS-H3	14.6	13	11.3	10	9.3	9	-
CBS-H4	11	9.6	9.6	9	8.6	-	-
CBS-H5	9.7	9.7	8.7	8.7	-	-	-
CBS-H6	11	9.6	9.3	9.3	-	-	-
AMW2-H7	18.7	16.7	12.7	11	9.7	9	-

[1]All data was taken as the average of three experimental results

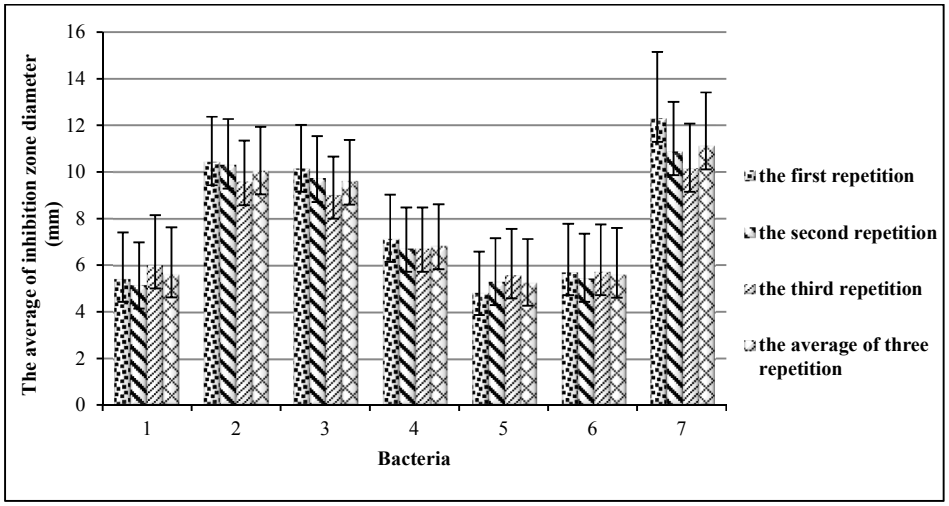

Fig.1. Statistical comparison of inhibition zone assay results among the all studied strains in MIC determination stage (confidence coefficient: p<0.05). 1: CBW-H1; 2: CBW-H2; 3: CBS-H3; 4: CBS-H4; 5: CBS-H5; 6: CBS-H6 and 7:AMW2-H7.

The results of optical density measurement of 7 isolate have showed in Table 3.

Table 3. Optical density of Hg^{2+}-resistant isolates at the stage of MIC determination[1]

Concentration (ppm) / Isolate name	OD_{600}						
	800	400	200	100	50	25	0
CBW-H1	0.0492	0.0713	0.0414	0.0552	0.0472	0.0411	1.2421
CBW-H2	0.0350	0.0354	0.0241	0.0394	0.0469	0.0396	1.6954
CBS-H3	0.0471	0.0916	0.0772	0.1020	0.1063	0.0796	1.5818
CBS-H4	0.0530	0.0395	0.9436	1.3097	1.0158	1.0124	1.1938
CBS-H5	0.0618	0.0815	1.3718	1.2782	1.1606	1.1474	1.2719
CBS-H6	0.0493	0.0361	0.9462	1.3406	1.2902	1.1470	1.2095
AMW2-H7	0.0405	0.0743	0.0550	0.0733	0.0741	0.0620	1.4493

[1]All data was taken as the average of three experimental results

Statistical analysis of OD_{600} determination results showed that there was meaningful differences between the gathered data of three experimental repetition for CBS-H4, CBS-H5, CBS-H6 isolates ($P<0.05$) (Fig. 2).

