Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.941304

56 (2015) 1037–1046
October

Taylor & Francis
Taylor & Francis Group

Isolation and characterization of Cd-resistant bacteria from industrial wastewater

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Received 7 January 2014; Accepted 27 June 2014

ABSTRACT

Three bacterial strains were isolated from industrial effluents of Penang, Malaysia. The strains RZ1, RZ2, and RZ3 were identified as *Pantoea* sp. RL32.2, *Salmonella enterica*, and *Enterobacter* sp. OCPSB1, respectively, based on morphological observation, biochemical, physiological characterization, and 16S rDNA sequence analysis. The strains RZ1, RZ2, and RZ3 removed 89.89, 82.10, and 89.14% of cadmium, respectively, when the 100 μg/mL of cadmium was added in the medium. The minimum inhibitory concentrations of strains RZ1, RZ2, and RZ3 were 750, 410, and 550 μg/mL, respectively. Cured strain showed resistance and sensitivity against some range of antibiotics. The molecular weights of induced proteins were 35 and 25 kDa in the presence of cadmium which points out a possible role of this protein in cadmium removal. Overall, these strains could be useful for the removal of cadmium in industrial wastewater.

Keywords: Absorbance; Antibiotics; Bioremediation; Cadmium; Isolation

1. Introduction

Cadmium (Cd) is a heavy metal contaminant in the environment. Extensive data suggest Cd is the most toxic heavy metal and it is included in the black list of several international agreements established to regulate the input of Cd into the environment [1]. It is extensively used in the industry for a number of applications, including electroplating, protection against corrosion, and stabilizing plastic [2]. Wastewaters of these industries contain Cd ranging from 10 to 100 mM [3]. Cd can enter the human food chain through plants, smoking materials, and diet [4]. Cd is carcinogenic, embryotoxic, teratogenic, and mutagenic and may cause hyperglycemia, reduced immunopotency, and anemia, due to its interference with iron metabolism [5]. The toxicity of Cd has also been well documented in selective types of almost all major phyla of eukaryotes [6].

Conventional methods such as precipitation, oxidation, or reduction have been commonly used to remove heavy metals from industrial wastewater [7]. They are ineffective and expensive. So, substitute methods of Cd removal and revival based on biological resources have been considered [8]. Among microorganisms, bacteria, yeast, and protozoa are generally the first category to be exposed to Cd present in the environment [9]. The Cd-resistant bacteria have been isolated in a number of studies which explain the mechanism of Cd uptake in bacteria [10]. Moreover, it

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is also indicated that Gram-negative bacteria are highly resistant to Cd ions and metabolize great amounts of Cd during growth. Gram-positive bacteria on the other hand, show a heterogeneous behavior and the intracellular pool reaches a plateau on increasing Cd concentration [11].

Bacterial genes that are involved in Cd-resistant are located on plasmids that harbor many genes against many metals [12]. In Cd-resistant bacteria four different mechanisms are present to resist the Cd. First one is efflux mechanism in which CadA and CadB genes are involved and they resist against zinc (Zn) and Cd. Second mechanism is based on enzymes that make the bacterial cell wall impermeable to Cd in which manganese transport system and zinc transport system are involved. Both systems are coded by chromosome. Third mechanism is the conversion of Cd into non-toxic form of enzymes. After bioconversion, the Cd undergoes valency change as a result less toxic and volatile compounds have been produced in many cases. Fourth, Cd-resistant bacteria have developed mechanisms to bind Cd to surface factor or intracellular binding. A mutant *Citrobacteri* isolated from metal-polluted area accumulates Cd inside the cell as insoluble cell-bound $CdHPO_4$ during the growth in the presence of glycerol 2-phosphate and Cd. The surface of the cell is most important for the precipitation of metal [13]. In some cases, Cd has been shown to bind to the capsular surface in *Klebsiella aerogenes* and in *Arthrobacter viscosus* [14]. Binding of Cd by thiols: this mechanism is important in the eukaryotic organisms but in case of bacteria, polythiols Cd binding peptide is analogous to metallothionein of animal cells [15].

Although different Cd-resistant bacterial species are used for the removal of Cd, the isolation of potential Cd-resistant bacteria and characterization of Cd removal properties are still required in order to remove the Cd contaminant from environment. The aim of this study was to isolate bacterial strains with high Cd-resistance. The physiological, biochemical, and molecular features were used to characterize the strains. Phylogenetic analysis, based on 16S rDNA gene sequence data, was also used to reveal the genetic relationship between the strains and others. The protein-profiling technique was also used to characterize strains on protein level.

