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The diversity and efficiency of sulfate-reducing bacteria in selected groundwater at West Bank, Palestine

Mays Nofal[a], Nasser J.Y. Sholi[b], Amer Marei[c], Saed Khayat[b,*]

[a]*Faculty of Graduate Studies, Palestine Technical University, Tulkarm, Palestine, email: mais_nofal@hotmail.com*
[b]*Faculty of Applied Science, Palestine Technical University – Kadoorie (PTUK), P.O. Box: 7, Yafa Street, Tulkarm, Palestine, emails: saed.khayat@gmail.com (S. Khayat), n.sholi@ptuk.edu.ps (N.J.Y. Sholi)*
[c]*Al Quds University, Jerusalem, West Bank, email: marei.amer@gmail.com*

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ABSTRACT

Sulfate-reducing bacteria (SRB) are unique groups of bacteria that can reduce sulfate to sulfide. They are considered an essential member of microbial communities and potentially be used in reducing the high concentration of sulfate in water or soil. So, this study aimed to isolate SRB species that survive and grow under severe saline conditions and test their efficiency in SO_4 reduction under lab conditions. Bacteria isolates were isolated from 7 water samples and two soil samples above Lisan white soil using a culture-dependent enrichment method. Molecular identification was carried out by amplification and sequencing of *dsrAB* gene from isolated instances. Depending on the sequence of *dsrAB* gene, five genotypes of bacteria with sulfate reduction ability were solitary and found. They belong to both traditional (*Desulfovibrio*) and non-traditional (*Enterobacter cloacae*, *Citrobacter werkmanii*, *Citrobacter freundii*, *Alcanivorax xenomutans*, *Pseudomonas aeruginosa*) SRB. In non-traditional SRB, the *dsrAB* gene was found to be active. All bacterial isolates were found to have a good ability in sulfate reduction, which reached up to 58% for *Desulfovibrio* while it was 45% and 41% for *Citrobacter* and *Enterobacter cloacae*, respectively. These results have shown the future potential of using these isolates to reduce sulfate in groundwater.

Keywords: Sulfate-reducing bacteria; *dsrAB* gene; Jericho

1. Introduction

Sulfate-reducing bacteria (SRB) are anaerobic bacteria that use hydrogen rather than oxygen in their metabolic activities [1]. They use sulfate (SO_4^{2-}) as a terminal electron acceptor in the degradation of organic compounds and generate hydrogen sulfide (H_2S) as a final product [2]. According to that, SRB is considered an essential member of microbial communities, which can be used to remove or reduce the high concentrations of sulfate in water or soil [3].

SRB plays a significant role in bioremediation. For instance, they can reduce and accumulate heavy metals in groundwater [3,4] and oil degradation [5]. Their essential role in the biogeochemical sulfur cycle makes it worthwhile to develop a simple, low cost and eco-friendly technique to reduce the salinity of groundwater by reducing SO_4^{2-} concentration. Specifically in Palestine, the Jericho area is suffering from degradation in groundwater quality due to high salinity levels [6]. The chloride and sulfate ratio in Arab project wells reach around 1,000–2,000 mg/L and 200–300 mg/L, respectively [7]. That means the salinity hazard is high in Jericho wells, and water needs treatment.

Additionally, Khayat et al. [8] and Sabieh [9] showed that high salinity in eastern Arab project wells is mainly due to the geological formations of the wells, which contain layers of both the Samara and Lisan formations of the

* Corresponding author.

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Pleistocene age. Gypsum of Lisan formation is considered the primary source of sulfate. Furthermore, it is owing to the rate of pumping, which is high and continuous throughout the year. Most of the groundwater samples were taken from shallow wells with aerobic environment, the effect of ascending deep brine that assumed to hold the SRB species is mixed during pumping process with the upper shallow layer where the corrected Redox potential value ranging between 300–500 mV. The dissolved organic carbon does not exceed 1.5 mg/L [10,11]. Both parameters indicate low bacterial activities with less competition that promote other rare extremophiles or nutrient selective bacteria to grow and functioning actively.

Moreover, SRB performs a valuable ecological role in the environment [12]. Since the SRBs are anaerobic bacteria that can degrade organic matter using sulfate as a terminal electron acceptor [13]. *Desulfovibrio* species can remove around 30% sulfate in wastewater Jing et al. [14]. Participating in H_2S production in swine wastes Karnachuk et al. [15]. While other species like *Citrobacter freundii* can reduce 12 mM of sulfate within 7 d under anaerobic conditions isolated from the sludge of a paper mill located in Tianjin, China, Zhou et al. [16].

