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Characterization and identification of cadmium-tolerant bacteria isolated from contaminated regions in Egypt

Rasha M. El-Meihy [a,b], Hamed E. Abou-Aly [a], Taha A. Tewfike [a], Eman A. El-Alkshar [a], Ahmed M. Youssef [c,*]

[a] Agricultural Microbiology Department, Faculty of Agriculture, Benha University, Moshtohor, Kaluybia, 13736, Egypt
[b] Microbial Biotechnology and Fermentation Laboratory, Moshtohor Research Park, Faculty of Agriculture, Benha University, Moshtohor, Kalyobiya, 13736, Egypt
[c] Packaging Materials Department, National Research Centre, 33 El Bohouth St. (former El Tahrir st.), Dokki, Giza, 12622, Egypt

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ABSTRACT

This study was designed under laboratory conditions to isolate, identify and characterize some cadmium-tolerant bacteria from heavy metals-contaminated regions of Egypt. These regions included three sites along Al-Rahawy drain, Giza Governorate and two agricultural soils from Kaluybia Governorate, Egypt. Three of sixty-nine cadmium-tolerant bacterial isolates were evident as the most tolerant isolates for cadmium according to the MIC and MTC tests. Results proved that MR99, MR100, and MR108 were able to grow on medium supplemented with cadmium at 2000 mg/l and inhibited at 2500 mg/l. After that, their ability to tolerate other heavy metals (copper, zinc, and lead) was evaluated and resulted in two highest tolerant isolates namely MR99 and MR108 which able to tolerate all four tested heavy metals at different concentrations. These isolates were identified as *Bacillus cereus* MG257494.1 (MR99) and *Alcaligenes faecalis* MG966440.1 (MR108) according to the morphological, cultural, biochemical characteristics and 16S rDNA partial sequence. Then, their biosorption potentials under different pH values in media supplemented with four heavy metals at 1000 mg/l were examined in addition to their enzymatic and non-enzymatic antioxidant activities.

1. Introduction

Heavy metals refer to a large group of elements which are biologically important (Peter, 2011), these metals can be categorized into three parts, first toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (such as Pt, Ag, Au, Ru etc.) and radionuclides (such as U, Th, Ra, Am, etc.) (Wang and Chen, 2006). The global pollution is increasing because of the variations in natural and anthropogenic activities, leading to contamination in various aquatic and terrestrial ecosystems with heavy metals, organic and inorganic chemical compounds. A wide variety of heavy metals such as zinc, lead, copper, and cadmium have been detected in soil presenting a major threat to the environment and population (Rajaganapathy et al., 2011).

The contamination of the environment with heavy metals has become a critical problem due to their absorption difficulty by microorganisms and become a threat to the different environments, moreover, they cannot be biologically degraded and indefinitely persist in the environment and transferred through the food chain causing serious hazard to human health (Goregaonkar et al., 2017). Generally, the toxic effects of heavy metals result from the interaction between metals and enzymes that cause inhibition of metabolic processes (Safahieh et al., 2012).

Among these metals, cadmium is one of the most toxic heavy metals and among the top ten in the blacklist of the World Health Organization (WHO) (Sharma et al., 2000). Additionally, cadmium is a naturally occurring toxic heavy metal that enters soil and water in several ways and has a high potential to precipitate in living tissues (microorganisms, plants, and animals) (Kharwar et al., 2017), this leads to several animal and human diseases such as kidney and lung disorders, destruction of red blood cells, mutations, DNA strand breaks, chromosomal damage, cell transformation and some cancers (Marzan et al., 2017) in addition to the chronic cadmium poisoning causes death in patients with Itai-Iitai disease (Nishijo et al., 2017).

In contaminated soil, microorganisms evolved several mechanisms by which they can survive in stress environment such mechanisms are metal exclusion by the barrier of permeability, cellular sequestration

* Corresponding author. Packaging Materials Department, National Research Center, Dokki, Cairo, Egypt.
E-mail address: amyoussef27@yahoo.com (A.M. Youssef).

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that is intracellular or extracellular, active efflux pumps, enzymatic reduction, metal volatilization and bio-precipitation (Dhuldhaj et al., 2013). Additionally, different types of resistance mechanisms have been developed by which microorganisms either secrete metal-binding protein or accumulate them in their cells (Jain and Bhatt, 2014). These heavy metals tolerant microorganisms can be used for bioremediation of contaminated soil (Yaseen et al., 2018); and treated wastewaters (Peter, 2011).

A laboratory experiment was conducted to isolate and identify some cadmium-tolerant bacteria from heavy metals-contaminated regions of Egypt to use them as heavy metals removal from contaminated sites. Then, these isolates were characterized for their ability to tolerate other three heavy metals (zinc, copper, and lead) depending on their absorption potential and antioxidant activities.

2. 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 samples of agricultural soils irrigated with wastewater were collected from Al-Gable Al-Asfar region (GS) and Kafr-Ilwan village (KI), Kaluybia Governorate, Egypt. All samples were stored at 4 °C until analysis.

2.2. Heavy metals analysis

The concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} in the collected samples were determined with an atomic absorption spectrophotometer (Buck Model 210 VGP).

2.3. Isolation of cadmium tolerant bacteria

The isolation of cadmium tolerant bacteria was carried out on Muller-Hinton broth medium (pH 7.3) (HIMEDIA Co., Germany) supplemented with 250 mg/l Cd^{+2} (2.04 g/l) as $CdCl_2$ (HIMEDIA Co., Germany) (Liu et al., 2004). 150 ml Erlenmeyer flasks containing 90 ml of the previous media were inoculated with 10 g of each sediment or soil samples and incubated at 30 °C ± 2 for 72 h under shaking (150 rpm/min.).

Enriched cultures showed turbidity after 3 days of incubation were diluted up to 10^{-6} using sterile distilled water, and then subcultured in Petri dishes containing a solidified medium. Appearing colonies on dishes and differing in shape, color and margins were purified and maintained in the previous medium and kept at 5 °C for further study (Moghannem et al., 2015).

2.4. Minimum inhibitory concentration (MIC) and maximum tolerance concentration (MTC) for cadmium

MIC and MTC of cadmium for sixty-nine bacterial isolates were determined using the agar plate dilution method as described by Malik and Jaiswal (2000). According the method described by Banerjee et al. (2015) and Khan et al. (2019), Muller-Hinton agar medium amended with different concentrations of Cd^{2+} namely, 500, 1000, 1500 and 2000 mg/l were used. Each isolate was streaked individually on Petri dishes containing the above medium and then incubated at 30 °C ± 2 for 72 h. 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.

