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Isolation, molecular characterization and extracellular enzymatic activity of culturable halophilic bacteria from hypersaline natural habitats

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Abstract. *Bin-Salman SA, Amasha RH, Jastaniah SD, Aly MM, Altaif K. 2018. Isolation, molecular characterization and extracellular enzymatic activity of culturable halophilic bacteria from hypersaline natural habitats. Biodiversitas 19: 1828-1834.* Saline habitats, like the Dead Sea, are unusual extreme environments, due to their extreme salinity. Most saline habitats originate from the evaporation of seawater, and have a nearly neutral to slightly alkaline pH (such as the Red Sea (pH8.3) and Arabian Gulf, pH8.3). Ten halophilic bacterial strains (two Gram-negative) belonging to the family of Halomonadaceae and (eight Gram-positive), belonging to the family of Bacillaceae, were isolated from the Red Sea, Arabian Gulf, and Dead Sea by subjecting the isolates to a high salinity medium, followed by identification using 16S rRNA gene sequencing. Four of isolates were designated on the basis of their tolerance to high salinity; SBR_1 exhibited 97% homology to *Halomonas aquamarina*, SBR_2 showed 97% homology to *Sediminibacillus* sp., (Red Sea), SBA_9 exhibited 94% homology to *Halobacillus* sp., (Arabian Gulf) and SBD_{17} gave 98% homology to *Halobacillus dabanensis* (Dead Sea). The isolates were also characterized by their physiological parameters, SBR_1 showed optimum growth at 30°C, pH8.5 and1.5M NaCl, SBR_2 at 30°C, pH6.0 and 1M NaCl. Optimum conditions for SBA_9 were 35°C, pH6.5 and 1M NaCl and for SBD_{17}, 37°C, pH7.0 and 1M NaCl.

Keywords: 16S rRNA gene sequence, Arabian Gulf, Dead Sea, extremophiles, halophiles, Red Sea

INTRODUCTION

Microorganisms living in extreme environments are referred to as extremophiles. So-called psychrophiles and thermophiles grow best at low and high temperature, respectively, alkaliphiles and acidophiles are adapted to alkaline and acidic conditions, barophiles grow best at high pressure, radioresistant organisms can live in high radiation environments while halophiles are salt-tolerant organisms (Rampelotto 2013). Extremophiles possess a number of strategies which allow them to live in such harsh environments, such as their ability to produce hydrolytic extremozymes which become increasingly attractive for modern biotechnology, industry, and medicine (Karray et al. 2018). For example, polymer-degrading enzymes and DNA polymerases are produced by thermophiles; these are stable and active at high temperatures. Proteases and lipases, found in psychrophiles are active at lower temperatures, while enzymes produced by acidophiles and alkaliphiles can be useful in the production of detergents (Babu et al. 2015). Halophiles or halophilic microorganisms grow in hyper-saline concentrations and include representatives of the eukarya, bacteria, and archaea (Mohammadipanah et al. 2015). The pink-red color of hypersaline environments worldwide, is due to halophilic microorganisms, and the most generally observed halophiles are either belong to the archaea or to some bacterial genera, such as *Haloquadratum*, *Halobacterium*, *Halomonas*, and *Salinibacter*, as well as the green alga, *Dunaliella salina* (Ma et al. 2010; Oren 2011; Waditee-Sirisattha et al. 2016). Halophiles can be divided into three main groups, due to their salt requirements; extreme halophiles prefer to grow at 5 M of NaCl, moderate halophiles at 3 M of NaCl and slight halophiles at 1 M of NaCl (Ventosa et al. 2015).