2. Materials and methods

2.1. Chemicals

Lauria bertani (LB) broth and LB agar were purchased from Hi-media laboratories (India), cadmium chloride ($CdCl_2$) was procured from Sigma Aldrich (USA), penicillin (10 IU), tetracycline (30 μg), amoxicillin (10 μg), gentamycin (10 μg), cephalexin (30 μg), erythromycin (15 μg), streptomycin (10 μg), ciprofloxacin (5 μg), and M9 acetate minimal medium (0.5 g/mL yeast extract, 0.2 g/mL $MgSO_4$, 5.0 g/mL sodium acetate, 0.001 g/mL $FeSO_4$, 0.001 g/mL $CaCl_2$ (as agglomerating agent), 0.5 g/mL K_2HPO_4 (potassium and phosphorous source), and 1.0 g/mL NH_4Cl).

2.2. Sampling

Wastewater effluent samples of Globetronics Industries Sdn. Bhd. and Ever-prosper Battery & Tyres Sdn. Bhd. Penang, Malaysia were collected from Bayan Lepas zone/area located at about 1 km away from the source of wastewater effluent. A total of four samples were taken from both localities in sterilized screw-capped bottles and brought to the microbiology laboratory of Universiti Sains Malaysia. The parameters such as pH, temperature, longitude, latitude, and Cd concentration were noted.

2.3. Isolation of Cd-resistant bacteria

LB agar plates (pH adjusted to 7 ± 7.5) containing $CdCl_2$ with Cd concentration 10 μg/mL were used to select Cd-resistant bacteria and 20 μL of each wastewater sample was spread on agar plates and incubates at 37°C for 24 h [16]. Bacterial colonies growing on agar plates were purified by streaking and re-streaking.

2.4. Identification of bacterial isolates based on classical taxonomy and 16S rDNA

Among the selected bacterial strains which can remove Cd, the morphological, physiological, and biochemical characteristics were evaluated. For morphological and physiological characterization like colony color was observed under 10X lens, shape and nature of strains either Gram-negative or Gram-positive were observed under light microscope after Gram's staining. The ability of bacterial isolates of spore forming or non-spore forming was checked by endospore staining. The motility of isolated strains was checked by motility test. In this test, semi-solid agar was prepared in the test tubes and stabbed by inoculating needle that was already charged with bacterial culture. These test tubes were incubated at 37°C for 24 h. Taxonomic identification of those isolates was carried out using Bergey's Manual of Determinative Bacteriology. Confirmation of the taxonomical status of the selected strains was done by molecular methods. For further

identification, the genomic DNA was extracted using Sigma's GenElute Bacterial Genomic Kit according to the manufacturer's instructions and the 16S rDNA gene was amplified by polymerase chain reaction (PCR) using the universal 16S rDNA primers 27F (5′AGAGTTTGATCMTGGCTCAG 3′) and 1492R (5′TACGGYTACCTTGTTACGACTT 3′) [17]. The reaction conditions were as follows: 95˚C for 3 min; 32 cycles: 94˚C for 1 min, 56˚C for 1 min and 72˚C for 2 min; 72˚C for 10 min and 4˚C pause. PCR products corresponding to the expected size of amplified 16S rDNA (1.5 kb) were purified with a Gel Extraction Kit (Invitrogen) and sent to Center of Chemical Biology, Universiti Sains Malaysia Co. Ltd. for sequencing.

2.5. Phylogenetic analysis

16S rDNA sequence was submitted to the database of GenBank and compared with similar sequences by BLAST analysis. Phylogenetic trees of 16S rDNA were constructed using the software Clustal W [3].

2.6. Cd removal

The 20 μL of bacterial culture was added in each flask which consisted of 100 μg Cd/mL. Subsequently, these flasks were placed on shaking incubator at 37˚C and aliquots (1 mL) were taken after every 4 h intervals up to 24 h for Cd estimation [18]. Each time the cultures were spun using centrifuge machine (Hermle, Ind Co. Ltd. China) at 6,000 rpm/min to remove bacterial cells, and the supernatant was used to determine Cd concentration by GBC932 atomic absorption spectrophotometry (Pantech Instruments, Blackburn, Victoria, Australia) at 228.8 nm using a Cd lamp. The amount of Cd in samples after various intervals of time was estimated using a standard curve, which was prepared by taking various known concentrations of Cd in the medium. Reduction in the amount of Cd in the medium after growth of bacteria was taken as the Cd uptake ability of the isolates.

2.7. Effect of pH and temperature on the removal of Cd

To see the effect of pH and temperature on the Cd removal the bacterial isolates. The 50 mL LB broth was taken in each conical flask to test the effect of different pH (1.0–13.0) and temperatures (5–45˚C) on Cd removal by bacterial isolates. These flasks were inoculated with 20 μL of bacterial culture followed by incubation at the desired temperatures and adjusted with particular pH. These experiments were performed in triplicates. At each pH and temperature, the bacterial cells and supernatant were separated to check the Cd concentration by using atomic absorption spectrophotometry at 228.8 nm using a Cd lamp.