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

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

2.6. Identification of the most cadmium tolerant bacterial isolates

The highest cadmium tolerant bacterial isolates were selected for identification. Morphological characteristics (the shape, spore formation and Gram stain) and biochemical characteristics (oxidase, catalase, and amylase enzymes, indole test, methyl red and Voges Proskauer test and sugar fermentation) as according to the key of Bergey's manual of systematic bacteriology (Brenner et al., 2005; Vos et al., 2009). The total DNA extraction was carried out by the CTAB method (Purohit et al., 2003). Bacteria were identified by a partial sequence of 16S rRNA gene following procedures reported earlier (Narde et al., 2004; Khan et al., 2019). A 1466bp product was amplified using 16S rDNA using the following primers:

(1)	Forward primer: 5′-AGAGTTTGATCMTGGCTCAG-3′
	Reverse primer: 5′-CGGYTACCTTGTTACGACTT-3′
(2)	Forward primer: 5′-AAACTYAAAKGAATTGACGG-3′
	Reverse primer: 5′-ACGGGCGGTGTGTRC-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 the 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: MR99: *Bacillus* MG257494.1 and MR108: *Alcaligenes* MG966440.1.

2.7. Effect of pH on growth and heavy metals biosorption potential for the most cadmium tolerant isolates

The most cadmium tolerant isolates were 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 h under shaking (150 rpm/min.). The optical density of bacterial growth measured at 600 nm (OD_{600}) using a spectrophotometer (Sco. Tech, SP UV-19) (Stevenson et al., 2016). Then, the bacterial culture was centrifuged at 5000 ×g for 20 min. Then, the following parameters were estimated:

- Bacterial dry weight was determined by harvesting the cells by centrifugation at 5000 ×g for 20 min. Harvested cells were washed twice with distilled water and desiccated in an oven at 80 °C for 48 h.
- 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) were 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):

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

2.8. Estimation of antioxidant activities

2.8.1. Preparation of cell free extract (CFE)

Tubes of Muller-Hinton broth medium (pH 7.3) 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 h using shaking incubator (150 rpm/min). CFE was obtained by centrifugation at 10,000 ×g for 5 min at 4 °C and kept at 4 °C for enzymatic and non-enzymatic antioxidant assays.

2.8.2. 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: 500 µL 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 500 µl 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:

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

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

2.8.3. Enzymatic antioxidant assay

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

2.8.3.1. Catalase activity (CAT) (EC 1.11.1.6). CAT 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.

2.8.3.2. Peroxidase activity (PO) (EC 1.11.1.9). PO was determined using 4-methyl catechol as substrate. The increase in the absorption caused by oxidation of 4-methyl catechol 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-methyl catechol, 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 changes in absorbance per min, under assay conditions (Onsa et al., 2004).

2.8.3.3. Polyphenol oxidase activity (PPO) (EC 1.10.3.1). PPO was carried out by measuring the increase in absorbance at 420 nm for 4-methyl catechol. The assay was performed with 100 µL of 100 m*M* sodium phosphate buffer (pH 7.0), 500 µL of 5 m*M* 4-methyl catechol and 500 µL of crude extract at room temperature. A total volume of the 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) (see Table 1).

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.

3. Results and discussion

3.1. Isolation of cadmium tolerant bacteria

Results in Table 2 and illustrated graphically by Fig. 1 indicated that sixty-nine cadmium tolerant bacterial isolates were obtained from different five contaminated sites. The lowest numbers of isolates (ten isolates) were obtained from Kafar Ilwan village soil that equivalent to 14.4% of total cadmium-tolerant isolates. These isolates were coded as KI346-KI355. Whereas the highest number of isolates were obtained from two sediment samples upstream and midstream Al-Rahawy drain with an equal number (seventeen isolates) that equivalent to 49.2% of the total cadmium-tolerant isolates, 24.6% by each sample. The isolates from the upstream Al-Rahawy drain were coded as UR16-UR32, while the isolates from the downstream Al-Rahawy drain sediment were coded as MR92-MR108. Additionally, eleven and fourteen isolates were isolated from downstream Al-Rahawy drain sediment and Al-Gabal Al-Asfar soil, respectively. The isolates from downstream Al-Rahawy drain were coded as DR202-DR212, while the isolates from Al-Gabal Al-Asfar soil were coded as GS272-GS285. In this trend, Verma et al. (2015) isolated twenty-seven bacterial isolates from eight soil samples on media amended with cadmium (100 ppm). Only one isolate has a high level of MIC (1000) for cadmium metal.

3.2. Minimum inhibitory concentration (MIC) and maximum tolerance concentration (MTC) for cadmium

Concerning the effect of different concentrations of cadmium on the isolated bacteria at medium supplemented with different concentrations of cadmium (500–2500 mg/l), data illustrated graphically by Fig. 2 showed that twenty-six bacterial isolates aren't able to tolerate more than 250 mg/l (the basic concentration in the isolation medium) and have MIC at 500 mg/l. Moreover, nineteen isolates have MIC and MTC at 1000 and 500 mg/l, respectively. Correspondingly, seventeen isolates have MIC and MTC at 1500 and 1000 mg/l, respectively. Additionally, three isolates (UR25, UR27, and MR98) were able to grow at 1500 mg/l and inhibited at 2000 mg/l. On the other hand, only three isolates (MR99, MR100, and MR108) were able to grow on medium supplemented with 2000 mg/l Cd^{2+} and inhibited at 2500 mg/l. So, these isolates were selected to further study.

Table 2
Number of cadmium-tolerant bacterial isolates from different contaminated sites.

Sediment samples			Soil samples	
Upstream Al-Rahawy drain (UR)	Midstream Al-Rahawy drain (MR)	Downstream Al-Rahawy drain (DR)	Al-Gabal Al-Asfr city (GS)	Kafr Ilwan village (KI)
17	**17**	**11**	**14**	**10**

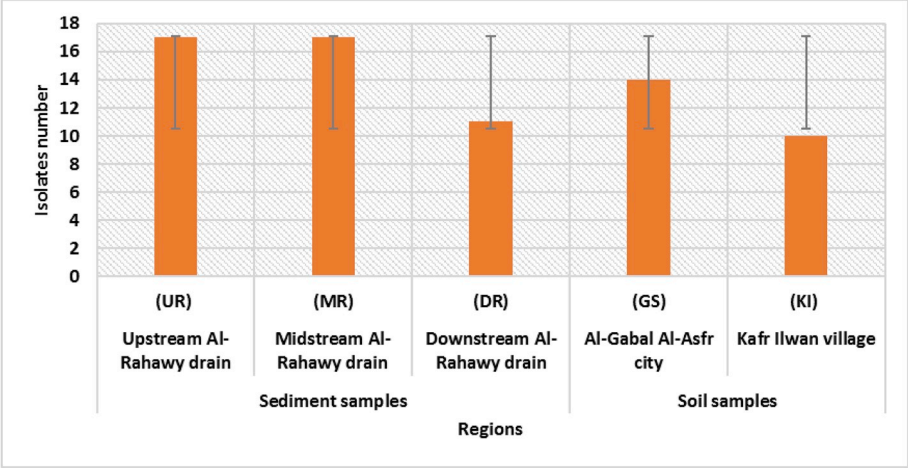


Fig. 1. Number of cadmium-tolerant bacterial isolates from different contaminated regions.

