BIODIVERSITAS
Volume 18, Number 3, July 2017
Pages: 1250-1257

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d180350

Iron (II) and other heavy-metal tolerance in bacteria isolated from rock varnish in the arid region of Al-Jafer Basin, Jordan

SULAIMAN ALNAIMAT[1,2,♥], SAQER ABU SHATTAL[1], OSAMA ALTHUNIBAT[2], EID ALSBOU[3], REDA AMASHA[4]
[1]Biological Department, Al-Hussein Bin Talal University, P.O. Box 20 Ma'an, Jordan. Mobile: 00962772229392, ♥email: s_alnaimat@ahu.edu.jo, sulaimanalnaimat2010@gmail.com
[2]Department of Laboratory Medical Sciences, Al-Hussein Bin Talal University, Jordan.
[3]ChemistryDepartment, Al-Hussein Bin Talal University, Jordan
[4]Biological Sciences Department, Faculty of Sciences, King Abdulaziz University. Jeddah, Saudi Arabia.

Manuscript received: 22 May 2017. Revision accepted: 29 July 2017.

Abstract. *Alnaimat S, Shattal SA, Althunibat O, Alsbou E, Amasha R. 2017. Iron (II) and other heavy-metal tolerance in bacteria isolated from rock varnish in the arid region of Al-Jafer Basin, Jordan. Biodiversitas 18: 1250-1257.* This study deals with enumeration, identification and molecular characterization of bacterial species tolerant of iron (II) and other heavy metals that isolated from rock varnish collected from the arid region of Al-Jafer Basin, Jordan. Based on conventional culture-dependent methods, six isolates exhibited a high degree of tolerance to iron (II) and other heavy metals with a minimum inhibitory concentration (550–600 mg/L) to iron, (500-550 mg/L) to lead, (350 mg/L) to copper, (400-450 mg/L) to chromium, (150-200 mg/L) to silver, and (150-200 mg/L) to mercury, in solid media. The results showed that all isolates could tolerate 2000 mg/L Fe^{2+} expressed as maximum tolerance concentration (MTC). The multiple metal resistances of these isolates were also associated with multiple antibiotic resistances. These iron tolerant isolates were shown to be capable of growth at temperatures ranging from 30 to 40°C and pH values ranging from 5 to 9 with some differences between the six isolates. On the basis of morphology and of 16S rDNA gene sequencing and phylogeny analysis, the iron-tolerant isolates were identified as *Bacillus zhangzhouensis* (DVCr1), *Staphylococcus warneri* (DVCr6), *Bacillus cereus* (DVCr7), *Staphylococcus pasteuri* (DVCu2), *Brevibacterium frigoritolerans* (DVPb) and *Bacillus altitudinis* (DVCr10). The 16S rDNA sequences from five of the six strains were submitted to GenBank and are available under the accession numbers KX781139, KX781140, KX781141, KX781145 and KX781148. The results highlight the high potential of a non-polluted habitat like rock varnish to provide new microbial communities that could be used for enhanced bioremediation of heavy metals from a contaminated site.

Keywords: Al-Jafer basin, rock varnish, bacteria tolerant of heavy metals, 16S rDNA

INTRODUCTION

Rock varnish, or desert varnish, is the dark thin outer layer substance of rock surfaces in arid and semiarid climates (Zhang et al. 2012). Its thickness rarely exceeds 200 μm (Parchert et al. 2012). It is normally composed of oxygen, silicon, and aluminum with trace elements, such as Fe, Mn, C, Ca, Na, K, N, P, Ti, Mg, S, Ba, and Cl (Perry et al. 2004).The black pigmented varnish skin has a high concentration of manganese oxides while the dark brown one is rich in iron (Fe) oxides (Parchert et al. 2012).

Al-Jafer basin is situated in the South Jordan, about 210 km south of Amman, the region is typically classified as an arid environment with mean annual rainfall of about 50 mm. The basin has an area of 13,500 square kilometers (Al Kuisi and El-Naqa 2013), scattered with plentiful rocks with iron-rich varnishes.

Due to their high persistence and toxicity in environments, heavy metals such as iron (Fe), lead (Pb), chromium (Cr), cadmium (Cd), mercury (Hg), cobalt (Co) and nickel (Ni) are considered as significant environmental pollutants, particularly in regions with high human activities (Gupta et al. 2012). Bacteria able to survive well in high concentrations of heavy metals have received growing attention in recent years. This characteristic makes them potentially useful for bioremediation (Gandhi et al. 2015).

There is a large body of work focused on microbial diversity associated with desert ecosystems, particularly rock varnishes (Northup et al. 2010; Parchert et al. 2012; Paulino-Lima et al. 2013; Zhang et al. 2012), and on heavy metal tolerance of bacteria isolated from different habitats. However, as far as we are aware, no study has dealt with the isolation of specific heavy metal tolerant microorganisms from desert rock varnish.

Iron oxides occur naturally in high concentration in rock varnish (Parchert et al. 2012)and are important products of rock weathering (Adams et al. 1992). The desert rocks scattered in Al-Jafer basin are predominantly covered by iron-rich varnish. Since microbial encounters with iron oxides are unavoidable in the rock varnish layer, we speculated that the indigenous rock varnish bacteria might develop tolerance strategies against the high concentration of these oxides. The present study is probably the first attempt of its kind to examine the tolerance levels of indigenous bacteria associated with rock varnish to iron (II) and other heavy metals, and to isolate and identify these indigenous bacterial strains.