The difficulty of studying microorganisms in natural environments via culturing and other traditional methods have hindered our full realization of microbial diversity (Alnaimat et al. 2017). One milliliter of seawater may contain 10^6 of microorganisms, which have not yet been identified, thereby making necessary the use of modern molecular methods for determining the vast variety and structure of microbial populations. These techniques and methods can be used on both culturable and non-culturable microorganisms isolated from a variety of environments including seawater and soil (Fakruddin and Mannan 2013). A number of molecular techniques have been devised for characterizing and identifying the phylogenetic and functional diversity of microorganisms, the most commonly used being the analysis of 16S rRNA genes for prokaryotes, which are selectively amplified by the Polymerase Chain Reaction (PCR) from the whole genomic DNA extracted from environmental sample, with or without, the need to culture microorganisms (Rastogi and Sani 2011; Li and Zhao 2013). The aim of this study was to isolate bacteria from the Red Sea, the Arabian Gulf

(also known as the Persian Gulf) in Saudi Arabia and the Dead Sea in Jordan, and then identify any halophilic bacteria isolated, using 16S rRNA gene sequencing. In addition, the study involved physiological characterization of halophilic bacterial isolates.

MATERIALS AND METHODS

Site description and sample collection

Samples were collected in May 2016 and September 2016. Three samples of water and three samples of sediment were aseptically collected from six different sites at the southern part of Red Sea (Site1, N: 21°29'14.8", E: 39°07'58.0"; Site2, N: 21°29'05.8", E: 39°08'004"; Site3, N: 21°28'50.2", E: 039°07'52.2"; Site4, N: 22.144268, E: 38.974901; Site5, N: 22.174521,E: 38.965919; Site6, N: 21° 29'23.5", E: 039°07'58.0"), at various depths (17m, 21m, 12m, 14m and 11m) with maximum distance estimated at nearly (1.8km), located in Jeddah city, Saudi Arabia. One sample of water and the other of sediment were collected from coast of Arabian Gulf, located in Khobar city (N: 28°24'01.2", E: 49°18'28.6"), Saudi Arabia. Three samples of water, three samples of sediment and three samples of saline mud were also collected from two different sites at the northern part of Dead Sea (N: 31°42'27.0", E: 35°34'52.7"), at two depths of (1.5m-3.0m), located in Balqa province, Jordan Recorded temperatures at the sampling sites varied between 34°C, 38°C and 30°C, respectively.

Isolation, purification, and preservation of halophilic bacteria

For isolation of halophilic bacteria,1.0g of both of sediment and mud were suspended in 9ml sterile dH_2O, followed by 9ml of sterile distilled water was poured aseptically into test tubes. For the preparation of the serial dilution series (up to 10^4), Culture media (saline nutrient, Zobell marine, casein, and seawater) were inoculated with 0.1ml of the diluted solutions of each sample and was spread on the surface of the medium (Nieto et al. 1989; Lee et al. 2003; Satbhai et al. 2015). All plates were incubated at 37°C and growth was monitored during 24h, 48h, and 72h. The different types of colonies were picked off and transferred to fresh medium in order to obtain pure cultures.

The cultures were finally purified on the same media from which they were isolated and all isolates were preserved at 4ºC. Simultaneously, the isolates were grown in broth, and 1ml of cultures were transferred with 1ml of 50% glycerol for long preservation at (-20ºC).

Phenotypic characterization of the selected halophilic bacteria

Selected halophilic bacteria were morphologically characterized using standard techniques (Gram stain, colony shape, size, and color on S.N agar plates, motility, and endospore-forming, etc.) according to Bergey's Manual of Determinative Bacteriology (Holt et al. 1994).

Physiological and biochemical characteristics

On saline nutrient agar plates, four genera of halophilic bacteria SBR_1, SBR_2, SBA_9, and SBD_{17} were purified. Biochemical tests of them were performed using API 20E® kit, and other tests for identification of each bacterial isolates. These tests include indole production, Voges Proskauer, methyl red, oxidase and catalase test, and their capacity for fermentation mannitol salt. Physiological tests were also performed, such as growth-temperature range, optimal pH, NaCl tolerance and growth of organic source. These tests were carried out as recommended by (Ventosa et al. 1982; Delgado-García et al. 2013).