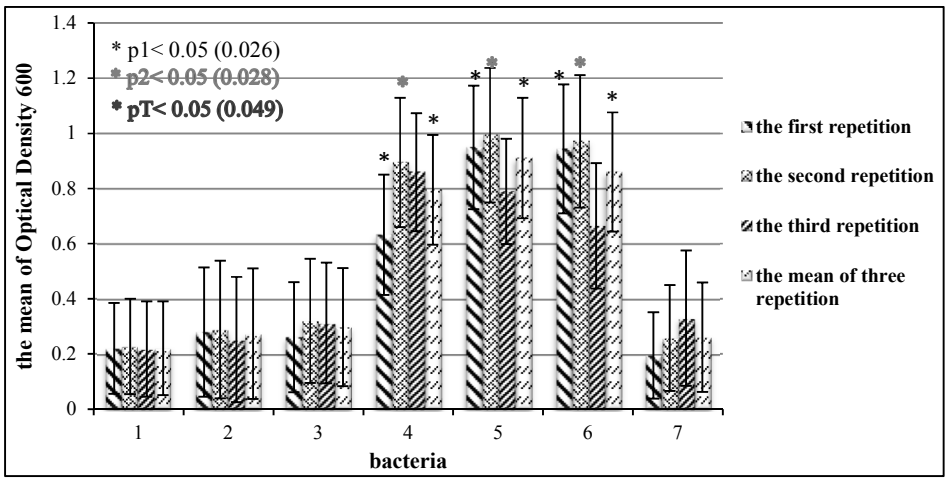

Fig.2. Statistical comparison between OD_{600} of all selenate-resistant isolates (confidence coefficient: $p<0.05$).1: CBW-H1; 2: CBW-H2; 3: CBS-H3; 4: CBS-H4; 5: CBS-H5; 6: CBS-H6 and 7: AMW2-H7.

Finally, the overall data and results showed that among 7 isolates, only CBS-H5 isolate has maximum MIC and MBC (equal to 400ppm) and minimum zone in 25-800ppm of metal concentration (Table 1 and 2). Therefore this isolate utilized for next experiments. In this regard, OD curve of CBS-H5 isolate has shown in Fig. 3.

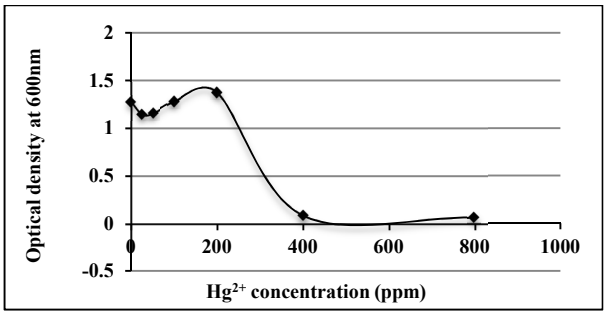

Fig.3. Optical density curve of CBS-H5 isolate

The results of growth pattern study:

Growth curve was drawn by the measurement of OD_{600}at predefined intervals until it was entered to stationary phase (Fig. 4).

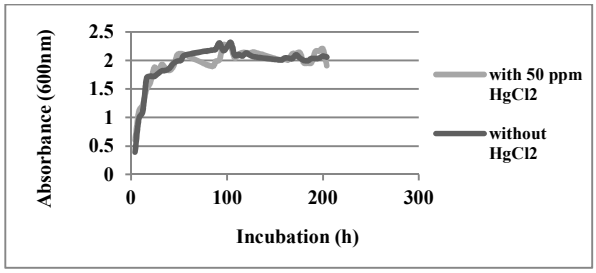

Fig.4. Growth curve of CBS-H5 isolate

Biosorption studies:

In this study, 50ppm of Hg^{2+} solution was interacted with pre-grown mid-exponential bacterial cells. The standard curve of Hg^{2+} adsorption has shown in Fig 5. The biosorption and %RE results are shown in Table 4 and Fig 6, 7**.**

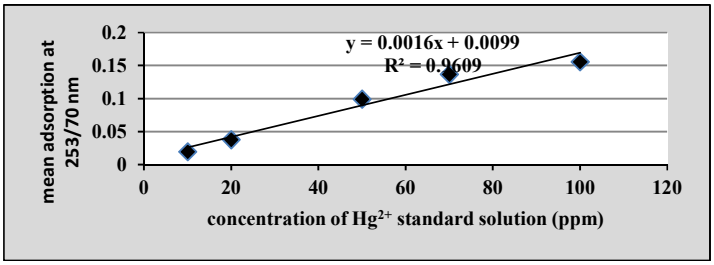

Fig.5. Standard curve of Hg^{2+} adsorption

Table 4: Adsorption capacity and Metal removal efficiency of selected strain[1]

Type of cell / parameter	Blank (Living cells)	Cells treated by autoclaving	Cells treated by heating at 100°C
Hg^{2+} Adsorption Capacity (mg/gdw)	23.56	19.75	22.06
Metal Removal Efficiency (%)	94.2%	78.9%	88.24%

[1]All data was taken as the average of three experimental results

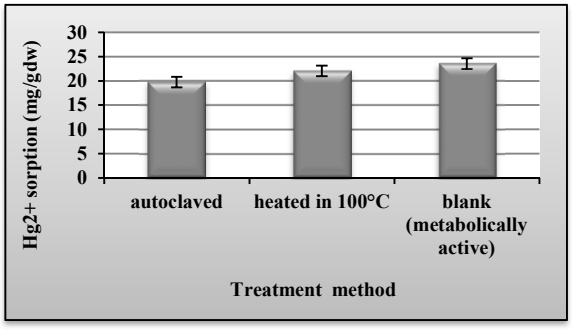

Fig.6. Mercury sorption capacity of active and inactive biomasses of CBS-H5 isolate