MATERIALS AND METHODS

Site description and sample collection

24 samples of iron-rich rock varnish were aseptically collected from two different sites situated at the western part of Al-Jafer basin desert, located near Ma'an province, Jordan. Only rocks with relatively flat surfaces were aseptically picked up and immediately placed in sterile aluminum foil for transport. At the lab and in a laminar flow bench, a grinding tool with a flame-sterilized coarse bit was used to harvest the varnish powder from the collected rock samples into a sterile container. The harvested powdered rock varnish (0.5 g)was then stored at -20 °C. (Zhang et al. 2012;Kuhlman et al. 2006).

Enumeration and Isolation of iron-tolerant bacteria

Powdered rock varnish samples (0.1 g) were directly plated on nutrient agar media (Sigma-Aldrich) supplemented with 300 mg/L of Fe^{2+}as iron (II) sulfate $FeSO_4 \cdot 7H_2O$. Cycloheximide at 50μg/mL was added to all media to inhibit fungal growth. After 72 hours of incubation at 37°C, morphologically different colonies were isolated and sub-cultured by streaking on the surface of nutrient agar (NA) plates for purification and preservation (Gandhi et al. 2015)

Determination of Optimal Growth Conditions (temperature, pH, and NaCl)

The optimum pH, temperature, and NaCl conditions for growth of each of the six iron tolerant isolates were determined using 96-well microplates. To find the pH range, the iron tolerant isolates were grown in nutrient broth (NB)media with different pH values (5, 6, 7, 8, and 9) and incubated at 35°C for 24 h. For temperature profiles, the iron tolerant isolates were incubated at temperature 25 C°, 30 C°, 35 C°,40 C°, and 45 C for 24 h. The optimal NaCl concentrations were evaluated through inoculation of iron tolerant isolates into NB medium supplemented with four different NaCl concentrations (1, 2,4 and 8 %). For all of the above parameters, the OD 600 values were measured after 24 h incubation using microplate reader (AccuReader M965, Metertech-Inc., Taiwan) (Gupta et al. 2012).

Minimal inhibitory concentrations of heavy metals of bacterial isolates

All purified strains were subsequently grown on nutrient agar amended with iron and other heavy metals (Cr, Pb, Hg, Cu, Hg and Ag) at the following concentrations: 150, 200, 250, 300, 350, 400, 450, 500, and 550 mg/L. Heavy metals (Cr, Pb, Hg, Cu, and Ag) were used as $CrCl_3$, Pb $(NO_3)_2$, $HgCl_2$, $CuSO_4$, and $AgNO_3$ respectively. The minimum inhibitory concentration (MIC) values were expressed as the lowest concentration of heavy metals that completely prevented the growth of isolated bacteria after three days incubation at 30°C (Gandhi et al. 2015).

Determination of maximum tolerable concentrations (MTCs) of iron ($FeSO_4$) in nutrient broth media

The maximum tolerable concentrations of Iron (II) of the presumptive tolerant isolates were evaluated in triplicate by using the broth microdilution method in 96-well microplates. The initial concentration of the Iron (II) used was2000 mg/L. Each well of a 96-well microplate was inoculated with 5 μL of one of the isolated bacteria suspensions adjusted to 0.5 McFarland standards with further 1:100 dilution to get a final density of approximately 5 x 10^5cfu and with 200 μL of each Iron (II) concentration diluted in sterile nutrient broth. Each Iron (II) concentration was tested in triplicate. Wells contained culture medium with either only $FeSO_4$ or only bacteria were used as controls. Following incubation at 30°C for 48 hours, (10 μL) from each well was spotted on nutrient agar plates and incubated at 37°C for 24-72 hours to test any appearance of growth. The maximum tolerable concentration (MTC) of iron (II) is defined as the highest concentration of iron (II) which allows growth after 48 h (Moghannem et al. 2015).

Determination of the co-resistance to antibiotics

Isolates were subjected to antibiotic resistance screening by the disc diffusion method in Mueller-Hinton agar (Oxoid) according to the CLSI procedures (Wayne 2009). The following antibiotics (Oxoid) were used: Novobiocin 30μg, Chloramphenicol 30μg, Penicillin 10μg, Erythromycin 15μg, Streptomycin 10μg, Kanamycin 30μg, Ampicillin 10μg, and Tetracycline 30μg (Gandhi et al. 2015).

Determination of the effect of iron ($FeSO_4$) growth kinetics of the bacterial isolates

Using 96-well microplates, Isolates were grown in a sterile NB medium, without (positive control sample) or with Iron ($FeSO_4$) at 125, 250, 500 and 1000 mg/L. During incubation at 35°C and with shaking (80 rpm), growth was recording at known intervals by measuring the optical density at 600 nm on a microplate reader (AccuReader M965, Metertech-Inc., Taiwan), and subtracting the signals measured for the metal salt alone (Françoiset al. 2012).

Molecular and phylogenetic analysis of the isolated strains

DNA extraction

DNA extraction of isolated samples was performed using the G-spin Total DNA Extraction Mini Kit (iNtRON Biotechnology, Suwon, Korea). The extracted DNA was used as a template for PCR to amplify 16S rDNA. The extraction method was performed according to the instructions of the manufacturers.

