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Detection of Heavy Metal Resistance Genes in an Environmental *Pseudomonas aeruginosa* Isolate

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Authors' contributions

This work was carried out in collaboration between all authors. Author AM designed the study. Author GEHO wrote the protocol. Author HHA wrote the first draft of the manuscript. Author ASAA managed literature searches. All authors managed the laboratory experiments and analyses of the study. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To detect heavy metals resistance genes in an environmental *Pseudomonas aeruginoas* (S 7) isolate.
Place and Duration of Study: Department of Biology, Faculty of Applied Science, Umm Al-Qura University, Makkah, Saudi Arabia, between January and March 2016.
Methodology: Detection of *PcoR* and *CzcD* genes involved in metal resistance by PCR. Cloning PCR products and insert the cloned genes into *E. coli* XL1-Blue. A PCR screening of the two genes was performed on *E. coli* XL1-Blue to confirm the detection of the two genes and finally amplified cloned *PcoR* and *CzcD* genes were sequenced for further confirmation.
Results: Using primers PcoR and CzcD, two PCR products of 636 bp and 389 bp respectively were detected in *P. aeruginosa* (S 7). A PCR screening of *E. coli* XL1-Blue that served as host for the cloned PCR products gave a 636 bp amplification with primer PcoR and a 389 bp amplification with primer CzcD. The inserted genes in *E. coli* XL-1 Blue were sequenced and confirmed the presence of *PcoR* and *CzcD* genes.

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Conclusion: The *Pseudomonas aeruginosa* (S 7) isolate reported in this study that showed remarkable tolerance to heavy metals by possessing genes involved in two types of efflux systems (P-type ATPase and Cation Diffusion Facilitator), may requires further investigation for its genetic structure and capability to be used in bioremediation of metals-contaminated environments.

Keywords: Pseudomonas aeruginosa; heavy metal resistance; bioremediation; public health.

1. INTRODUCTION

Heavy metal pollution poses serious ecological risks, and consequently public health concerns. Some heavy metal ions such as Zn^{2+} and Cu^{2+} might be required by living cells as micronutrients, copper for instance could play a role in cellular redox reactions [1], however, excessive concentrations of these metals can be toxic to viable cells. Because of its toxic nature, heavy metals are not as amenable to bioremediation as organics [2].

Pseudomonas aeruginosa is a ubiquitous, saprophytic Gram-negative short rod, with remarkable ability to adapt in various environments. *Pseudomonas aeruginosa* is a very important bacterium in bioremediation due to its ability to degrade a large scale of pollutants as aliphatic and aromatic hydrocarbons and its ability to detoxify and tolerate wide range of toxic heavy metals [3-6].

To cope with unfavorable metal concentrations, *P. aeruginosa*, as well as other metal-tolerant bacteria, develop various detoxification and/or tolerance mechanisms, such metal reduction, precipitation as metal salts, intracellular sequestration, binding to metallothionins and the removal of excessive metal ions out of the cell by active transport (efflux pump) [2]. Removal of excessive metal ions out of the cell by efflux pump is achieved by various proteins driven by ATP hydrolysis (ATPases) and cation diffusion transporter that acts as chemiosmotic ion-proton exchanger and the resistance nodulation division (RND) transporters that mediate proton driven efflux [7-9]. Generally, more than one type of protein efflux are found in metal-tolerant bacteria; the CBA transporter, which include the *CzcCBA* gene region. The *CzcCBA* gene region encodes outer membrane factors CzcC, membrane fusion protein CzcB and CzcA protein of the resistance-nodulation-cell division protein family, that play a role in zinc and copper tolerance [7-9]. The other efflux protein, the copRS is a two-component system that are identified as key genes involved in copper resistance, the representative member of the cupper efflux system include copA (ATPase); CusCFBA and multi-copper oxidase CueO [8].

Previously we managed to isolate a bacterium from industrial wastewater with multi-heavy metal tolerance capacity [6], this isolate was named (S7), later was identified as *P. aeruginosa* by 16s rDNA sequencing. The aim of this work is to detect and identify the genes of metal tolerance possessed by this isolate.