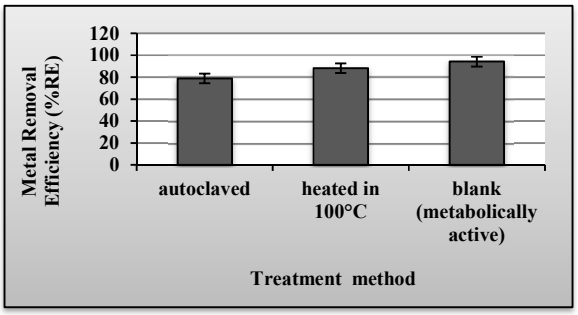

Fig.7. Mercury removal efficiency of active and inactive biomasses of CBS-H5 isolate

Chi-square test analysis of adsorption capacity and %RE results didn't show any meaningful differences between all inactivation treatment method and metabolically active biomass ($X^2_{0.05,2}<5.991$). Thus it could be said that this isolate don't have any preference for using biosorption or bioaccumulation to removal of metal.

Morphological and biochemical characterization of the selected isolate:

One bacterial isolate namely CBS-H5 was isolated from carbon-black industry sludge of Ahwaz, Iran. The preliminary characterization of this isolate was done on the basis of its morphology and gram stain (Fig. 8). This isolate was Gram-negative short bacilli. Biochemical characterization was done in terms of different biochemical abilities according to Bergey's manual (Table 5).

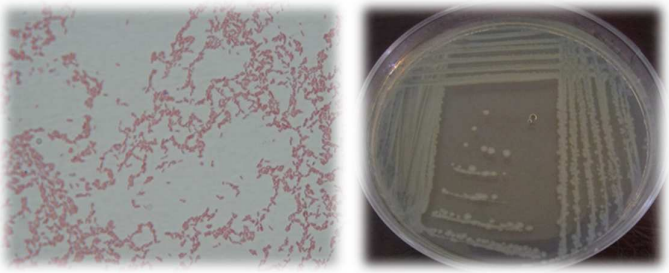

Fig.8. Microscopic and morphological shape of the best isolate

Table 5. Morphological and biochemical characteristics of CBS-H5

Cell morphology	Gram reaction	Catalase activity	Oxidase activity	Oxidative/Fermentative (OF)	Urease activity	Hydrolysis of:		Utilization of:							Growth feature on MacConkey	Methyl red test	V/P test	Nitrate reduction	Reaction in SIM medium	Reaction in Triple Sugar Iron (TSI) agar
						Starch	Gelatin	Citrate	Glucose	Xylose	Lactose	Arabinose	Sorbitol	Manitol	Maltose						
Short rod	negative	+	-	+/+	+	-	-	+	+	+	-	-	+	+	+	Lactose Positive	+	-	+	Motility - Indole - H_2S -	Alkaline/Alkaline

Molecular characterization and nucleotide sequence accession number:

DNA of the promising isolate was extracted and amplified; the produced amplicons was analyzed using agarose gel electrophoresis as shown in Fig. 9. The BLAST database (http://www.ncbi.nlm.nih.gov/BLAST/) was used to make sequence comparisons and a neighbor-joining tree was constructed with MEGA 4.0 software (Fig. 10). The 16SrRNA gene sequence was deposited as accession JQ965666 in the GenBank database.