Amplification of 16S rRNA gene and phylogenetic analysis

The SSU rRNA gene was amplified with the bacterial forward primer 27F (3'-AGRGTTYGATYMTGGCTCAG-5') and 1492R (5′-RGYTACCTTGTTACGACTT-3′).Amplification of 16S rRNA was performed in a total volume of 50 μL containing 2.0 μL Genomic DNA, 25μL of 2x PCR Master mix Solution i-MAX II (iNtRON), 1.0 μL Forward Primer, 1.0 μL Reverse Primer, and 31μL of sterile distilled water (sdH_2O). The PCR reaction mixtures, after incubation at

94°C for 3 minutes as an initial denaturation, were cycled 30 times through the following temperature profile: denaturation for 1 minute at 94°C; annealing for1 minute at 60°C; and elongation for 5 minutes at 72°C with final incubation for 5 minutes at 75°C, after which 10 µL of each PCR amplification mixture was mixed with 2 µL of Blue/Orange 6x loading dye and analyzed by 1% agarose gel electrophoresis. In addition, 6µL of 1 Kb Hyper Ladder loading was used to confirm the correct sized product. A PCR quick-spin PCR Product Purification Kit was used to purify PCR products according to the manufacturer's protocol. The purified PCR products were sequenced using the commercial service of MACROGEN, Korea.

The resulting 16S rRNA gene sequences were compared with those in GenBank using the blast program (NCBI) and the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net; (Kim et al. 2012). The 16S rRNA gene sequences of isolated strains were aligned with corresponding sequences of closely related type species (retrieved from the GenBank/EMBL/DDBJ database) using Clustal x1.83 (Thompson et al. 1997).

Phylogenetic trees were reconstructed using the neighbor-joining method (Saitou and Nei, 1987)contained in MEGA6software (Tamura et al. 2013). Bootstrap values (more than 40%) based on 1000 replications were listed at nodes.

Nucleotide sequence accession number

The nucleotide sequences of 16S rRNA documented in this study were deposited in (National Center for Biotechnology Information NCBI)nucleotide sequence databases under Accession Numbers listed in Table 4.

Statistical analysis

Data are expressed as mean ± standard error of the mean (S.E.M) of at least three independent samples as described above. Graphs were constructed and statistical analysis performed with GraphPad Prism (version 7.02 for Windows; GraphPad Software, La Jolla, CA, USA).

RESULTS AND DISCUSSION

Isolation of the iron-tolerant bacteria from rock varnish powder

Iron-tolerant strains isolated from the rock varnish samples collected from the western part of Al-Jafer basin desert, grew well on a nutrient agar medium enriched initially with 300 mg/ L Fe^{+2} (Figure 1). Only six different colonies were isolated and subcultured on nutrient agar media on the basis of colony morphology. Strains were designated as DVPb, DVCr10, DVCr6, DVCr7, DVCu2, and DVCr1.

Minimal inhibitory concentrations of heavy metals for the bacterial isolates

In order to evaluate their multi-metal tolerance capacity, all purified presumptive strains were subsequently grown on nutrient agar amended with iron and other heavy metals (Pb, Cu, Cr, Ag, and Hg) at different concentrations. As shown in (Table1),all the bacterial isolates exhibited a high degree of tolerance to Fe^{2+} with minimum inhibitory concentration (MIC) ranging from 550mg/L to 600 mg/L. The results showed that all isolates could tolerate 2000 mg/L Fe^{2+} expressed as maximum tolerable concentration (MTC). In addition to iron, all isolates showed a high degree of multi-metal tolerance based on MIC values; isolated strains showed tolerance (500-550), (350), (400-450), (150-200), and (150-200) mg/L for lead, copper, chromium, silver and mercury, respectively.

Determination of the co-resistance to antibiotics

Except for the DVCr10 strain, all iron-tolerant isolates tested for antibiotic susceptibility were shown to be multi-drug resistant. The results are summarized in (Table 2).

Morphological and physiological characteristics, and the optimal growth parameters of iron tolerant isolates

As listed in Table 3, all isolates were Gram-positive and had a white or off-white colony color. Also, they were irregular in shape except for DVCr6 and DVCr10 strains, which produced circular and filamentous colonies, respectively. The colony elevation in most of these strains was flat except DVPb and DVCr10 (Raised). The colony margins were found to be lobate for DVCr10, DVCu2, and DVPb, undulate forDVCr7 and DVCr1 strains, and entire for the DVCr6 strain. Microscopic examination revealed that DVPb, DVCr1, DVCr7, and DVCr10 strains were rod cell morphology, whileDVCr6 and DVCu2 strains had a spherical cell morphology. As summarized in Table 3 and detailed inFigure 2 and Figure 3,the optimal temperature for all isolated bacteria was 30C°. The best growth pH for DVCr1, DVCr6, DVCr7, and DVCu2 was 6, while for DVPb and DVCr10 it was8 and 7, respectively. The optimal concentration of NaCl was determined to be 2 % for DVCr1, DVCr6, DVCr7, and DVCu2 and to be 1% for DVPb and DVCr10.

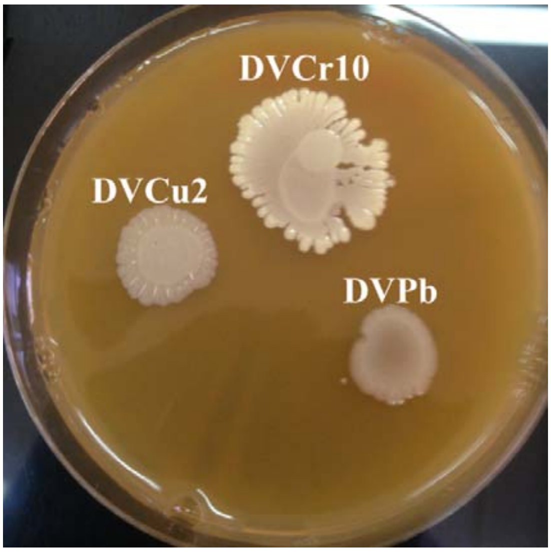

Figure 1. Successful growth of some isolates grown on nutrient agar plate containing 300mg/L Fe^{2+}

Table 1. Minimum inhibitory concentration (MIC mg/L) of different metals obtained for the six isolates from rock varnish and the maximum tolerable concentration of Fe $^{2+}$ (MTC mg/L).

