REP-PCR Analysis to Study Prokaryotic Biodiversity from Lake Meyghan

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Abstract. Repetitive extragenic palindromic elements-polymerase chain reaction (rep-PCR) with 16S ribosomal ribonucleic acid (16S rRNA) genes sequences successfully used for the analysis of microbial community. In this study, the prokaryotic community in Lake Meyghan described by using rep-PCR analysis along with 16S rRNA gene sequencing. The water samples were collected from Lake Meyghan in November 2013. All samples were diluted and cultured on three different media. To estimate the number of prokaryotes per milliliter of the lake we used quantitative real-time PCR (qPCR). Rep-PCR combination with 16S rRNA gene sequencing was performed to investigate prokaryotes biodiversity in the lake. 305 strains were isolated in this work; 113 isolates for green region, 102 isolates for red region, and 90 isolates for white region. The dendrograms generated 10, 7, and 9 clusters for a 70 % similarity cut-off for green, red, and white regions, respectively. Based on rep-PCR and 16S rRNA gene sequencing, the recovered isolates were dominated by (77.5 %) *Halobacteriacae* and many isolates were related to the genera *Halorubrum*, *Haloarcula*, *Haloterrigena*, *Natrinema*, and *Halovivax* in the white region. In the red region more isolated strains (57.5 %) belonged to *Bacillaceae* and the remaining 42.5 % of isolates belonged to archaea domain, *Halorubrum*, and *Haloarcula*. In the green region members of *Gammaproteobacteria* were recovered, this region was dominant with *Pseudoalteromonas*, *Salinivibrio*, and *Aliidiomarina*.

Introduction

Microorganisms despite their small size have a vast impact on our lives. In the 1960s, microbiologists began exploring the impact of biodiversity on the structure and function of microbial communities [1, 2]. Understanding patterns of prokaryotes (bacteria and archaea) biodiversity is of particular importance for the reason that prokaryotes may well comprise the majority of the earth's species diversity, many processes responsible for sustaining life on earth are mediated by them therefore their diversity is practically very important in bioprospecting (the search for novel biochemicals for use in medicine, agriculture, and industry) and bioremediation (the biological degradation of pollutants) [3-5].

Saline and hypersaline environments are very useful in studies on prokaryotes diversity. On the one hand, prokaryotes diversity decreases with increasing salinity [5-8]. On the other hand, aquatic hypersaline systems have proved to be an excellent source of new culturable microorganisms [9-12]. Hypersaline environments can be separated into two types, thalassohaline and athalassohaline [13]. Thalassohaline aquatic environments have an ionic compositions similar

to seawater with NaCl as dominant salt [14, 15]. A Thalassohaline aquatic environments are formed by dissolution of mineral salt deposits of continental origin that are dominated by K^+, Mg^{2+}, Na^+, and CO_3^{2-} ions such as can be found in the soda lakes [15, 16]. Many ecological studies were performed with useful molecular techniques in both athalassohaline and thalassohaline hypersaline habitats [17-21].

To date, repetitive extragenic palindromic elements-polymerase chain reaction (rep-PCR) has become one of the highly reliable and widely used techniques for studies of molecular diagnostics, microbial ecology, environmental, and medical microbiology. This unique technique provides a very easy, quick, and inexpensive way to detect diversity of the microbial genomes being studied [22, 23]. Albeit, other genomic DNA methods are also useful to group and identify microbes, the rep-PCR is a method known for its simplicity, rapidity, and cost effectiveness which enjoys the essential resolving power need for microbial identification [24]. This technique is often used for epidemiological analyses, genotyping, and strain tracking purposes but some researchers recently used this technique with/without 16S ribosomal ribonucleic acid (16S rRNA) genes sequences successfully for analyzing microbial community [25-27].

Microbial genomes include many different types of repetitive DNA elements, make up 5 % of the whole genome [28-30]. Albeit, some repetitive elements (e.g. *Mycobacterium leprae*-specific repetitive element) are particularly existent in a limited group of bacteria, others, such as the rep DNA sequences, have been seen among many groups of phylogenetically diverse archaea and bacteria [30-32]. Since each microbial strain has repetitive elements located in distinct positions around their genome, the PCR method, performed using these repetitive sequences as primers and genomic DNA as template, generates banding patterns and can act as a barcode for a specific strain of microorganisms [33]. Rep-PCR is a method known for its simplicity, rapidity, and cost effectiveness and when this technique is coupled with 16S rRNA gene sequencing technique could have reliable results and give us excellent idea about the diversity of the regions being studied. [25-27, 32].

