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Isolation and Characterization of Zinc Tolerant Bacteria from Contaminated Sediments and Soils in Egypt

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ABSTRACT

A total of 71 zinc tolerant bacteria were ,isolated from Four heavy metals contaminated sediments and soils in Egypt, three sediment samples (Upstream, midstream, downstream Al-Rahawy drain, Giza Governorate) and two agricultural soil samples (Al-Gabal Al-Asfar region and Kafr-Ilwan village, Qalubia Governorate). The minimum inhibitory concentration (MIC) and maximum tolerance concentration (MTC) under zinc (Zn^{+2}) concentrations ranged between 500-4500 mg/l. The most zinc tolerant isolate UR45 with MIC 4500 mg/l and MTC 4000 mg/l was identified by partial sequence of 16S rRNA genes as *Alcaligenes faecalis* MG257493.1 (UR45). Tolerance of *A. faecalis* to cadmium (Cd^{2+}), copper (Cu^{2+}) and lead (Pb^{2+}) under different concentrations (1000-4500 mg/l) was evaluated. Growth of the strain and its biosorption activities (metal uptake, metal residual, metal biosorption) at 1000 mg/l of Zn^{2+}, Cd^{2+}, Cu^{2+} and Pb^{2+} under different pH values (5-9) was studied. pH 8 was optimum for growth in media supplemented with Zn^{2+} or Cu^{2+}. Whereas, the biosorption potentials were differed according to the examined metal. Finally, enzymatic (catalase, peroxidase, polyphenol oxidase) and non-enzymatic (inhibition 2,2-DiPhenyl-2-Picrylhydrazyl hydrate (DPPH)) antioxidant activities of the *A. faecalis* MG257493.1 in presence of four heavy metals individually or in mixture at 1000 and 1500 mg/l were estimated. The obtained data showed that most antioxidant activities of the strain was increased with the increasing of heavy metals concentrations up to 1500 mg/l.

Keywords: Heavy metals, zinc, *Alcaligenes faecalis*, biosorption, antioxidant activities.

Introduction

Contamination of sediments and soils with heavy metals is a major environmental problem all over the world. These inorganic pollutants are released by effluents generated from various industries **(Ezzouhri *et al.*, 2009)**. Even essential biological trace elements, such as Zn^{2+}, can be toxic at high concentrations **(Fang *et al.*, 2016).** Heavy metals in soils cannot be mineralized or broken down to less toxic forms **(Chen *et al.*, 2014)**. A large proportion of heavy metals are generally bound to organic and inorganic soil components or exist as insoluble precipitates. Therefore, developing appropriate strategies for the remediation of heavy metal contaminated soils demands urgent attention from the perspectives of environmental conservation **(Aboushanab *et al.*, 2006)**. In naturally polluted environments, the microbe's response to heavy metals toxicity depends on the concentration and the availability of metals and on the action of factors such as the type of metal, the nature of medium and microbial species **(Ezzouhri *et al.*, 2009)**.

Generally, the contaminated sites are the sources of metal resistant microorganisms **(Vadkertiova and Slavikova, 2006)**. Similar findings of occurrence of Zn^{2+} metal resistant bacteria in contaminated soils, wastewater effluents, river water and sediments have been reported **(Ahemad and Malik, 2012; Jackson *et al.*, 2012; Mgbemena *et al.*, 2012)**. Additionally, microbial biomass have evolved various measures to respond to heavy metals stress via processes such as transport across the cell membrane, biosorption to cell walls, entrapment in extracellular capsules as well as precipitation and transformation of metals **(Malik, 2004)**. Also, he proved that microorganisms isolated from contaminated sites have an excellent ability of removing significant quantities of metals both from different environments. Therefore, it is important to explore microorganisms from contaminated sources for the bioremediation of heavy metals since conventional processes such as chemical precipitation; ion exchange and reverse osmosis are uneconomical and inefficient for treating effluents of dilute metal concentrations **(Gavrilesca, 2004)**. Heavy metal resistant bacteria have been demonstrated to exhibit high metal biosorption or bioaccumulation capacity in the laboratory setting **(Bautista-Hernández *et al.*, 2012)** and some heavy metal resistant strains have been successfully applied in remediating contaminated sites elsewhere in the developed world **(Ansari and Malik, 2007)**.

Free radicals can be generated under different stress conditions included heavy metals in the form of reactive oxygen species (ROS), such as superoxide anion radicals (O^{2-}), hydroxyl radicals (OH^{1-}), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). These ROS are an entire class of highly reactive molecules derived from the normal metabolism of oxygen or from exogenous factors and agent **(Chung *et al.* 2006).** Antioxidants are compounds formed inside the cell to preserve it from oxidative damage of

DNA, protein, lipid, and other molecules caused by ROS **(Heo *et al*., 2006).**

The aim of this study was to isolate, identify and characterize zinc tolerant bacteria from heavy metals contaminated sites in Egypt. MIC and MTC of heavy metals (zinc) for bacteria were determined and selected of the most resistant strain. Also, study the other heavy metals tolerance (Cd^{2+}, Cu^{2+}, Pb^{2+}) and the biosorption potential of the selected isolate. Moreover, study the antioxidant activities which might be useful in heavy metals bioremediation of the environment.