2. MATERIALS AND METHODS

2.1 Preparation of Chromosomal DNA from Bacterial Isolate

Genomic DNA was prepared as described according to Assaeedi et al. [10] and Abulreesh et al. [11]. A single bacterial colony was transferred into 5.0 ml of LB broth. The culture was incubated overnight at 37℃ with vigorous shaking. A volume of 1.0 ml of the culture was centrifuged at 13,000-16,000 *g* for 2 min to pellet the cells; the 240 µl of 10 mg ml^{-1} lysozyme (Sigma-Aldrich, St. Louis, USA) was added gently to mix. The purpose of this pretreatment is to weaken the cell wall so that efficient cell lyses can take place. The sample was incubated at 37℃ for 30–60 min, centrifuged for 2 min at 13,000 – 16,000 *g* and the supernatant was removed. An aliquot of 600 µl of nuclei lyses solution was added gently until the cells were re-suspended. The samples were incubated at 80℃ for 5 min to lyse the cells, and then cool at room temperature. A volume of 3.0 µl of RNAase solution (10 mg ml^{-1}) was added. The tube was inverted 2 - 5 times to mix, incubated at 37℃ for 30 min, then cool at room temperature. A volume of 200 µl of protein precipitation solution was added to the RNAase-treated cell lysate. The mixture was vortexed vigorously for 20 seconds to mix the protein precipitation solution with the cell lysate. The sample was incubated on ice for 5 min and centrifuged at 13,000 *g* for 3 min. The supernatant was transferred to a clean 1.5 ml micro centrifuge tube containing 600 µl of room temperature isopropanol, gently mixed by

inversion until the thread-like strands of DNA form a visible mass and centrifuged at 13,000 - 16,000 *g* for 2 min. The supernatant was carefully poured and the tube dried on clean absorbent paper. An aliquot of 600 µl of room temperature 70% ethanol was added and the tube gently inverted several times to wash the DNA pellet, then centrifuged at 13,000 - 16,000 *g* for 2 min; carefully aspirate the ethanol. The tube was drain on clean absorbent paper and the pellet allowed to air–dry for 10 –15 min, after that the genomic DNA was dissolved in 100 µl of DNA hybridization solution and stored at – 20℃.

2.2 Oligonucleotide Primers

The Oligonucleotide primers were synthesized at the Agricultural Genetic Engineering Research Institute (AGERI), Cairo, Egypt. The primers used were PCOR [12] and CzcD [13]. The primers sequence and exact length of amplified region are shown in Table 1.

2.3 Polymerase Chain Reaction

The gene amplification PCR reagent consisted of *Taq* DNA Polymerase (1.0 unit) (Bioneer, Daejeon, Korea); deoxyribonucleoside triphosphates (dATP, dTTP, dCTP, and dGTP, 10 mM each), $MgCl_2$ (25 mM), PCR buffer 10 X contains 500 mM KCl, 100 mM Tris-Hcl pH 9.0 and 1.0% triton X-100 buffer. After the initial denaturation at 96℃ for 1 min, 30 cycles each cycle consisted of denaturation at 95℃ for 30 sec, annealing at 50℃ for 30 sec, and extension for 72℃ for 2 minutes [14].

2.4 Purification of PCR Products

Wizard PCR preps DNA purification system for rapid purification of DNA was used to purify the PCR products, including primer-dimmers and amplification primers. The procedure was performed according to the manufacturer specifications (Promega, Madison, USA). For each 100 µl of completed PCR reaction, was transferred to a clean micro centrifuge tube and 100 µl of direct purification buffer was added and vortexed. An aliquot of 1.0 ml of resin was added then vortexed briefly 3 times over a one–minute period. For each PCR product, one Wizard Minicolumn was used. The plunger from a 3.0 ml disposable syringe was removed and set aside then the resin/DNA mix was pipetted into the syringe barrel and the syringe plunger was inserted slowly and gently, the slurry was pushed into the Minicolumn with the plunger. The syringe was detached from the Minicolumn and the plunger was removed from the syringe. The syringe was reattached to the Minicolumn and 2.0 ml of 80% isopropanol was pipetted into the syringe to wash the column. The Minicolumn was removed from the syringe and transferred to a 1.5 ml micro centrifuge tube. Centrifugation was done for 20 s at 13,000 *g* to dry the resin after that the Minicolumn was transferred to a new micro centrifuge tube and 50 µl of water was applied to a new micro centrifuge tube and left 5 min then centrifuged for 20 s at 13,000 *g* to elute the bound DNA product. The Minicolumn was removed and discarded and the purified DNA stored in micro centrifuge tube at –20ºC.