2.8. Determination of optimum growth conditions

For optimum growth of the bacterial isolates, two parameters, i.e. temperature and pH, were considered in both control and Cd-stressed conditions. For each bacterial isolate, 5 mL LB broth was added into 18 sets, each set consisted of three test tubes. The tubes were autoclaved and inoculated with 20 μL of the freshly prepared culture of each bacterial isolate grown over night at 37˚C. The nine sets of tubes without Cd-stress and nine sets of tubes with Cd-stress (100 μg/mL) were incubated at 5, 10, 15, 20, 25, 30, 35, 40, and 45˚C, respectively. After an incubation period of 12 h, their absorbance was measured at 600 nm using a LAMBDA 650 UV/vis spectrophotometer (Perkin Elmer, New York, USA). To determine the optimum pH, test tubes having 5 mL LB broth was prepared in 12 sets, each containing 3 test tubes and their pH was adjusted to 1.0, 3.0, 5.0, 7.0, 9.0, and 13.0 and then autoclaved. These tubes were inoculated with 20 μL freshly prepared cultures of each bacterial isolate. The six sets of test tubes were incubated with Cd-stress (100 μg/mL) and six sets of test tubes were incubated without Cd-stress. After an incubation period of 12 h, their absorbance was measured at 600 nm.

2.9. Effect of Cd on bacterial growth

The growth curves of bacterial isolates were determined in LB broth with 100 μg/mL of Cd and in control medium. For each bacterial isolate, 50 mL medium was taken in one set consisting of three flasks, autoclaved and then inoculated with 20 μL of the freshly prepared inoculums. The cultures were incubated at 37˚C in a shaker at 80–100 rotation/min. An aliquot of culture was taken out in an oven-sterilized tube at regular intervals of 0, 4, 8, 12, 16, 20, 24, and 32 h. Absorbance was measured at 600 nm and growth was plotted graphically.

2.10. Determination of Cd-tolerance

The growth measurement is used to evaluate the resistant properties of bacterial strains. The Cd-resistance of bacterial isolates was determined using stock solutions of 10 μg/mL of $CdCl_2$. The Cd-resistance was checked by increasing the concentration of

respective Cd in a stepwise manner with 50 μg/mL of Cd-checked resistance in the M9 acetate minimal medium Culture flasks containing 100 mL of medium and Cd^{2+} ions were inoculated with 20 μL overnight bacterial cultures and incubated at 37˚C for 24 h. Growth was measured as optical density at 600 nm.

2.11. Determination of antibiotic resistance

These strains were tested for their sensitivity against eight antibiotics that were given above. The 0.1 mL bacterial culture was plated onto LB agar plates. The antibiotic disks were placed on the plates and incubated at 37˚C for 1–2 d.

2.12. Protein profiling

In conical flasks, 20 mL LB broth was taken in triplicates and steam sterilized. Bacterial isolates were stressed with concentrations of Cd 350 μg/mL with control and were incubated for 16 h at 37˚C in shaking incubator and cells were harvested by centrifugation. The pellet was dissolved in 100 μL of 1X loading dye and then heat shock was given for 5 min, eppendorf was shifted on ice for 2 min and then was centrifuged at 12,000 rpm for 10 min. Supernatant was transferred to a new eppendorf, then the final centrifugation was done at 12,000 rpm for 10 min, and the supernatant was shifted to a new eppendorf. The bacterial proteins were resolved by SDS-PAGE. Electrophoresis was performed in vertical mini-slab gel (Mini-Protean III; BioRad) with a gel thickness of 0.75 mm and gel size 8 × 7 cm. The gels were composed of 10% resolving gel and 4% stacking gel and run at constant voltage of 200 V for 50 min. Amounts of 10 μL of bacterial protein extracts were loaded into each well of the gel. After electrophoretic separation, the gel was stained with Coomassie blue solution (0.01% Coomassie brilliant blue R250, 45% (v/v) methanol and 10% (v/v) glacial acetic acid) for 30 min at room temperature and subsequently placed in the destaining solution (50% (v/v) methanol and 2% (v/v) acetic acid) for 1 h. The gel image was captured and analyzed using VersaDoc Imaging System (BioRad) [19].

3. Results and discussion

3.1. Sampling and isolation of Cd-resistant bacteria

The parameters of industrial wastewater samples were noted as shown in Table 1. On the basis of morphology and color, three Cd-resistant bacterial strains were isolated and named as strains RZ1, RZ2, and RZ3.