Isolated strains	MIC mg/L						MTC mg/L
	Fe (II)	Pb	Cu	Cr	Ag	Hg	Fe (II)
DVPb	600	500	350	400	150	150	>2000
DVCr1	550	500	350	400	200	150	>2000
DVCr6	550	550	350	400	200	200	>2000
DVCr7	550	550	350	400	150	200	>2000
DVCu2	550	500	350	450	150	150	>2000
DVCr10	550	500	350	450	200	200	>2000

Table 2. Antibiotics resistance pattern of the isolated strains

Isolated strains	Resistance pattern*
DVPb	$C^{30}E^{15}N^{30}P^{10}S^{10}T^{30}$
DVCr1	$C^{30}E^{15}P^{10}S^{10}T^{30}$
DVCr6	$C^{30}E^{15}N^{30}P^{10}S^{10}T^{30}$
DVCr7	$C^{30}K^{30}N^{30}P^{10}T^{30}$
DVCu2	$C^{30}N^{30}P^{10}S^{10}T^{30}$
DVCr10	$P^{10}T^{30}$

Note: C^{30} = Chloramphenicol 30μg, E^{15} = Erythromycin 15μg , K^{30} = Kanamycin 30μg, P^{10} = Penicillin 10μg, N^{30} = Novobiocin 30μg, S^{10}= Streptomycin 10μg, T^{30} = Tetracycline 30μg

Table 3. Morphological and physiological characteristics of iron tolerant isolates from rock varnish

Isolates	Colony color	Gram reaction	Elevation	Margin	Form	Cell morphology	Optimum pH	Optimum NaCl %	Optimum temp. (C°)
DVPb	White	G+	Raised	Lobate	Irregular	Rod	8	1	30
DVCr1	White	G+	Flat	Undulate	Irregular	Rod	6	2	30
DVCr6	White	G+	Flat	Entire	Circular	Spherical	6	2	30
DVCr7	Off-White	G+	Flat	Undulate	Irregular	Rod	6	2	30
DVCu2	White	G+	Flat	Lobate	Irregular	Spherical	6	2	30
DVCr10	White	G+	Raised	Lobate	Filamentous	Rod	7	1	30

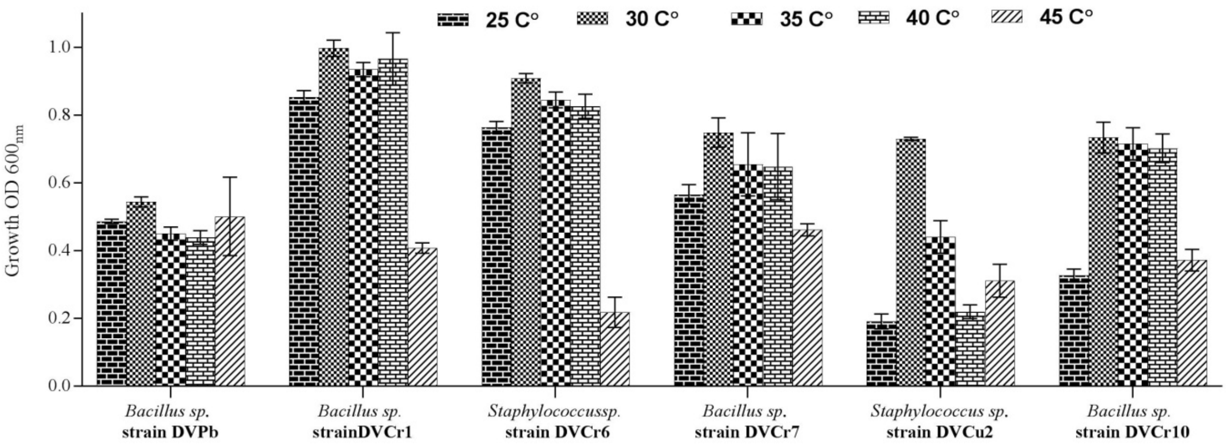

Figure 2. Effect of different temperatures on the growth of the isolated strains

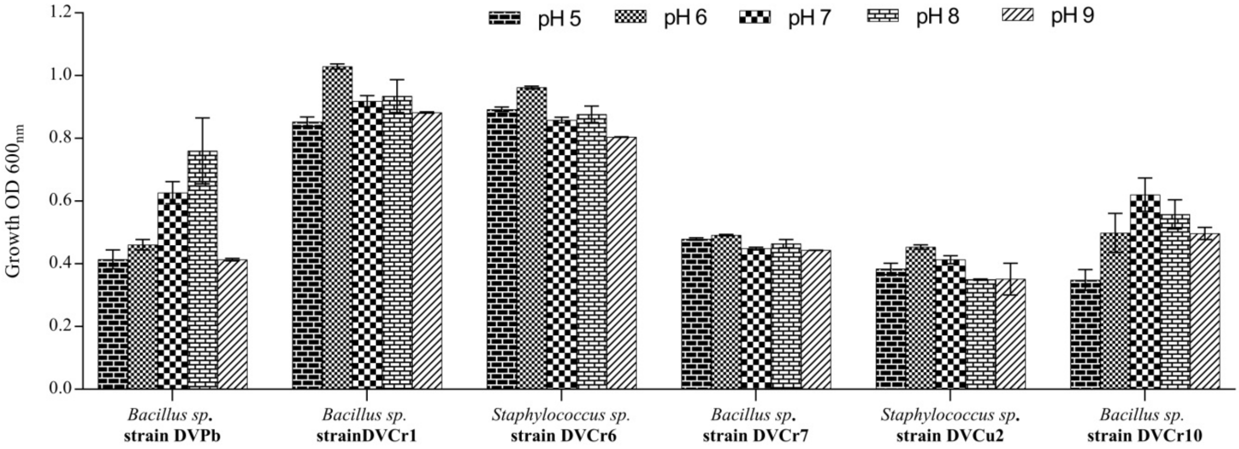

Figure 3. Effect of different pH values on the growth of the isolated strains

Determination of the effect of iron ($FeSO_4$) on the growth kinetics of bacterial isolates