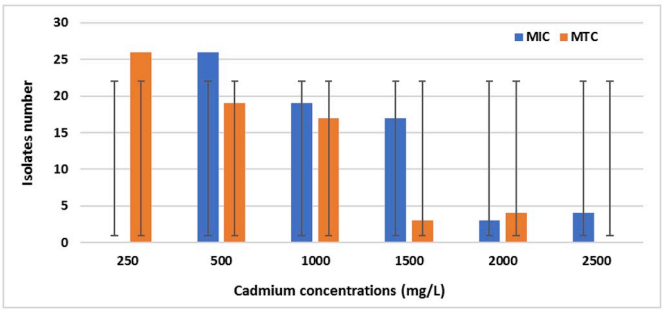


Fig. 2. Minimum inhibitory concentration (MIC) and maximum tolerance concentration (MTC) of cadmium for cadmium-tolerant bacteria.

3.3. Tolerance of the heaviest metals tolerant bacterial isolates to four heavy metals (Cu^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+})

From the MIC and MTC results, three isolates were chosen as the most tolerant isolates for Cd^{2+}, these isolates namely MR99, MR100, MR108 and recovered from midstream Al-Rahawy drain sediment. In this experiment, the three selected isolates were examined under four heavy metals at different concentrations ranged between (1000–2000) mg/l for Cu^{2+} and Cd^{2+}, and between (2000–4000) mg/l for Zn^{2+} and 1000–3000 mg/l for Pb^{2+}.

Results in Table (3) showed that two isolates MR99 and MR108 were able to tolerate all four tested heavy metals at different concentrations expect Cu^{2+} at 2000 mg/l whereas, the isolate MR100 wasn't able to tolerate copper or lead at any concentration. Therefore, these two isolates (MR99 and MR108) were selected for identification and selected to the following experiments. These results are in harmony with those obtained by Silva et al. (2009) who found that *P. aeruginosa* AT18 was resistant to high concentrations of four heavy metals (Zn^{2+}, Cu^{2+}, Cr^{2+}, and Mn^{2+}) and they suggested that this strain was suitable as a bio-removal agent for heavy metals. Also, according to the MICs of many metals on three bacterial isolates that showed tolerance to these elements reported that the cellular mechanisms for metal tolerance in these bacteria were mainly specific for cadmium and/or zinc. Moreover, Ledin (2000) explained the differences between microbial tolerance and resistance; he defined tolerance as the ability to cope with metal toxicity by employing intrinsic properties of the microorganisms, whereas resistance is the ability of microbes to detoxify heavy metals by being activated in direct response to the high heavy-metal concentrations.

Additionally, Sharma and Archana (2016) reported that to avoid toxicity, metals must be quickly and efficiently either eliminated from any cell or converted into a biologically inactive form. In general, there are two basic mechanisms of resistance to heavy metal ions, intracellular or extracellular complexation of toxic metal ions and reduced accumulation based on the active efflux of the cations. The second one is the primary mechanism developed in prokaryotes. However, enzymatic transformations of metal ions (oxidation, reduction, methylation, and demethylation) are also defense mechanisms in bacteria.

3.4. Identification of the most heavy metals tolerant isolates MR99 and MR108

Morphological, cultural, biochemical characteristics and 16S rDNA partial sequences were used for identification and characterization of the selected isolates as the most heavy metals-tolerant bacteria (Khan et al., 2019).

3.4.1. Morphological and biochemical characteristics

According the microscopic morphology, isolate MR108 was straight short rods, Gram-negative and non-spore forming. Whereas, the isolate MR99 was consider as a straight long rod, gram-positive, spore-forming, and motile. These three bacterial isolates were aerobic, non-pigmented and don't able to grow on nitrogen-free medium (Table 4).

The phenotypic characterization of MR99 presented in Table (4) showed that it was catalase and oxidase-positive. It utilized glucose, xylose, sucrose, arabinose, mannitol and ribose as sole carbon source and acid derived from them which caused indicator color change, but don't able to use lactose. This isolate was positive to indole, Voges Proskaure and methyl red tests. Although it was able to hydrolysis starch and gelatin, it isn't able to hydrolysis casein or lipids.

Table 3
Tolerance of the heaviest metals tolerated bacterial isolates to four heavy metals (Cu^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}).

Isolates Code.	Cu^{2+} (mg/l)				Cd^{2+} (mg/l)				Zn^{2+} (mg/l)				Pb^{2+} (mg/l)			
	1000	1500	2000	2500	1000	1500	2000	2500	2000	3000	4000	4500	1000	2000	3000	3500
MR99	+	+	–	–	+	+	+	–	+	+	+	–	+	+	+	–
MR100	–	–	–	–	+	+	+	–	+	+	–	–	–	–	–	–
MR108	+	+	–	–	+	+	+	–	+	+	+	–	+	+	+	–

Table 4
Morphological and biochemical characteristics of the most heavy metals tolerant isolates MR99 and MR108.

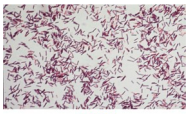
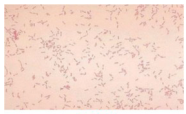
Characteristics		MR99	MR108
Shape		Long rod	Short rod
Gram staining		+	–
Spore formation		+	–
Pigmentation	of colony	–	–
	of medium	–	–
Respiration		Aerobic	Aerobic
Growth on N_2-free medium		–	–
Catalase production		+	+
Oxidase production		–	+
Indole production		+	+
V.P. test		+	–
Methyl Red		+	±
Carbon source and acid derived	Glucose	+	+
	Xylose	+	–
	Arabinose	+	–
	Mannitol	+	–
	Lactose	–	–
	Sucrose	+	–
	Ribose	+	–
Hydrolysis of	Starch	+	+
	Gelatin	+	–
	Casein	–	–
	Lipids	–	–
Shape under microscope		 ×1200	 ×1200

Table 5
Effect of pH on bacterial growth and biosorption potential under different heavy metals by *B. cereus* MG257494.1

Heavy metals	pH	Residual metals cons. mg/l	Efficiency of biosorption (%)	Biomass dry weight (mg/g)	Metals uptake (mg/g dry weight)	Metals con. in biomass mg/g
Cd^{2+}	**5**	500	50.0	0.109	41.28	90.36
	6	390	61.0	0.101	54.36	226.52
	7	240	76.0	0.100	68.40	279.49
	8	440	56.0	0.116	43.47	103.00
	9	530	47.0	0.108	39.16	80.12
Cu^{2+}	**5**	420	58.0	0.112	46.62	96.69
	6	390	61.0	0.100	54.90	200.00
	7	180	82.0	0.100	73.80	250.88
	8	491	50.9	0.110	41.65	290.13
	9	455	54.5	0.123	39.89	189.33
Zn^{2+}	**5**	430	57.0	0.110	46.64	144.63
	6	370	63.0	0.100	56.70	207.54
	7	310	69.0	0.100	62.10	276.99
	8	520	48.0	0.115	37.58	100.39
	9	600	40.0	0.103	34.96	95.66
Pb^{2+}	**5**	430	57.0	0.105	48.88	105.21
	6	270	73.0	0.100	65.70	232.56
	7	200	80.0	0.100	72.00	266.44
	8	369	63.1	0.114	49.88	210.33
	9	510	49.0	0.100	44.10	80.70

* Initial conc. 1000 mg/l.