Pseudomonas aeruginosa can play a role in the bioremediation of the environment. In reference studies [17,18], the authors shown that *P. aeruginosa*, which was isolated from a soil sample, could degrade 96% of 1,500 mg sodium dodecyl sulfate, molecular identification of traditional and non-traditional SRB was based on 16S rRNA and *dsrAB* gene in many studies. For example, *Citrobacter freundii* Zhang et al. [19]. *Desulfosarcina* and *Desulfobacterium* species within *Desulfobacteraceae* [20]. *Desulfohalobiaceae* and *Desulfobacteraceae* [21]. *dsrAB* gene was also used in identification and classification of SRB bacteria as *Desulfococcus* [22] and *Desulfobulbaceae* [23].

Therefore, understanding the biological diversity of SRB is critical for us to use it in a sustainable manner and to benefit from its metabolic activity, as some of them can degrade environmental pollutants.

In Palestine, the diversity of SRB in a hypersaline environment is not well known. Only a few studies have shown the existence of SRB in the West Bank. Additionally, most of these studies did not characterize or sequence the bacterial strain, and none tried to test the efficiency of isolated SRB in sulfate reduction. This study aimed to isolate SRB species that are well adapted to survive and grow under severe conditions (severe high saline conditions) and test their efficiency in SO_4 reduction.

This study will be the first step toward establishing an advance, cost-effective and suitable method for groundwater treatment and decreasing the salinity concentration, mainly the high sulfate ratio in water using SRB rather than the expensive existing desalination techniques.

2. Materials and methods

2.1. Sample collection

Seven water samples were collected from seven wells (Olive, Makatib, Hamra, Seder, Da'bes, Mazra'h, Awael wells) in the Arab Construction Project Association in Jericho city, Palestine. Moreover, two soil samples were taken from above Lisan white soil. The date of sampling was the 15th of August 2020. For each instance, the pH, electrical conductivity (EC), and dissolved oxygen (DO) were measured at the biotechnology lab at Palestine Technical University.

2.2. Isolation procedures

The culture medium used to grow the sulfate-reducing bacteria was the Postgate C Media, prepared according to Cortás et al. [24]. Solid and semisolid media contained 0.5 g potassium phosphate, 1.0g ammonium chloride, 4.5 g sodium sulfate, 0.04 g calcium chloride, 0.06 g magnesium chloride, 9.4 mL sodium lactate (50% m/v), 1.0 g yeast extract, 0.1 g ascorbic acid, 0.04 g ferrous sulfate, 35 g sodium chloride with 15 and 1.9 g agar for each media respectively in 1 L of distilled water. While liquid media prepared with 0.5 g potassium phosphate, 1.0 g ammonium chloride, 4.5 g sodium sulfate, 0.04 g calcium chloride, 0.06 g magnesium sulfate, 6.0 mL sodium lactate (50% m/v), 1.0 g yeast extract, 0.04 g ferrous sulfate and 0.3 g sodium citrate in 1 L of distilled water [24,25]. The pH of the media was adjusted to 7.5 then the media autoclaved at 121°C for 15 min.

For soil samples, 200 g of sieved Lisan soil was mixed with 3–5 g of calcium sulfate in a bowl. The filter paper was torn into small pieces and combined with the soil. Distilled water was added to the soil mixture until it reached a cream-like consistency.

The enriched soil was applied to the bottom of a measuring cylinder in a thickness of about 2–5 cm. Then the column was filled uniformly with mud at the height of about 15–25 cm, and after 24 h, about 0.5 cm layer of water was added to cover the mud; then, the top of the measuring cylinder was closed with plastic film. The column was placed for about a month at room temperature.

For the first culturing step, 150 mL of water was taken from each water sample and centrifuged at 6,000 rpm for 5 min in a 15 mL falcon tube. Then, the pellet for water samples was taken, inoculated on Medium C, and incubated at 37°C in anaerobic conditions.

A unique bag called (An aero GenTM2.5L) was used for the anaerobic condition for more than 2 d. Furthermore, 50 mL of water were filtered in sterile filter paper (pore size 0.2 μm, diam. 47 mm). "Whatman membrane filters mixed cellulose ester". The whole filter paper and filter papers isolated from the soil sample were inoculated on medium C in Petri dishes and kept as a previous condition.

After 4 to 6 d of culturing on selective media (Postgate C semisolid and liquid media), bacterial growth was detected for the first time. Generally, the biological activity of bacteria and the production of sulfides were detected by changing the color from pink to colorless and forming black precipitates. Sub-culturing was done on weekly bases during the experiment. Moreover, morphological identification based on gram stain and microscopic examination is also made.