3.2. Taxonomical identification and 16S rDNA

All the selected bacterial strains RZ1, RZ2, and RZ3 were morphologically, physiologically, and biochemically characterized. The investigated bacteria form mostly round colonies onto the agar surface. The colors of colonies vary from off-white to white and were slightly yellow. The cells of the isolated bacteria were mostly cocci shape. Most of the part of the isolates were Gram-negative, non-spore-forming bacteria and mostly were motile. After physiologo-biochemical tests, that were carried out, it comes to the next more important characteristics of the tested bacteria as shown in Table 2. All three strains could not degrade the hydrogen peroxide and urea and were also unable to hydrolyze gelatin. The strain RZ1 failed to ferment carbohydrate but strains RZ2 and RZ3 were able to ferment carbohydrate. Both strains RZ1 and RZ2 showed negative on MRVP test but strain RZ3 showed positive results for mixed acid fermentation. The strains RZI and RZ2 were unable to use citrate as a carbon source while strain RZ3 was metabolized easily. All the three strains were non-pathogenic, fastidious, and lactose fermenting. The taxonomic status of these isolates was determined using Bergey's Manual of Determinative Bacteriology [20], and showed that

Table 1
Collection of water samples

S. no.	Industries	Samples	pH	Temp. (˚C)	Longitude	Latitude	Cadmium concentration (mg/L)
1	Globetronics Industries Sdn. Bhd.	Sample 1	6.0	30	5˚.07617′N	100˚.386119′E	0.36
		Sample 2	7.0	29	5˚.38400′N	100˚.302104′E	0.52
2	Ever-prosper Battery & Tyres Sdn. Bhd.	Sample 3	6.0	35	5˚.41264′N	100˚.325307′E	0.67
		Sample 4	7.0	33	5˚.329379′N	100˚.482284′E	0.82

Table 2
Morphological and biochemical characteristics of bacterial isolates

	RZ1	RZ2	RZ3
Morphological			
Colony color	Off-white	Yellow	White
Gram nature	Negative	Negative	Negative
Cell morphology	Cocci	Cocci	Cocci
Motility	Positive	Positive	Positive
Colony shape	Round	Round	Round
Spore formation	Negative	Negative	Negative
Biochemical tests			
Catalase test	Negative	Negative	Negative
Urease test	Negative	Negative	Negative
Gelatin hydrolysis test	Negative	Negative	Negative
Carbohydrate test	Negative	Positive	Positive
MRVP test	Negative	Negative	Positive
Citrate test	Negative	Negative	Positive
Blood agar test	Positive	Positive	Positive
Chocolate agar test	Positive	Positive	Positive
MacConkey agar test	Positive	Positive	Positive

strains RZ1, RZ2, and RZ3 belong to genus *Pantoea*, *Salmonella*, and *Enterobacter*, respectively.

"Universal" eubacterial primers were used to amplify 1.5 kb of 16S rDNA of the strains RZ1, RZ2, and RZ3. The PCR products obtained were purified and sequenced by using BLAST to compare its sequence to all sequences in the NCBI database (http://www.ncbi.nlm.nih.gov). The 16S rDNA genes of strains RZ1, RZ2, and RZ3 were 97% homologous to *Pantoea* sp. RL32.2 (Accession number: GU056355), 97% homologous to *Salmonella enterica* (Accession number: KF056927), and 95% homologous to *Enterobacter* sp. OCPSB1 (Accession number: JN119828), respectively.

3.3. Phylogenetic analysis of 16S rDNA

Nucleotide sequence analysis of the 16S rRNA gene has been considered a fast and accurate method to identify the phylogenetic position of bacterial strains. The full-length 16S rDNA of strains RZ1, RZ2, and RZ3 were sequenced and used to construct phylogenetic development trees as shown in Fig. 1. We found that strain RZ1 was classified in the branch of *Pantoea* sp. It has 97% similarity with *Pantoea* sp. RL32.2 as shown in Fig. 1(a). The RZ2 strain has 97% similarity with *S. enterica* subsp. *Entericaserovar typhi*, which is classified under the family of *Enterobacteriaceae* 1736 as shown in Fig. 1(b). The strain RZ3 was classified under *Enterobacter*, it has 95% similarity with *Enterobacter* sp. OCPSB.

3.4. Cd estimation

These results showed that all the three strains were efficient in the removal of Cd as shown in Fig. 2. The three different strains showed different efficiency in removing Cd. The strains RZ1 and RZ2 were more efficient during the late log phase and less efficient during the early log phase, while strain RZ3 was less efficient in the late log phase and more efficient in the early log phase. Within 4 h of inoculation, strain RZ3 removed 52% while 8% removal in the presence of strain RZ1 and 10% removal in the presence of strain RZ2. After 8 h of incubation, strains RZ1 and RZ2 showed 58 and 40% reduction in the Cd, respectively, while strain RZ3 showed only 1% Cd removal. After 24 h, this reduction ranged between 82 and 89% in the three strains. Generally, the process of micro-organism removal of heavy metals includes biosorption and bioaccumulation. Biosorption process means metal ions are adsorbed by micro-organisms through the biochemical reactions including complex, chelate, ions exchange, and adsorption. Zeng et al. [3] described that *Pseudomonas aeruginosa* strain E1 uses these two mechanisms for the removal of Cd from industrial wastewater. Both living and non-living cells have biosorption. Bioaccumulation happens only in living cells. It is an active process and needs energy provided by the metabolism of micro-organisms [21].