On the other hand, the phenotypic characterization of the other isolate MR108 showed that it was catalase-positive and oxidase-negative. Among the previously mentioned carbon sources, it able to utilize glucose only as sole carbon source and acid derived from it which caused indicator color change. This isolate was negative to indole, Voges Proskaure, and methyl red tests. Although it able to hydrolysis starch and don't able to hydrolysis gelatin, casein or lipids.

3.4.2. DNA and phylogenetic analysis

The sequence of the selected isolates was compared with related species of bacteria to check the similarity of 16S rRNA gene and their phylogenetic lineage using the alignments 16s rRNA gene forward sequence of each isolate. The bacterial 16S rRNA gene was increasingly used to discover and identify the species of unknown bacteria. These small 16S rRNA sequences are highly conserved regions (Raza and Ameen, 2016).

From the 16S rRNA gene sequencing results illustrated by the phylogenetic tree, the nearest bacterial species to MR99 isolate was *Bacillus cereus* strain JEM-2 subsp. HCBD, with 99% matching. Additionally, the nearest bacterial species to MR108 isolate was *Alcaligenes faecalis* strain L48, with 96% matching (Figs. 3 and 4). The sequence was submitted to the NCBI website with the accession number GenBank: MG257494.1 for MR99 and GenBank: MG966440.1 for Mr108.16S rRNA can discover the species and subspecies of bacteria (Ziaei-Nejad et al., 2006). These 16S rRNA housekeeping genes are widely used to identify bacteria because these regions are conserved (Raza and Ameen, 2016). Same 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 16S rRNA gene sequences, four of them are *Alcaligenes* spp. A similar *Alcaligenes* sp. was isolated from soil by Osborne and Erlich (1976) and was shown to oxidize arsenite to arsenate.

3.5. Effect of pH on heavy metals tolerant bacterial growth and their biosorption potential

In this experiment, the identified strains *Bacillus cereus* MG257494.1 and *Alcaligenes faecalis* MG966440.1 were examined for their biosorption potentials under different pH values in media supplemented with four heavy metals (Cu^{2+}, Cd^{2+}, Zn^{2+}, and Pb^{2+}) at 1000 mg/l. These potentials included determinations in cultural media (residual metals concentration (mg/l)), and determinations in microbial biomass, efficiency of biosorption (%), metals uptake (mg/g dry weight) and metals concentration in biomass (mg/g)).

Generally, *B. cereus* MG257494.1 has higher biosorption efficacy when developed in media supplemented with Cu^{2+} (82.0%) followed by media with Pb^{2+} (80%) at pH 7. The lowest biosorption efficiency was recorded at pH 9 when the bacterial strain was grown in media supplemented with Zn^{2+} at 1000 mg/l. Moreover, the accumulation of Pb^{2+} ions by *B. cereus* MG257494.1 was enhanced with increasing pH up to 7 and then decreased.

These results are in agreement with those demonstrated by Paul et al. (2006), the adsorption of lead by *B. cereus* M116 was improved with the increase in pH value from 3.6 to 7.0, beyond which the adsorption could not be carried out due to the precipitation of metal. Green-Ruiz et al. (2008) reported that the maximum value of Zn^{2+} uptake occurred at pH 6 and 7 by B. *circulans* 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%.

Respecting the effect of different pH values on Al. *faecalis* MG966440.1, data presented in Table (6) indicated that this strain was capable of absorbing different heavy metals under different pH values. This may be because *Alcaligenes faecalis* has been reported as heavy metals tolerant bacteria as Gupta and Nirwan (2015) and Abbas et al. (2015) demonstrated.

The optimum pH value that gave higher biosorption activity in media supplemented with any of all heavy metals was 6 compared to other pH values. Also, at the same pH value, the highest and the lowest biosorption efficiency by *Al. faecalis* was observed in media supplemented with Cd^{2+} and Zn^{2+}, respectively. Whereas, the biosorption potentials were equal in media supplemented with either Cu^{2+} or Pb^{2+}. These results agree with those reported by Ray et al. (2005) that the adsorption of metals decreases at low pH values because of the