2.3. DNA extraction and PCR amplification

The genomic DNA was extracted from a single colony of bacteria using the GenElute bacterial genomic DNA kit. The *dsrAB* gene was amplified using universal primer

DSR1-F (5′-ACSCACTGGAAGCACG) and DSR4-R(5′-GTGTAGCAGTTACCGCA) [26]. Each PCR contained 30 ng of template DNA and 12 pmol of both primers and the amplification conditions were 95°C for 7 min, 30 cycles of 95°C for 1 min, 50°C for 30 s, and 72°C for 30 s. and final extension step of 72°C for 7 min. Subsequently, aliquots of 10 ul of the reactions t were analysed by 2% agar-gel electrophoresis and visualized using a UV Trans-illuminator.

2.4. Sequence analysis and phylogenetic analyses

The bands were excised from the gel and then purified using a QIAquick gel extraction kit to be ready for the sequencing. Moreover, a Nanodrop spectrophotometer was used to quantify and assess the purity of DNA and sequenced using Sanger's sequencing method (model 3730 DNA Analyzer Thermo Scientific Fisher).

The sequences will be compared with sequences stored in Gen-Bank using the BLAST (basic local alignment search tool) algorithm for species identification. Consequently, a phylogenetic tree will be constructed using the neighbor-joining algorithms with the Molecular Evolutionary Genetics Analysis software (MEGA X).

2.5. Sulfate measurement

Whitly Jar Gassing System has been used to create an anaerobic condition to measure the efficiency of SRB isolates in sulfate reduction. 20 mL of bacterial culture (specific species) with a concentration 4.0×10^8 CFU/mL mixed with Postgate C liquid media and sulfate (200 mg/L). The volume was brought to 250 mL with autoclaved water; the pH was adjusted to 7.5. Postgate C media with the same sulfate concentration have been used for control. The sulfate concentrations were measured every 2 h using HACH advanced spectrophotometer and a specific sulfate kit (SulfaVer® 4 sulfate reagent powder pillows) at OD_{450} for all samples.

3. Results and discussion

3.1. Sulfide production

SRB represent themselves as a class of anaerobic bacteria that can reduce sulfate to sulfide for obtaining energy. Notably, the detection of SRB stains in our samples was reported based on the ability of this bacterium to reduce sulfate concentration and production of H_2S.

A few requirements must be achieved to determine the maximum growth of reducing bacteria. Firstly, the carbon source must be suitable for growth. Secondly, isolation of sulfate-reducing bacteria needs selective growth media. The commonly used media for the SRB growth is Postgate C Media. Postgate C Media was prepared according to Cortás et al. [24] study. The chemical composition in this media is highly recommended for the cultivation of SRB. Moreover, the biological activity of bacteria and the production of sulphide were observed by changing in media color from pink to colorless and by formation of black precipitates especially, in the column of the soil sample and this due to presence of S^{2-} in the grown colonies and Fe^{2+} in the medium.

3.2. Amplification of dsrAB gene

The superb method to detect SRB and provide information about their metabolic capabilities is functional genes, which encode enzymes that play an essential part in the sulfate reduction pathway. Notably, *dsrAB* gene encodes α and β subunits of an enzyme that catalysis the six-electron reduction step of sulfite to sulfide [27]. *dsrAB* marker gene was successfully amplified in both soil and water-tested samples. The survival; of these isolates in a specific medium, color change, and presence of precipitate were good indicators that these SRB reduce sulfate through dissimilatory pathways.

The PCR results of amplification of *dsrAB* gene are using a specific primer which demonstrated by Wagner et al. [26], shows a presence of a highly diverse bacterial population (Fig. 1). Furthermore, the dominant band observed in most water and soil samples were corresponding to *Enterobacter cloacae*. Interestingly, the bands were at a different intensity than band number 8, which was found more intense, suggesting that this bacterium was metabolically active.

3.3. Diversity of SRB based on dsrAB

The composition of SRB communities isolated from water and soil samples collected from Jericho city was determined by the DNA sequencing of the *dsrAB* gene. All thirteen sequences were submitted to GenBank, NCBI (National Center for Biotechnology Information). Moreover, the sequences were compared with sequences stored in GenBank using the BLAST (primary local alignment search tool) algorithm. Their accession numbers, systematic position, and percentage of identity are presented in Table 1.