3.5. Effect of pH and temperature on Cd removal

Maximum removal of Cd was observed 89.89, 82.10, and 89.14% by strains RZ1, RZ2, and RZ3 at pH 7.0 because pH 7.0 was the optimum pH of these bacterial strains, and at low and high pH no Cd removal was noted as shown in Figs. 3–5. The medium pH affects the solubility of metals and the ionization state of the functional groups (carboxylate, phosphate, and amino groups) of the microbial cell [22]. The Cd removal was decreased at higher and lower than optimum temperature 35˚C because at optimum temperature the biomass of bacteria was high as shown in Figs. 4 and 6. Bioaccumulation of Cd^{2+} ions decreased with increasing temperature. At low temperatures, the binding of heavy metal ions to micro-organism is by passive uptake [23]. Similar conclusions were also found by El-Deeb [24] and Mohamed [25]. The thermal-resistant bacteria effectively removed the Cd due to the production of enzymes like aldolase, RNA, and DNA polymerases which can perform their normal metabolism at the extremely higher temperature. Chang et al. [26] isolated *Vibrio parahaemolyticus* which showed higher thermal stability and survived in the

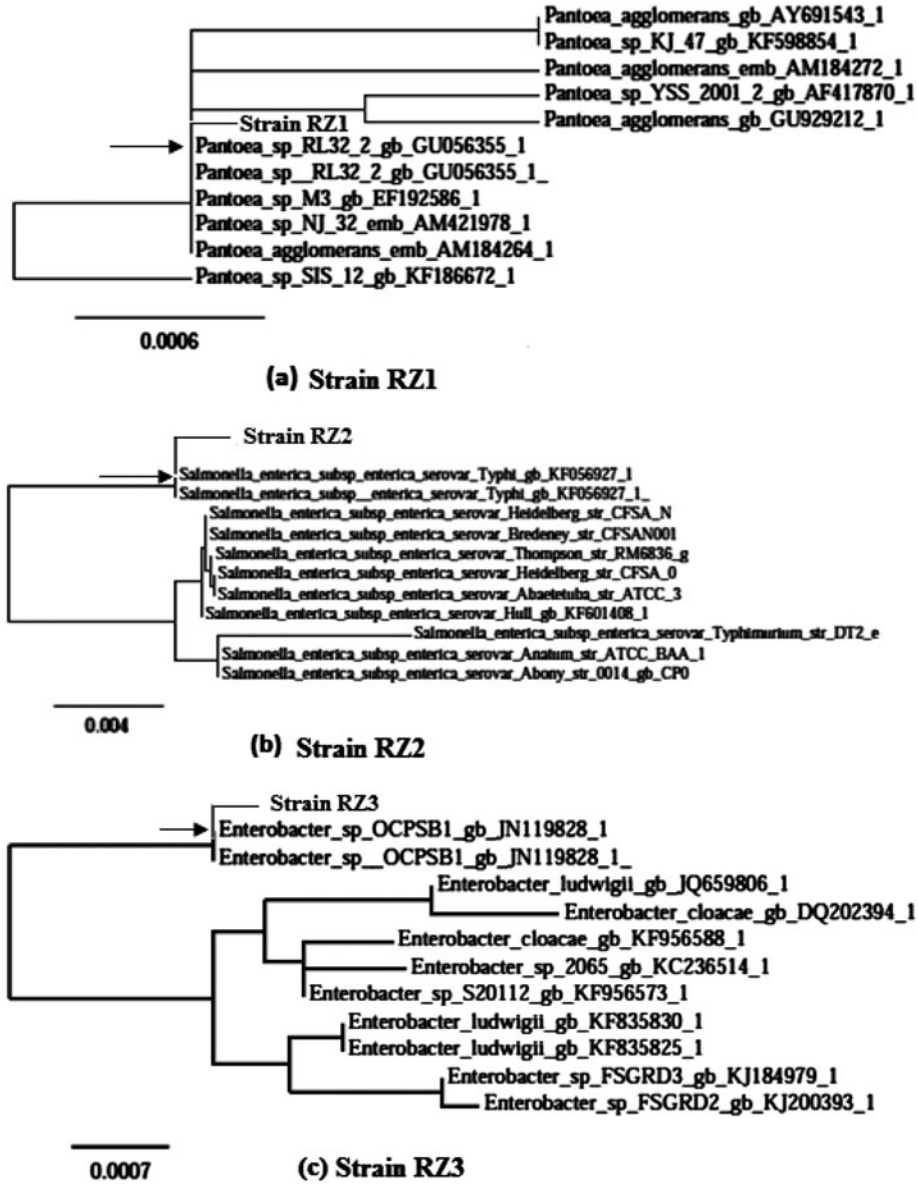

Fig. 1. Phylogenetic development trees based on 16S rDNA analysis: (a) strain RZ1, (b) strain RZ2 and (c) strain RZ3.

range of 0–70˚C and also removed 85% of Cd from wastewater.

3.6. Bacterial growth patterns against Cd