Materials and Methods

2.1. Collection of samples

Sediment samples were collected from three heavy metals contaminated sites upstream (UR), midstream (MR) and downstream (DR) of Al-Rahawy drain, Giza Governorate, Egypt and two agricultural soils irrigated with waste water were collected from Al-Gable Al-Asfar region (GS) and Kafr-Ilwan village (KI), Qalubia Governorate, Egypt. All samples were stored at 4ºC until analysis.

1.2. Heavy metals analysis

This method is described by **(Burau, 1982).** The concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} in the final solutions were determined with an atomic absorption spectrophotometer (Buck Model 210 VGP)**.**

Table 1. Heavy metals concentrations in sediment and soil samples

Heavy metals		Samples				
		UR	MR	DR	GS	KI
Cd^{2+}	**mg/l**	0.431	0.035	0.001	4.44	3.28
Cu^{2+}		0.411	0.170	0.013	5.85	0.70
Zn^{2+}		0.224	0.195	0.033	4.63	3.20
Pb^{2+}		0.070	0.005	0.004	0.25	0.15

UR: Upstream Al-Rahawy drain sediment MR: Midstream Al-Rahawy drain sediment
DR: Downstream Al-Rahawy drain sediment GS: Al-Gable, Al-Asfar
KI: Kafr Ilwan soil

2.3. Isolation of zinc of tolerant bacteria

The isolation of zinc tolerant bacteria was carried out on Muller-Hinton broth medium (HIMEDIA Co., Germany) supplemented with 250 mg/l Zn^{+2} (1.10g/l) as $ZnSO_4.7H_2O$ (HIMEDIA Co., Germany) **(Liu *et al*., 2004).** 150 ml Erlenmeyer flasks containing 90 ml of the previous media were inoculated with 10g of each sediment or soil samples and incubated at 30°C±2 for 72 hr. under shaking (150 rpm/min.). Enriched cultures showed turbidity after 3 days of incubation were diluted up to 10^{-6} using sterile distilled water, then subculture onto Petri dishes containing the same solidified medium. Appearing colonies on dishes and differing in shape, color and margins were purified and maintained on the previous medium and kept at 5ºC for further study **(Moghannem *et al*., 2015).**

2.3. Minimum inhibitory concentration (MIC) and maximum tolerance concentration (MTC) for zinc

MIC and MTC of zinc for 71 bacterial isolates were determined using agar plate dilution method as described by **Malik and Jaiswal (2000).** Muller-Hinton agar medium amended with different concentrations of Zn^{2+} namely, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500 mg/l were used. Each isolate was streaked individually on Petri dishes containing the abovementioned medium and then incubated at 30°C±2 for 72 hr. MIC is the lowest concentration that completely inhibits the visible growth of microorganisms while, the highest concentration which bacterial isolates able to grow was considered as MTC. **(Banerjee *et al*., 2015)**.

2.4. Identification of the most zinc tolerant bacterial isolate

The highest zinc tolerant bacterial isolate was selected for identification. Total DNA extraction was carried out by CTAB method **(Purohit *et al*., 2003)**. Bacteria were identified by partial sequence of 16S rRNA gene following procedures reported earlier **(Narde *et al*., 2004)**. A 1466bp product was amplified using 16S rDNA using primers:

Forward primer:

5′-AGAGTTTGATCMTGGCTCAG-3′

Reverse primer:

5′-CGGYTACCTTGTTACGACTT-3′

Samples were identified through BLAST analysis of the partial sequences and deposited in NCBI GenBank. The sequences are deposited at GenBank for accession numbers. PCR products (1.5 kb) of 16S rRNA genes were used for DNA sequencing. Sequences of related bacteria with greatest similarity to the 16S rRNA sequence of the Selected bacterial isolate were extracted from nucleotide sequence databases and aligned using CLUSTAL W (1.81) Multiple Sequence. A phylogenetic tree was constructed using the neighbor-joining distance method with the MEGA4 software **(Tamura *et al*.,**

2007) and the reliability of the bootstrap consensus inferred from 1000 replicates. The 16S rRNA gene sequences of the bacterial isolate reported in this paper were deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with the Accession numbers: UR45: BankIt2056333 Alcaligenes MG257493.1

2.5. Tolerance of the potent strain for Cd^{2+}, Cu^{2+} and Pb^{2+}

The most zinc tolerant strain was grown on Muller-Hinton agar medium containing different concentrations of three heavy metals (HIMEDIA Co., Germany) in addition to zinc as follow: cadmium at 1000, 1500, 2000 mg/l as $CdCl_2$; lead at 1000, 2000, 3000 mg/l as $Pb(CH_3COO)_2$, copper at 1000, 1500, 2000 mg/l as $CuSO_4$ and zinc at 2000, 3000, 4000 mg/l as $ZnSO_4$; to find out the most zinc tolerant against the above mentioned metals **(Congeevaram *et al.*, 2007).**

2.6. Effect of pH on growth and heavy metals biosorption potential for the most zinc tolerant isolate

The most zinc tolerant isolate was grown in Muller-Hinton broth medium individually supplemented with four heavy metals Zn^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+} at 1000 mg/l and adjusted to different pH values (5.0, 6.0, 7.0, 8.0 and 9.0) and was kept constant during the experiment by the addition of 0.1*N* HCl or 1*N* NaOH as required. The tubes were incubated at 30°C±2 for 72 hr. under shaking (150 rpm/min.). The optical density of bacterial growth measured at 600 nm (OD_{600}) using spectrophotometer (Sco. Tech, SP UV-19) **(Stevenson *et al.*, 2016)**. Then, the bacterial culture was centrifuged at 5000 xg for 20 min. Then, the following parameters were estimated:

- Bacterial dry weight was determined by harvesting the cells by centrifugation at 5,000 xg for 20 min. Harvested cells were washed twice with distilled water and desiccated in an oven at 80°C for 48 hr.
- The residual ion concentration of metal was detected in the supernatants using the atomic absorption spectrophotometer (Buck Model 210 VGP).
- The amounts of metal uptake (mg/l) was calculated according to the equation by **Shetty and Rajkumar (2009)**:

$$\text{Heavy metal uptake (mg/l)} = \frac{V(CI - CF)}{\text{dry biomass weight (g)}}$$

Where:

V: volume of reaction CI: Initial metal concentration CF: Final metal concentration (Residual)

- Heavy metal biosorption (%) was calculated according to the equation by **Shetty and Rajkumar (2009)**:

$$\text{Efficiency of biosorption (\%)} = \frac{(CI - CF)}{CI} \text{ x } 100$$

2.7. Estimation of antioxidant activities

2.7.1. Preparation of cell free extract (CFE)

Tubes of Muller-Hinton broth medium (pH 7.5±0.2) individually amended with four heavy metals (Zn^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+}) at 1000 and 1500 mg/l and in mixtures from them, then inoculated with 1 % of the overnight grown zinc tolerant isolate culture and incubated at 30°C±2 for 18 hr. using shaking incubator (150 rpm/min). CFE was obtained by centrifugation at 10,000 xg for 5 min at 4°C and kept at 4°C for enzymatic and non-enzymatic antioxidant assays.

2.7.2. Non-enzymatic antioxidant assay

DPPH free radical scavenging assay was measured as non-enzymatic assay using the procedure described by **Heo *et al.* (2005)** as follows: 500µL of CFE, 3000 µL of a freshly prepared solution of 2,2-DiPhenyl-2-Picryl hydrazyl hydrate (DPPH) at a concentration of 5mg/100 ml ethanol was added. Control was prepared using 500µl of ethanol addition to 3000 µL DPPH solution, mixed and incubated for 30 min in dark. Absorbance (As) was measured at 517 nm after 30 min. The percentage of radical scavenging activity was calculated according to the following equation:

$$\text{\% Residual of DPPH after 30 min} = \frac{\text{As517 control} - \text{As517 sample}}{\text{As517 control}} \text{ x } 10$$

$$\text{\% Inhibited of DPPH after 30 min} = \text{\% Residual of DPPH} - 100$$

2.7.3. Enzymatic antioxidant assay

Three oxidative enzymes (catalase, peroxidase and polyphenol oxidase) were determined spectrophotometrically as follows:

- **Catalase activity (CAT) (EC 1.11.1.6)** was determined by monitoring the decrease in absorbance at 240 nm resulting from the decomposition of H_2O_2. A complete reaction mixture was including 1500 µL of 100 m*M* potassium phosphate buffer (pH 7.0), 500 µL of 75 m*M* H_2O_2, 200 µL of enzyme extract and 800 µL of double distilled water (DDW), in quarts cuvettes as described by **Aebi (1984)**. One unit of enzyme activity was defined as absorbance per min.
- **Peroxidase activity (POD) (EC 1.11.1.9)** was determined using 4-methylcatechol as substrate. The increase in the absorption caused by oxidation of 4-methylcatechol by H_2O_2, was measured at 420 nm. The reaction mixture contained 100 µL of 100 m*M* potassium phosphate buffer (pH 7.0), 500 µL of 5 m*M* 4-methylcatechol, 500 µL of 5 m*M* H_2O_2 and 500 µL of crude extract in a total volume of 4000 µL by DDW at room temperature. One unit of enzyme activity was defined as 0.001 change in absorbance per min, under assay conditions **(Onsa *et al.*, 2004)**.
- **Polyphenol oxidase activity (PPO) (EC 1.10.3.1)** was carried out by measuring the increase in absorbance at 420nm for 4-methylcatechol. The assay was performed with 100 µL of 100 m*M* sodium phosphate buffer (pH 7.0), 500 µL of 5 m*M* 4-methylcatechol and 500 µL of crude extract at room

temperature. Total volume of reaction mixture was 3000 µL with DDW. One unit (U) of enzyme activity was defined as the amount of the enzyme that caused a change of 0.001 in absorbance per min **(Oktay *et al.*, 1995).**

Results and Discussion

1.1. Isolation of zinc tolerant bacteria

A total of 5 samples (3 sediments and 2 soils) were collected from heavy metals contaminated sites in Egypt. A total of 71 zinc tolerant bacterial isolates were recovered on Muller-Hinton agar supplemented with 250 mg/l concentration of zinc sulphate heptahydrate by pouring plate method **(Fig. 1)**. These results were in accordance with **Sen and Joshi (2016)** who collected 12 soil samples from contaminated sites and isolated 23 indigenous bacterial strains on nutrient agar medium supplemented with 1000 mg/l concentration of $ZnSO_4.7H_2O$.