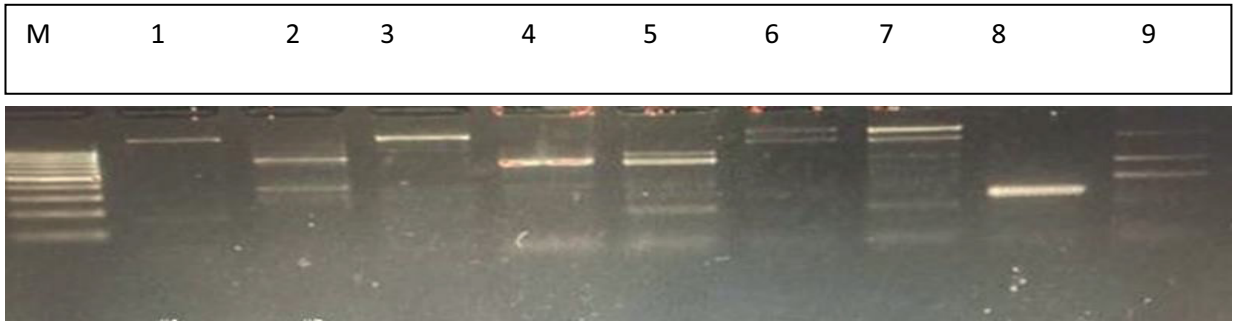

Fig. 1. PCR amplification of *dsrAB* gene using DSR1-F, DSR4-R primers; M, DNA marker, lane 1–9 represent test samples.

The results show members of SRB belong to classes *Gammaproteobacteria* and *Deltaproteobacteria*. Furthermore, the type *Gammaproteobacteria* had eight isolates belonging to the *Enterobacterales* order and the four belonging to *Pseudomonadales* and *Oceanospirillales* (Fig. 2).

Based on the sequence obtained, a neighbor-joining phylogenetic tree was constructed to visualize the relationship among the isolated strains (Fig. 2). The tree contains two major clades: one consisting of members of SRB belonging to class *Deltaproteobacteria* and *Gammaproteobacteria*.

Table 1
Systematic positions of the isolated SRB from water and soil samples

DNA samples/ bands num.	Phylum	Class	Order	Genus	Species	Gene bank accession no.	Per. ident.
1	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacter*	*Enterobacter cloacae*	CP046116.1	91.80%
2	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacter*	*Enterobacter cloacae*	CP046116.1	92.59%
3	*Proteobacteria*	*Deltaproteobacteria*	*Desulfovibrionales*	*Desulfovibrionaceae*	*Desulfovibrio*	CP023415.1	92.69%
4	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacter*	*Enterobacter cloacae*	CP046116.1	93.28%
5	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacter*	*Enterobacter cloacae*	CP046116.1	88.14%
6	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Citrobacter*	*Citrobacter freundii*	AP022378.1	97.30%
7	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Citrobacter*	*Citrobacter werkmanii*	CP044101.1	85.48%
8	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacter*	*Enterobacter cloacae*	CP046116.1	93.46%
9	*Proteobacteria*	*Gammaproteobacteria*	*Oceanospirillales*	*Alcanivoracaceae*	*Alcanivorax*	CP012331.1	94.89%
10	*Proteobacteria*	*Gammaproteobacteria*	*Oceanospirillales*	*Alcanivoracaceae*	*Alcanivorax*	CP012331.1	91.95%
11	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas aeruginosa*	CP034908.2	91.84%
12	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacter*	*Enterobacter cloacae*	CP046116.1	95.00%
13	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas aeruginosa*	CP034908.2	90.04%

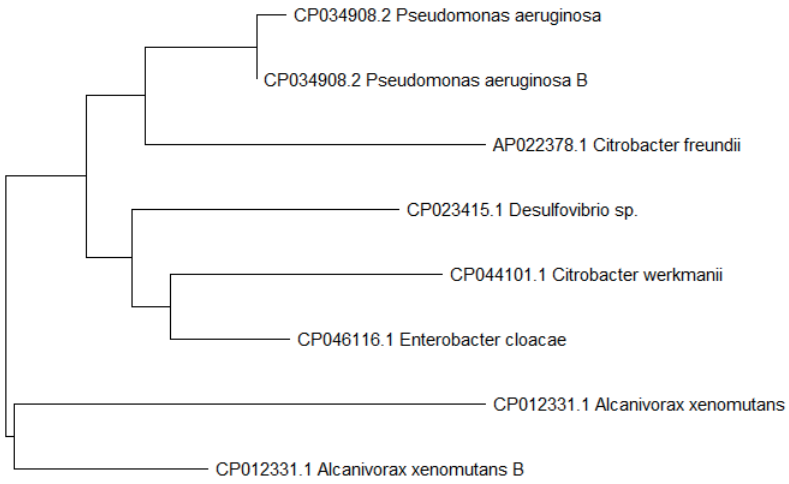

Fig. 2. Phylogenetic tree based on *dsrAB* gene sequences of SRB species using (MEGA X).

