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Isolation of heavy metal resistance bacteria from lake sediment of IIUM, Kuantan

Zaima Azira Zainal Abidin[a,*], Puteri Nur Ezzati Badaruddin[b], Ahmed Jalal Khan Chowdhury[b]

[a]*Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, email: zzaima@iium.edu.my (Z.A.Z. Abidin)*
[b]*Department of Marine Science Kulliyyah of Science, International Islamic University Malaysia*

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ABSTRACT

The present study deals with isolation and identification of heavy metal resistance bacterial strains from lake sediment located within the University of International Islamic University Malaysia (IIUM) campus with the aim to assess possible heavy metal pollution of the lake and in search of bacterial strains that can be utilized for bioremediation purposes. In a total of 80 isolates were obtained and 24 isolates were randomly selected for heavy metal resistance test using the following heavy metals - chromium (Cr), nickel (Ni), copper (Cu), cobalt (Co), cadmium (Cd) and mercury (Hg). Heavy metal resistance pattern associated with IIUM lake was in the form of Cr > Ni > Cu > Co > Cd > Hg. Isolates (I3, I5, I13 and I22) that displayed high heavy metal resistance tests were identified through the 16S rRNA gene sequence. Isolates I3 and I5 were closely related to *Bacillus megaterium* (99%) and *Bacillus cereus* (98%) respectively, while Isolate I13 was highly similar to *Pseudomonas stutzeri* (99%) and I22 I22 fit in *Staphylococcus pasteuri* (99%).

Keywords: Heavy metal resistance; Bacteria; MIC; 16S rRNA gene

1. Introduction

Heavy metals are a group of metals with a density greater than 5 g/cm^3. It is non-biodegradable and considered as a key to an environmental contaminant which related to potential toxicity. Some heavy metals such as nickel, iron, copper and zinc are essential to metabolic reactions and are required as trace elements by the organisms. Other heavy metals such as mercury, silver and cadmium have no biological role in organisms, and they detrimental even at very low concentrations [1]. In an aquatic environment, heavy metals usually accumulate in the sediment. Heavy metal will accumulate and the level of accretion can act as an indicator for monitoring of pollution levels. As it is rapidly discharged into the environment, it will associates with particulates and finally settles at the bottom of the sediments [2]. The release of wastewater, for example, from various resources containing substantial metals increases the occurrence of heavy metal resistant bacteria population. Bacteria will be evolved to tolerate elevated concentration of heavy metals and this adaption will result in the development of protection mechanisms to survive in such an environment. Several protection mechanisms that relate to heavy metal resistance capability by bacteria include an extracellular barrier, active transport of metal ions (efflux), extracellular sequestration, intracellular sequestration with low molecular weight, cysteine-rich proteins and reduction of metal ions [3,4].

Predominantly heavy metal tolerant microorganisms are isolated from heavy metal contaminated sites such as industrial wastewaters [5], mine tailings, [6] soils and water

* Corresponding author.

contaminated by heavy metals [7,8]. However, there are reports on the presence of heavy metal resistant bacteria from uncontaminated locations [9,10]. These findings exemplified the emergence of heavy metal resistance long before the anthropogenic pollution of the environment. An artificial lake located in International Islamic University Malaysia (IIUM), Kuantan is an example of such a place. This lake is used for recreational activities such as kayaking and fishing. Moreover, this lake also harbors fishes like the snakehead fish, perch fish and catfish. This study attempts at isolating heavy metal resistant bacteria from IIUM Lake which can be considered as an uncontaminated site. The finding of this study will ascertain the status of the lake, whether the lake had been exposed to possible heavy metal pollution and this information is vital for the wellbeing of IIUM communities.

2. Materials and methods

2.1. Sampling site

The sampling area was the artificial lake located in IIUM, Kuantan. This lake is near to the mosque, hostels, main road and main entrance of IIUM (Fig. 1). Sediment was collected by using Ponar grab at the center of the lake and from three different points. Sediment samples were kept into the sterile plastic and processed immediately.

2.2. Isolation of bacteria

Bacteria were isolated and enumerated using serial dilution technique on nutrient agar. About 1 g of sediment sample was placed into a 10 ml of falcon tube. Then 10 ml of saline water was added and mixed thoroughly followed by dilution 10^{-1} to 10^{-6}. About 100 µl of each dilution was plated onto nutrient agar in duplicate. Plates were then incubated at 37°C for 48 h. Colonies obtained were enumerated and the morphology was noted. All isolates were subjected to Gram staining.