Iran has a great diversity of hypersaline habitats such as Lake Urmia, Lake Meyghan, Aran-Bidgol salt lake, and Howz-e Soltan salt lake. Lake Meyghan is one of the most important hypersaline lakes in Iran, because of its large mineable sodium sulfate deposits, the largest in the Middle East [34]. Lake Meyghan covers an area of 110 km^2 in the central part of Iran (Markazi province, north of Arak city). The lake itself is located in an area with an arid to semi–arid continental climate and temperature ranges from +40 °C in summer to -30 °C in winter [34].

Since rep-PCR is a simple, rapid and cost effective and inexpensive technique to investigate and obtain quick and basic information about microbial communities, we decided to describe the prokaryotic community in Lake Meyghan by using rep-PCR analysis along with 16S rRNA gene sequencing.

Materials and Methods

Sample sites, samples collection and growth conditions

The Lake Meyghan (34°11'–27.91"N, 49°50'–26.70"E) was sampled in November 2013. We sampled the lake at three different sites named according to brine color. These were G (green, 34°11'–21.59"N, 49°50'–45.73"E), R (red, 34°11'–20.78"N, 49°50'–21.87"E), and W (white, 34°11'–35.31"N, 49°50'–18.25"E). The samples were collected aseptically and transferred to the lab within four hours. The pH and salinity of the samples were measured *in situ* with portable meter PT-10P (Sartorius, Germany). Prokaryotes isolated under aerobic conditions on three growth media. The Modified Growth Medium (MGM) medium contained (g L^{-1}): NaCl 184.8, $MgSO_4.7H_2O$ 26.9, $MgCl_2.6H_2O$ 23.1, peptone 10.0, KCl 5.4, yeast extract 2.0, $CaCl_2.2H_2O$ 0.8, and agar 15.0; pH 7.2 at 25 °C. Moderately Halophilic medium (MH) contained of (g L^{-1}): NaCl 81.0, $MgSO_4.7H_2O$ 9.6, $MgCl_2.6H_2O$ 7.0, KCl 2.0, $CaCl_2$ 0.54, glucose 1.0, proteose peptone 5.0, yeast extract 10.0, agar 15.0, NaBr 0.026, and $NaHCO_3$ solution 10.0 mL (Add 0.06 g $NaHCO_3$ to 10.0 mL of deionized water); pH 7.5 at 25 °C. Marine medium contained of (g L^{-1}): NaCl 19.45, $MgCl_2$ (anhydrous)

5.90, peptone 5.0, Na_2SO_4 3.24, $CaCl_2$ 1.80, yeast extract 1.0, KCl 0.55, $NaHCO_3$ 0.16, Fe (III) citrate 0.10, KBr 0.08, $SrCl_2$ 0.034, H_3BO_3 0.022, Na_2HPO_4 0.008, Na-silicate 0.004, NaF 0.0024, $(NH_4)NO3$ 0.0016, and agar 15.0; pH 7.6 at 25 °C. All samples were serially diluted up to 10^{-6} and plated according to Burns et al. [35]. The plates were incubated aerobically at two different temperatures 30 and 40 °C for 8 weeks.

DNA extraction and Quantitative real-time PCR (qPCR)

Environmental DNA was extracted as described previously [35, 36]. Bacterial and archaeal genomic DNA was extracted by the DNP^{TM} kit (Cinnagen, Iran), according to the manufacturer's recommendations. DNA concentration and purity were spectrophotometrically assessed by reading A_{260} and A_{280} and confirmed by visualization on 1 % agarose gel.

The standard curves and qPCR were performed as described previously [37]. Plasmid DNA possessing a full-length copy of 16S rRNA gene belonging either to the *Halorubrum chaoviator* (DSM 19316) and *Escherichia coli* (ATCC 25922) were used as DNA standards in qPCR. The target DNA for standard curves were amplified using the species-specific primer sets 27F and 1492R for bacteria and 20F and 1530R for archaea (Table 1) [38, 39]. Copy number per µL of extracted DNAs were calculated using formula

$$\text{Copy number per µL} = \frac{\text{concentration of plasmids (gm/µL)} \times 6.023 \times 10^{23}}{\text{length of recombinant plasmid (bp)} \times 660}$$