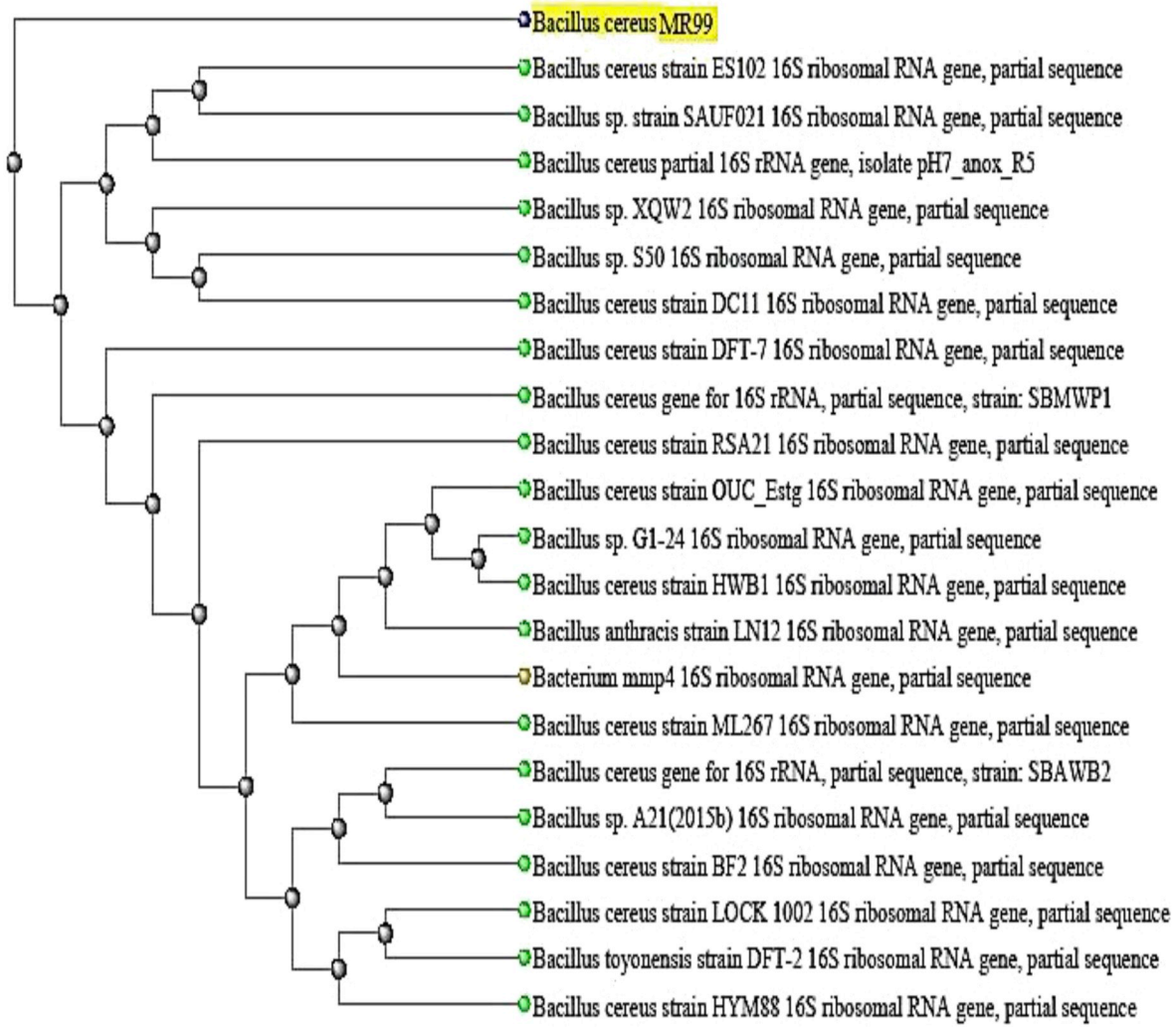


Fig. 3. Phylogenetic trees recovered from maximum likelihood analyses of the 16S rRNA gene partial sequence for the obtained isolates, *Bacillus* sp. MR99 (MG257494.1). The trees show the phylogenetic position of recovered *Bacillus* species within the phylogenetic branches of same genus. Average bootstrap values, of compared algorithms, are indicated at the branch roots. The bar represents 0.02 changes per nucleotide. Accession numbers of database extracted sequences are in brackets. It could be explained that the biosorption process consists of two phases: one phase is solid (biomass, sorbent, biosorbent, biological material) and another is a liquid phase (solvent) containing dissolved metal ions (Abbas et al., 2015). Regarding the effect of pH values on *B. cereus* activities, data in Table (5) showed that pH 7 was the optimum value for most activities (efficiency of biosorption and metal uptake) in medium supplemented with any of the tested metals (Cu^{2+}, Cd^{2+}, Zn^{2+}, and pb^{2+}) at 1000 mg/l.

competition for binding sites between cations and protons. The highest and the lowest biomass dry weight (0.130 and 0.098 mg/g) were recorded in media supplemented with Pb^{2+} at pH 8 and Zn^{2+} at pH 5, respectively. Additionally, data clearly indicated that pH 6 was the optimum for metal uptake and metal concentration in biomass. This trend was true in media individually supplemented with all heavy metals. Although the highest metal uptake by *Al. faecalis* was recorded in media amended with Cd^{2+} the highest metal concentration in biomass was recorded in the media supplemented with Cu^{+2}. Also, it was clear that the lowest metal uptake and metal concentration in biomass were recorded in media supplemented with Zn^{2+} and Cu^{2+} at pH 5, respectively.

Additionally, pH 8 was the optimum value for biomass dry weight in media supplemented with any of heavy metals (except Zn^{2+} was at pH 7). This result was following Tanveer (2011) who reported that *Al. faecalis* subsp. *phenolicus* demonstrated tolerance to micropollutants including heavy metals (up to 250 µg/ml for zinc in LB medium).

3.6. Cadmium-tolerant bacteria as antioxidant agents

Enzymatic and non-enzymatic antioxidant activities of *B. cereus* MG257494.1 and *Al. faecalis* MG966440.1 were estimated at two concentrations of heavy metals 1000 and 1500 mg/l individually. The ability to inhibit 2,2-DiPhenyl-2-Picryl hydrazyl hydrate (DPPH) under heavy metals stress was assayed for this purpose while three enzymes namely catalase (CAT), peroxidase (PO) and polyphenol oxidase (PPO) were assayed as enzymatic antioxidant activities.

Data indicated that the maximum residual DPPH (minimum inhibition) by the two strains were observed in culture filtrate free of heavy metals. The highest percentage was observed by *Al. faecalis* MG966440.1 followed by *B. cereus* MG257494.1 at the rate of 49.4 and 40.6%, respectively. This may be because of their low ability to inhibit DPPH under normal conditions without stress. Heo et al. (2006) confirmed that DPPH is a free radical generating compound and has been widely used to evaluate the free radical scavenging ability of various antioxidative compounds under stress conditions.

Generally, the inhibition of DPPH by *Al. faecalis* MG966440.1 was increased with the increase of heavy metals concentrations. In the