2.3. Determination of the minimal inhibitory concentration (MIC) of heavy metals

The heavy metal resistance of the bacterial strains was determined using Mueller Hinton agar supplemented with various concentrations of six different heavy metals (Cd^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Hg^{2+}, Ni^{2+}) in the form of chloride salts. The initial concentration of the heavy metal was at 20 µg/ml and the concentration of the heavy metals was gradually increased at 10 µg/ml until the isolates failed to grow. MIC was noted when the isolates failed to grow on plates even after a maximum of 5 d of incubation. The test was conducted in duplicate.

2.4. Molecular identification using 16S rRNA gene

Polymerase chain reaction (PCR) amplification of the 16S rRNA gene was performed using the following set of primers: 27F 5′–AGAGTTTGATCCTGGCTCTCAG–3′ and 1492R 5′–GGTTACCTTGTTACGACTT–3′. The PCR reactions were performed in a final volume of 50 µl which consist of 200 ng DNA template, 25 µl of MyTaq™ Mix 2X (Bioline, UK) and 0.4 µM primers under the following conditions: initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 55°C for 60 s and 72°C for 4 min; and extension step at 72°C for 10 min. The amplification products were confirmed using 1% agarose gel and sent to 1st Base Laboratory, Malaysia for purification and sequencing. The resultant 16S rRNA gene sequences were manually verified and edited using BioEdit sequence alignment editor. The partial nucleotide sequences analysis of the isolates was carried out via the GenBank BLASTn search tool.

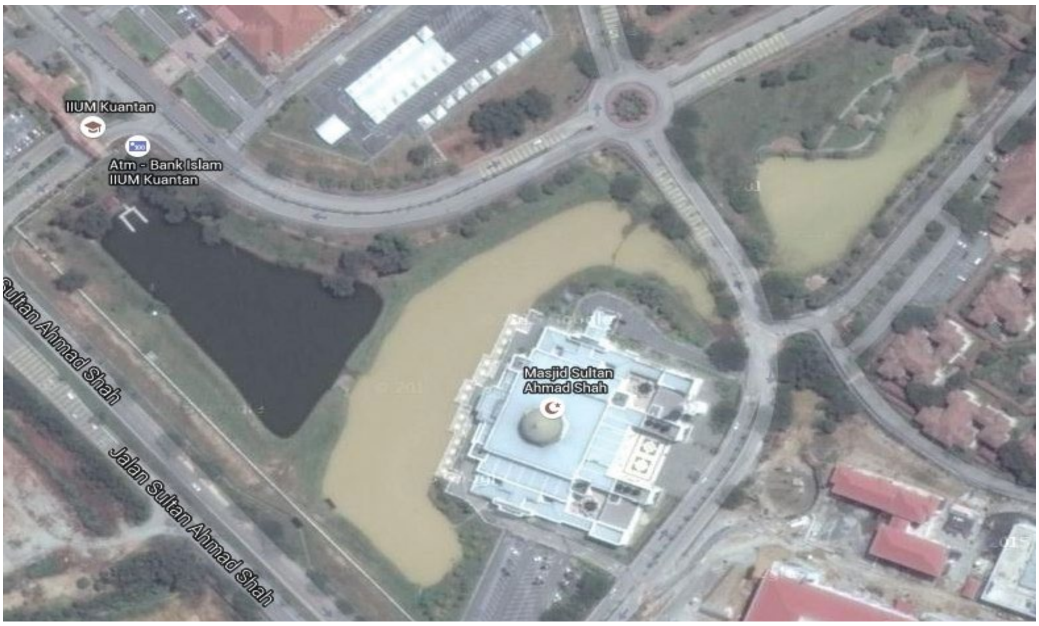

Fig. 1. Overview of IIUM Kuantan Lake.