(660 = Molecular weight of one basepair in double-stranded DNA, 6.023×10^{23} = Avogadro's number) [40]. qPCR was performed with Rotor-Gene^{TM} 6000 (Corbett Research Biosciences, Sydney, Australia) using the species-specific primer sets Eub338 and Eub518 for bacteria samples and Parch519F and ARC915R for archaea samples (Table 1) [38, 41-43]. Amplification were performed in a total volume of 15 µL, containing 30 ng of template DNA, 7.5 µL of 2X Maxima SYBR Green qPCR Master Mix (Fermentas, France), 1.5 µL of each primer (10 pmol/µL) and sterile H_2O. The amplification program involved an initial denaturation step at 95 °C for 15 min, followed by 40 cycles of 94 °C 30 s, 60 °C for 30 s and 72 °C 20 s for both bacteria and archaea. For all standard curves, the coefficients of determination (R^2 value) were higher than 99.0 %. The number of target genes per mL of sample was calculated using formula [40]

$$\text{Number of gene copies per mL samples} = \frac{\text{(gene copies per reaction mix)} \times \text{(volume of DNA [µL])}}{\text{(3 µL DNA per reaction mix)} \times \text{(mL sample used)}}.$$

Table 1. List of oligonucleotides used in this study.

Primer Name	Primer Sequence (5' to 3')	Reference
27F	AGAGTTTGATCMTGGCTCAG	38
1492R	GGTTACCTTGTTACGACTT	38
20F	TCCGGTTGATCCTGCCG	39
1530R	AAGGAGGTGATCCAGCC	39
Eub338	ACTCCTACGGGAGGCAGCAG	38
Eub518	ATTACCGCGGCTGCTGG	41
Parch519F	CAGCCGCCGCGGTAA	42
ARC915R	GTGCTCCCCCGCCAATTCCT	43
REP1R	IIIICGICGICATCIGGC	44
REP2I	ICGICTTATCIGGCCTAC	44

Rep-PCR analysis

The primers used for the rep-PCR reaction were REP1R and REP2I (Table1) [44]. DNA amplification was carried out using approximately 100 ng genomic DNA. The amplification program involved an initial denaturation cycle for 7 min at 95 °C, followed by 35 cycles of 30 s at 90 °C, 1 min annealing at 40 °C, 8 min extension at 72 °C and 16min final extension at 72 °C. The reaction mixture contained 12.5 µL GoTaq[®] G2 Master Mix (Promega, Madison, USA), 1 µL of each primer (10 pmol/µL). The PCR product underwent 1% agarose gel electrophoresis for 3 h under constant 80 V in 0.5 X TBE, gels were stained with ethidium bromide, visualized under ultraviolet (UV) radiation and photographed.

The rep-PCR were analyzed using the BioNumerics software package version 6.6.11 (Applied Maths NV, Sint-Martens-Latem, Belgium). Using the Jaccard algorithm [23]. Clusters were considered at a 70 % similarity cut-off [24].

PCR amplification of 16S rRNA genes and sequence analysis

After generating the dendrograms based on the rep-PCR analysis, the 34 representative isolates for all regions and each cluster were subjected to 16S rRNA genes sequencing analysis. These isolates were amplified using either bacteria domain-specific primer 27F and 1492R or archaea-specific primer 20F and 1530R (Table 1) [38, 39]. The PCR conditions were as follows for bacteria: 94°C for 3 min, followed by 25 cycles of 95 °C for 45 s, 55 °C for 45 s and 72 °C for 90 s, with final 10 min extension at 72 °C and for archaea: 94 °C for 3 min, followed by 30 cycles of 94 °C for 15 s, 52 °C for 30 s and 72 °C for 50 s, with final 7 min extension at 72 °C.

Relevant sequences were extracted from GenBank (www.ncbi.nlm.nih.org) using BLASTN and through the EzBioCloud server (www.ezbiocloud.net/eztaxon) [8]. The sequences were considered to belong to an operational taxonomic unit (OTU) if they shared ≥97% sequence identity.

Maximum likelihood searches under a General-Time-Reversible (GTR) substitution model with gamma distributed rate heterogeneity and a proportion of invariable sites (GTR + Γ + I) were calculated using MEGA version 5 [45, 46].

Nucleotide sequence accession numbers

The 16S rRNA gene sequences determined in this study have been deposited in the GenBank sequence database under accession numbers KX351831–KX351864.

Results

The physicochemical properties of water samples collected are presented in Table 2. The temperature of all sites were the same. The salinity of green, red, and white sites were 5%, 18%, and 30%, respectively. With a pH of 8.8, 7.9, and 7.7 in green, red, and white sites, respectively. We identified Na^+ and Cl^- major ions in the all samples. Total prokaryote cells per mL of samples determined by qPCR were ranged 3.6–8.1 × 10^6. The abundance of bacterial and archaeal cells determined with bacteria-specific primers and archaea-specific primers represent 15.7%–79% and 21%–84.3% of target genes per mL of samples, respectively. The abundance of bacterial cell was more significant in green site (79%), while the abundance of archaeal cell was more dominant in white and red sites (84.3% and 78.1%).

