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Heavy metals detection using biosensor cells of a novel marine luminescent bacterium *Vibrio* sp. MM1 isolated from the Caspian Sea

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ABSTRACT

Monitoring and assessing toxic materials which are being released into the environment along with wastewater is a growing concern in many industries. The current research describes a highly sensitive and rapid method for the detection of toxic concentrations of heavy metals in aquatic environments. Water samples were collected from southern coasts of the Caspian Sea followed by screening of luminescent bacteria. Phylogenetic analysis, including gene sequence of *16S rRNA*, and biochemical tests were performed for identification of the isolate. Luminescence activity was tested and measured after treatment of the isolate with different concentrations of heavy metals and reported as EC_{50} value for each metal. A luminous, gram negative bacterium with the shape of a curved rod was isolated from the Caspian Sea. Biochemical tests and *16S rRNA* gene sequence analysis indicated that the isolate MM1 had more than 99% similarity to *Vibrio campbellii.* The novel isolate is able to emit high levels of light. Bioluminescence inhibitory assay showed that the *Vibrio* sp. MM1 had the highest sensitivity to zinc and the lowest sensitivity to cadmium; EC_{50} values were 0.97 mg l^{-1} and 14.54 mg l^{-1}, respectively. The current research shows that even low concentrations of heavy metals can cause a detectable decline in luminescence activity of the novel bacterium *Vibrio* sp. MM1; hence, it makes a good choice for commercial kits for the purpose of monitoring toxic materials.

1. Introduction

"Heavy metal" is defined as the group of metals and metalloids with density greater than 4 ± 1 g/cm^3 or 5 times or more, greater than water (Hawkes, 1997). This term is widely recognized and usually applied to the general contaminants of both terrestrial and aquatic ecosystems. Heavy metals generally involve; lead, cadmium, zinc, mercury, arsenic, silver, chromium, copper, iron, and the platinum group elements (Duruibe et al., 2007). Exposure to these toxicants especially through food and beverages, drinking water, and air, has adverse effects on human health (Jarup and Akesson, 2009). The toxicity of heavy metals to mammals is due to the chemical reactivity of its ions with cellular proteins, membrane and enzymatic systems. Usually the organs in the body that accumulate heavy metals with high concentration are targets of specific metal toxicity. This is often associated with exposure conditions and the chemical complex of the metal, as in its valiancy state, volatility, lipid solubility etc. (Kampa and Castanas, 2008).

Anthropogenic discharge of heavy metals into the environment is increasing due to the growth of industrialization all over the world. Since heavy metals cannot be degraded or destroyed, they persist in the environment. Therefore, the presence of heavy metals in effluents as a result of their uses in industries is an ever-growing concern (Mohammed et al., 2011).

It is widely accepted that the simple measurement of pollution concentration, using available regulatory rules, is not an accurate and reliable way to measure the environmental noxiousness. These analyses are very general and are able to show only the nature of the pollutants but do not give us any data about the biological effects of these pollutants. Hence, biological sensors and detectors have attracted interests to be applied as a more sensitive and rapid method to detect eco-toxicants. (Girotti et al., 2008). Many scientists have studied to establish rapid and sensitive bioassays to track and evaluate the process of toxic materials discharge. Previously, crustaceans, fish and algae were used for the measurement of toxicants in aquatic environments, but using these organisms as a biosensor was very time consuming and needed a large sample volume, which is not cost effective. Hence, using microorganisms to detect toxicants has been developed which is rapid, highly sensitive and user-friendly (Parvez et al., 2006). Using microbial cells to detect toxic materials and to monitor diverse chemicals is a unique method in the field of ecotoxological assessment (Ivask et al., 2009).