3. Results and discussion

In a total of 80 isolates were successfully recovered and the majority of the isolates belong to Gram-negative bacteria. This was followed by a random selection of 24 isolates for the next test that is, heavy metal resistance test. Table 1 depicts the MICs of bacterial isolates against tested heavy metals namely, Cd^{2+}, Cu^{2+}, Co^{2+}, Cr^{2+}, Ni^{2+}, Hg^{2+} in the form of chloride salts. Almost all isolates were found to have MIC > 400 µg/ml for Cr except for isolate I6 (MIC = 380 µg/ml) and a similar observation was recorded for Ni except for I6 (MIC = 390 µg/ml) and I7 (MIC = 390µg/ml). Several studies indicated that some of the bacteria isolated may possibly tolerate a concentration of Cr until 1,000 µg/ml [11–13]. An abnormal state of resistance towards Cr usually happened in the sediment that may be tainted with Cr in the area [14]. Plausible reasons for Ni presence in the IIUM Lake may happen due to the atmospheric deposition, land application of fertilizers, paints from the buildings, wastewater irrigation and sewage sludge. The presence of a high concentration of Ni will produce a lot of nickel-resistant strains in the bacterial community [15]. The majority (62.4%) of the isolates had a MIC of 200 µg/ml for Cu^{2+} indicating low tolerance of the isolates towards Cu. The highest tolerance to Cu recorded was in the range 300–320 µg/ml by isolates I5, I12 and I15. The major existence of Cu was influenced by the wastewaters, urban runoff or effluents from the industry [16]. Contamination of Cu usually occurred because of the application of agriculture input. It is an essential micronutrient that compulsory for the growth of plants specifically in disease resistance and production of seed. The fertilizer and pesticides added at the surrounding of the lake for the growth of trees caused the surpass of copper in the sediment [17]. As in this case, it can be said that the IIUM Lake is not contaminated with Cu. A Similar trend was also observed for Co whereby the majority of the isolates possessed MIC of 200 µg/ml. The highest MIC recorded for Co was in the range of 250–300 µg/ml indicating low tolerance of isolates to Co. This was probably due to low contamination of Co at the lake environment. Co pollution usually occurred because of industrial waste and Co burning [18]. Almost all isolates showed low tolerance towards Cd with MIC < 200 µg/ml. The highest MIC recorded was 290 µg/ml by isolates I13 and I15. The utmost substantial use of Cd is in batteries either rechargeable or secondary power sources [16]. Acid rain will also increase soils and surface waters acidification that upturn geochemical mobility of cadmium. Cd was not promptly assimilated by microorganisms. This substantial metal can harm the cell layers and the structure of the DNA. The resistance of the bacteria towards the heavy metal happened when there was a metal displacement from the local binding site of bacteria or the ligand interaction

Table 1
MIC of heavy metal in µg/ml

Isolates	MIC of heavy metal (µg/ml)					
	Chromium	Nickel	Copper	Cobalt	Cadmium	Mercury
I1	>400	>400	50	140	90	50
I2	>400	>400	140	120	50	50
I3	>400	>400	240	250	240	200
I4	>400	>400	250	280	50	50
I5	>400	>400	300	150	290	70
I6	380	390	140	140	120	100
I7	>400	390	120	80	60	90
I8	>400	>400	130	120	50	50
I9	>400	>400	170	300	50	50
I10	>400	>400	160	100	50	50
I11	>400	>400	230	140	150	60
I12	>400	>400	320	300	50	50
I13	>400	>400	170	170	290	230
I14	>400	>400	150	110	50	50
I15	>400	>400	300	130	130	230
I16	>400	>400	70	100	70	90
I17	>400	>400	240	90	50	60
I18	>400	>400	270	150	140	100
I19	>400	>400	170	100	60	120
I20	>400	>400	200	100	70	70
I21	>400	>400	160	90	50	70
I22	>400	>400	170	90	70	220
I23	>400	>400	150	80	50	80
I24	>400	>400	160	100	80	120

Table 2
Molecular identification of isolates exhibiting high resistance to heavy metals

Isolate	Heavy Metal MIC (μg/ml)						Closest relative	Identity
	Cd^{2+}	Cu^{2+}	Co^{2+}	Cr^{2+}	Ni^{2+}	Hg^{2+}		
I3	240	240	250	>400	>400	200	*Bacillus megaterium* strain NBRC 15308	99%
I5	290	300	150	>400	>400	70	*Bacillus cereus* ATCC 14579	98%
I13	290	170	170	>400	>400	230	*Pseudomonas stutzeri* strain ATCC 17588	99%
I22	70	170	90	>400	>400	220	*Staphylococcus pasteuri* ATCC 51129	99%