Hydrolytic activities of the halophilic bacterial isolates

In order to detect the enzymatic production for halophilic bacteria, isolates were tested on agar plates. Amylase production was performed on starch media according to (Amoozegar et al. 2003), protease was applied to skim milk media according to (Amoozegar et al. 2008), lipase was carried out on Luria-Bertani media with Tween-80 according to (Martin et al. 2003), nuclease was determined by DNase media according to (Onishi et al. 1983), L-asparaginase was on modified M9 medium according to (Shirazian et al. 2016), phosphatase was on National Botanical Research Institute's-phosphate media according to (Nautiyal 1999), for chitinase was used to colloidal chitin according to (Shaikh and Deshpande 1993).

Antibiotic resistance profile

Isolates were exposed to antibiotic resistance screening by the disc diffusion method on Mueller-Hinton agar medium, supplemented with 10% NaCl for halophilic bacteria. The following antibiotics (Mast Group Ltd, Merseyside, U.K.) were used: Cephalothin (30 µg), Cotrimoxazole (25 µg), Imipenem (10 µg), Erythromycin (15 µg), Teicoplanin (30 µg), Ciprofloxacin (5 µg), Vancomycin (30 µg), Ampicillin (10 µg), Augmentin (30 µg), Cefoxitin (30 µg), Gentamicin (10 µg), Amikacin (30 µg), Cefepime (30 µg), Piperacillin (100 µg), Ticarcillin (75 µg), PenicillinG (10 units), Clindamycin (2 µg), Ceftazidime (30 µg), Aztreonam (30 µg) and Tobramycin (10 µg) (Andrews 2008).

Molecular and phylogenetic analysis of the isolated strains

DNA extraction of isolated samples was performed using (Key prep-Bacterial DNA Extraction Kit) (QIAamp® DNA Blood Mini Kit 50). The extracted DNA was used as a template for PCR to amplify 16S rRNA. The extraction method was performed according to the instructions of the manufacturers. 16S rRNA gene was amplified with the bacterial forward primer 27 F (5`-ATG GAG AGT TCG ATC CTG GC-3`) and reverse primer 1303 R (5`-TCC CTC ATT ACG AGC TTG TAC ACA-3`) (MACROGEN). Amplification of 16S rRNA was performed in a total volume of 25µl containing 2.0µL Genomic DNA, 12.5µL of 2x Green MasterMix (Go Taq®),1.0µL Forward Primer, 1.0µL Reverse Primer, and 8.5µl of sterile distilled water. The PCR reaction mixtures, after incubation at 95°C for 5 minutes as an initial

denaturation, were cycled 33 times through the following temperature profile: denaturation for 30sec at 95°C; annealing for 1 minute at 61°C; and elongation for 1 minutes at 70°C with final incubation for 7 minutes at 70°C, after which 10µL of PCR amplification analyzed by 1% agarose gel electrophoresis. In addition, 3µl of 100bp ladder loading was used to confirm the correct sized product. PureLink® Quick Gel Extraction Kit (Invitrogen™) 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 phylogenetic trees were reconstructed.

Statistical analysis

Statistical analyses were performed using the statistical package of a scientific data (SigmaPlot software, version 14.0). The triplicate data were represented as means and error bars showing standard errors of the means. Graphs were constructed and statistical analysis performed with GraphPad Prism (version 6.01f or Windows; GraphPad Software, La Jolla, CA, USA).

RESULTS AND DISCUSSION

Fifty-eight halophilic bacterial strains were isolated under aerobic conditions of seventeen samples taken from diverse high-saline environments varying between water, sediment and mud, i.e. from The Red Sea and Arabian Gulf-Kingdom of Saudi Arabia, which are saline and alkaline environments (pH 8.39-pH 8.35) and the Dead Sea in Jordan, which is a hyper-saline and acidic environment (pH 6.03) making them harsh environments even for microorganisms. During early summer May, twenty-four bacterial isolates were obtained from the Red Sea samples. In the same time, nine bacterial isolates were obtained from the Arabian Gulf samples. At the end of the summer September, twenty-five bacterial isolates were obtained from Dead Sea samples. For the specific isolation of halophilic bacteria, a range of media containing a range of NaCl was used. Saline nutrient medium (1M NaCl) yielded ten strains of halophilic bacteria. In this study, it was decided to fully characterize only four of the ten strains, since these were most suited to grow at high concentrations of NaCl.