Thirteen isolates were isolated from Upstream Al-Rahawy drain sediment (UR33-UR45), nineteen isolates were isolated from Midstream Al-Rahawy drain sediment (MR108-MR127), ten isolates were isolated from Downstream Al-Rahawy drain sediment (DR213-DR222), fourteen isolates were isolated from Al-Gabal Al-Asfar region soil (GS286-GS299) and fifteen isolates were isolated from Kafr-Ilwan village soil (KE356-KE370). Also, data showed that the highest and the lowest numbers of isolates were obtained from Midstream and Downstream Al-Rahawy drain sediments, respectively.

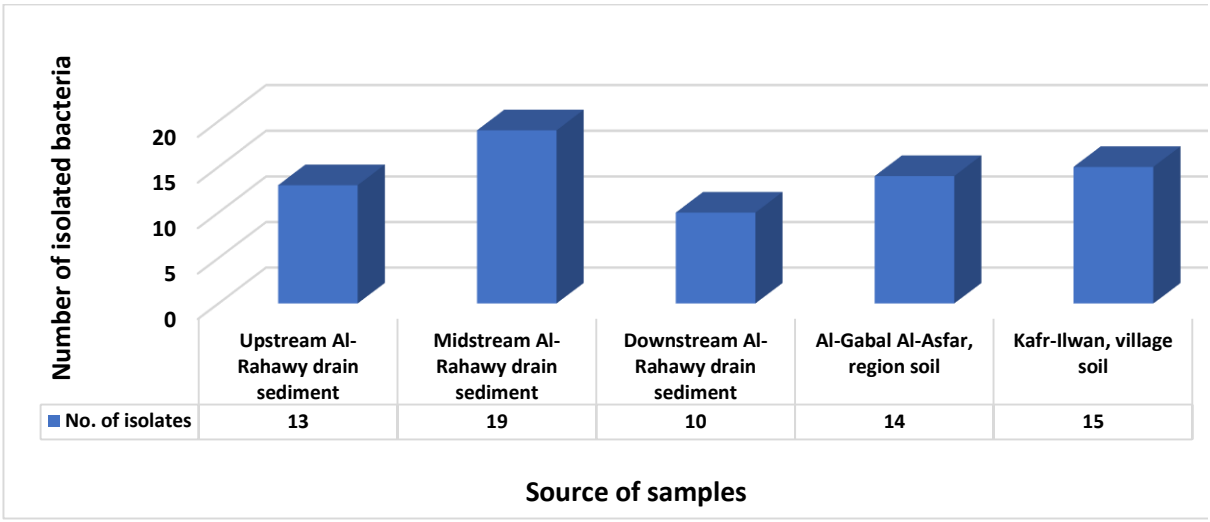

Fig. 1. Number of isolated bacteria from five contaminated sites under 250 mg/l of zinc.

1.2. Minimum inhibitory concentration (MIC) and maximum tolerance concentration (MTC) for zinc

All the 71 bacterial isolates were subjected to Muller-Hinton supplemented with different concentrations (500, 1000, 1500, 2000, 2500, 3000, 3500, 4000 and 4500 mg/l) of Zn^{2+} for determination of MIC and MTC **(Fig2).** The MIC values for all isolates ranged between 1000 mg/l to 4500 mg/l. Among these isolates, only three isolates namely, GS 296, KE364 and KE365 showed low MIC values 1000 mg/l. Moreover, 14.88% of isolates have MIC value at 1500 mg/l; 6.2% of isolates have MIC value at 2000 mg/l; 2.48% of isolates have MIC value 2500 mg/l; 21.08% of isolates have MIC value at 3000 mg/l; 18.6% isolates have MIC value at 3500 mg/l; 19.84% of isolates have MIC value at 4000 mg/l while, 1.24% (only one isolate, UR45) has MIC value at 4500 mg/l. These results were in harmony with **Sen and Joshi (2016)** who demonstrated that the MIC values for 23 zinc resistant isolates ranged between 2000 mg/l to 3100 mg/l. Respecting the MTC values, all tested isolates have MTC values of zinc ranged between 500 mg/l to 4000 mg/l. The highest MTC value was observed with the isolate UR45 which isolated from Upstream Al-Rahawy drain, Giza Governorate at 4000 mg/l zinc sulphate heptahydrate. Therefore, the isolate UR45 was selected for further identification on the basis of 16S rRNA and characterization.

Owolabi and Hekeu (2015) estimated the MTC for Zn^{2+} by zinc-resistant bacteria, they found that MTC value was ranged between 300 and 2000 mg/l, depending on the bacterial strains. Also, they reported that the method for MTC examination of heavy metals for resistant bacteria have been inconsistent from study to study; while some have used liquid media **(Hassen *et al.*, 1998)**, most have conducted the determinations in solid media **(Xu *et al.*, 2014)** as was done in the present study. It is generally considered that heavy metals are more toxic in liquid than in solid media due to more dispersion in the culture **(Haferburg *et al.*, 2007)**.