2.5 Cloning of PCR Products

Cloning was carried out with pGem cloning according to the manufacturer manual (Promega, Madison, USA). The following components were added to micro centrifuge tube; 1.0 µl of cloning vector (10 ng μl^{-1}), 1.5 µl of insert DNA, 1.0 µl of T4 DNA Ligase (Sigma-Aldrich, St. Louis, USA), 1.5 µl of T4 DNA Ligase buffer 10 X and distilled water to final volume of 15 µl. The components were mixed gently and incubated for 3 h at 16ºC, then incubated at 4ºC overnight [15].

2.6 Bacterial Transformation

The ligated reaction should be transformed into bacterial cells as a host for further analysis. *E. coli* is the domestic host for that purpose.

2.7 Preparation of *E. coli* Competent Cells

Competent cells of *E. coli* XL1-Blue were prepared for transformation of recombinant plasmid by Trituration Procedure Protocols and Application Guide (Promega, Madison, USA).

Table 1. Primers sequence and expected product size

Primer	Forward sequence	Reverse sequence	Product size	Reference
PcoR	5 CAGGTCGTTACCGCAGG 3	5 CTCTGATCTCCAGGACATATC 3	636	[12]
CzcD	5'CAGGTCACTGACACGACCAT 3'	5'CATGCTGATGAGATTGATGATC 3'	398	[13]

E. coli XL1-Blue strain was inoculated into 25 ml of LB medium then incubated at 37℃ shaking incubator for about 4 h until growth reaches optical density of 0.4 at 600 nm. The culture was then chilled in ice water for 2 h and cells were collected by centrifugation at 2,500 *g* for 15 –20 min at 4℃ The harvested cells were re-suspended in ice-cold trituration buffer and then diluted to 500 ml with the same solution. The cells were incubated on ice for 45 min. The cells were collected again by centrifugation at 1,800 *g* for 10 min and gently re-suspended in 10 ml of ice–cold trituration buffer. The cells were pooled and added 80% glycerol with gentle swirling to a final concentration of 15% (v/v). Finally, cells were aliquoted in 50 µl quantities.

2.8 Transformation of the Competent Cells

The cells were thawed on ice and a volume of 50 µl was transferred into a pre-chilled eppendorf tube and the ligation mixture was added to the cells contains 7.0 µl of ligated DNA. The tube was swirled gently and incubated on ice for 30 min, then heat pulsed in 42℃ water bath for 30 s followed by incubation on ice for 2 min. After that 500 µl of preheated 42℃ LB medium was added and incubated at 37℃ for 1 h with shaking at 250 rpm. A volume of 100 µl of transformation mixture was plated using a sterile spreader onto LB plates containing 100 µg ml^{-1} ampicillin, 40 µg ml^{-1} X-gal (Promega, Madison, USA), and 0.5 mM IPTG. The plates were incubated overnight at 37℃.

2.9 Screening of Transformed Cells

In the case of transformation of competent cells with pGem, that has a ligated fragment at its Multiple Cloning Site (MCS), detection of recombinants is by blue/white colony screening. The MCS of vector lies within the *Lac*Z gene, which when expressed, in response to the presence of synthetic inducer IPTG, produces the enzyme β–galactosidase. This releases an indigo dye from the chromomeric substrate, X-gal resulting in the formation of a blue colony. Insertion of a DNA fragment within the MCS would result in the failure of *Lac*Z expression, and the consequent formation of white colony; thus, cells transformed with pGem derivatives were plated on LB agar, to identify recombinants.