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Many toxic materials, such as heavy metals, pesticides and industrial wastes can make a significant effect on the bacterial light emitting system, which is currently an applied method to test the toxicity of water and soil samples (Hong et al., 2010) and is known as bioluminescence inhibition assay. Bioluminescence inhibition assay has been employed as a rapid and reliable detecting tool to determine the toxicity of effluents in different industries, such as textile and paper mill industries (Devare and Bahadir, 1994; Rigol et al., 2004). The bioluminescence inhibition assay has been developed by means of a marine gram negative bacterium, *Vibrio fischeri* (strain NRRL B-11177), and is widely used to measure acute toxicity. Light emitting process is closely related to the metabolic activity of the bacterial population and any inhibition of enzymatic activity causes a sensible decrease in the amount of bioluminescence. This assay allows us to measure sub-lethal concentrations of toxic substances. This test has gained popularity due to the fact that a toxic substance often has similar toxic effects on different organisms, despite the diverse toxicity mechanisms for various species (Kaiser, 1998). Hence, luminescence inhibition in microorganisms can efficiently display noxious effects on higher organisms. Due to the adverse effects of environmental pollution on humans and increased rates of illness, it is critical to develop sensitive tools for rapid assessment of toxic and genotoxic effects of environmental pollutants. The bioluminescence inhibition assay, due to its high accuracy, simple handling and low cost, can be applied as a reliable reporter for the screening and monitoring of various aquatic environments. These environments may contain toxicants such as heavy metals, pesticides, aromatic hydrocarbons, fuels, and alkanes (Thakre and Shanware, 2015).

The aim of this study is to represent the potential uses of native strains, isolated from the Caspian Sea, to increase the sensitivity of heavy metal biosensors for the screening of heavy metal pollutants in synthetic environmental samples.

2. Material and methods

2.1. Sea water samples and growth condition

Water samples were collected from 5, 10 and 15 m depths in sterile plastic containers, kept at ambient temperature until being processed in the laboratory. Location of sampling was southern coasts of the Caspian Sea in Babolsar, Iran. Sea Water Broth (SWB) culture medium was used for enrichment and initial cultivation of luminous bacteria. SWB contained (per liter): peptone, 5 g; yeast extract, 5 g; meat extract, 3 g; NaCl, 20 g; KCl, 0.7 g; $MgCl_26H_2O$, 5.3 g; $MgSO_4$, 7 g; $CaCl_2$, 0.1 g and pH 6.8–7.2. Collected sea water samples were inoculated into a flask containing 100 ml of SWB medium and incubated at 28 °C on a rotary shaker with 180 rpm for 24 h. Enriched culture was serially dilution plated on SWA medium (SWB containing 1.5% agar) and incubated at 28 °C for 24 h. Isolation of luminous colonies was carried out in a dark room. Single colonies of luminescent bacteria were subcultured on fresh SWA plates and incubated at 28° C. Luminescent isolate was stored in 15% glycerol at −85 °C for future studies.

2.2. Morphological and physiological characteristics

Colonial morphology was investigated for shape, margin, elevation etc. Cell morphology of the bacterial isolate was studied by light microscopy and standard gram staining methods (Coico, 2005). Biochemical tests were done in accordance with Alsina's keys (Alsina and Blanch, 1994) for the purpose of initial identification and differentiation. Arginine dihydrolase test along with decarboxylation of lysine and ornithine were evaluated on the Moller base medium. Cytochrome oxidase, catalases, citrate, methyl-red, Voges-Proskauer, indole production, gelatin liquefaction, hydrogen sulphide production and motility tests were performed. Growth of the isolate was monitored on TCBS medium and carbon utilization was investigated for glucose, sucrose, arabinose, mannose, mannitol, inositol, sorbitol and fructose.