The other consists of bacteria belonging to the class *Gammaproteobacteria*, mainly, *Alcanivorax*, which has the most significant genetic distance.

This study showed a high diversity of traditional and non-traditional SRB with a high degree of similarity to different species. The class *Gammaproteobacteria* had eight isolates belonging to the *Enterobacterales* order, and six showed a high degree of similarity (91%–95%) to *Enterobacter cloacae*. However, two isolates belonged to *Citrobacter*, *Citrobacter freundii* and *Citrobacter werkmanii*. In this study, we isolated *Citrobacter freundii* and *Citrobacter werkmanii* species from water samples with a similarity up to 97.3% and 85.48%, respectively.

Interestingly, the low similarity between bacterial species may be related to evolution under extreme conditions and positive selection responsible for nucleotide variation. Also, microorganisms are exposed to develop numerous special adaptations to survive under harsh conditions [28]. Additionally, the evolution of survival strategy may provide an adaptive allele with some improved function, leading to increased fitness of the organism under extreme environments [29].

Adaptation to environmental changes, microorganisms can sense and translate extracellular stimuli into specific cellular signals resulting in altered gene expression and protein activities. The transformation may lead to the expression of a previously unexpressed gene or the activation of an active expression gene [30] as in this study *dsrAB* gene was involved in *Citrobacter*. Similar results were reported by Yang et al. [31], who isolated a sulfate-reducing *Citrobacter*, which contains a dissimilatory sulfite reductase gene (*dsr*). On the contrary, the *Citrobacter* strains isolated by Zarasvand and Rai [32] lacked *dsrAB* gene, indicating that *Citrobacter* species may have some other strategies for sulfate reduction.

Adaptation can allow the prokaryotes to function better at high salt and low oxygen level. Also, the organism can access valuable new resources, such as the non-traditional SRB that uses hydrogen rather than oxygen in its metabolic activities and sulfate (SO_4^{2-}) as a terminal electron acceptor in the degradation of organic compounds.

P. aeruginosa is widely found in soil and water. In this study, *Pseudomonas* was isolated from the water sample. Furthermore, *P. aeruginosa* is well adapted to grow in anaerobic environments in the presence of nitrogen oxides [17]. *P. aeruginosa* is characterized by a high level of metabolic adaptability. Moreover, this type of bacteria responds flexibly to any environmental change, such as limitation in specific nutrients. This is due to a high number of genes which encode transcription regulators and transport systems to uptake organic compounds into the cell [33]. Tralau et al. [33] tested the growth rate of *P. aeruginosa* with different sulfur sources and found that it grow faster when sulfate was the source of sulfur compared with other sources of sulfur like cyclamate. Also, a small pulse of growth was observed when sulfate in the media was exhausted.

Two isolates showed 91%–94% similarity with *Alcanivoracaceae Alcanivorax*. This genus is an essential member of the hydrocarbonoclastic clade. *Alcanivorax* using hydrocarbon substrates such as alkanes and a variety of alkyl-benzene derivatives are compounds as a source of carbon and energy [34]. Zadjelovic et al. [34] did genomic characterization of *Alcanivorax*, which was isolated from marine plastic debris, and showed that this type of bacteria has different enzymes that could play an important role in biodegradation processes.

A member of traditional SRB (*Desulfovibrio*) belongs to the class *Deltaproteobacteria* isolated from Lisan white soil. *Desulfovibrio* is an anaerobic cell with high oxygen tolerance. This may explain its presence in soil samples.

The most frequent group among isolated SRB was *Enterobacter cloacae*, found in both water and soil samples. This result can be explained by the easy and rapid growth of *Enterobacter*, which excludes other SRBs while competing for the same substrate. Furthermore, a previous study by Mpongwana et al. [35] isolated *Enterobacter* sp. that can do their metabolic functions at high salinity conditions up to 4% (w/v) NaCl. The chloride and sulfate concentrations in our study area reach 1,000–2,000 mg/L and 200–300 mg/L, respectively [7]. This means that our isolates can reduce sulfate under high saline conditions. Additionally, isolating bacteria from non-traditional SRB with sulfate reduction capability shows that SRB can still be expanded.