2.10 PCR Screening

White colonies were chosen for examination and screening using PCR technique. Bacterial colony was touched with toothpick and re-suspended in 50 µl sterile water then boiled for 5 min. To a PCR tube 4.0 µl of cell extract was added to 25 µl PCR reaction containing the following components; 2.5 µl 10X buffer, 10 pmol forward primer, 200 µM dNTPs, 10 pmol reverse primer, 1.0 unit *Taq* Polymerase, and water to 25 µl volume. The PCR cycle was carried out at 95℃ for 30 s, 50℃ for 30 s and 72℃ for 2 min, for 30 cycles.

2.11 Restriction Endonuclease Digestion

Plasmids were digested with the required restriction enzymes as described by the manufacturer for optimal reaction conditions (New England Biolabs, Hitchin, UK). A total volume of 20 µl reaction mix containing 1.0 µg DNA, 2.0 µl of the 10X buffer 2 and 10 unit of *Eco*RI restriction Endonuclease (New England Biolabs, Hitchin, UK). The reaction mix was incubated at 37℃ for 2 h followed by heat inactivation at 65℃ for 10 min. Agarose gel (1.2%) (Sigma-Aldrich, St. Louis, USA) was prepared and run as described by Green and Sambrook [16]. Ethidium bromide was added to the gel solution at a concentration of (0.1 µg ml^{-1}), to stain the DNA for visualization after the run using a long wave UV Transilluminator (λ=375). A one–tenth volume of sample loading buffer was added to the samples before loading it onto the gel.

2.12 Plasmid DNA Purification

Plasmid DNA preparation was prepared as described by Green and Sambrook [16]. A single bacterial colony was transferred into 2.0 ml of LB medium, containing 100 µg ml^{-1} ampicillin. The culture was incubated overnight at 37ºC with vigorous shaking. A volume of 1.5 ml of culture was centrifuged at 12,000 *g* for 30 s at 4ºC. The bacterial pellet was re-suspended in 100 µl ice-cold solution I (150 mM glucose, 25 mM Tris-Hcl, pH 8.0 and 10 mM EDTA, pH 8.0) by vigorous vortex. A volume of 200 µl of freshly prepared solution П (0.2 N NaOH, 1% SDS) was added and the tube was inverted gently 3 times, after that 150 µl of ice–cold solution Ш (5 M potassium acetate glacial acetic acid) was added, centrifugation at 12,000 *g* for 5 min at 4ºC in a micro centrifuge was carried out and the aqueous phase was transferred to a new tube, then equal volumes of phenol: chloroform were mixed and centrifuged at 12,000 *g* for 2 min. The aqueous phase was transferred to a new tube, and the DNA was precipitated from solution by adding one-tenth volume of 3.0 M sodium

acetate and 2 volumes of ethanol. Following 30 min incubation at –20ºC, the pellet was washed with 1.0 ml of 70% ethanol and vacuum dried, after that step the nucleic acid was dissolved in 50 µl of TE pH 8.0 or H_2O and stored at –20℃.

2.13 Sequencing of the Cloned Genes

The automated DNA sequencing reactions was performed using ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster city, USA) in conjunction with ABI PRISM 310 Genetic Analyzer. Cycle sequencing was performed using the Gene Amp 2400 Thermal Cycler, the reaction was conducted in a total volume of 20 µl containing 8.0 µl of terminator ready reaction mix, 1.0 µg of plasmid DNA, and 3.2 pmol of M13 universal forward primer (provided with the kit). The cycle sequencing program was 96℃ for 10 s, 50℃ for 5 s, and 60℃ for 4 min, repeated for 25 cycles with rapid thermal ramping [17]. The nucleotide sequence was determined automatically by the electrophoresis of the cycle sequencing reaction product on ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster city, USA). The data were provided as fluorometric scans from which the sequence was assembled using the sequence analysis software.