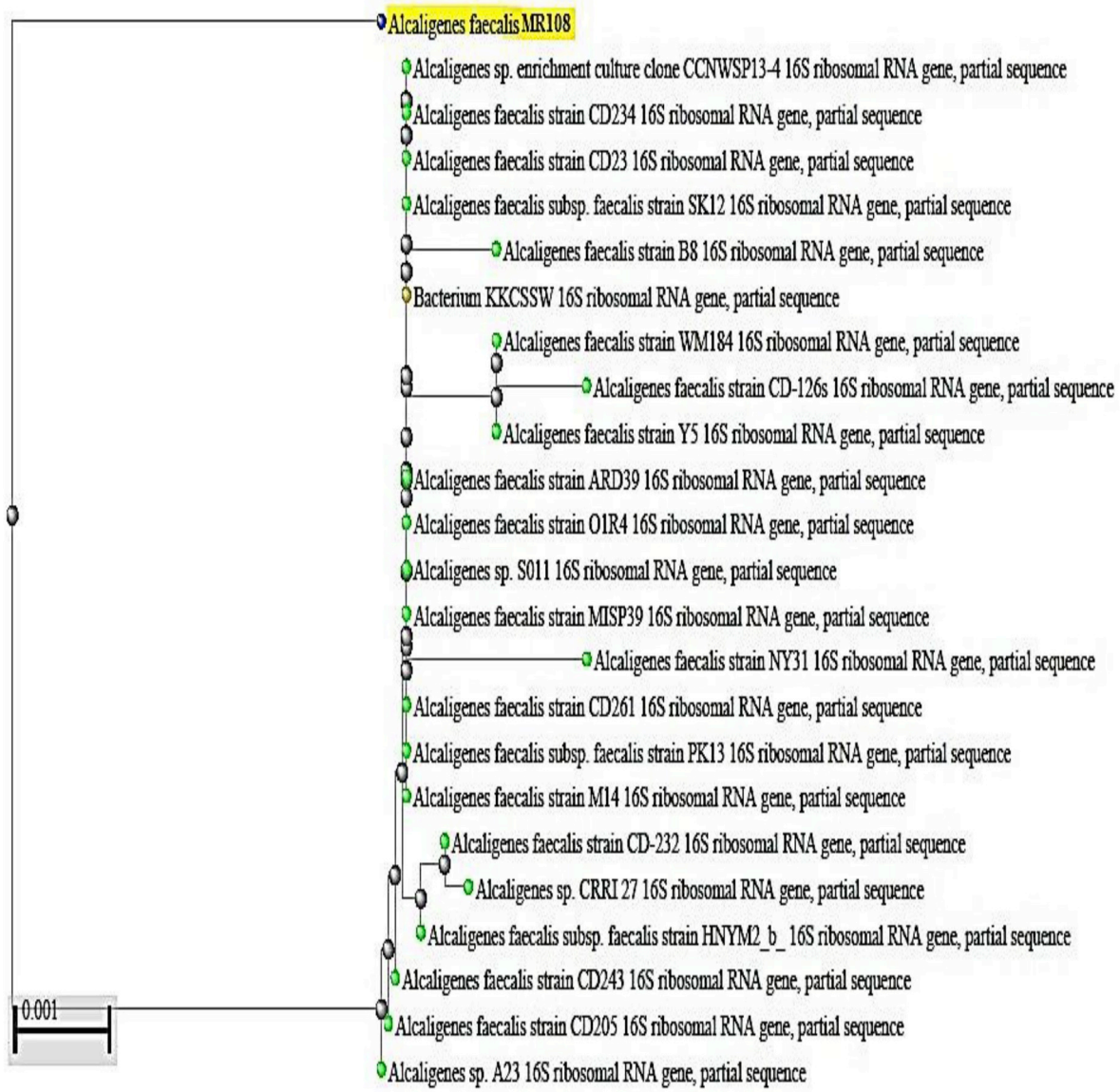


Fig. 4. Phylogenetic trees recovered from maximum likelihood analyses of the 16S rRNA gene partial sequence for the obtained isolates, *Alcaligenes* sp. MR108 (MG966440.1). The trees show the phylogenetic position of recovered Alcaligenes species within the phylogenetic branches of same genus. Average bootstrap values, of compared algorithms, are indicated at the branch roots. The bar represents 0.02 changes per nucleotide. Accession numbers of database extracted sequences are in brackets.

Table 6
Effect of pH on bacterial growth and biosorption activities under different heavy metals by *Al. faecalis* MG966440.1

Heavy metals	pH	Residual metals cons. mg/l	Efficiency of biosorption (%)	Biomass dry weight (mg/g)	Metals uptake (mg/g dry weight)	Metals con. in biomass mg/g
Cu^{2+}	**5**	520	48.0	0.112	38.58	100.00
	6	230	77.0	0.100	69.30	300.00
	7	300	70.0	0.109	57.80	230.45
	8	410	59.0	0.126	42.14	150.69
	9	480	52.0	0.103	45.45	189.20
Cd^{2+}	**5**	337	66.3	0.123	48.53	120.33
	6	115	88.5	0.100	79.65	270.69
	7	133	86.7	0.110	70.94	210.89
	8	353	64.7	0.126	46.21	190.33
	9	460	54.0	0.103	47.20	160.63
Zn^{2+}	**5**	590	41.0	0.098	37.68	136.96
	6	355	75.0	0.099	68.18	292.52
	7	369	63.1	0.113	50.27	200.69
	8	420	58.0	0.104	50.21	130.00
	9	470	53.0	0.099	48.18	120.89
Pb^{2+}	**5**	510	49.0	0.111	39.74	145.36
	6	230	77.0	0.100	69.30	270.55
	7	290	71.0	0.100	63.90	210.44
	8	350	65.0	0.130	45.01	170.49
	9	405	59.5	0.125	42.93	110.23

* Initial conc. 1000 mg/l.

contrast, opposite results were recorded with *B. cereus* MG257494.1 when inoculated in media supplemented with Cu^{2+}, Cd^{2+} and Zn^{2+} individually (Table 7). Additionally, the maximum inhibition of DPPH in media amended with Pb^{2+} or Zn^{2+} was recorded at 1500 mg/l by *B. cereus* MG257494.1 and *Al. faecalis* MG966440.1, respectively. The reduction capability of DPPH radical was determined by the decrease induced by antioxidative compounds (Athukorala et al., 2003). The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability (Ilhami et al., 2004). Also, data presented in Table (7) indicated that proved that the highest and lowest inhibition of DPPH in media supplemented with heavy metals mixture was observed by *Al. faecalis* MG966440.1 at 1500 mg/l and *B. cereus* MG257494.1 at 100 mg/l, respectively. This may be means that *Al. faecalis* MG966440.1 has high ability to savaged free radicals under a high-stress condition.

Data in Table (7) indicated that the highest values of all estimated enzymes (CAT, PO, PPO) by *B. cereus* MG257494.1 were recorded in media with Cu^{2+} at 1500 mg/l, Cd^{2+} at 1000 mg/l and Cd^{2+} at 1500 mg/l, respectively. Moreover, CAT and PPO values were higher in media supplemented with heavy metals mixture at 1500 than 1000 mg/l. While the PO was higher at 1000 than 1500 mg/l. Generally, CAT values were lower in media supplemented with Zn^{2+} at two concentrations than other heavy metals. Whereas, PO values were lower in media with Pb^{2+} at two concentrations than other heavy metals.

The lowest and the highest CAT values by *Al. faecalis* MG966440.1 were recorded in media supplemented with Pb^{2+} at 1000 mg/l and media with heavy metals mixture at the same concentration. Moreover, PO values were higher in media supplemented with Cd^{2+} than other heavy metals. Whereas, lower values were recorded in media amended with Pb^{2+} at two concentrations. Additionally, higher PPO values were recorded in media amended with Cu^{2+} than other heavy metals individually. Many studies were in harmony with our results, Schutzendubel and Polle (2002) demonstrated that bacteria could increase their antioxidant activity to decrease the negative consequences of heavy metal stress.

Moreover, the obtained results are in good agreement with the previous results which confirmed that the total amount of metal biosorption in a multiple metal system is lower than that in a single metal system (Utigikar et al., 2000). The microorganism can develop resistance to heavy metal by adsorption via extracellular polysaccharides, cell exclusion, sequestration as insoluble phosphates, or ion efflux to the cell exterior (Jarosławiecka and Piotrowska-Seget, 2014). Enzymatic detoxification of metal ions (oxidation, reduction, methylation, and demethylation) to less toxic forms is also defense mechanisms in bacteria. Metal (ox) reducing-PGPRs can decrease metal mobility and/or toxicity (Chatterjee et al., 2009).

4. Conclusion

The current study aimed to isolate, identify and characterize cadmium tolerant bacterial isolates from heavy metals contaminated sites in Egypt. Only three of sixty-nine isolates were recovered as the most tolerant isolates for cadmium. Among them, two isolates MR99 and

Table 7
Antioxidant activities of two bacterial filtrates under different heavy metals concentrations.