The results of growth kinetic studies of the isolated strains, which are displayed in Figure 4, show that the strains were able to survive and even multiply in various Fe^{2+} concentrations. Optical densities of growing cultures were reported after 6, 18, 24, and 40 hrs of incubation. The growth of bacterial isolates declined in response to raising the concentration of iron as compared to control, but still they were still able to grow at a higher Fe^{2+}concentration (500 mg/L). No significant effects were observed when the isolated strains were grown in the presence of Fe^{2+} at 125 mg/L concentration as compared with control, but increasing the concentration to 1000 mg/L was, however, toxic to all isolated bacteria strains.

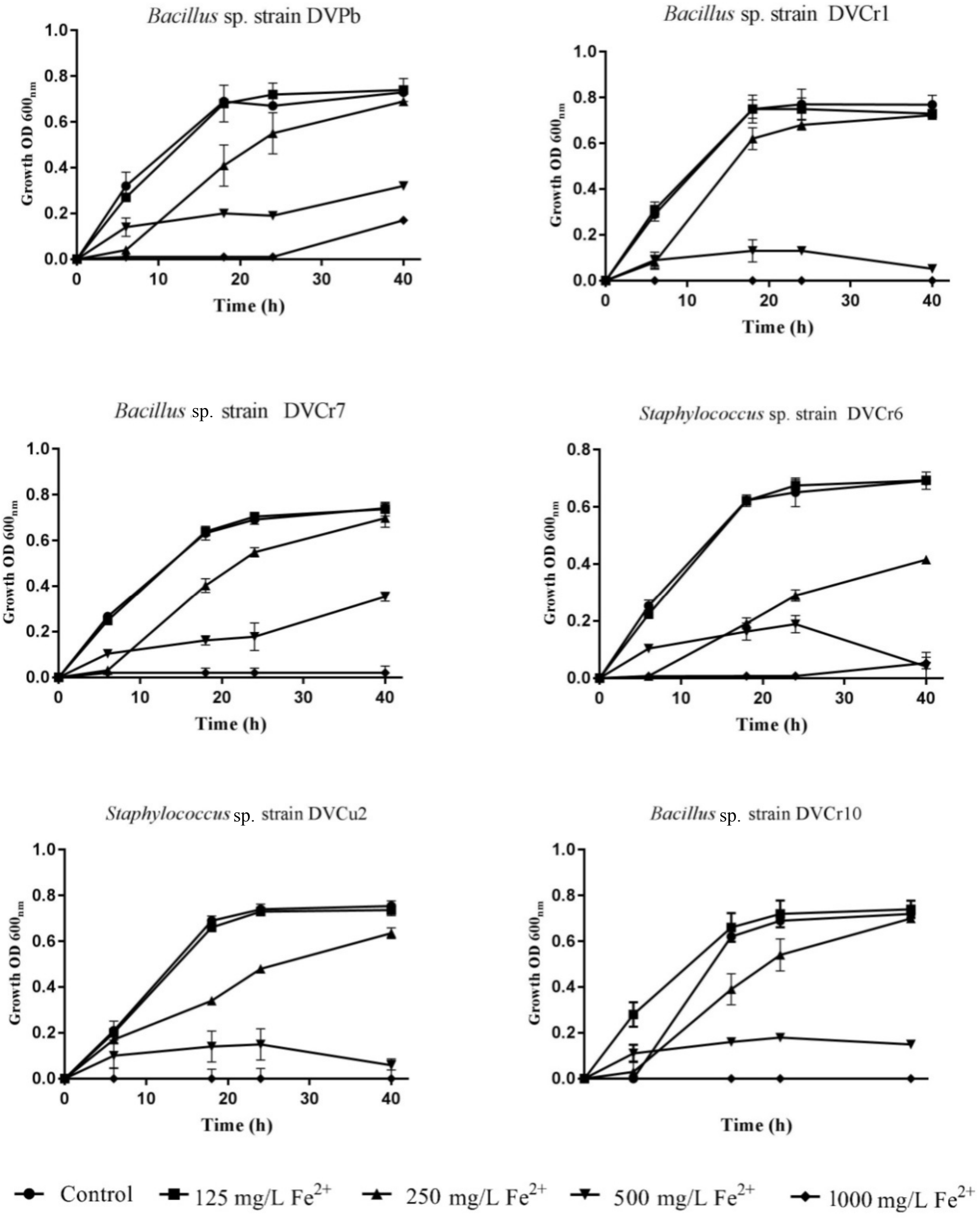

Figure 4. Growth kinetics of the bacterial isolates in the absence and presence of different concentrations (125-1000 mg/L) of Fe $^{2+}$