3. RESULTS

3.1 Detection of *PcoR* and *CzcD* Resistance Genes

A partial length of *PcoR* and *CzcD* resistance genes have been detected and amplified from *Pseudomonas aeruginosa* (S7) which previously showed high levels of resistances to various concentrations of heavy metals [6]. Amplification of 636 bp fragment was performed using primers PcoR1 and PcoR2 by using total genomic DNA As shown in (Fig. 1) the size of the amplified product is identical to the amplified 636 bp fragment encompassing the *PcoR* gene. Amplification of 389 bp fragment was performed using the primer pair CzcD1 and CzcD2 by using total genomic DNA. As shown in the (Fig. 2) the size of the amplified product is identical to the expected amplified fragment of a 398 bp encompassing the *CzcD* gene.

3.2 Cloning and Transformation of the PCR Products

The PCR fragments of the putative *PcoR* and *CzcD* gens were purified and ligated into pGem T easy vector in the multiple cloning sites, following ligation, plasmids were transformed into *E. coli* XL 1-Blue, the transformants were plated on LB ampicillin agar containing X-gal and IPTG for selection of recombinant white/blue colony screening. White colonies were picked up and plated onto master plate as well as being cultured in liquid media for DNA minipreps.

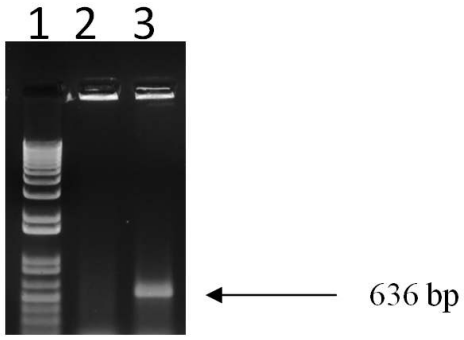

Fig. 1. Agarose gel electrophoresis of the PCR amplification product of *PcoR* gene, lane 1: DNA marker and lane 3: the amplified fragment from *Pseudomonas aeruginosa* (S 7)

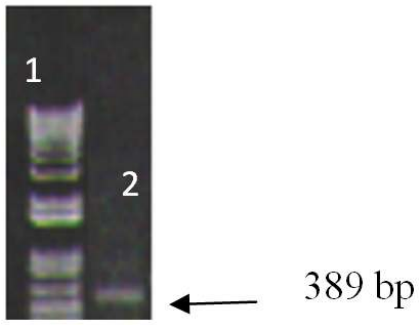

Fig. 2. Agarose gel electrophoresis of the PCR amplification product of *CzcD* gene, lane 1: DNA marker and Lane 2: the amplified fragment from *Pseudomonas aeruginosa* (S 7)

3.3 Insert Release

Cloned samples were grown over night in order to prepare plasmid minipreps for the restriction digestion. *EcoR1* restriction endonuclease was used for double digestion on both sides of multiple cloning sites on the vector on both sides of cloning insert DNA fragment. Insert were released at the expected size for *CzcD* gene a 389 bp product and a 636 bp for *PcoR* gene. (Fig. 3 A and B).

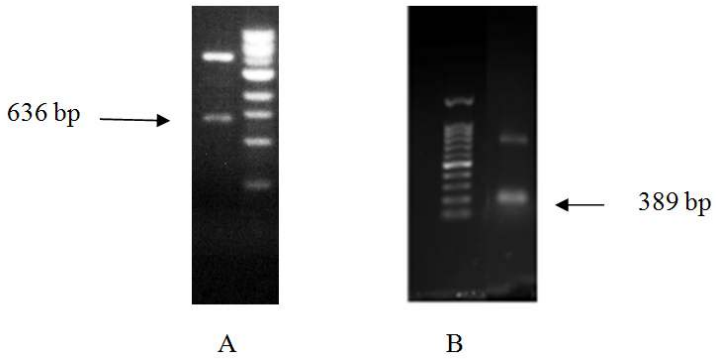

Fig. 3. Single digestion to the cloned genes to release the insert, A: *PcoR* insert, B: *CzcD* insert

3.4 PCR Screening

White colonies were screened by PCR to confirm the cloning step. White colonies were picked up and used as a template in the PCR reaction, using the PcoR and CzcD primers. White colonies gave positive PCR product at the exact size while blue colonies showed no band indicating therefore no combination (Fig. 4).