2.3. 16S rRNA gene analysis and molecular identification

To identify the bacterial isolate, genomic DNA was extracted using standard phenol chloroform method (Wilson, 2001). Amplification of the *16S rRNA* gene was performed using universal primers PA: (5′-AGAGTTTGATCCTGGCTCAG-3′) and PH: (5′-AGAGTTTGATCCTGGCTCAG-3′) (Mohseni et al., 2014). The PCR cycling program used to amplify *16 S rRNA* gene was: 5 min at 94 °C, 30 cycles of 30 s at 94 °C, 30 s at 56 °C, 90 s at 72 °C and a final extension step for 7 min at 72 °C. Amplified fragments of *16S rRNA* gene was sequenced by GATC (Germany). Chromas Lite (2.1.1) software package was used to analyze anomalies of *16S rRNA* gene sequences then assembled using the CAP contig program in BioEdit (7.1.3.0) software (Hall, 1999). The identification of phylogenetic neighbors was initially carried out by the BLASTN (Altschul, 1997) program against the database containing type strains with validly published prokaryotic names and representatives of uncultured phylotypes (Kim et al., 2012). The top thirty sequences with the highest similarity were then selected for the calculation of pairwise sequence similarity using the global alignment algorithm (Myers and Miller, 1988), which was implemented at the EzTaxon server (Kim et al., 2012). Phylogenetic tree was constructed using the neighborhood-joining method with 1000 bootstrap replicons in MEGA 7 software. (Tamura et al., 2013).

2.4. Accession number

The accession number provided by GenBank nucleotide sequence databases for the *16S rRNA* gene of MM1 isolate was KP732105.

2.5. Bacterial luminescence evaluation

Bacterial isolate was cultured in SWB medium with a range of 0–10%NaCl and pH of 5–8 at 28 °C and the relation of growth and luminescence intensity was investigated after 12 h. Bacterial growth was recorded at OD_{600}, using a spectrophotometer (E-Chrom Tech, CT-2200) with an interval of two hours. Simultaneously, luminescence intensity was measured at the same time intervals using a Berthold luminometer and reported as RLU, which stands for Relative Light Units. Luminescence intensity adjusted on 8×10^6 RLU s^{-1} and standardized for toxicity tests.

2.6. Heavy metal toxicity assay

For toxicity assay, 22 h cultured cells with an OD_{600} of 0.9 were harvested by centrifugation then washed in 2% NaCl solution. Heavy metal cations including $Cd(NO_3)_2$, $Pb(NO_3)_2$, $CuSO_4$, $NiSO_4$, $CoCl_2$ and $ZnSO_4$ (Merck, Germany) were dissolved in HPLC grade water and kept in the dark at 4 °C as a resource of cations. Different concentrations of each metal were prepared in 2% NaCl solution as follows (mg l^{-1}) Cd: 0–36.0; Pb: 0–12.43; Cu: 0–7.62; Ni: 0–7.04; Co: 0–20.0 and Zn: 0–5.23. In order to perform the cytotoxicity test, 0.1 ml of bacterial cell suspension was added to 0.9 ml of heavy metal solutions with 2% NaCl. Luminescence of the cell suspension was evaluated after 15 min at 15 °C. Finally the toxicity of samples was calculated by EC_{50} value with Microsoft Excel (Liengme, 2015). The EC_{50} is defined as a value in which a specific concentration of a certain toxicant causes a 50% decline in bioluminescence (Brovko, 2007).

3. Results

3.1. Isolation and characterization of the luminescent bacterium

From twelve isolated colonies, a glowing blue-green colony was selected in the dark room and subcultured in SWA medium at 28 °C for

24 h. The luminescent isolate was designated as MM1. The characteristics of colony morphology were circular, convex, mucoid with entire margin and smooth surface. Its color was cream with a pale yellow tint. The results of cell morphology of the MM1 isolate using standard gram staining showed that it was slightly curved and Gram-negative.