The growth patterns of these bacterial strains were not significantly different from those of controls, but the growth rate was lower in the presence of Cd^{2+} ions. The growth patterns were shown in Fig. 7. This could be explained by the toxicity of Cd, which causes retardation in the growth of the strains due to less enzymatic activities. Similar conclusions were reached by Filali et al. [27].

3.7. Cd-resistance

The strain RZ1 was found resistant against Cd up to a concentration of 750 μg/mL, but strain RZ2 can tolerate the Cd-stress up to 410 μg/mL and resistance level of strain RZ3 was 550 μg/mL. A common property of many bacteria capable of growth in the presence of Cd is their ability to prevent accumulation

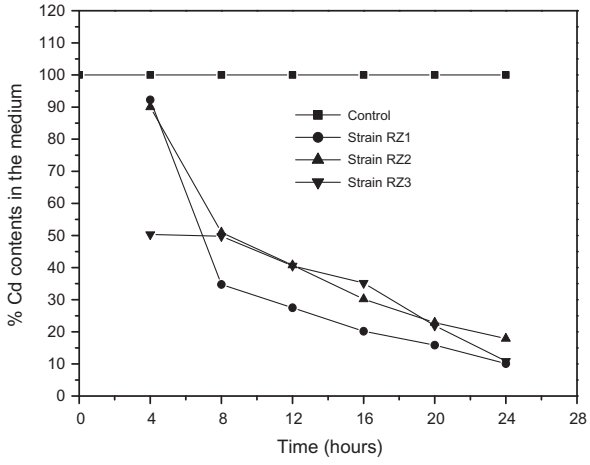

Fig. 2. Removal of Cd after inoculation of the three bacterial isolates. The medium containing the same original amount of Cd but without inoculation was taken as control. The initial concentration of Cd^{2+} ions in the medium was 100 μg/mL.

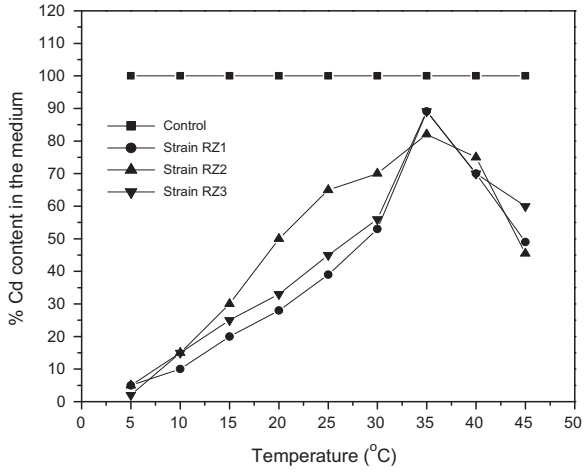

Fig. 4. The effect of various temperatures on the removal of Cd^{2+} ions by three bacterial isolates. The initial concentration of Cd^{2+} ions in the medium was 100 μg/mL.

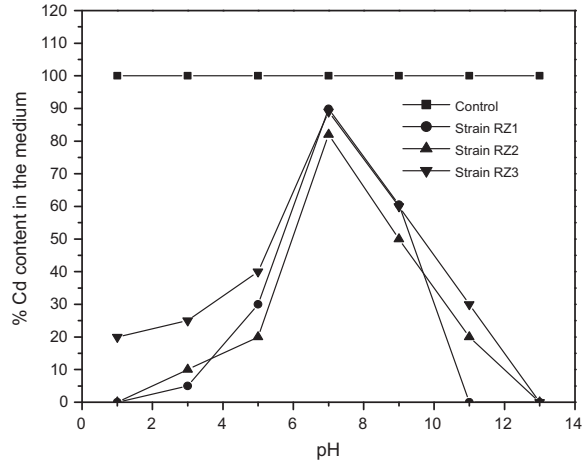

Fig. 3. The effect of various pH on the removal of Cd^{2+} ions by three bacterial isolates. The initial concentration of Cd^{2+} ions in the medium was 100 μg/mL.