3.5 Sequencing of the *PcoR* and *CzcD* Genes

To confirm that we get the right genes, both genes were sequenced. Purified plasmids were used as template in the sequencing reaction using big dye terminator ready sequencing kit and M13 forward primer (Figs. 5 and 6).

4. DISCUSSION

Pseudomonas aeruginosa is a Gram-negative short rod that are wide spread in the environment and also considered as opportunistic human pathogen. The bacterium has remarkable ability to adapt in various environmental conditions and capable of utilizing wide range of carbon sources such as aliphatic, aromatic, and poly aromatic hydrocarbons, it also possesses unique genetic structure that allows it to detoxify and/or tolerate high concentrations of various heavy metals [3-5].

In a previous study, we managed to isolate a Gram-negative bacterium (isolate S 7) from industrial wastewater, this isolate showed remarkable ability to persist in various concentrations of zinc, copper, cadmium and cobalt [6]. It was the only isolate that was able to show persistence in all four tested metals, and therefore we carried out biochemical and molecular identification tests for isolate (S 7). The biochemical traits of that isolate revealed it was *Pseudomonas aeruginosa*, and that was further confirmed by sequencing of its 16s rDNA [6]. Thus the aim of this work was to isolate and identify the heavy metal tolerance genes possessed by *Pseudomonas aeruginosa* (S 7).

In this study we focused on detecting metal tolerance genes involved in transport system mechanism, i.e. P-type ATPases and cation diffusion facilitator (CDF), such as the Pco and Czc systems [1,8]. The Pco system is the first genetic determinants of bacterial copper resistance, this system was first identified in *E. coli* and *Pseudomonas syringae*, while the Czc system was first described in *C. metallidurans* with the CzcD Cu^{2+} and Zn^{2+} efflux system [13].

Using primers PcoR and CzcD we manage to detect these genes in *Pseudomonas aeruginosa* (S 7) that was previously isolated from industrial wastewater and showed high tolerance to various concentrations of Cu^{2+}, Zn^{2+}, Co^{2+} and Cd^{2+} [6]. Initially we detected these genes from the tested isolate by PCR, then we cloned PCR products and transferred those cloned genes into *E. coli* XL1-Blue, and ran a PCR screening to ensure that we detected the correct genes, finally we sequenced the amplified genes for final confirmation. The results confirmed that isolate *Pseudomonas aeruginosa* (S7) was able to tolerate various concentrations of Cu^{2+}, Zn^{2+}, Co^{2+} and Cd^{2+} due to its possession of both *PcoR* gene (P-type ATPase) and *CzcD* gene (Cation Diffusion Facilitator – CDF).

There are four genes involved in copper resistance in *Pseudomonas* species, these genes are *pocA*, *pocB*, *pocC* and *pocD* that encodes for multi-copper oxidase (MCO), and *pcoR* genes that codes for the DNA binding repressor protein [18]. Generally in Gram-

negative bacteria the transcription of ATPase gene is activated with increase of copper concentrations [8] and that perhaps explained the ability of our *P. aeruginosa* (S 7) to persist even in higher concentrations of copper [6]. This assumption is further confirmed by the detection of a 636 bp product with primer PcoR from the same isolate in this study.

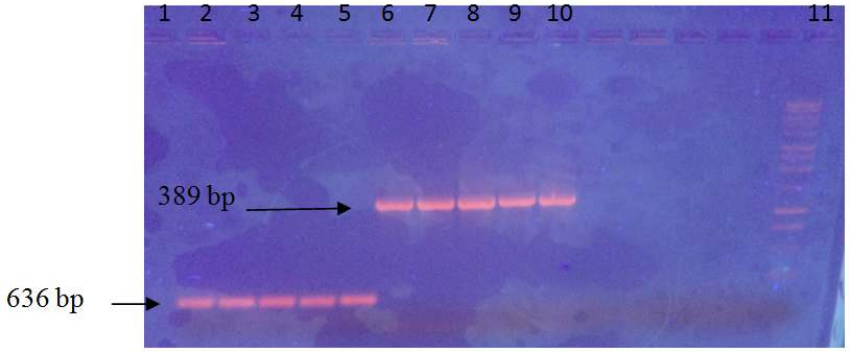

Fig. 4. PCR amplification fragments from the clones. Lanes from 1-5 are positive clones from *CzcD* gene. Lanes from 6-10 are positive clones from *PcoR* gene. Lane 11: DNA marker