Bacterial strains	Heavy metals	Conc. (mg/l)	Non-enzymatic as (%) DPPH after 30 min.		Enzymatic as absorbance per min.		
			Residual	Inhibited	CTA	PO	PPO
Bacillus cereus	**Without any heavy metals**		40.6	59.4	0.001	0.078	0.098
MG257494.1	**Cu^{2+}**	**1000**	9.50	90.5	0.141	0.060	0.195
		1500	37.8	62.2	0.208	0.094	0.216
	Cd^{2+}	**1000**	10.2	89.8	0.066	0.095	0.202
		1500	17.6	82.4	0.093	0.348	0.323
	Pb^{2+}	**1000**	8.40	91.6	0.015	0.030	0.123
		1500	6.90	93.1	0.055	0.057	0.100
	Zn^{2+}	**1000**	16.7	83.3	0.007	0.013	0.085
		1500	37.8	62.2	0.014	0.027	0.137
	H. M. mixture	**1000**	46.1	53.9	0.052	0.423	0.101
		1500	34.9	65.1	0.089	0.152	0.209
Alcaligenes faecalis **MG966440.1**	**Without any heavy metals**		49.4	50.6	0.010	0.053	0.078
	Cu^{2+}	**1000**	22.8	77.2	0.105	0.092	0.287
		1500	15.9	84.1	0.024	0.059	0.237
	Cd^{2+}	**1000**	24.0	76.0	0.008	0.704	0.208
		1500	21.9	78.1	0.066	0.522	0.207
	Pb^{2+}	**1000**	11.8	88.2	0.003	0.025	0.108
		1500	7.80	92.2	0.018	0.039	0.075
	Zn^{2+}	**1000**	17.6	82.4	0.021	0.042	0.143
		1500	13.2	86.8	0.010	0.060	0.183
	H. M. mixture	**1000**	33.6	66.4	0.079	0.188	0.131
		1500	23.9	76.1	0.095	0.044	0.354

H. M.: Heavy metals, CTA: catalase, PO: peroxidase, PPO: polyphenol oxidase.

MR108 can tolerate four heavy metals (copper, cadmium, zinc, and lead) at different concentrations so, identified according to the morphological, cultural, biochemical characteristics and 16S rDNA partial sequence as *Bacillus cereus* MG257494.1 and *Alcaligenes faecalis* MG966440.1. Then, these two strains were examined for their biosorption potentials under different pH values in media supplemented with four heavy metals at 1000 mg/l. These potentials included determinations in cultural media (residual metals concentration (mg/l)), and determinations in microbial biomass, efficiency of biosorption (%), metals uptake (mg/g dry weight) and metals concentration in biomass (mg/g)). Results showed that pH 7 and 6 were optimum for *B. cereus* MG257494.1 and *Al. faecalis* MG966440.1, respectively. After that, it was estimated their characterization as antioxidant agents by enzymatic and non-enzymatic methods. Results indicate that the highest percentage was observed by *Al. faecalis* MG966440.1 followed by *B. cereus* MG257494.1 at the rate of 49.4 and 40.6%, respectively. Moreover, the enzymatic antioxidant activities by the two strains proved that the values of the three estimated enzymes (catalase, peroxidase, polyphenol oxidase) were higher in media supplemented with heavy metals mixture at different concentration (1500 and 1500 mg/l).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101299.

References

Abbas, S., Ahmed, I., Iida, T., Lee, Y.J., Busse, H.J., Fujiwara, T., Ohkuma, M., 2015. A heavy metal tolerant novel bacterium, *Alcaligenes pakistanensis* sp. nov., isolated from industrial effluent in Pakistan. Antonie Leeuwenhoek 108, 859–870.

Aebi, H.B., 1984. Isolation, purification, characterization, and assay of antioxygenic enzymes: 13: catalase in vitro. Methods Enzymol. 21–126.

Ansari, R.A., Qureshi, A.A., Ramteke, D.S., 2016. Isolation and characterization of heavy metal resistant microbes from industrial soil. Int. J. Environ. Sci. 6 (5), 670–680.

Athukorala, Y., Lee, K.W., Shahidi, F., Heu, M.S., Kim, H.T., Lee, J.S., Jeon, Y.J., 2003. Antioxidant efficacy of extracts of an edible red Alga *(Grateloupia filicina)* in linoleic acid and fish oil. J. Food Lipids 10, 313–327.

Banerjee, S., Gothalwal, R., Sahu, P.K., Sao, S., 2015. Microbial observation in bioaccumulation of heavy metals from the Ash Dyke of thermal power plants of Chhattisgarh, India. Adv. Biosci. Biotechnol. 6, 31–138.

Brenner, D.J., Krieg, N.R., Staley, J.T., Garrity, G.M., 2005. Bergey's Manual of Systematic Bacteriology, second ed., vol. 2. Springer-Verlag, New York, NY. parts A, B and C.

Chatterjee, S., Sau, G.B., Mukherjee, S.K., 2009. Plant growth promotion by a hexavalent chromium reducing bacterial strain, *Cellulosimicrobium cellulans* KUCr3. World J. Microbiol. Biotechnol. 25, 1829–1836.

Congeevaram, S., Dhanarani, S., Park, J., Dexilin, M., Thamaraiselvi, K., 2007. Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. J. Hazard Mater. 146, 270–277.

Dhuldhaj, U.P., Yadav, I.C., Singh, S., Sharma, N.K., 2013. Environmental contamination and human exposure to lead in Brazil. Rev. Environ. Contam. Toxicol. 224, 1–38. https://doi:10.1007/978-1-4614-5882-1_1.

Goregaonkar, S.S., Tiwari, S.R., Doifode, S.H., Prashar, K., Warkhade, B.B., 2017. Isolation and characterization of cadmium resistant bacteria from industrial waste water and soil. Int. J. Life Sci., Special Issue A8, 22–27. http://oaji.net/articles/2017/736-1506255979.pdf.

Green-Ruiz, C., Rodriguez-Tirado, V., Gomez-Gil, B., 2008. Cadmium and zinc removal from aqueous solutions by *Bacillus jeotgali*: pH, salinity and temperature effects. Bioresour. Technol. 99, 3864–3870.

Gupta, S., Nirwan, J., 2015. Evaluation of mercury biotransformation by heavy metal-tolerant *Alcaligenes* strain isolated from industrial sludge. Int. J. Env. Sci. Technol. 12, 995–1002.

Heo, S., Park, E., Lee, K., Jeon, Y., 2006. Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresour. Technol. 96, 1613–1623.

Heo, S., Park, P., Park, E., Kim, S., Jeon, Y., 2005. Antioxidant activity of enzymatic extracts from a brown seaweed *Ecklonia cava* by electron spin resonance spectrometry and comet assay. Eur. Food Res. Technol. 221, 41–47.

Ilhami, G., Sat, I.G., Beydemir, S., Elmastas, M., Kufrevioglu, O.I., 2004. Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L.). Food Chem. 87, 393–400.

Jain, S., Bhatt, A., 2014. Molecular and in situ characterization of cadmium resistant diversified extremophilic strains of *Pseudomonas* for their bioremediation potential. Biotechnology 4, 297–304. http://doi:10.1007/s13205-013-0155-z.

Jarosławiecka, A., Piotrowska-Seget, Z., 2014. Lead resistance in microorganisms. Microbiology 160, 12–25.

Khan, M.H., Unnikrishnan, S., Ramalingam, K., 2019. Bactericidal potential of silver-tolerant bacteria derived silver nanoparticles against multi drug resistant ESKAPE pathogens. Biocatalysis Agric. Biotechnol. 18, 100939.

Kharwar, R.N., Srivastava, P.K., Singh, M., 2017. Role of rhizospheric mycobiota in remediation of arsenic metalloids. Phytoremed. Environ. Pollutants 137–158. http://doi:10.1016/j.chemosphere.2016.12.116.