Phenotypic characterization of halophilic bacteria

Morphological characters of the isolates SBR_1, SBR_2, SBA_9, and SBD_{17} were described after growth on saline nutrient agar medium within 24h and represented in Table 1. The colonies of SBR_1were purple-colored, opaque, surface of smooth, raised with a regular-circular edge, the colonies of SBR_2 had pale pink colored, opaque, surface of smooth, flat, with a regular-circular edge, the colonies of SBA_9 had yellowish-orange colored, opaque, surface of smooth, raised with a regular-circular edge, and the colonies of SBD_{17} had yellowish-orange colored, opaque, surface of smooth, flat, with a regular-circular edge. Examination of the isolates under light microscope showed that the cells of SBR_1 from overnight culture were Gram-negative, motile, none spore-forming, they have capsule and monobacilli, while cells of SBR_2 were Gram-positive, motile, none spore-forming, they have capsule and diplobacilli or monobacilli, SBA_9 cells were Gram-positive, motile, spore-forming, they have capsule and diplobacillior monobacilli, whereas cells of SBD_{17} were Gram-positive, motile, spore-forming, they have capsule and diplobacillior monobacilli.

A range of biochemical and physiological tests was carried out to identify the genus of isolates SBR_1, SBR_2, SBA_9 and SBD_{17}, the results are shown in Table 1.

Table 1. Summary of the morphological and the biochemical characteristics of SBR_1, SBR_2, SBA_9, and SBD_{17}

Morphological and biochemical characteristics	SBR_1	SBR_2	SBA_9	SBD_{17}
Source of isolate	Sediment (Red Sea)	Sediment (Red Sea)	Water (Arabian Gulf)	Mud (Dead Sea)
Form	Regular-Circular	Regular-Circular	Regular-Circular	Regular-Circular
Margin	Entire	Entire	Entire	Entire
Elevation	Raised	Flat	Raised	Flat
Surface	Smooth	Smooth	Smooth	Smooth
Color	Purple	Creamy	Yellowish orange	Yellowish orange
Opacity	Opaque	Opaque	Opaque	Opaque
Gram's reaction	Negative	Positive	Positive	Positive
Cell shape	Bacilli	Bacilli	Coccobacilli	Bacilli
Spore-forming	Negative	Negative	Positive	Positive
Capsule	Positive	Positive	Positive	Positive
Motility	Motile	Motile	Motile	Motile
Pigment production	Negative	Negative	Negative	Negative
Catalase	Positive	Positive	Positive	Negative
Oxidase	Negative	Negative	Negative	Negative
Indole production (IND)	Positive	Positive	Positive	Positive
Citrate(CIT)	Negative	Positive	Positive	Positive
Methyl red (MR)	Negative	Positive	Positive	Positive
Voges-Proskauer (VP)	Negative	Negative	Negative	Negative
Fermentation of Mannitol salt	Negative	Positive	Positive	Positive

Negative patterns of SBR_1 results were obtained in the oxidase, Voges-Proskauer, citrate, and methyl red tests, while it was positive for catalase and indole production. Negative results of SBR_2 and SBA_9 were obtained in the oxidase and Voges-Proskauer tests but they were positive for catalase, indole production, citrate, and methyl red. Negative responses of SBD_{17} were for catalase, oxidase, and Voges-Proskauer tests while positives were for indole production, citrate and methyl red.

As illustrated in Figure 1, the physiological characteristics for SBR_1, SBR_2, SBA_9 and SBD_{17} were showed that they grow under anaerobic conditions, the growth temperatures were SBR_1 = (4-40°C), SBR_2 = (25-45°C), SBA_9 = (25-40°C) and SBD_{17} = (35-45°C) The other clear difference between the strains was their ability to grow at different pH levels, SBR_1= (pH 7.0-9.0), SBR_2= (pH 5.5-7.5), SBA_9= (pH 6.0-8.0) and SBD_{17}= (pH 6.5-8.0) as clearly shown in Figure 2. Whereas the different NaCl concentration showed that SBR_1, SBR_2, SBA_9, and SBD_{17} are moderate halophiles (i.e., they have an absolute requirement for NaCl in the growth medium) (Figure 3), no significant effects were observed when the isolated strains were grown in the presence of 1M of NaCl concentration. The organic source gave the highest growth of strains with yeast extract compared to peptone or casein.