Molecular and phylogenetic analysis of the isolated strains

PCR amplification of the 16S rDNA gene produced fragments of approximately 1400 base pairs in size (Table .4). The resulting 16S rRNA gene sequences from iron-tolerant isolates were compared and the closest match was detected using BLAST program (NCBI) and the EzTaxon-e database. The highest sequence similarities for the iron-tolerant isolates were as follows: Strain DVCr1 showed 99.93% similarity with *Bacillus zhangzhouensis,* accession numberJOTP01000061; Strain DVCr6 showed 99.93% similarity with *Staphylococcus warneri* ATCC 27836; Strain DVCr7 showed 100.00%similarity with *Bacillus cereus,* accession numberAE016877;Strain DVCu2 showed 100.00% similarity with *Staphylococcus pasteuri* ATCC 51129;Strain DVPb showed 100.00% similarity with *Brevibacterium frigoritolerans* DSM 8801 while Strain DVCr10 showed 99.79% similarity with *Bacillus altitudinis*, accession number ASJC01000029. As clearly shown in Figure 5, the neighbor-joining phylogenetic tree based on 16S rRNA gene sequences confirms that iron-tolerant isolates fell into two genera; *Bacillus* and *Staphylococcus.* The 16S rDNA sequences from the 5 strains were submitted to GenBank and are available under the accession numbers KX781139, KX781140, KX781141, KX781145 and KX781148.

Table 4. Comparative analysis of 16S rRNA gene sequences of iron tolerant isolates from the desert varnish, using highly matched species available in EzBioCloud's database (Yoon et al. 2016)

Isolated strains	Sequence length	Genbank accession no.	Highly matched bacteria accession no.	% Similarity
Bacillus sp. strain DVCr1	1411	KX781139	*Bacillus zhangzhouensis* JOTP01000061	99.93
Staphylococcus sp. strain DVCr6	1409	KX781140	*Staphylococcus warneri* ATCC27836 $^{(T)}$	99.93
Bacillus sp. strain DVCr7	1394	KX781141	*Bacillus cereus* AE016877	100.00
Staphylococcus sp. strain DVCu2	1374	KX781145	*Staphylococcus pasteuri* ATCC51129	100.00
Bacillus sp. strain DVPb	1390	KX781148	*Brevibacterium frigoritolerans* DSM8801 $^{(T)}$	100.00
Bacillus sp. DVCr10	1422	****	*Bacillus altitudinis* ASJC01000029	99.79

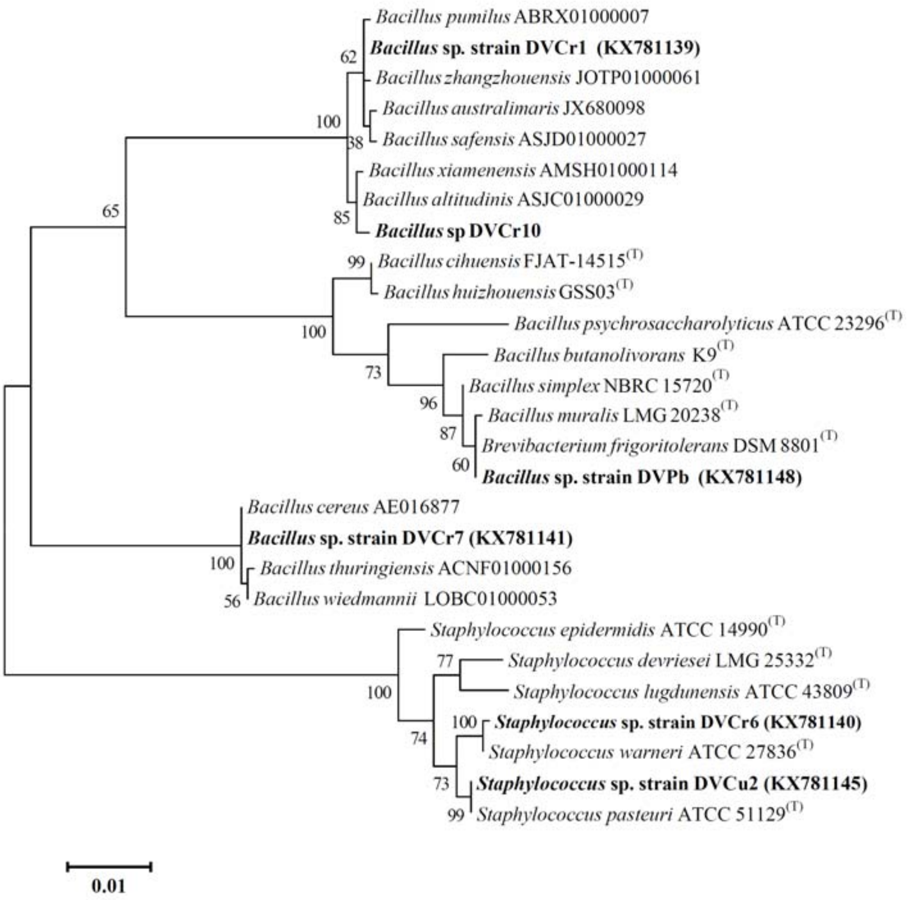

Figure 5. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the interrelationships of the isolated bacterial strains. The GenBank accession number of each strain is shown in parenthesis. The tree was generated using the neighbor-joining (NJ) method (Saitou and Nei 1987) contained in the MEGA6 software package (Tamura et al. 2013). Bootstrap values based on 1000 replications are listed at nodes. Scale bar represents 0.01substitutions per nucleotide position

Discussion

Culture-dependent techniques are widely used to investigate microbial diversity in a variety of environments, particularly extreme habitats that might contain potentially new bacterial species. It is well known that the microbial world represents a wide gene pool that may be explored for several useful applications, such as bioremediation and biosorption abilities (Gandhi et al. 2015). This study is a part of a large-scale project aimed to investigate the microbial diversity associated with the rock varnish in the arid region of Al-Jafer Basin, Jordan. In the current study, focus was placed on characterization and identification of bacteria tolerant of iron (II) and other heavy-metals, that we isolated from the iron-rich varnish. Most previous studies aiming to isolate heavy metal tolerant bacteria have focused on metal-contaminated habitats, whereas in this work we investigated a non-polluted habitat, rock varnish, for the same purpose.