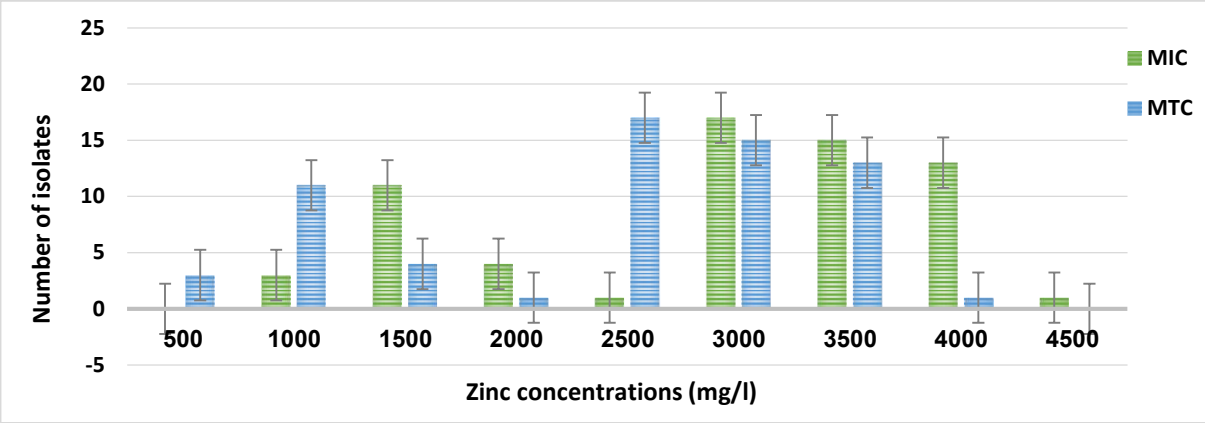

Fig. 2. Minimum inhibitory concentration (MIC) and maximum tolerance concentration (MTC) tolerance of zinc on bacterial isolates from different contaminated sites.

1.3. Identification of the most zinc tolerant isolate UR45

Amplification of 16S rRNA of the most zinc tolerant isolate UR45 and its sequencing were carried out and BLAST analysis of the complete sequences of 16S rRNA revealed the isolate to be *Alcaligenes faecalis* (GenBank accession nos.: MG257493.1). Distribution of the various strains on the phylogenetic tree showed that the isolate was closely related to the reference standard strains. The phylogenetic tree **(Fig. 3)** showed that the identified bacterium was *Alcaligenes faecalis* MG257493.1 which classified as: phylum: Proteobacteria, class: Betaproteobacteria, order: Burkholderiales, family: Alcaligenaceae. Similar trend of results was observed by **Sen and Joshi (2016)** who reported that proteobacteria were identified as the dominant group of bacteria with tolerance to heavy metals. Also, **Ansari *et al*. (2016)** isolated six bacterial isolates from contaminated soil and characterized based on 16SrRNA gene sequences four of them are *Alcaligenes* spp.

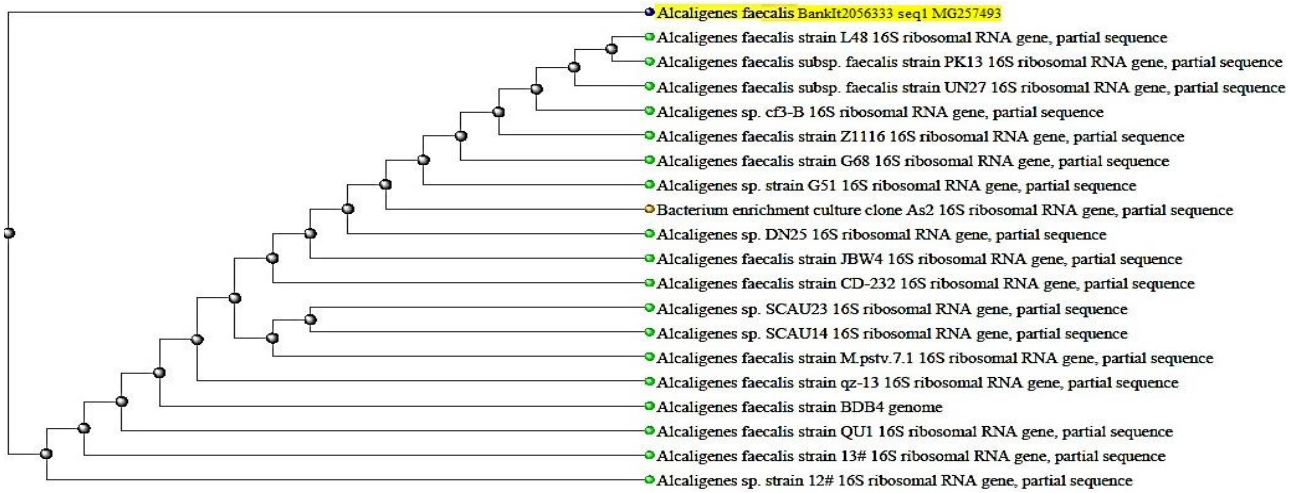

Fig.3. Phylogenetic tree showing interrelationships of the zinc tolerant isolate based on 16SrRNA sequences.