3.2. Biochemical and physiological characterization of MM1

Biochemical tests, according to the scheme of Alsina & Blanch, demonstrated that the MM1 isolate is a member of *Vibrio* genus (Alsina and Blanch, 1994). Round colonies (2–3 mm) of a bright cream shade were observed on TCBS agar. Growth occurred in the presence of 1–8% NaCl (w/v), but not at 0% or 10%NaCl. The minimum, maximum and optimum temperatures for growth were 12–15 °C, 35–37 °C and 28 °C, respectively. Catalase, oxidase, motility and indole tests were positive while citrate utilization, urease, gelatinase and MR-VP tests were negative. In addition, the result in SIM medium showed that MM1 was not able to produce H_2S. Assimilation of glucose, mannitol and mannose were positive, while assimilation of arabinose, inositol, sucrose, sorbitol and lactose were negative. Furthermore, Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase were (-,+,-), respectively. Eventually, growth occurred on TCBS agar and the colony showed a green shade.

3.3. Molecular identification and phylogenetic analysis

16S rRNA gene sequence analysis revealed that the MM1 isolate was a member of the genus *Vibrio* and the closest relative to *Vibrio campbellii* with 99% similarity. Phylogenetic dendrogram drawn for *16S rRNA* gene, using neighbor-joining method is provided in Fig. 1. According to the mentioned characters and similarity of the hypervariable region in the *16S rRNA* gene sequence, the isolate was identified as *Vibrio* sp. MM1.

3.4. Bioluminescence measurement

Growth and luminescence intensity measurement for MM1 isolate indicated that optimum concentration of NaCl and pH for luminescence was 2% and 7.0, respectively. Fig. 2 shows that luminescence intensity increased from 394.44 × 10^3 to 731.63 × 10^3 RLU after 18–20 h of growth. Then it was fixed on 784.64 × 10^3 RLU after 22 h at 28 °C. Therefore, a 22-h culture with high luminescence intensity was used as cell suspension for toxicity assessment of heavy metals.

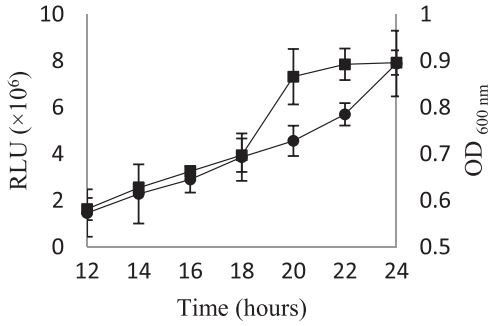

Fig. 2. Growth and luminescence intensity of MM1 in SWB medium with 2% NaCl and pH = 7.0 at 28 °C.

3.5. Heavy metal toxicity assessment

Effect of various concentrations of heavy metal cations including Cd (II), Pb (II), Cu (II), Ni (II), Co (II) and Zn (II) on the luminescence of MM1 showed that even a low concentration of these compounds could make a considerable decline in luminescence intensity. The results demonstrated that luminescence intensity had a sharp fall when the concentration of heavy metals increased (Fig. 3). Cytotoxicity effect on MM1 was determined for each heavy metal by means of EC_{50} value (Table 1). When the MM1 isolate was exposed to 5.23 mg l^{-1} of Zn, luminescence reduced from 892.522 × 10^3 RLU to less than 193.780 × 10^3 RLU with a 21% decrease of the initial luminescence. When luminescence intensity decreased to half, EC_{50} value was measured 0.97 mg l^{-1} Zn (II). This was the lowest concentration of the tested toxic metals that decreases luminescence of MM1 to half. The results in Table 1 showed that the EC_{50} values was 0.97 mg l^{-1} for Zn, 3.00 mg l^{-1} for Ni, 3.62 mg l^{-1} for Cu, 5.75 mg l^{-1} for Pb, 6.16 mg l^{-1} for Co and 14.54 mg l^{-1} for Cd. Results demonstrated that the MM1 isolate had the highest sensitivity to Zn (0.97 mg l^{-1}) and the lowest sensitivity to Cd (14.54 mg l^{-1}).