Liu, H.L., Chen, B.Y., Lan, Y.W., Cheng, Y.C., 2004. Biosorption of Zn (II) and Cu (II) by the indigenous *Thiobacillus thiooxidans*. Chem. Eng. J. 97, 195–201.

Malik, A., Jaiswal, R., 2000. Metal resistance in *Pseudomonas* strains isolated from soil treated with industrial wastewater. World J. Microbiol. Biotechnol. 16, 177–182.

Marzan, L.W., Hossain, M., Mina, S.A., Akter, Y., Chowdhury, A.M.A., 2017. Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: bioremediation viewpoint. The Egypt. J. Aqua. Res. 43 (1), 65–74. http://doi.org/10.1016/j.ejar.2016.11.002Get.

Moghannem, S.A., Refaat, B.M., El-Sherbiny, G.M., El-Sayed, M.H., Elsehemy, I.A., Kalaba, M.H., 2015. Characterization of heavy metal and antibiotic resistant bacteria isolated from polluted localities in Egypt. Egypt. Pharm. J. 14 (3), 158–165.

Narde, G.K., Kapley, A., Purohit, H.J., 2004. Isolation and characterization of *citrobacter* strain HPC255 for broad-range substrate specificity for chlorophenols. Current microbial 48, 419–423.

Nishijo, M., Nakagawa, H., Yasushi, S.K.N., Teruhiko, K., 2017. Causes of death in patients with Itai-itai disease suffering from severe chronic cadmium poisoning: a nested case–control analysis of a follow-up study in Japan. BMJ Open 7, e015694. http://doi:10.1136/bmjopen-2016-015694.

Oktay, K., Schenken, R.S., Nelson, J.F., 1995. Proliferating cell nuclear antigen marks the initiation of follicular growth in the rat. Biol. Reprod. 53, 295–301.

Onsa, G.H., Saari, N.B., Selamat, J., Bakar, J., 2004. Purification and characterization of membrane bound peroxidases from *Metroxylon Sagu*. Food Chem. 85, 365–376.

Osborne, F., Erlich, H., 1976. Oxidation of arsenite by a soil isolate of *Alcaligenes*. J. Appl. Bacteriol. 41, 295–305.

Paul, S., Bera, D., Chattopadhyay, P., Ray, L., 2006. Biosorption of Pb (II) by *Bacillus cereus* M116 immobilized in calcium alginate gel. J. Hazard. Sub. Res. 5 (2), 1–13.

Peter, A.O., 2011. Biological Remediation of Hydrocarbon and Heavy Metals Contaminated Soil. Soil Contamination, 1281-142.

Purohit, P.K., Kondev, J., Phillips, R., 2003. Mechanics of DNA packaging in viruses. Proc. Natl. Acad. Sci. 100 (6), 3173–3178.

Rajaganapathy, V., Xavier, F., Sreekumar, D., Mandal, P.K., 2011. Heavy metal contamination from soil, water and fodder and their presence in livestocks and Products: a Review. J. Environ. Sci. Technol. 4, 234–249.

Ray, L., Paul, S., Bera, D., Chattopadhyay, P., 2005. Bioaccumulation of Pb(II) from aqueous solutions by *Bacillus cereus* M1. J.hazard. Subs. res. 5, 1–21.

Raza, S., Ameen, A., 2016. Comparison of 16S rRNA gene of *Bacillus cereus* with different bacterial Species. J, Advances Biol. Biotechnol. 7 (2), 1–6.

Safahieh, A., Abyar, H., Roostan, Z., Zolgharnein, H., Mojoodi, F., 2012. Isolation and characterization of *Pseudomonas* resistant to heavy metals and poly aromatics hydrocarbons (PAHs) from Persian Gulf sediments. Afr. J. Biotechnol. 11, 4418–4423. http://doi:10.5897/AJB11.3450.

Schutzendubel, A., Polle, A., 2002. Plant responses to abiotic stresses: heavy metal induced oxidative stress and protection by mycorrhization. J. Exp. Bot. 53, 1351–1365.

Sen, M., Joshi, H., 2016. Determination of minimum inhibitory concentration and antibiotic susceptibility of zinc tolerant bacteria isolated from mine soil of zawar mines. Indian. J. Fund. Appl. Life Sci. 6 (2), 23–29.

Sharma, P.K., Balkwill, D.L., Frenkel, A., Vairavamurthy, M.A., 2000. A new Klebsiella planticola strain (Cd-1) grows anaerobically at high cadmium concentrations and precipitates cadmium sulfide. Appl. Environ. Microbiol. 66 (7), 3083–3087. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC92115/.

Sharma, R., Archana, G., 2016. Cadmium minimization in food crops by cadmium resistant plant growth promoting rhizobacteria. Appl. Soil Ecol. 107, 66–78.

Shetty, R., Rajkumar, S., 2009. Biosorption of Cu (II) by metal resistant *Pseudomonas* sp. Int. J. Environ. Res. 3 (1), 121–128.

Silva, R.M.P., Rodríguez, A.A., De, J.M., Oca, G.M., Moreno, D.C., 2009. Biosorption of chromium, copper, manganese and zinc by *Pseudomonas aeruginosa* AT18 isolated from a site contaminated with petroleum. Bioresour. Technol. 100, 1533–1538.

Stevenson, K., McVey, A.F., Clark, I.B., Swain, P.S., Pilizota, T., 2016. General calibration of microbial growth in microplate readers. Sci. Rep. 6, 1–7.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Tanveer, F., 2011. Bioaccumulation of Selected Heavy Metals by Various Bacterial Strains from Tannery Effluents. Masters Dissertation. Fatima Jinnah Women University, Rawalpindi.

Utigikar, V.P., Chen, B.Y., Tabak, H.H., Bishop, D.F., Govind, R., 2000. Treatment of acid mine drainage: equilibrium biosorption of zinc and copper on non-viable activated sludge. Int. Biodeterior. Biodegrad. 46, 19–28.

Verma, C., Singh, P., Kumar, R., 2015. Isolation and characterization of heavy metal resistant PGPR and their role in enhancement of growth of wheat plant under metal (cadmium) stress condition. Arch. Appl. Sci. Res. 7 (7), 37–43.

Vos, P., Garrity, G., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.H., Whitman, W.B. (Eds.), 2009, Bergey's Manual of Systematic Bacteriology, second ed., vol. 3. Springer-Verlag, New York, NY.

Wang, J.L., Chen, C., 2006. Biosorption of heavy metals by *Saccharomyces cerevisiae*. Biotechnol. Adv. 24, 427–451.

Yaseen, R.Y., Abd El-Aziz, S.M., Eissa, D.T., Abou-Shady, A.M., 2018. Application of biosurfactant producing microorganisms to remediate heavy metal pollution in El-Gabal El-Asfar area. Alexandria Sci. Exch. J. 39, 17–34.

Ziaei-Nejad, S., Rezaei, M.H., Takami, G.A., Lovett, D.L., Mirvaghefi, A.R., Shakouri, M., 2006. The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp Fenneropenaeus indicus. Aquaculture 252, 516–524.