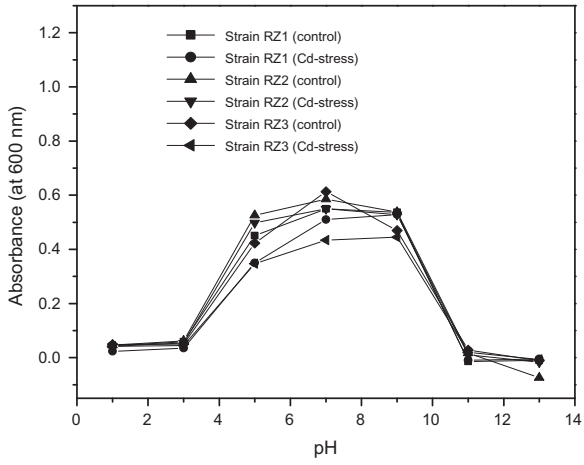

Fig. 5. The optimum pH of bacterial isolates in the presence and absence of Cd.

of free intracellular metal. At least two alternative mechanisms are possible. One is that the membrane of resistant bacteria acts as an impermeable barrier for toxic metals while allowing the passage of physiological cations. Cobbett and Goldsbrough [28] described such differences in spheroplasts prepared from Cd-sensitive or Cd-resistant strains of *S. aureus*. He suggested that exclusion of toxic ions was dependent upon conformational changes in the membrane. Alternatively, specific sites for the continuous efflux of Cd may operate and hence minimize the intracellular Cd concentration. Such a system operated in Cd-resistant *S. aureus* cells, since only the resistant strain effluxed the metal when cells were transferred from a medium containing Cd to a Cd-free medium [29]. Based on our results, the possible mechanism existed in these strains was accumulation of Cd^{2+} ions inside bacterial cells, and formed $CdHPO_4$. The formation of $CdHPO_4$ occurred during the growth of bacteria in the presence of glycerol 2-phosphate and Cd. This was confirmed by the decrease in the concentration of Cd at different time intervals in the medium.

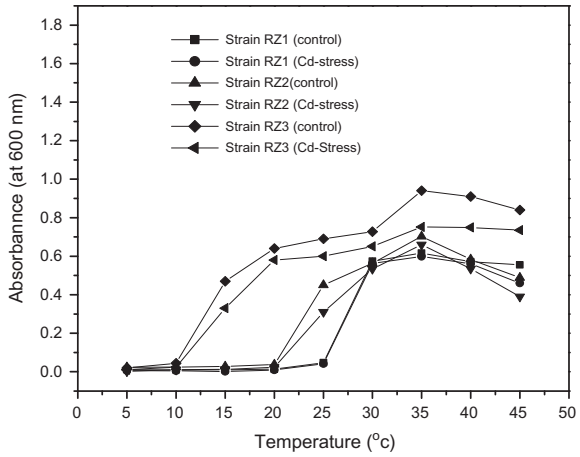

Fig. 6. The optimum temperature of bacterial isolates with and without Cd-stress.

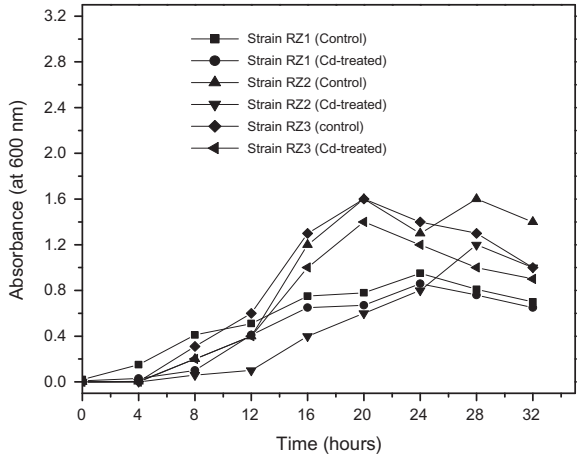

Fig. 7. The effect of Cd on the growth patterns of bacterial isolates.

3.8. Antibiotic susceptibility

In order to determine the resistance to antibiotics, the reactions of eight antibiotics were examined by the disk diffusion method. All three strains were sensitive against tetracycline, gentamycin, streptomycin, and ciprofloxacin but resistant against amoxicillin, erythromycin, and penicillin. Against cephalexin, strain RZ1 was resistant while strains RZ2 and RZ3 were sensitive as shown in Table 3. Under environmental conditions of Cd-stress, micro-organisms might have developed various mechanisms to resist antibiotics and tolerate metals. Microbes surviving in polluted water usually change intrinsic biochemical and structural properties, physiological, and genetic adaptation. According to some findings, metal resistance is associated with multiple antibiotic resistances on R plasmid [30,31].