In total, 305 strains were isolated; 113 isolates for the green region, 102 isolates for the red region, and 90 isolates for the white region. Out of these isolates, 120 isolated strains (40 isolates for each region) were randomly selected to generate dendrogram based on rep-PCR analysis. The abundance of bacterial and archaeal in all samples, determined by qPCR, was reported as the number of target genes per mL of samples (Table 2). For all standard curves, the coefficients of determination (R^2 value) were higher than 99.0 % (Fig. 1).

Table 2. Physico-chemical properties of water samples, total cell count and relative percentages of bacterial and archaeal cells in each sample with species-specific primers by using qPCR.

	Green	Red	White
Parameter:			
Salinity (%)	5	18	30
pH	8.8	7.9	7.7
Temperature (°C)	15	15	15
Ion concentration (g L^{-1}):			
Na$^+$	8.6	64.7	111.5
Ca^{2+}	0.38	0.56	0.55
K$^+$	0.06	0.19	0.20
Cl$^-$	11.6	92.6	164.1
HCO$_3$$^-$	0.16	0.76	0.22
Number of bacteria per mL of samples (%)	6.4×10^6 (79%)	1.2×10^6 (21.9%)	5.6×10^5 (15.7%)
Number of archaea per mL of samples (%)	1.7×10^6 (21%)	4.3×10^6 (78.1%)	3.0×10^6 (84.3%)
Total (number of prokaryote cells per mL of samples)	8.1×10^6	5.5×10^6	3.6×10^6
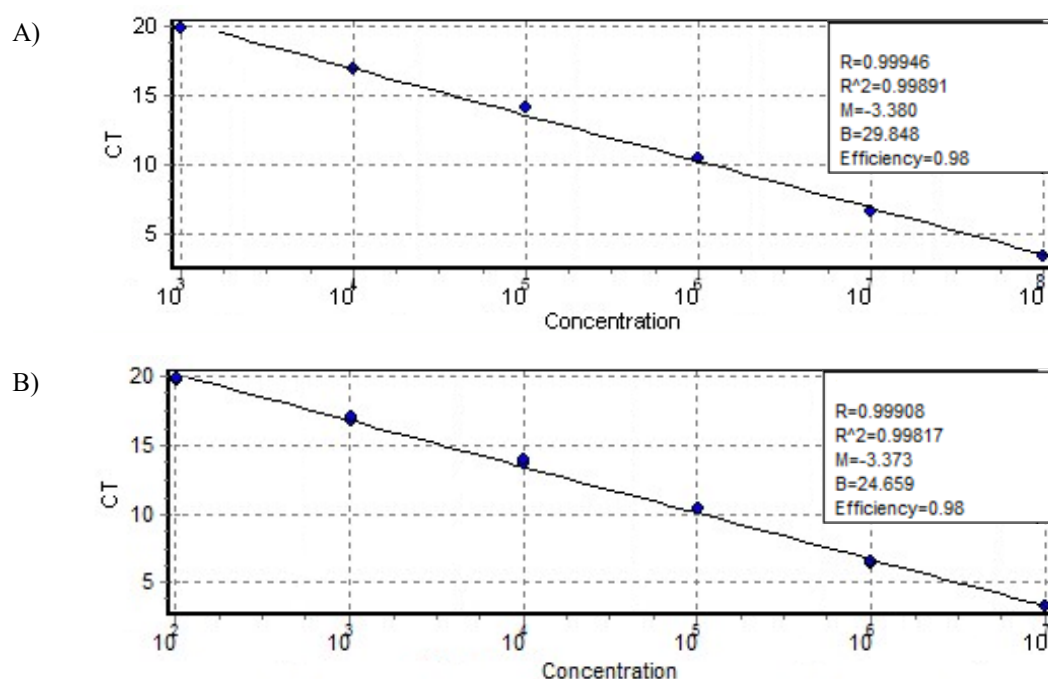
Figure 1. Standard curves with the CT plotted against the concentrations of the target DNAs (*Halorubrum chaoviator* (DSM 19316) for archaea and *Escherichia coli* (ATCC 25922) for bacteria). A standard curve was generated using a 10-fold serial dilution of target DNAs amplified on the Rotor-Gene^{TM} 6000. A) for bacteria B) for archaea. CT: cycle threshold; R: square root of correlation coefficient; R^2: R2: correlation coefficient; M: slope; B: intercept.