After the powdered rock varnish samples had been initially screened, six iron-tolerant bacteria with different morphology showed noticeable growth in the presence of 300 mg/ L Fe^{+2}; these strains were designated as DVPb, DVCr10, DVCr6, DVCr7, DVCu2, and DVCr1. Encouraged by these preliminary findings, the multi-metal tolerance of isolates against Pb, Cu, Cr, Ag, and Hg at different concentrations was evaluated using MIC technique which is a powerful and easy technique for determination of heavy metal resistance (Neethu et al. 2015) (Table1). Beside high iron tolerance capacity with MIC values up to 550-600 mg/L and MTC larger than 2000 mg/L, isolates were found to exhibit different multiple heavy metal tolerance characteristics to Pb, Cu, Cr, Ag, and Hg, this phenomenon is common among isolated heavy metal tolerance microbes (Alboghobeishet al. 2014; Moghannem et al. 2015). The high level of iron oxides that occur naturally in rock varnish (Parchert et al. 2012) appears to have given the indigenous bacterial strains a remarkable level of tolerance to iron. In addition, the results reveal that all iron tolerant isolates tested for antibiotic susceptibility had multiple antibiotic resistances (Table 2). Association of antibiotic resistance with metal tolerance is common and well documented in many other studies (Gandhi et al. 2015; Neethu et al. 2015; Shammi and Ahmed 2016).This could be as a result of genes for metal tolerance and antibiotic resistance genes existing together on the same R-plasmid and or transposons in bacteria. Closely associated genes would likely be distributed together in the environment (Shammi and Ahmed, 2016; Gandhi et al. 2015). Recently, Feßler and his co-workers analyzed R-plasmids obtained from a bovine methicillin-resistant *Staphylococcus aureus* (MRSA) isolate. Their findings revealed that five different antibiotic resistance genes were co-located on a plasmid with heavy metal resistance genes (Feßler et al. 2017).

The combination of morphological characterization and genetic analysis (Table 3, Table 4) enabled the identification of iron-tolerant isolates as follows: strain DVCr1, strain DVCr10, strain DVCr7 and strain DVPb belonging to the genus *Bacillus*; and strains DVCr6 and DVCu2 belonging to the genus *Staphylococcus*. All of the isolates were Gram-positive bacteria. As shown in Figure 5, the phylogenetic tree based on 16S rRNA gene sequences confirms that the iron-tolerant isolates fell into two genera; *Bacillus* and *Staphylococcus*. It seems that because the genus *Bacillus* is ubiquitous in terrestrial areas and is extensively distributed in nature, *Bacillus* species are dominant bacteria in heavy metal-polluted areas (Zampieri et al. 2016; Shammi 2016; Alboghobeish et al. 2014). Also, the ability of *Staphylococcus* species to tolerate heavy metals has been confirmed in other research (Feßler et al. 2017). For example, Zampieri et al. (2016) isolated metal-tolerance *Staphylococcus* sp. from the polluted sediments of the Araça Bay on the São Paulo coast of Brazil.

The growth capacities of iron tolerant isolates at temperatures between 30 and 40°C and at pH values ranging from 5 to 9 were demonstrated in our results (Figure 2-3), suggesting that the isolates we obtained might be suitable for bioremediation of metals under a wide range of temperatures and pH values. This is consistent with the results of the previous study where the growth of selected metal-tolerance strains was only slightly affected by different temperatures and pH values within a biological range (Moghannem et al. 2015).

The growth rate of the iron tolerant isolates in the presence of Fe^{2+}at 125 mg/L concentration was not significantly different from the growth rate of the control, but raising the Fe^{2+} concentration prolonged the lag phase and reduced the growth rate. Similar results have been reported by others (Raja et al. 2009; Moghannem et al. 2015; Khanet al. 2016).This behavior could be explained by the fact that exposure to metal stress likely directs the energy budget of the exposed bacteria away from growth towards the maintenance of other functions that enable the bacteria to resist metal toxicity (Alboghobeish et al. 2014).

As stated above, the isolation of bacteria tolerant of iron (II) and other heavy metals from rock varnish in the arid region of Al-Jafer basin was predicable based on results of research elsewhere. Samples of rock varnish from dry areas in continental United States, Hawaii, and Australia have been found to contain biochemical compounds (siderophores) released by microbes to mobilize a variety of different iron oxides that could be concentrated by precipitation onto bacterial cell walls (Adams et al. 1992).Taken along with the results of our study, this demonstrated ability of rock varnish indigenous bacteria to produces iderophore and to use their cell walls to precipitate iron oxides, suggests that bacterial isolates from rock varnish could be a promising tool in the biogeochemical cycling of iron and other toxic heavy metals, and in biosorption applications for metal-contaminated environments. Further investigations are needed to optimize the conditions for metal removal from polluted aqueous solutions by current isolates.

ACKNOWLEDGEMENTS

The authors would like to thank Al-Hussein Bin Talal University of Jordan for providing the financial support and services to facilitate this research.