1.4. Tolerance of zinc tolerant *Alcaligenes faecalis* MG257493.1 to different heavy metals

Obtained results from the MIC analyses revealed that *Al. faecalis* MG257493.1 was the most tolerant bacteria to Zn^{2+}. For this reason, it was tested for tolerance against different heavy metals Cd^{2+}, Pb^{2+} and Cu^{2+} at different concentrations ranged from 1000 mg/l to 4500 mg/l. The degree of tolerance was measured by minimum inhibitory concentration (MIC) and maximum tolerance concentration (MTC) in the presence of each metal. In this respect, data graphically illustrated by **Fig (4)** clearly indicated that *Al. faecalis* MG257493.1 was more sensitive to copper than other metals with MIC value (2000 mg/l) followed by cadmium with MIC value (2500 mg/l). Moreover, MIC of Zn and Pb against *Al. faecalis* MG257493.1 was 4500 mg/l and 3500 mg/l, respectively. This high incidence and multiple nature of metal resistance might be the result of continuous exposure of these isolates to more than one heavy

metal. Additionally, MTC values for the Cd, Zn, Pb and Cu were 2000 mg/l, 4000 mg/l, 3000 mg/l and 1500 mg/l, respectively.

These metal-resistant bacteria can play important role in the cleanup of contaminated environment because resistance to the metals is a general approach that occurs in the biological management of contaminated surroundings **(Filali *et al*., 2006)**. Based on the ability of identified strain to resist four heavy metals **(Fig 4)**, *Al. faecalis* MG257493.1 was further tested for their potential used in biosorption. Biosorption of heavy metals by microorganisms has received rapid attention in recent times to clean up the contaminated surroundings **(Farhadian *et al*., 2008)**. Moreover, **Owolabi and Hekeu (2015)** reported that some of the Zn^{2+} resistant bacterial isolates showed resistance to Pb^{2+}. Also, in results by **Ahemad and Malik (2012)**who found that zinc resistant bacteria belonging to genus *Pseudomonas* sp. exhibited co-resistance against Cu^{2+}, Hg^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Cr^{3+} and Cr^{6+} in addition to Zn^{2+}. Additionally, efflux transporters play an important role in heavy metal homeostasis of different heavy metals such as Zn^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+} and Ag^{2+} **(Owolabi and Hekeu, 2015)**. An alternative resistance mechanism of Zn^{2+} and Pb^{2+} dependent upon metabolic energy of microorganisms is the bioaccumulation of both heavy metals **(Augusto da Costa and Duta, 2001)**.

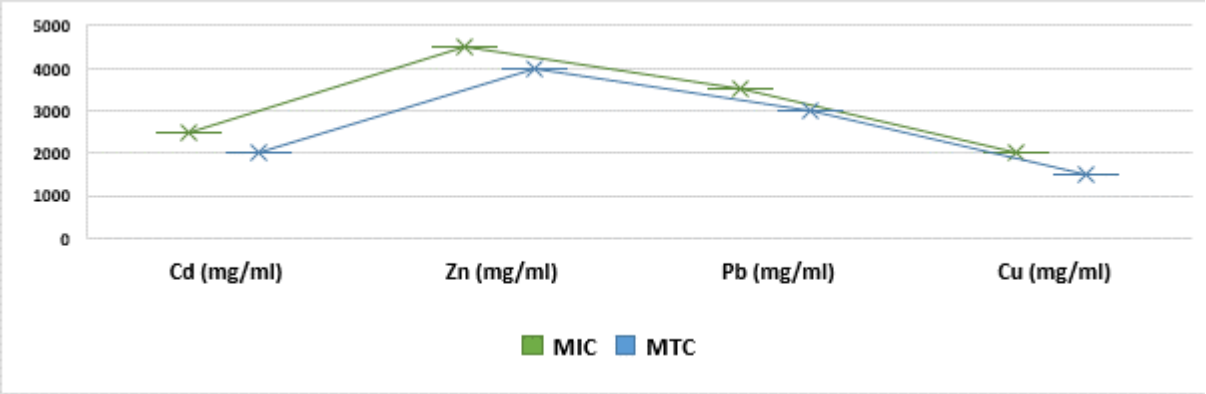

Fig.4. The MIC and MTC values for *A. faecalis* MG257493.1 under different concentrations of heavy metals.

1.5. Effect of pH on biosorption potential by *Al. faecalis* in presence of different heavy metals

In this experiment, the identified strain *Al. faecalis* MG257493.1 was examined under different pH values in media supplemented with four heavy metals (Cd^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}) at 1000 mg/l. In this respect, pH effects on *Al. Faecalis* MG257493.1, data presented in **Table (2)** indicated that the efficiency of biosorption and metal uptake were higher at pH 7 than other pH values. This trend of results was true under four examined heavy metals. Moreover, the optimum pH value for biomass dry weight was varied according to the heavy metal presence in media. Data indicated that the highest biomass dry weight was recorded at pH 6 and 8 in media supplemented with Cu^{2+} and Pb^{2+}, respectively.

Whereas, pH 9 was the optimum for biomass dry weight in media supplemented with Cd^{2+} or Zn^{2+}.

Additionally, data recorded in **Table (2)** also showed that the optimum pH values which gave higher Cd^{2+} and Pb^{2+} concentrations in biomass were 6 and 9, respectively. While, pH 8 was the optimum value for highest Cu^{2+} and Zn^{2+} concentrations in biomass.