Enzymatic production of halophilic bacteria

The halophilic bacterial isolates SBR_1, SBR_2, SBA_9, and SBD_{17} were investigated for their ability to hydrolyze extracellular enzyme substrates on solid medium supplemented with 1M NaCl (Table 2). The SBR_1 strain produced lipase, L-asparaginase, and chitinase enzymes, while the SBR_2 strain produced proteinase and nuclease. While the two strains SBA_9 and SBD_{17} produced amylase, proteinase, lipase, and nuclease.

Determination of the resistance to antibiotics

Halophilic isolate tested for antibiotic susceptibility was shown to be resistant (Table 3).

Molecular and phylogenetic analysis of the isolated strain

PCR amplification of the 16S rRNA gene produced fragments of approximately 1500 base pairs in size (Table 4). The resulting 16S rRNA gene sequences from halophilic isolates were compared and the closest match was detected using BLAST program (NCBI). The highest sequence similarities for the halophilic isolates were as follows: Strain SBR_1 showed 97% similarity with *Halomonas aquamarina*, accession number EU684464.1; Strain SBR_2 showed 97% similarity with *Sediminibacillus* sp., accession number KM199865.1; Strain SBA_9 showed 94% similarity with *Halobacillus* sp, accession number FJ477402.1 while strain SBD_{17} showed 98% similarity with *Halobacillus dabanensis*, accession number KT008293.1. As clearly shown in Figure 4, the neighbor-joining phylogenetic tree based on 16S rRNA gene sequences confirms that halophilic isolates fell into three genera; *Halomonas*, *Sediminibacillus*, and *Halobacillus*.

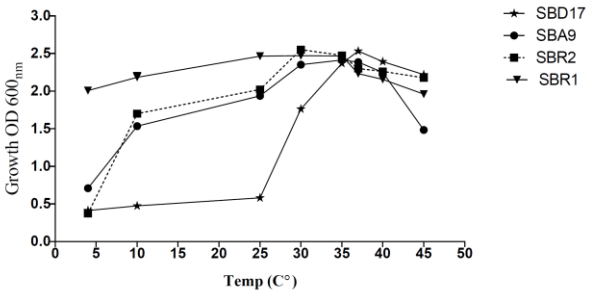

Figure 1. Effect of different temperatures on the growth of the isolated strains

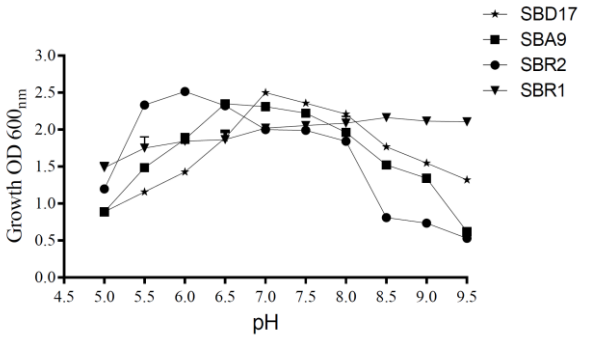

Figure 2. Effect of different pH values on the growth of the isolated strains

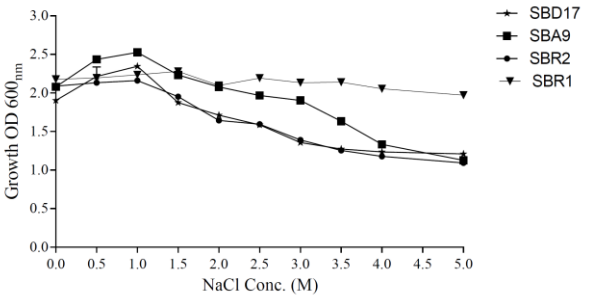

Figure 3. Effect of different NaCl Concentrations on the growth of the isolated strains