3.4. Sulfate reduction

Investigation of the isolated SRB can reduce sulfate; changes in sulfate concentration in the three samples prepared as mentioned before containing three strains of isolated SRB (A: *Desulfovibrio*, B: *Citrobacter*, C: *Enterobacter*) under anaerobic conditions are measured every 2 h for all samples including the control. Fig. 3 shows the amount of sulfate reduced by each isolate. For *Desulfovibrio*, sulfate concentration gradually decreased from 66 to 28 mg/L during the experiment's 10–48 h. While *Citrobacter* sulfate concentration progressively reduced from 64 to 34 mg/L during the 18 to 48 h of the investigation. Also, *Enterobacter cloacae* sulfate concentration dropped from 65 to 38 mg/L within 48 h.

The highest efficiency of sulfate elimination during the experiment was for *Desulfovibrio*, at around 58%. It is higher than the efficiency of non-traditional SRB *(Citrobacter* and *Enterobacter*) isolate*d fro*m the study area, which reached approximately 45% and 41%, respectively.

Different genera of *Enterobacteriaceae* can reduce sulfate, the major one is *Citrobacter*, and in this study, we isolated two species of it. Additionally, *Enterobacter cloacae* had shown an excellent capacity to reduce sulfate, which was 41%. Few studies discussed the role and the importance of lowering sulfate in *Enterobacter cloacae* metabolic activity. Also, neither of them tried to test the efficiency of *Enterobacter* in sulfate reduction.

Few studies have shown the existence of SRB in the West Bank. Significantly lower Jordan Valley. Also, the diversity of SRB is not well known. In this study, we isolated six types of bacteria from water and soil samples using the cultural method. Furthermore, through PCR, we checked the presence of *dsrAB* gene in the isolated bacteria to confirm that they reduce sulfate through the dissimilatory pathway. The *dsrAB* gene was presented in all of them. Importantly, this study has shown a presence of *dsrAB* gene in a non-traditional SRB as *Citrobacter freundii*, *Citrobacter werkmanii*, *Alcanivorax*, and *P. aeruginosa*, and this gene may

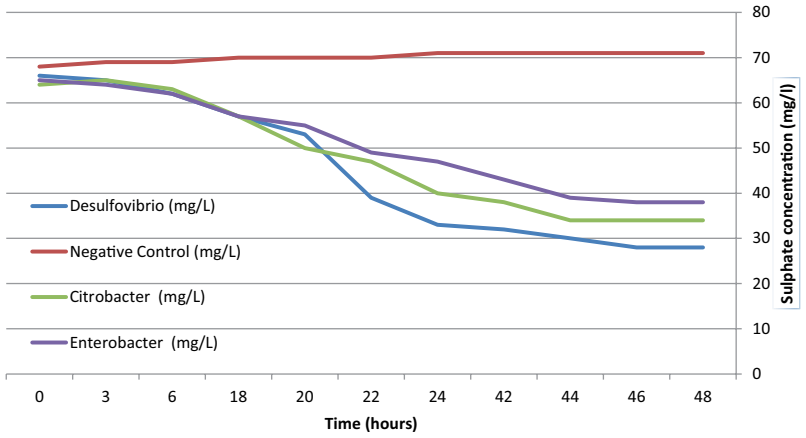

Fig. 3. Sulfate reduction efficiency for *Desulfovibrio*, *Citrobacter*, and *Enterobacter*.

be active in the isolates as a result of adaptation under severe conditions [36].

The isolated bacteria from Arab project wells can live under a high saline environment (1%–2% (w/v) NaCl) and high sulfate concentration (200–300 mg/L). The results reflected an excellent efficiency for both traditional and non-traditional SRB for sulfate reduction. The efficiency for *Desulfovibrio*, *Citrobacter*, and *Enterobacter cloacae* reached around 58%, 45%, and 41% for each one, respectively. Notably, the remaining isolated bacteria *P. aeruginosa* and *Alcanivorax* were tested, but no change in sulfate concentration was observed.

4. Conclusion

The application of molecular biological methods to investigate the distribution of bacteria in a unique area like Jericho has the advantage of providing direct information on community structure. This study showed a high diversity of traditional and non-traditional SRB with an excellent capacity to reduce sulfate concentration. Moreover, all isolated strains adapted to survive under severe high saline conditions. SRB can be applied beneficially to reduce groundwater salinity by reducing SO_4^{2-} and this is considered a safe, simple, economical alternative to using any desalination techniques, either thermal or membrane technologies that need the energy to operate and produce fresh water [37].