Generally, Cd^{2+} was absorbed higher than other heavy metals by *Al. Faecalis* MG257493.1 at pH 6 and 7. Also, the biosorption efficiency was increased with the increasing of pH value and reach at maximum at pH 7 then decreased with the increasing of PH value. This trend of results was observed in metal uptake by the same bacterial strain. These results are in agreement with those obtained by **Green-Ruiz *et al.* (2008)** who reported that the maximum value of Zn (II) ions uptake occurred at pH 6 and 7 by *Bacillus circulans* (D21) reaching to 25.40 and 25.97% of the initial metal concentration, respectively, however the lowest biosorption value was observed at pH 5 which showed 11.86%. **Mohamed and Abo-Amer (2012)** observed similar results using as bioremediation agents, accumulating metal well in comparison to HM-6 i.e *Bacillus cereus*. HM-7 i.e *Alcaligenes* sp showed highest percentage of metal accumulation i.e 38.24% biosorption and only 37.48% was left in supernatant, as well as **Ting and Choong (2009)** in their comparison between the ability of a *Trichoderma* isolate to bioaccumulate and bio-absorb.

These results revealed that utilization of the metal-resistant bacteria for biosorption is a successful approach for the removal of heavy metals **(Filali *et al*., 2006)**. Microorganisms able to achieve different transformation and immobilization processes, one of them is bioaccumulation, based on the incorporation of metals inside the biomass and another process is biosorption, in which metallic ions remain at the cellular surface by different mechanisms

(Vijayaraghavan and Yun ,2008). The effects of metals on the growth of *Alcaligenes* spp. were investigated by determining the OD_{600}. The growth of the strains was responses towards media supplemented with metals at 4000 mg/l. The highest percent of accumulation viz. biosorption (in cell wall) and bioaccumulation (in intercellular space) was observed in *Alcaligenes* sp. heavy metal resistant strain in supernatant **(Ansari *et al*., 2016)**. According .Also, **Issazadeh *et al*. (2013)** reported that microbial exposure to heavy metals selects and maintains microbial variants able to tolerate the harmful effects of metals. Various and efficient metal resistance mechanisms have been identified in diverse species of bacteria. Moreover, **Augusto da Costa and Duta (2001)** reported that the maximum zinc bioaccumulations were 4.3 mol/g biomass for *B. sphaericus*, 4.6 mol/g biomass for *B. cereus*, 4.8 mol/g biomass for *Bacillus* sp. and 5.0 mol/g biomass for *B. subtilis.*

Table 2. Effect of pH on bacterial growth and biosorption potentials under different heavy metals by *Al. faecalis* MG257493.1.

Heavy metals	PH	Residual metals cons. mg/l	Efficiency of biosorption (%)	Biomass dry weight (mg/g)	Metals uptake (mg/g dry weight)	Metals cons. in biomass mg/g
Cu	5	420	٥٨.0	0.102	51.32	150.5
	6	340	٦٦.0	0.113	52.58	280.4
	7	210	٧٩.0	0.099	71.81	290.9
	8	362	٦٣.0	0.10	56.70	483.5
	9	550	٤٥.0	0.101	40.10	360.6
Cd	5	410	٥٩.0	0.122	43.54	100.0
	6	169	83.1	0.100	74.79	379.4
	7	151	84.9	0.100	76.41	300.1
	8	390	61.0	0.114	48.18	189.3
	9	450	55.0	0.125	36.62	130.6
Zn	5	530	47.0	0.122	34.68	160.3
	6	366	63.4	0.100	57.06	200.0
	7	300	70.0	0.100	63.00	190.7
	8	520	48.0	0.105	41.16	465.6
	9	569	43.1	0.112	34.64	380.5
Pb	5	370	63.0	0.103	55.06	200.0
	6	350	65.0	0.103	56.81	90.36
	7	200	80.0	0.100	72.00	179.3
	8	422	57.8	0.113	46.05	150.0
	9	400	60.0	0.100	54.0	290.9
* Initial conc. 1000 mg/l						

3.6. Estimation of antioxidant activities

In this experiment, the antioxidant activities of *Al. faecalis* MG257493.1 filtrate under different heavy metals individually or in mixture were estimated at two concentrations (1000 and 1500 mg/l). Regarding this trend, data in **Table (3)** clearly indicated that the highest residual of DPPH in *Al. faecalis* MG257493.1 filtrate was observed in filtrate without any heavy metals followed by filtrate contained the mixture of heavy metals at 1500 mg/l. Moreover, it was clear that the inhibition of DPPH was higher in filtrate amended with all heavy metals either individually or in mixture at 1500 mg/l than 1000 mg/l.

The highest inhibition of DPPH was recorded in media supplemented with copper at 1500 mg/l followed by cadmium at the same concentration. Although this strain was isolated as zinc-tolerant bacteria, the lowest inhibition of DPPH was observed in media amended with zinc than other metal. DPPH is a free radical generating compound and has been widely used to evaluate the free radical scavenging ability of various antioxidative compounds **(Heo *et al*., 2006)**. Also, they mention that the reduction capability of DPPH radical was determined by the decrease induced by antioxidative compounds. The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability **(Ilhami *et al*. 2004)**. Many reports have recently described the ability to scavenge DPPH free radical on some microorganisms **(Athukorala *et al*., 2003)**.

Table 3. Enzymatic and non-enzymatic antioxidant activities of *Al. faecalis* MG257493.1 filtrate under different heavy metals concentrations.