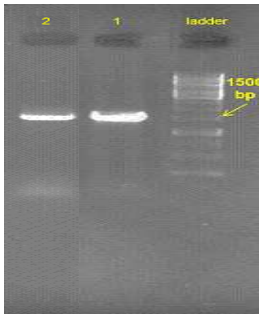

Fig.9. Result of electrophoresis: 1. Control positive; 2. CBS-H5 isolate

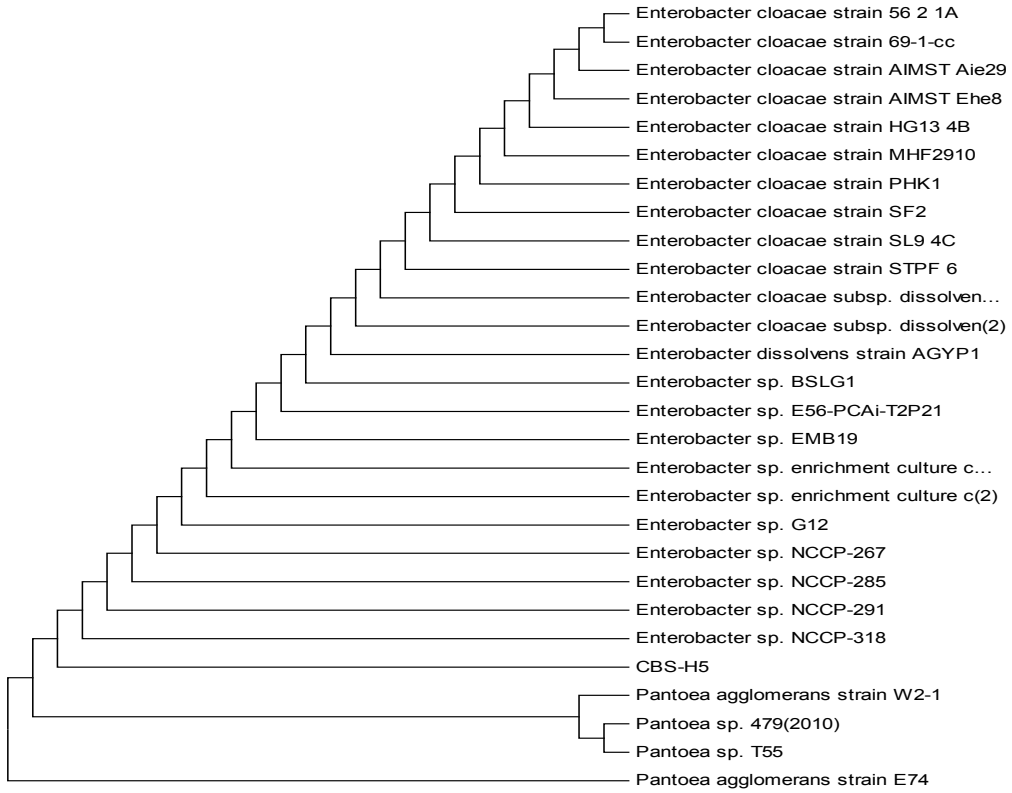

Fig.10. Phylogenetic relationships among representative experimental strain (CBW-S1) and the most closely related *Enterobacter* sp.. The dendrogram was generated using MEGA 4.0 program

DISCUSSION

While toxic effects of mercury have been proved for centuries, environmental pollution to this element arises from natural and anthropogenic activities, yet. This matter can cause universal pollution including lithosphere, hydrosphere, atmosphere and biosphere on a global scale[6,22]. At all environmental backgrounds (sediment/soil/biota/water), this contamination is due to this fact which a sets of chemical reactions cause to cycle different redox forms of mercury in the environment. Therefore, several complex compounds and forms of mercury are found in these situations [43]. In such environments, also even in uncontaminated sites, we can find mercury-resistant bacteria (MRB) which have been developed resistant systems. These resistant-determinants have been found in both gram-positive and negative bacteria and mainly attributed to *mer* operon that provides the ability of enzymatic reduction of Hg^{2+} to metallic mercury for resistant-bacteria [6, 42].

As we noted above, in this study we gathered 87 isolates at the first screening stage. There are various methods for selection of more resistant isolates toward multiple antimicrobial agents, toxic

substance and heavy metals in literatures. Therefore in order to access more resistant isolates, we used MIC, MBC and Disc diffusion methods.

It should be noted that in the field of bacterial resistance to the mercuric compounds, there are great differences between literatures. For example, in our study, the highest amount of MIC and MBC (400ppm) was gathered for CBS-H5 isolate.

On the other hand, at some literatures, the threshold of bacteria against mercury was very low. For example, in the study of Figueiredo et al., the results of micro dilution broth method for MIC determination showed that their isolates exhibited MIC values from 0.16 - 140µg/ml for Hg^{+2}. Moreover, it is worth nothing to say that the most resistant isolates (from aerobic, anaerobic and SRB bacteria) were isolated from sediments which proved that mercuric compounds are distributed in all environments [11]. In the study of Ruiz-Diez et al. (2012), the highest MIC level of the most tolerant isolates was reported as ≥12.5µM [32]. François et al. (2012) used from microdilution method for MIC determination at the presence of 2µM-1mM $HgCl_2$. Gathered results showed that 105 strains showed tolerance to 10µM $HgCl_2$. Moreover, 7 strains revealed higher tolerance level in the 20-100µM $HgCl_2$ and enhanced mucoid characteristic (as an indicator to produce EPS for mercury sorption) when grown on the suitable medium. Study on the biosorption capacity by killed and living biomass of isolates showed that killed biomass have more sequestration capacity (40-120 mg/gdw) and it have higher biosorption capacity than live bacteria (1-2 mg/gdw) [12]. In the scope of susceptibility determination of isolates to mercury toxicity, Giriby using direct culture method and metal dilution in liquid medium, gathered 20 mercury-resistant isolates which among of them *Pragia fontium* had highest MIC equal to 100ppm [14]. Moreover, the method of dilution on solid medium, utilized by many researcher e.g. Priyadarshini. He introduced the four most resistant isolates which in the range 1.5625-200ppm had MIC equal to 25ppm and the lowest zone diameter [29]. In the study of Dzairi et al., published data showing that *Klebsiella pneuomoniae* and *Pseudomonas aeruginosa* have the most mercury resistance level which is equal to 2400µM[10].