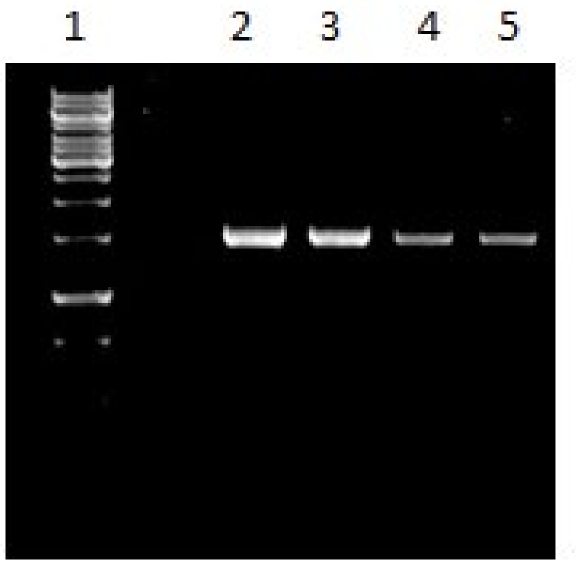

Fig. 2. Agarose gel electrophoresis of PCR amplification of the 16S rRNA gene. Lane 1: 1 kb ladder; Lane 2: I3; Lane 3: I5; Lane 4: I13; Lane 5: I22.

[19]. As for Hg, MIC for the majority of the isolates did not exceed 120 μg/ml with the exception of isolates I3, I13, I15 and I22 (MIC: 200–230 μg/ml). The presence of mercury is usually related to cross-contamination. It can occur through filtration of water either from drains, streams or tailings lakes profoundly polluted during raining seasons which went along with the overflowing and flooding of the contaminated water [20]. Overall, almost all isolates showed high resistance to Cr and Ni but low tolerance to Cu, Co and Hg. Findings in this study may suggest that IIUM Lake is not heavily polluted with heavy metals. Moreover, the occurrence of heavy metal resistance bacteria indicated the possibility of obtaining such bacterial strains from an uncontaminated location as reported by others [7–9]. The resistance pattern associated with IIUM lake was in the following order Cr > Ni > Cu > Co > Cd > Hg.

Four isolates were selected for molecular identification using 16S rRNA gene-based on their strong heavy metal resistance profiles. Isolate I3 showed high MIC readings ≥ 200 μg/ml for Cd^{2+}, Cu^{2+}, Co^{2+}, Cr^{2+}, Ni^{2+}, Hg^{2+} while I5 gave MIC > 200 μg/ml for Cd^{2+} and Cu^{2+} but low MIC values for Co^{2+} and Hg^{2+}. MIC values for I13 for Cd^{2+} and Hg^{2+} were 290 μg/ml and 230 μg/ml respectively and lower values of MIC = 170 μg/ml for 200 μg/ml Cu^{2+} and Co^{2+}. On the other hand, I22 gave high MIC value (>200 μg/ml) for Cr^{2+}, Ni^{2+} and Hg^{2+} but low MIC for the remainder heavy metals. PCR amplification of the 16S rRNA gene (~1,500 bp) for these isolates was successfully obtained as depicted in Fig. 2. Following sequencing, partial sequences of the 16S rRNA gene were compared with the NCBI database and results were as illustrated in Table 2. Isolate I3 showed high similarity to *Bacillus megaterium* while I3 is closely related to *Bacillus cereus*. Several studies have indicated that *Bacillus* sp. can show high resistance towards heavy metal toxicity and possessed the capability to eradicate metal [21–23]. For example, *B. megaterium* was applied in the bioremediation of heavy metals due to its ability to bioaccumulate and biosorption potentials [24,25]. Isolate I13 was highly similar to *Pseudomonas stutzeri* and I22 was closely related to *Staphylococcus pasteuri*. This finding is in agreement with other findings [26–33] that indicated *Bacillus* sp., *Pseudomonas* sp. and *Staphylococcus* sp. strains possess the multiple-metal resistance ability. These bacterial strains (I3, I5, I13 and I22) with heavy metal resistance capability are perfect for tests at the metal-contaminated sites as well as candidates for bioremediation purposes.

4. Conclusion

Findings from this study showed that IIUM Lake may be regarded as an uncontaminated location as indicated by low MIC values obtained against several metals. Four isolates (I3, I5, I15 and I22) demonstrated that the presence of heavy metal resistance bacteria could be recovered from an uncontaminated site. Furthermore, these bacteria can be utilized for future application in bioremediation on heavy metal polluted sites.

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