4. Discussion

In this study, a novel bacterial strain, MM1, with high bioluminescence intensity was isolated from the Caspian Sea. Bioluminescence inhibitory assay showed that the isolate was highly sensitive to low

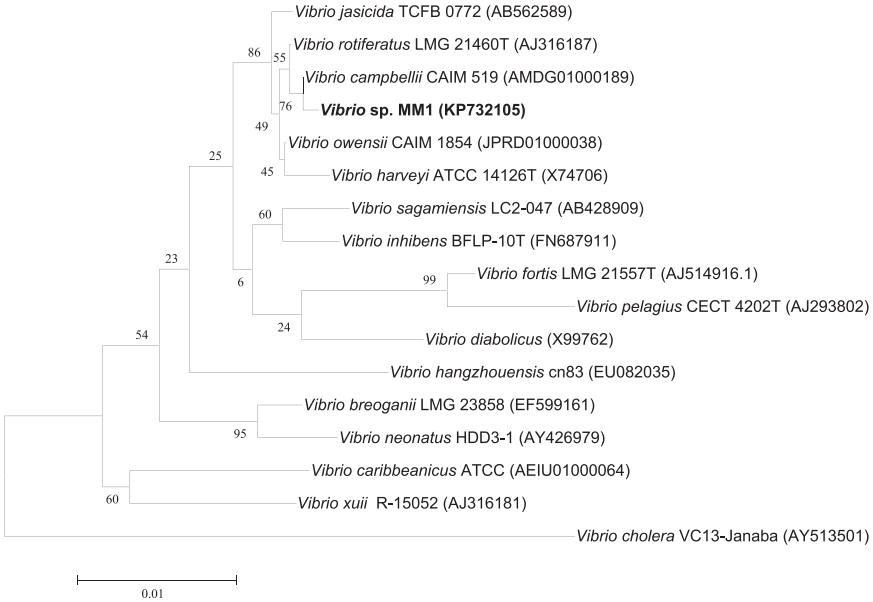

Fig. 1. Phylogenetic dendrogram based on partial *16S rRNA* gene sequences using neighbor-joining method showing the relatedness between MM1 isolate (in bold) and close species. *Vibrio cholera* VC13-Janaba was used as outgroup. Digits at nodes stand for the level of bootstrap support values after 1000 simulations. Scale bar = 1% sequence divergence.