Pepi et al. (2013) published that by testing mercury resistance of isolated bacteria in the range 2.715-81.449 ppm, *Psychrobacter* sp. ORHg1 showed the highest resistance (27.150ppm) to this compound. Moreover, these researchers reported that the capacity of *Psychrobacter* sp. ORHg1, *Pseudomonas* sp. ORHg4 and *Pseudomonas* sp. ORHg5 to volatilize $HgCl_2$, was the most within 5-10 minutes of the contact time between the selected bacterial biomass and metal stress [27].

On the other hand, some published data have been reported higher resistance levels. For example, Five strains of *Bacillus cereus* in the study of Kannan and Krishnamoorthy (2006) showed the high resistance level to $HgCl_2$ (~500ppm). They reported that this resistance level may appear to higher than some isolated bacteria which obtained from aquatic ecosystems [21]. Irawati et al. (2012) used from two resistant bacterial isolates (*Brevundimonas* sp. HgP1 and *Brevundimonas* sp. HgP2) which have MIC equal to 575ppm of $HgCl_2$. Their studies the effect of mercuric toxicity on the growth and morphological changes of the selected isolates. Besides, strain HgP1 showed the accumulation capacity up to 1.09 and 2.7mg/gdw and the removal efficiency of 64.38 and 57.10% of mercuric ions from the metal solution containing 50 and 100ppm $HgCl_2$, respectively [18].

This various behavior of bacteria to mercuric toxicity which resulting in differences in MIC, depends upon several factors like (i) diffusion rate (ii) composition of used medium (iii) complexation and (iv) availability of metals to the bacteria [21].

This subject has been widely reported that natural material such as microorganisms are inexpensive, therefore, this feature is a great advantage (such as rapid production of microbial biomass, simple requirements of nutrients, high biosorption capacity of pollutants, recovery of valuable metal ions from the biosorbents, microbial adaptation to toxic levels of heavy metals and etc.) for using them for removal and accumulating heavy metals from contaminated environments [2]. In the other hand, various microbial mechanism for mercury detoxification have introduced such as biotransformation, bioprecipitation and biosorption [7]. Among them, biosorption has studied extensively and has reported that there are two biosorption mechanisms: inactively and actively [2].According to this information, herein the biomass of selected isolate encountered with metal solution to determine the biosorption mechanism.

As we said earlier**,** after living cells, those cells which treated by heating in 100ºC has the most efficiency in Hg^{2+} biosorption from medium. However, Serinath et al. observed that Cr(VI)-biosorption by the dead cells was higher than the living cells and it increased significantly ($P<0.001$) to 39.9 and 30.7 mg Cr/gdw [35]. They said this increase in biosorption capacity was due to this fact that dead cells have adapted to the conditions of pH [35]. According to Halttunen, dual behavior of different treatment methods using EDTA, heat, salts, acids and different organic solvents may be due to that these methods cause weight loss of biomass. In other words, this contradictory behavior is associated with reduced metal binding capacity (as the time of the binding sites destruction) or increased metal binding capacity (it is a

consequence of partial degradation of cell wall and therefore production new binding sites in its surface) [16]. Junlian et al. reported that severity of the cell surface destruction can effect on the biosorption capacity of biomass. It is due to this phenomenon that interaction between cell surface anionic/ cationic groups resulted in the biosorption of heavy metals ions. For this reason, various treatment methods have the different biosorption efficiency [20].

With regard to different tolerance value between introduced isolates in our study and other published data, it should be noted that this diversity in metal tolerance and sorption capacity could be explained by different origin of isolation sites (wastewater or sediments), the type of studied strains and etc. therefore, it is suggested that comprehensive studies perform to evaluate the various resistant microorganisms from different sources and challenge them by multiple compounds and chemical forms of heavy metals (especially mercury).

With considering attained results of this study, can introduce CBS-H5 isolate as a possible efficient biosorbent with MIC and MBC equal to 400ppm, high sorption capacity and metal removal efficiency, which expected to be helpful in removing mercury contamination from environment.

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