Table 2. Hydrolytic activities of the halophilic bacterial isolates

Enzyme	SBR1	SBR2	SBA9	SBD17
Hydrolysis of starch	-	-	+	+
Hydrolysis of skim milk	-	+	+	+
Hydrolysis of fat	+	-	+	+
Hydrolysis of DNA	-	+	+	+
Hydrolysis of L-asparagine	+	-	-	-
Hydrolysis of phosphate	-	-	-	-
Hydrolysis of chitin	+	-	-	-

Table 3. Antibiotics resistance pattern of the isolated strains

Isolated strains	Resistance pattern*
SBR_1	AP^{10}, AUG30, FOX30, GM^{10}, KF^{30}, TS^{25}, AK^{30}, CAZ^{30}, ATM^{30}, CIP^{5}, VA^{30}, PRL^{100}, IMI^{10}.
SBR_2	AP^{10}, AUG^{30}, FOX^{30}, GM^{10}, KF^{30}, TS^{25}, AK^{30}, CPM^{30}, TC^{75}, PG^{10}, E^{15}, CD^{2}, TEI^{30}, CAZ^{30}, ATM^{30}, CIP^{5}, TN^{10}, VA^{30}, PRL^{100}, IMI^{10}.
SBA_9	AP^{10}, AUG^{30}, FOX^{30}, GM^{10}, KF^{30}, TS^{25}, AK^{30}, CPM^{30}, TC^{75}, PG^{10}, E^{15}, CD^{2}, TEI^{30}, , CAZ^{30}, ATM^{30}, CIP^{5}, TN^{10}, VA^{30}, PRL^{100}, IMI^{10}.
SBD_{17}	AP^{10}, AUG^{30}, FOX^{30}, GM^{10}, KF^{30}, TS^{25}, AK^{30}, CPM^{30}, TC^{75}, PG^{10}, E^{15}, CD^{2}, TEI^{30}, CAZ^{30}, ATM^{30}, CIP^{5}, TN^{10}, VA^{30}, PRL^{100}, IMI^{10}

Note: * 10 µg Ampicillin (AP), 30 µg Augmentin (AUG), 30 µg Cefoxitin (FOX), 10 µg Gentamicin (GM), 30 µg Cephalothin (KF), 25 µg Cotrimoxazole (TS), 30 µg Amikacin (AK), 30 µg Cefepime (CPM), 75 µg Ticarcillin (TC), 10units PenicillinG (PG), 15 µg Erythromycin (E), 2 µg Clindamycin (CD), 30 µg Teicoplanin (TEI), 30 µg Ceftazidime (CAZ), 30 µg Aztreonam (ATM), 5 µg Ciprofloxacin (CIP), 10 µg Tobramycin (TN), 30 µg Vancomycin (VA), 100 µg Piperacillin (PRL), 10 µg Imipenem (IMI).

Table 4. Comparative analysis of 16S rRNA gene sequences of halophilic isolates from the Red Sea sediment, the Arabian Gulf water, the Dead Sea mud, using highly matched species available in BLASTN

Isolated strain	Sequence length	Highly matched bacteria accession no.	GenBank accession no.	Similarity (%)
SBR_1	1470	*Halomonas aquamarina*	EU684464.1	97%
SBR_2	1475	*Sediminibacillus* sp.	KM199865.1	97%
SBA_9	800	*Halobacillus* sp.	FJ477402.1	94%
SBD_{17}	1519	*Halobacillus dabanensis*	KT008293.1	98%