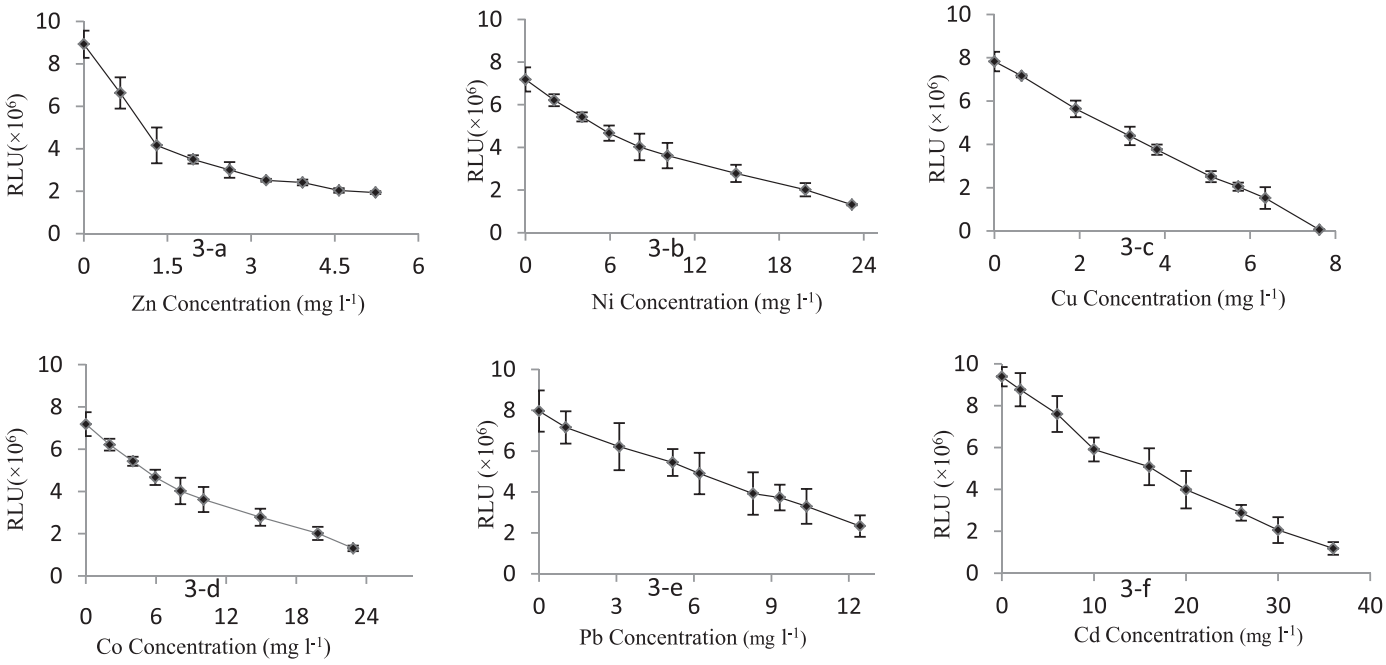

Fig. 3. Heavy metal sensitivity profile of MM1 against different concentrations of various heavy metals (mg l^{-1}). The lowest and highest concentrations of heavy metals that could decrease luminescence to 50% were 0.97 mg l^{-1} for Zn (3-a) and 14.54 mg l^{-1} for Cd (3-f), respectively.

Table 1
The phenotypic characteristics of *Vibrio* sp. MM1 compared with closest related species.

Phenotypic characteristics	**Bacteria**					
	MM1	***V. campbelli***	***V. rotiferianus***	***V. owensii***	***V.fischery***	***V. harveyi***
Cell morphology	Curved rods	Curved rods	Curved rods	Curved rods	Curved rods	Rods
Luminescence	+	–	–	–	+	v
Gram	–	–	–	–	–	–
Growth on NaCl:						
0%[a]	–	–	–	–	–	–
0.8%	+	+	+	+	+	+
2.0%	+	+	+	+	+	+
10.0%	–	–	–	–	–	–
Production of:						
Arginin dihydrolase[a]	–	–	–	–	–	–
Lysin decarboxylase	+	+	+	+	+	+
Ornithin decarboxylase	–	–	+	+	–	+
Catalase	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+
Utilization of Citrate[a]	–	–	–	–	v	–
Urease	–	–	+	–	+	v
Indole	+	+	+	+	–	+
Motility	+	+	+	+	+	+
H_2S formation	–	–	–	–	–	–
Gelatinase	–	+	+	+	–	+
Voges–Proskauer[a]	–	–	–	–	–	–
Glucose (Gas)	–	–	–	–	–	–
Assimilation of:						
D-Glucose	+	+	+	+	V	+
L-Arabinose	–	–	+	–	–	–
D-Lactose	–	–	–	ND	–	–
D-Mannitol	+	+	–	+	V	+
D-Sucrose[a]	–	–	+	+	–	V
myo-Inositol	–	–	–	–	–	–
D-Sorbitol	–	–	–	–	–	–
Mannose	+	**v**	+	+	+	+
Colony color on TCBS agar	G	–	Y	Y	Y	Y

V. campbelli; V. fischery and *V. harveyi* (Alsina and Blanch, 1994); *V. rotiferianus*: (Gomez-Gil et al., 2003); *V. owensii*,(Cano-Gomez et al., 2010).
+, positive; -, negative; v, variable between strains; ND, no data.
[a] Test used to differentiate A-, L+, O- *Vibrio* species according to the scheme of Alsina and Blanch (1994).

Table 2
Comparison of MM1 EC_{50} values (mg l^{-1}) determined for the metals in this study with toxicity values from other investigators.