REFERENCES

Adams JB, Palmer F, Staley JT. 1992. Rock weathering in deserts: mobilization and concentration of ferric iron by microorganisms. Geomicrobiol J 10 (2): 99-114.

Al Kuisi M, A El-Naqa.2013. GIS based spatial groundwater recharge estimation in the Jafr basin, Jordan– application of WetSpass models for arid regions. Rev Mex Cienc Geol 30 (1): 96-109.

Alboghobeish H, Tahmourespour A, Doudi M. 2014. The study of Nickel Resistant Bacteria (NiRB) isolated from wastewaters polluted with different industrial sources. J Environ Health Sci Eng 12: 44. DOI: 10.1186/2052-336X-12-44

Feßler AT, Zhao Q, Schoenfelder S,Kadlec K, Michael GB, Wang Y, Ziebuhr W, Shen J,Schwarz S. 2017. Complete sequence of a plasmid from a bovine methicillin-resistant Staphylococcus aureus harbouring a novel ica-like gene cluster in addition to antimicrobial and heavy metal resistance genes. Vet Microbiol 200: 95-100.

François F, Lombard C, Guigner J-M, Soreau P,Brian-Jaisson F, Martino G, Vandervennet M, Garcia D, Molinier A-L, Pignol D. 2012. Isolation and characterization of environmental bacteria capable of extracellular biosorption of mercury. Appl Environ Microbiol 78 (4): 1097-1106.

Gandhi V, Priya A, Priya S, Daiya V, Kesari J, Prakash K, Kumar Jha A, Kumar K, Kumar N. 2015. Isolation and molecular characterization of bacteria to heavy metals isolated from soil samples in Bokaro Coal Mines, India. Pollution 1 (3): 287-295.

Gupta K, Chatterjee C, Gupta B. 2012. Isolation and characterization of heavy metal tolerant Gram-positive bacteria with bioremedial properties from municipal waste rich soil of Kestopur canal (Kolkata), West Bengal, India. Biologia 67 (5): 827-836.

Khan I, Aurangzeb N, Zia M, Nisa S.2016. Isolation and identification of heavy metal tolerant bacteria from volta battery effluent, Hattar, Haripur Pakistan. Pure Appl Biol 5 (4): 719-726.

Kim OS, Cho Y-J, Lee K, Yoon S-H, Kim M, Park S-C, Jeon YS,Lee J-H, Yi H. 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Intl J Syst Evol Microbiol 62 (3): 716-721.

Moghannem SA, Refaat BM, El-Sherbiny GM, El-Sayed MH, Elsehemy IA, Kalaba MH. 2015. Characterization of heavy metal and antibiotic-resistant bacteria isolated from polluted localities in Egypt. Egypt Pharmaceut J 14 (3): 158-165.

Neethu C, Mujeeb RahimanK, Saramma A, Mohamed HathaA. 2015. Heavy-metal resistance in Gram-negative bacteria isolated from Kongsfjord, Arctic.Can J Microbiol 61 (6): 429-435.

Northup DE, Snider JR, Spilde MN, Porter ML, van de Kamp JL, Boston PJ, Nyberg AM, Bargar JR.2010. Diversity of rock varnish bacterial communities from Black Canyon, New Mexico. J Geophys Res 2010: 115. DOI: 10.1029/2009JG001107.

Parchert KJ, Spilde MN, Porras-Alfaro A, Nyberg AM, Northup DE. 2012. Fungal communities associated with rock varnish in Black Canyon, New Mexico: casual inhabitants or essential partners? Geomicrobiol J 29 (8): 752-766.

Paulino-Lima IG, Azua-Bustos A, Vicuña R,González-Silva C, Salas L, Teixeira L, Rosado A, da Costa Leitao AA, Lage C. 2013. Isolation of UVC-tolerant bacteria from the hyperarid Atacama Desert, Chile. Microb Ecol 65 (2): 325-335.

Perry RS, Dodsworth J, Staley JT,Engel MH. 2004. Bacterial diversity in desert varnish. In Third European workshop on exo/astrobiology. Mars: the search for life. SP-545. ESA Publications, Noordwijk, The Netherlands.

Raja CE, Selvam G,Omine K. 2009. Isolation, identification and characterization of heavy metal resistant bacteria from sewage. In: Intl Joint Symp on Geodisaster Prevention and Geoenvironment in Asia, 205-211.

Saitou N, Nei M.1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4 (4): 406-425.

Shammi T, Ahmed S. 2016. Heavy metal tolerance and antibiotic resistance of *Bacillus* spp. isolated from two major rivers in Bangladesh. Mol Biol Evol 30 (1-2): 17-22.

Tamura K,Stecher G,Peterson D, Filipski A,Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30 (12): 2725-2729.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25 (24): 4876-4882.

Wayne P.2009. Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial disk diffusion susceptibility tests 19th ed. approved standard. CLSI document M100-S19: 29.

Yoon S-H, Ha S-M, Kwon S,Lim J, Kim Y, Seo H, Chun J. 2016. Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. Intl J Syst Evol Microbiol. DOI: 10.1099/ijsem.0.001755.

Zampieri BDB, Pinto AB,Schultz L, de Oliveira MA, de Oliveira AJFC. 2016. Diversity and distribution of heavy metal-resistant bacteria in polluted sediments of the Araça Bay, São Sebastião (SP), and the relationship between heavy metals and organic matter concentrations. Microb Ecol 72 (3): 582-594.

Zhang T, Liu M, Sun J, Shi Y, Zeng J,Lou K.2 012. Bacterial diversity in rock varnish of extreme arid region of Turpan Basin. Acta Ecol Sin 32 (5): 265-270.