Table 3
Resistance of antibiotics by cadmium resistant bacterial strains

Antibiotics	RZ1	RZ2	RZ3
Tetracycline (30 μg)	21 mm (S)	24 mm (S)	25 mm (S)
Amoxicilin (10 μg)	R	R	R
Penicillin (10 units)	R	R	R
Gentamycin (10 μg)	20 mm (S)	16 mm (S)	20 mm (S)
Cephalexin (30 μg)	R	14 mm (S)	16 mm (S)
Erythromycin (15 μg)	R	R	R
Streptomycin (10 μg)	17 mm (S)	13 mm (S)	15 mm (S)
Ciprofloxacin (5 μg)	30 mm (S)	35 mm (S)	17 mm (S)

Notes: S: Sensitive; R: Resistant.

3.9. Protein bands

To study the protein profile of bacteria under stressed and non-stressed conditions, total cell proteins of bacteria were isolated after 16 h of Cd exposure. The Cd-stress to bacteria was given after their optical density reached at 0.3 indicating that bacteria have entered into a log phase. The protein gel of stress organisms indicated new protein bands in front of 35 and 25 kDa. This indicated that the Cd-resistance proteins in bacteria were inductive proteins and they expressed only in the stressed conditions as shown in Fig. 8. The nature of the 35 and 25 kDa proteins system remains unknown and may be the result of Cd-resistance or its cause. The 35 kDa protein appeared upon adaptation to Cd and may therefore be a protein which controls intracellular Cd concentrations. It may be a new form of a preexisting transport protein (33 kDa protein) with altered specificity such that Cd uptake is reduced. Alternatively, it may be a new protein capable of exchanging internal Cd^{2+} ions for external cations and hence preventing Cd accumulation. These results were in agreement with SDS-PAGE analysis by Chovanová et al. [32]. They studied the membrane proteins of adapted and non-adapted bacterial cells and found that in adapted cells, inducible proteins were present in membrane with molecular weight of 34.5 and 25 kDa.

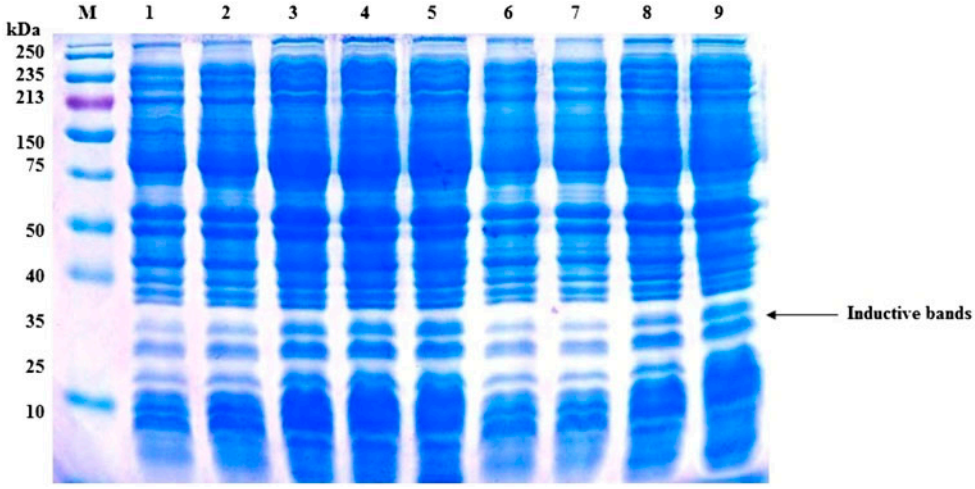

Fig. 8. M indicating the marker 1, 2, 6 and 7 representing non-stress protein bands of strains RZ1, RZ2 and RZ3 respectively while 3, 4 signing stress protein bands of strain RZ1, 8 indicating stress protein bands of strain RZ2 and 9 showing the inductive protein bands of strain RZ3.

4. Conclusions

The isolated bacterial strains were Gram-negative, coccus, and highly resistant to Cd. Based on the strains characterizations and the 16S rDNA sequence comparison, those bacterial strains were identified as *Pantoea* sp. RL32.2, *S. enterica*, and *Enterobacter* sp. OC-PSB1. They also showed excellent Cd removal ability, i.e. 89.89, 82.10, and 89.14%, respectively. Thus, these bacterial isolates can be exploited for the bioremediation of Cd containing wastes due to high resistance against Cd and potential to remove the Cd from industrial wastewater.

Acknowledgement

The authors acknowledge the research grant provided by the Universiti Sains Malaysia under the Short Term Grant Scheme (Project No. 304/PTEKIND/6312008).

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