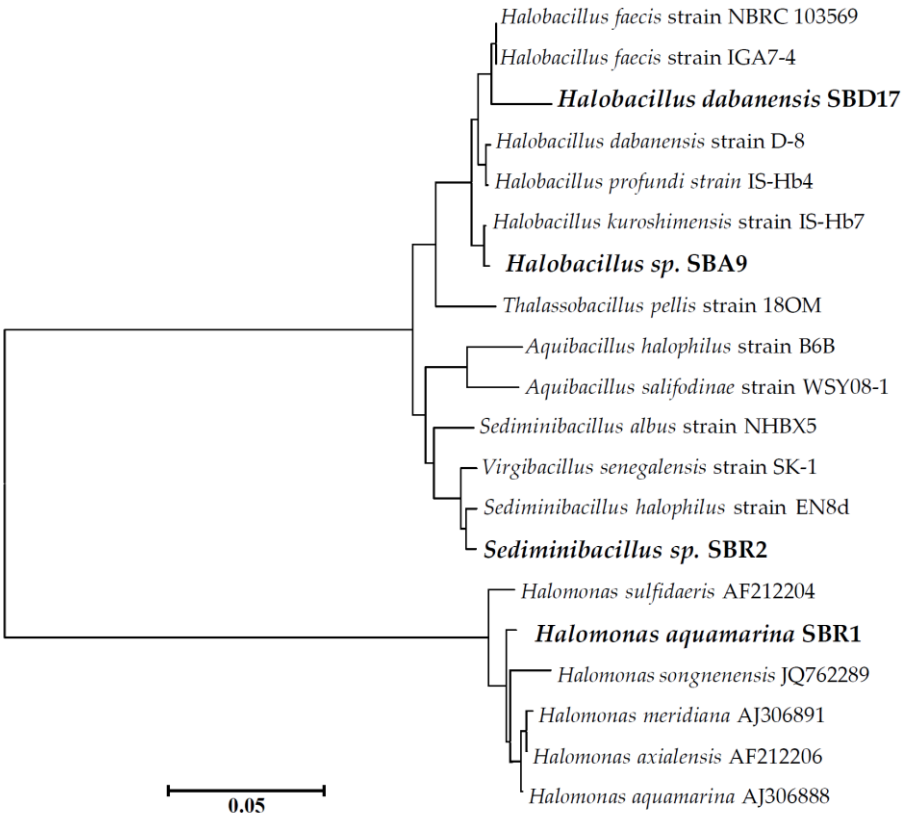

Figure 4. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the interrelationships of the isolated bacterial strains. The GenBank accession number of each strain is shown in parenthesis. The tree was generated using the neighbor-joining (NJ) method (Saitou and Nei 1987) contained in the MEGA6 software package (Tamura et al. 2013). Bootstrap values based on 500 replications are listed at nodes. Scale bar represents 0.05 substitutions per nucleotide position

Discussion

Traditional methods of bacterial cultivation have been widely used to investigate microbial diversity in a range of environments, including extreme habitats that might include potentially new bacterial species. It is well known that the microbial community represents a wide gene pool that can be explored for several beneficial applications, such as the production of biomolecules and bioremediation (Poli et al. 2017). Generally, halophilic microorganisms are organisms that grow optimally at high salt concentrations. For optimal growth, extreme halophiles require 2.5-5.2 M (15-30%) NaCl (Siglioccolo et al. 2011). This work aimed to investigate the microbial diversity discovered in the Red Sea region, the Arabian Gulf, Saudi Arabia and in Dead Sea region, Jordan. In the current study, focus was placed on identification and characterization of halophilic bacteria that they were isolated from sediment, mud and water samples. After initial characterization of the isolates showed them to be halophilic bacteria. Based on the reported results, the morphological and biochemical characteristics of halophilic bacterial isolates were displayed in Table 1. And the halophilic bacterial isolates at the different concentrations of NaCl was evaluated by growing in (SNB) at 37°C for determination of the growth range of salinities. Figure 3 showed the salinities range of isolates was at the moderate level (0.5-3.0 M NaCl). Beside the high salt tolerance capacity, that up to 3M, the growth capacity of halophilic bacterial isolates at different temperature, SBR_1(4-40°C), SBR_2 (25-45°C), SBA_9 (25-40°C) and SBD_{17} (30-45°C) and at pH values, SBR_1 (7.0-9.0), SBR_2 (5.5-7.5), SBA_9 (6.0-8.0) SBD_{17} (6.5-8.0) were demonstrated in the results (Figures 1 and 2), suggesting that the isolates obtained might be tolerant for growth not only with salt but with a wide range of temperature and pH values. the ability of halophilic isolates for producing some enzymes like amylase, protease, lipase, DNase, L-asparaginase and chitinase is shown in Table 2.