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References

[1] D.R. Cullimore, Standard Methods for the Application of BART Testers in Environmental Investigations of Microbiological Activities, Droycon Bioconcepts Inc., Canada, 2013.
[2] K. Daly, R.J. Sharp, A.J. McCarthy, Development of oligonucleotide probes and PCR primers for detecting phylogenetic subgroups of sulfate-reducing bacteria, Microbiology (Reading), 146 (2000) 1693–1705.
[3] Y.J. Chang, A.D. Peacock, P.E. Long, J.R. Stephen, J.P. McKinley, S.J. Macnaughton, A.K. Hussain, A.M. Saxton, D.C. White, Diversity and characterization of sulfate-reducing bacteria in groundwater at a uranium mill tailings site, Appl. Environ. Microbiol., 67 (2001) 3149–3160.
[4] G. Muyzer, A.J.M. Stams, The ecology and biotechnology of sulfate-reducing bacteria, Nat. Rev. Microbiol., 6 (2008) 441–454.
[5] J. Guan, L.-P. Xia, L.-Y. Wang, J.-F. Liu, J.-D. Gu, B.-Z. Mu, Diversity and distribution of sulfate-reducing bacteria in four petroleum reservoirs detected by using 16S rRNA and *dsrAB* genes, Int. Biodeterior. Biodegrad., 76 (2013) 58–66.
[6] S. Khayat, H. Hötzl, S. Geyer, W. Ali, Hydrochemical investigation of water from the Pleistocene wells and springs, Jericho area, Palestine, Hydrogeol. J., 14 (2006) 192–202.
[7] A. Marie, A. Vengosh, Sources of salinity in ground water from Jericho area, Jordan Valley, Groundwater, 39 (2001) 240–248.
[8] S. Khayat, H. Hotzl, S. Geyer, W. Ali, Hydrochemical investigation of water from the Pleistocene wells and springs, Jericho area, Palestine, Hydrogeol. J., 14 (2006) 192–202.
[9] M. Sabieh, Ice Age and Groundwater System Jericho Region, AL-Quds University, 2009 (In Arabic).
[10] S. Khayat, S. Geyer, A. Marei, Tracing the Inorganic Carbon System in the Groundwater From the Lower Jordan Valley Basin (Jericho/Palestine), P. Birkle, Ed., In the Water Rock Interaction XIII (ED.), Taylor and Francis Group, 2010, p. 1008.
[11] S. Khayat, M. Ghanem, A. Tamimi, M. Haddad, Hydrochemistry and Isotope Hydrogeology in the Jericho Area/Palestine, The Water of the Jordan Valley, Springer, Berlin Heidelberg, 2009, pp. 325–348.
[12] K.U. Kjeldsen, A. Loy, T.F. Jakobsen, T.R. Thomsen, M. Wagner, K. Ingvorsen, Diversity of sulfate-reducing bacteria from an extreme hypersaline sediment, Great Salt Lake (Utah), FEMS Microbiol. Ecol., 60 (2007) 287–298.
[13] S. Dar, Diversity and Activity of Sulfate-Reducing Bacteria in Sulfidogenic Wastewater Treatment Reactors, 2007.
[14] Z. Jing, Y. Hu, Q. Niu, Y. Liu, Y.-Y. Li, X.C. Wang, UASB performance and electron competition between methane-producing archaea and sulfate-reducing bacteria in treating sulfate-rich wastewater containing ethanol and acetate, Bioresour. Technol., 137 (2013) 349–357.
[15] O.V. Karnachuk, I.I. Rusanov, I.A. Panova, M.A. Grigoriev, V.S. Zyusman, E.A. Latygolets, M.K. Kadyrbaev, E.V. Gruzdev, A.V. Beletsky, A.V. Mardanov, N.V. Pimenov, N.V. Ravin, Microbial sulfate reduction by *Desulfovibrio* is an important source of hydrogen sulfide from a large swine finishing facility, Sci. Rep., 11 (2021) 10720, doi: 10.1038/s41598-021-90256-w.