Heavy metals	Conc. (mg/l)	Non-enzymatic as (%) DPPH after 30 min.		Enzymatic (absorbance per min.)		
		Residual	Inhibited	CAT	POD	PPO
Without any heavy metals		56.3	43.7	٠.٠٤٥	٠.٠٧٨	٠.٠٦٤
Zn^{2+}	1000	26.8	73.2	٠.٠٤١	٠.٠٦٣	٠.١٣٧
	1500	17.1	82.9	0.071	0.028	0.121
Cu^{2+}	1000	20.9	79.1	٠.٠٦١	٠.٠٧٧	٠.٢٦٢
	1500	7.70	92.3	٠.٠٥٥	٠.٠٤٠	٠.١٧٠
Pb^{2+}	1000	10.1	89.9	0.047	0.039	0.203
	1500	9.90	90.1	0.046	0.036	0.063
Cd^{2+}	1000	24.0	76.0	٠.٠٧٣	0.556	٠.٢٩٨
	1500	9.80	90.2	٠.٠٤٩	0.522	٠.١٣٥
Heavy metals mixture	1000	40.7	59.3	٠.٠٥٠	٠.١١٩	٠.٢١٢
	1500	26.9	73.1	٠.٠٦٨	٠.٢٦٦	٠.٢٠٨

Respecting the enzymatic antioxidant activities of *Al. faecalis* MG257493.1, data in **Table (3)** showed that CAT activity was increased with the increasing of Zn^{2+} concentration in *Al. faecalis* MG257493.1 filtrate. The same trend was observed in filtrate contained mixture of heavy metals. Reverse results were observed in filtrate amended with Cu^{2+}, Pb^{2+}, Cd^{2+}. Whereas, POD and PPO activities were higher in filtrate amended with each heavy metal at 1000 mg/l. Also, the activities of POD and PPO were increased and decreased with increasing of concentration of heavy metals mixture, respectively.

Conclusion

Out of the 71-zinc tolerant bacterial isolates from heavy metals contaminated sites in Egypt, UR45 isolate was found to show highest MIC and MTC for zinc at 4500 and 4000 mg/l, respectively. This isolate was identified as *Alcaligenes faecalis* MG257493.1 by partial sequence of 16S rRNA genes and showed metal tolerance to cadmium, lead and copper This study revealed the biosorption potential of the identified strain for different heavy metals under different pH values. Also, the antioxidant activities for metal tolerance by this strain represents a point of interest for possible environmental applications to remove the toxic effect of heavy metals from the contaminated environments.

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عزل وتوصيف البكتريا المتحمله للزنك من الرواسب والتربه الملوثه فى مصر

فى هذا البحث تم عزل ٧١ عزله من البكتريا المتحمله للزنك من الرواسب والتربة الملوثه بالعناصر الثقيلة وذلك من ثلاث عينات من رواسب مصرف الرهاوى بمحافظة الجيزة (أعلى المصب – منتصف المصب – أسفل المصب) وكذلك تربة زراعية من منطقتى الجبل الأصفر وكفر علوان بمحافظة القليوبية. فى هذه الدراسه تم انتخاب أفضل عزلة وذلك بتقدير أقل تركيز مثبط وأعلى تركيز لتحمل البكتريا للزنك من خلال تركيزات تتراوح من ٥٠٠– ٤٥٠٠ ملليجرام/لتر. ولقد أوضحت النتائج أن أكثر العزلات تحملا هى العزلة رقم UR٤٥ حيث كان تركيز ٤٥٠٠ ملليجرام/لتر هو أقل تركيز مثبط لها بينما أعلى تركيز من الزنك تحملته هذه العزله كان ٤٠٠٠ ملليى جرام/لتر. وقد تم تعريف العزلة (UR45) بتقنية RNA ١٦S حيث أوضحت النتائج أنها *Alcaligenes faecalis* MG257493.1 . كذلك تم دراسة تأثير درجات مختلفة من الـ pH (٥–٩) على نمو السلالة *Alcaligenes faecalis* MG257493.1 وذلك بهدف تحديد درجة pH المثلى والدنيا لمدى قدرتها على الامتصاص الحيوى للعناصر الثقيلة من خلال تقديرامتصاص المعدن والمعدن المتبقى عند تركيز ١٠٠٠ ملليى جرام /لتر لكل من الرصاص والنحاس والكادميوم والزنك، وقد أوضحت النتائج أن8 pH هو الأمثل للنمو في البيئة المضاف لها عنصر الزنك والنحاس في حين اختلفت قيمة الامتصاص الحيوى وفقا للمعادن التى تم تقديرها. أيضا تم تقدير نشاط مضادات الأكسده الانزيمية للسلالة *Alcaligenes faecalis* MG257493.1 مثل (إنزيم الكتاليز والبيروكسيديز والبولى فينول أوكسيديز) وكذلك مضادات الأكسدة غير الإنزيمية (٢،٢ داى فينيل– بيكريل هيدرازيل هيدرات (DPPH)) وذلك فى وجود تركيزات مختلفة من العناصر الثقيلة ١٠٠٠–١٥٠٠ ولقد أوضحت النتائج نشاط مضادات الأكسدة الانزيمية غير الإنزيمية يزاد بزيادة تركيز المعادن الثقيلة قيد الدراسة.