In addition, the results reveal that halophilic bacterial isolates tested for antibiotic susceptibility had multiple antibiotic resistances (Table 3). In order to identify the strains, molecular methods were used. In the current study, four moderately halophilic bacterial isolates were identified using 16S rRNA analysis, which is acknowledged as the method of choice for identifying novel isolates to the genus and particularly species level. The results were compared with those described in a range of identification schemes and the literature in general (Hotl et al. 1994; Amoozegar et al. 2003; Liu et al. 2005; Tamegai et al. 2006; Wang et al. 2009; Guzmán et al. 2010). The halotolerant bacteria, *Halobacillus salinus* isolated from a salt lake in Korea can, for example, grow at 23% NaCl (Echigo et al. 2005). In the last decade, a few studies have been demonstrated the ability of halophiles to grow in non-saline habitats. Usami et al. (2007) for example, have isolated two strains of halophilic bacteria from non-saline soil in Japan, designated $BM2^T$ and HN2. The cells of strain $BM2^T$ were found to be Gram-positive, rod-shaped, aerobic, and motile. Growth occurred at 5-25% (w/v) NaCl, with optimal growth occurring at 10-15% (w/v) NaCl at 20-50 °C and pH of 7-10. 16S rRNA gene sequencing, used to analyze the phylogeny of strain $BM2^T$showed 98% sequence similarity to *Alkalibacillus haloalkaliphilus* $DSM5271^T$ (Al'Abri 2011).

In an earlier study, conducted by Savage et al. (2008), the occurrence of novel halophilic archaeon, has been reported in the Zodletone Spring in south-western Oklahoma, USA, this being characterized by its low-salt, as well as rich-sulfide content. A novel halophilic archaeon of strain $BZ256^T$ was isolated, and the cells were found to occur as non-flagellated, non-motile, cocci, and forming *Sarcina*-like clusters. They grew at a 1.3-4.3 M salt concentration and with optimum growth at almost 3.5M NaCl and required at least 1 mM Mg^{+2}, a pH range of 5.0-8.5 and a temperature range of 25-45°C. The 16S rRNA gene sequence of strain $BZ256^T$ revealed that it was related to *Halogeometricum borinquense*. Strain $BZ256^T$ stands for a member of a novel genus and species within the family Halobacteriaceae, proposed as *Halosarcina pallida* gen. nov, sp.nov. (Savage et al. 2008). Phylogenetic analyses are derived from the comparison of the nucleotide sequences unknown with already identified ones, found in GenBank, that are accessible worldwide (Clarridge 2004). Phylogenetic analysis is represented using phylogenetic tree construction (Brinkman and Leipe 2001). In this study, the sequences data were used to produce a phylogenetic tree providing the basis for efficient phylogenetic investigation of each genus. Figure 4 is an example of phylogenetic analysis of *Halomonas aquamarina* strain, *Sediminibacillus* sp. strain, *Halobacillus* sp. strain, and *Halobacillus dabanensis* strain. Phylogenetic analysis of SBR_1, SBR_2, SBA_9, SBD_{17} by using the BLASTN algorithm at NCB1 indicated that the halophilic bacterial isolate SBR_1 belongs to the genus *Halomonas* and is closely similar to *Halomonas aquamarina* (i.e., the halophilic bacterium obtained here from the Red Sea sediment is *Halomonas aquamarina*). SBR_2, from the Red Sea sediment, belongs to the genus *Sediminibacillus* sp, while SBA_9 was shown to be belonging to the genus, hence, the halophilic bacterium obtained from Arabian Gulf water in the present study could be identified as *Halobacillus* sp. Finally, SBD_{17} isolated from Dead Sea mud, was shown to belong to the genus *Halobacillus* and closely similar to *Halobacillus dabanensis*.

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