[16] W. Zhou, M. Yang, Z. Song, J. Xing, Enhanced sulfate reduction by *Citrobacter* sp. coated with Fe_3O_4/SiO_2 magnetic nanoparticles, Biotechnol. Bioprocess Eng., 20 (2015) 117–123.
[17] M.J. Filiatrault, K.F. Picardo, H. Ngai, L. Passador, B.H. Iglewski, Identification of *Pseudomonas aeruginosa* genes involved in virulence and anaerobic growth, Infect. Immun., 74 (2006) 4237–4245.
[18] P. Ambily, M. Jisha, Biodegradation of anionic surfactant, sodium dodecyl sulfate by *Pseudomonas aeruginosa* MTCC 10311, J. Environ. Biol., 33 (2012) 717–720.
[19] H. Zhang, M. Li, Z. Yang, Y. Sun, J. Yan, D. Chen, Y. Chen, Isolation of a non-traditional sulfate reducing-bacteria *Citrobacter freundii* sp. and bioremoval of thallium and sulfate, Ecol. Eng., 102 (2017) 397–403.
[20] A. Gittel, M. Mussmann, H. Sass, H. Cypionka, M. Könneke, Identity and abundance of active sulfate-reducing bacteria in deep tidal flat sediments determined by directed cultivation and CARD-FISH analysis, Environ. Microbiol., 10 (2008) 2645–2658.
[21] K.U. Kjeldsen, A. Loy, T.F. Jakobsen, T.R. Thomsen, M. Wagner, K. Ingvorsen, Diversity of sulfate-reducing bacteria from an extreme hypersaline sediment, Great Salt Lake (Utah), FEMS Microbiol. Ecol., 60 (2007) 287–298.
[22] J. Leloup, H. Fossing, K. Kohls, L. Holmkvist, C. Borowski, B.B. Jørgensen, sulfate-reducing bacteria in marine sediment (Aarhus Bay, Denmark): abundance and diversity related to geochemical zonation, Environ. Microbiol., 11 (2009) 1278–1291.
[23] M. Bahr, B.C. Crump, V. Klepac-Ceraj, A. Teske, M.L. Sogin, J.E. Hobbie, Molecular characterization of sulfate-reducing bacteria in a New England salt marsh, Environ. Microbiol., 7 (2005) 1175–1185.
[24] L. Cortás, M. Carreira, A. Costa, Biogenic production of sulfides in water-oil samples and its correlation with the deterioration of storage tanks, Braz. J. Pet. Gas, 6 (2012).
[25] M. Ismail, N. Yahaya, A. Abu Bakar, N.M. Noor, Cultivation of sulfate-reducing bacteria in different media, Malays. J. Civ. Eng., 26 (2014) 456–465.
[26] M. Wagner, A.J. Roger, J.L. Flax, G.A. Brusseau, D.A. Stahl, Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration, J. Bacteriol., 180 (1998) 2975–2982.
[27] J.W. Moreau, R.A. Zierenberg, J.F. Banfield, Diversity of dissimilatory sulfite reductase genes (*dsrAB*) in a salt marsh impacted by long-term acid mine drainage, Appl. Environ. Microbiol., 76 (2010) 4819–4828.
[28] S.-J. Li, Z.-S. Hua, L.-N. Huang, J. Li, S.-H. Shi, L.-X. Chen, J.-L. Kuang, J. Liu, M. Hu, W.-S. Shu, Microbial communities evolve faster in extreme environments, Sci. Rep., 4 (2014) 6205, doi: 10.1038/srep06205.
[29] R.D. Barrett, H.E. Hoekstra, Molecular spandrels: tests of adaptation at the genetic level, Nat. Rev. Genet., 12 (2011) 767–780.
[30] E.V. Koonin, M.Y. Galperin, Sequence - Evolution - Function: Computational Approaches in Comparative Genomics, Kluwer Academic, Boston, 2003.
[31] L.-p. Yang, X.-h. Zheng, G.-q. Zeng, M.-y. Xu, G.-p. Sun, Isolation and characterization of a sulfate reducing *Citrobacter* sp. strain SR3, Huan Jing Ke Xue., 31 (2010) 815–820.
[32] K.A. Zarasvand, V.R. Rai, Identification of the traditional and non-traditional sulfate-reducing bacteria associated with corroded ship hull, 3 Biotech, 6 (2016) 1–8.
[33] T. Tralau, S. Vuilleumier, C. Thibault, B.J. Campbell, C. Anthony Hart, M.A. Kertesz, Transcriptomic analysis of the sulfate starvation response of *Pseudomonas aeruginosa*, J. Bacteriol., 189 (2007) 6743–6750.
[34] V. Zadjelovic, M.I. Gibson, C. Dorador, J.A. Christie-Oleza, Genome of *Alcanivorax* sp. 24: a hydrocarbon degrading bacterium isolated from marine plastic debris, Mar. Genomics, 49 (2020) 100686, doi: 10.1016/j.margen.2019.05.001.
[35] N. Mpongwana, S.K.O. Ntwampe, L. Mekuto, E.A. Akinpelu, S. Dyantyi, Y. Mpentshu, Isolation of high-salinity-tolerant bacterial strains, *Enterobacter* sp., *Serratia* sp., *Yersinia* sp., for nitrification and aerobic denitrification under cyanogenic conditions, Water Sci. Technol., 73 (2016) 2168–2175.
[36] E. Darmon, D.R.F. Leach, Bacterial genome instability, Microbiol. Mol. Biol. Rev., 78 (2014) 1–39.
[37] H.J. Krishna, Introduction to Desalination Technologies, Texas Water Development, 2004.