Chemicals	MM1 15 min. EC_{50} mg l^{-1}	Microtox™ 15 min. EC_{50} mg l^{-1}	TOXcontrol® 15 min. EC_{50} mg l^{-1}	Other Literatures 15 min. EC_{50} mg l^{-1}
Zn (II)	0.97	2.5[a],17[b]	ND	1.56[c]
Ni (II)	3.00	32[k]	317.2[d]	251[e]
Cu (II)	3.62	8[a]	10.61[d]	2.39 ± 0.44[f]
Pb (II)	5.75	11[a]	70–110[d]	1.97 ± 0.36[g]
Co (II)	6.16	16[h]	ND	45.9 ± 4.59[i]
Cd (II)	14.54	59.3 ± 6.92[j]	ND	21.9[f]

NF: No Data.
EC_{50} values are cited
[a] (Bulich and Isenberg, 1981).
[b] (Kahru, 1993).
[c] (Greene et al., 1985).
[d] (Lopez-Roldan et al., 2012).
[e] (Liu and Dutka, 1984).
[f] (Newman and McCloskey, 1996).
[g] (Hong et al., 2010).
[h] (Munkittrick et al., 1991).
[i] (Fulladosa et al., 2005).
[j] (Mowat, 2002).
[k] (Villaescusa et al., 1998)

concentrations of heavy metals, which makes it a good candidate for the detection of low levels of heavy metal pollutants in water and wastewater. Physiological and molecular characterization showed that the isolated bacterium belonged to *Vibrio* genus and had close similarities to *V. campbellii*, *V. rotiferianus* and *V. owensii* and was placed in the harveyi clade (Sawabe et al., 2013). The isolate MM1 had differences in biochemical characteristics and luminescence ability with the harveyi clade. The results of biochemical tests showed that arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase (A,L,O), which are parts of the main key in the Alsina key classification method, were (-,+,-) respectively which indicates that MM1 is in the same cluster as *V. campbellii* and *V. fischeri* but in a different cluster from *V. harveyi* (Alsina and Blanch, 1994). Indole production, motility, oxidase and catalase activity were positive for MM1 as well as other luminescent species whilst citrate utilization and MR-VP test were negative as in other strains. Despite these similarities, some differences were also observed in biochemical characteristics, which distinguish the MM1 isolate from other species. A comparison of the MM1 isolate with other species shows that despite close phylogenetic relationships between species of *Vibrio* (more than 99%), the isolate had some unique characteristics; thus, it could be classified as a novel species. MM1 isolate had intensive luminescence ability while no such ability was reported for *V. campbellii* (Alsina and Blanch, 1994), *V. rotiferianus* (Gomez-Gil et al., 2003) and *V. owensii* (Cano-Gomez et al., 2010) which had the closest similarity to the isolate.

MM1 isolate was used as a luminescent biosensor to evaluate the toxicity of heavy metals by means of EC_{50}. Results demonstrated that MM1 is highly sensitive to Zn, Ni, Cu, Pb and Co ions, and a low concentration of these heavy metals can cause a tangible decrease in bioluminescence intensity. First toxicity monitoring system based on luminescent bacteria was introduced by Azur Environmental (formerly Microbics Corporation) (Bulich, 1979) and since then, numerous commercial toxicity monitoring kits have been developed which used luminescent bacteria to detect toxic materials. Microtox, ToxControl, LUMIStox, and ToxScreen are some known kits which use *V. fischeri* or *Photobacterium phosphoreum* (Farré and Barceló, 2003; Kokkali and van Delft, 2014; Lopez-Roldan et al., 2012). These kits can be categorized in three groups: laboratory-based assays, portable devices and online biomonitors. Laboratory-based assays are usually applied for performing risk assessment of chemicals, regular analysis of samples and research purposes. They are user-friendly methods, but the organism must be cultured in advance, so it is time consuming and needs a laboratory to work in. MicroTox500 and LUMIStox are laboratory-based kits. Portable devices are those that can be carried to the polluted site and measure the toxicity of the sample in situ. So they can provide possibility of early detection of pollutants in emergency circumstances like contaminant leakage, pollution source detection etc. Some examples of portable devices are Check Light-Field and BioTox. Another type of toxicity assessment method is known as online bio-monitors, which is the frontier technology in the field of environmental toxicity monitoring. TOXcontrol, and Microtox CTM are well known examples of this technology. The advantage of this technology is that they can monitor samples in real time and record variations in toxicant concentration. Furthermore, a user can control the device and check the data provided by this technology, but weekly maintenance is required to keep the instruments working properly (Kokkali and van Delft, 2014).