ACCGGGCCCCCCGTCGAGGTCGACGGTATCGATAAGCTTGATCAGGTCGTTA

CGTGCAGCAGGGACTGGTTGAGGAAGGCTATCAGGCCGATCTCTTTAATAAT

GGCCGCGATGGTCTCGGGGCCGCGTCGAAGCGACAGTATGATTTGATAATAC

TGACGTGATGCTGCCTTTCCTCCACGGGTGGCAAATCATCAGCGCACTGAGG

GAGTCCGGGCACGAACAACCGGTCCTGTTTTTAACCGCAAAGGACAACGTGC

GGGAGAAAGTGAAAGGACTGGAGCTTGGCGCAGATGACTACCTACCATTAA

GCCCTTTGATTTTACGGAGCTGGTTCACGTGTAAGAACCGTACTGCGCCGGGC

ACGCTCGCAGGCCGCAACAGTCTGCACCATGGCCGATATGACCGTTGATATG

GTGCGCCGGACCGTGATCCGTTCCGGGAAGAAGATCCATCTCACCGGTAAAG

AATACGTTCTTCCTTCAGTTGCTGCTGCAACGCACCGGAGAAGTGTTACCCAG

GACTCTTATCTCGTCCCTGGTCTGGAACATGAATTTTGACAGTGATACGAATG

TGATTTGATGTCGCCCCGTGAGACGTCTGAGAAGTAAAATTCATGATGACTTT

GAGCAAACTGATCCATACCGTTCCCGGTCCCGGATCTGTCCTGGAGATC

AGAAAATCGAATTCCTGCACCCG

Fig. 5. Nucleotide sequence of *PcoR* gene detected in *P. aeruginosa* (S 7)

CCGGTACGTTCCTGATGCCGAAAGGTCCGGAGGGGGGGGAGGAATGAATATT

TTGGCCGTTGCTCTCCTGCACGCACGCCGCCACATCGCCTCCACGGACACCCC

GTCGCACTGGGCCATTCGCACAGGGCCTGGCTTATTGCGGATTCCGCCAAGC

CGTACCCGCGGACAAGAAGCGGGTCATTTTGGCTACTACCGTTTTGGAGGAT

TCTTGCCGCGGCCTTTATCGCATTGCTGCTGTTTCGGTGTTGGCTTATCTACAT

TCCTGTACGAAAGCCAACCTTCCCGGCTTGGAAATTCGCCACCTCAGATTAG

AGTTCAAACCGGGGCTTTGTTTCCGTCGTTGGGCTGTGGCTAGGGGCC

Fig. 6. Nucleotide sequence of *CzcD* gene detected in *P. aeruginosa* (S 7)

The Czc system mediates resistance to cobalt, zinc and cadmium through ion effluxed catalyzed by the $CzcCB_2A$ cation-proton antiporter. The CzcD protein is a membrane-bound protein that is involved in the regulation of the Czc system. The CzcD does not only protect the cells against toxic Co^{2+}, Zn^{2+} and Cd^{2+}, but also involved in the regulation of expression of the $CzcCB_2A$ efflux system [19]. The detection of *CzcD* gene in our *P. aeruginosa* (S 7) in this study further confirms the observation made by Mihdhir et al. [6] who observed high level of tolerance to Zn^{2+}, Co^{2+} and Cd^{2+} by the same isolate.

5. CONCLUSION

The *Pseudomonas aeruginosa* (S7) reported in this study that showed remarkable tolerance to heavy metals by possessing *PcoR* and *CzcD* genes involved in removing heavy metals through active transport system, and also tested for its ability to degrade crude oil and other hydrocarbons derivatives and showed notable ability to utilize various hydrocarbons (unpublished data). This isolate requires further investigation for its genetic structure and capability to be used in bioremediation of metals and hydrocarbon-contaminated environments.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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