Too many studies used these tests in the last decades and reported different results in various conditions. A 15 min comparison of EC_{50} value for MM1 and commercial kits, based on data reported from other studies is shown in Table 2. These results demonstrated that the EC_{50} value of the MM1 isolate toward Zn^{2+} was very low and less than 1 mg l^{-1} in 15 min. However, other studies reported that the value was at a range of 2.50–17.00 mg l^{-1} in Microtox™ and 4.30 mg l^{-1} in LUMIStox kit, but results of a study by Hong et al. was similar to this study. (Bulich and Isenberg, 1981; Hong et al., 2010; Kahru, 1993). The MM1 isolate showed to be extremely more sensitive to Ni^{2+} than other tests; with an EC_{50} value of 3.00, while EC_{50} values reported for Microtox™ and TOXcontrol® kits were 32.00 and 317.20 mg l^{-1} respectively. (Villaescusa et al., 1998). The MM1 isolate showed similar sensitivity to *Photobacterium* sp. LuB-1 isolated from china for the assessment of Cu, but it was more sensitive than Microtox™ and TOXcontrol® kits. EC_{50} value in 15 min with 2% NaCl concentration, was determined 3.62 mg. l^{-1} for MM1 while it was reported to be 2.29 mg l^{-1} for *Photobacterium* sp. LuB-1 (Hong et al., 2010) and 8 mg l^{-1} for Microtox™ test (Bulich and Isenberg, 1981). This study showed that MM1 isolate was less sensitive than *Photobacterium* sp. LuB-1 when assessing Pb which was reported 1.197 mg l^{-1} (Hong et al., 2010), but it was more sensitive than Microtox™ and TOXcontrol® kits (Table 2). For Co, MM1 was more sensitive than Microtox™ and other studied tests. An amount of 6.16 mg. l^{-1} of cobalt was enough to decrease luminescence intensity to 50% but this concentration was once reported 16 mg l^{-1} (Munkittrick et al., 1991) and another time reported 32.71 mg l^{-1} (Ince et al., 1999) for Microtox™ test. EC_{50}

measured for Cd was 14.54 mg l^{-1} while it was reported 59.3 ± 6.92 mg l^{-1} for Microtox™ test (Mowat, 2002).

Hong and colleagues reported that no luminescence was observed in the isolated bacterium LuB-1 under distilled water treatment, but it displayed stable luminescence intensity when the medium contained 0.2–5% (w/v) NaCl. In these conditions, the bacterium could detect toxicants in a wide range of salt concentration (Hong et al., 2010). Similarly, we found that MM1 could not grow on 0% NaCl and the optimum concentration of salt for bioluminescence was determined 2% NaCl, which is related to the characteristics of the habitat and sea water composition. More studies are needed to determine the sensitivity of the isolated bacterium to pesticides and other hazardous compounds and the possibility of using it as a commercial kit.

5. Conclusion

It can be concluded from this study that the novel bacterium *Vibrio* sp. MM1, which was isolated from the Caspian Sea, has the potential to be used as an alternative microorganism in the bioluminescence inhibition assay. In fact, it can be applied as an in situ bioassay for polluted sites, due to its high sensitivity in detecting environmental toxicants, especially heavy metals.

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