The dendrograms generated 10, 7, and 9 clusters for a 70 % similarity cut-off for the green, red, and white regions, respectively (Fig. 2-4). This same cut-off enabled the observation of a large clusters in different regions; cluster H in the green region with 13 isolates, clusters K, M, O in the red region with 10, 8, and 11 isolates, respectively, and clusters R, T, and U in the white region with 9, 7, and 9 isolates, respectively. The similarity between the most distant isolates for the green, red, and white regions were 44 %, 32 %, and 46 %, respectively (Fig. 2-4). Site green presented the highest diversity with 10 clusters (between 100 % and 44 %), designated A, B, C, D, E, F, G, H, I, and J (Fig. 2). Site red had lower diversity, with 7 clusters (between 100% and 32%), designated K, L, M, N, O, P, and Q (Fig. 3). Site white had 9 clusters (between 100% and 46%), designated R, S, T, U, V, W, Y, Z, and & (Fig. 4).
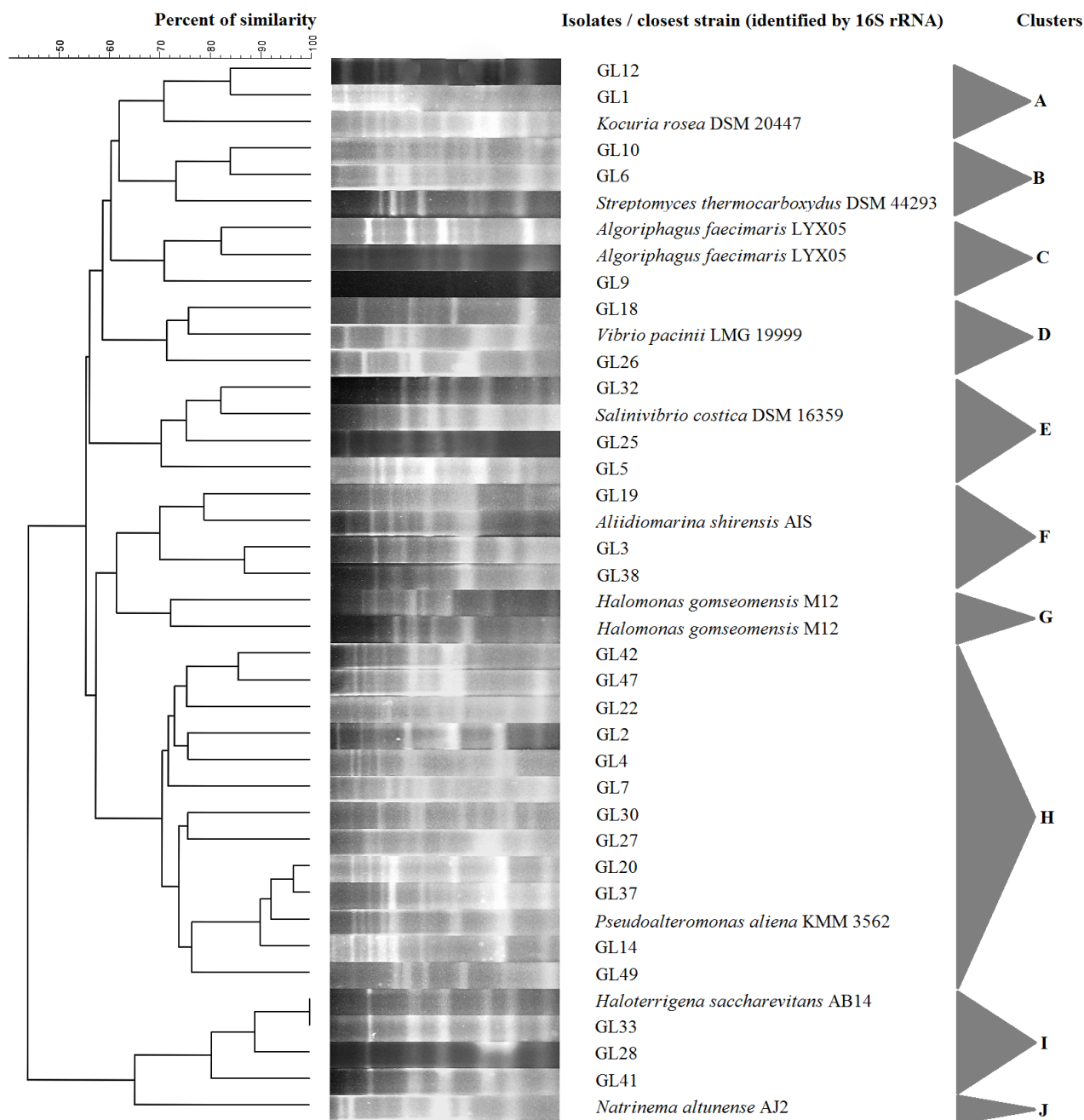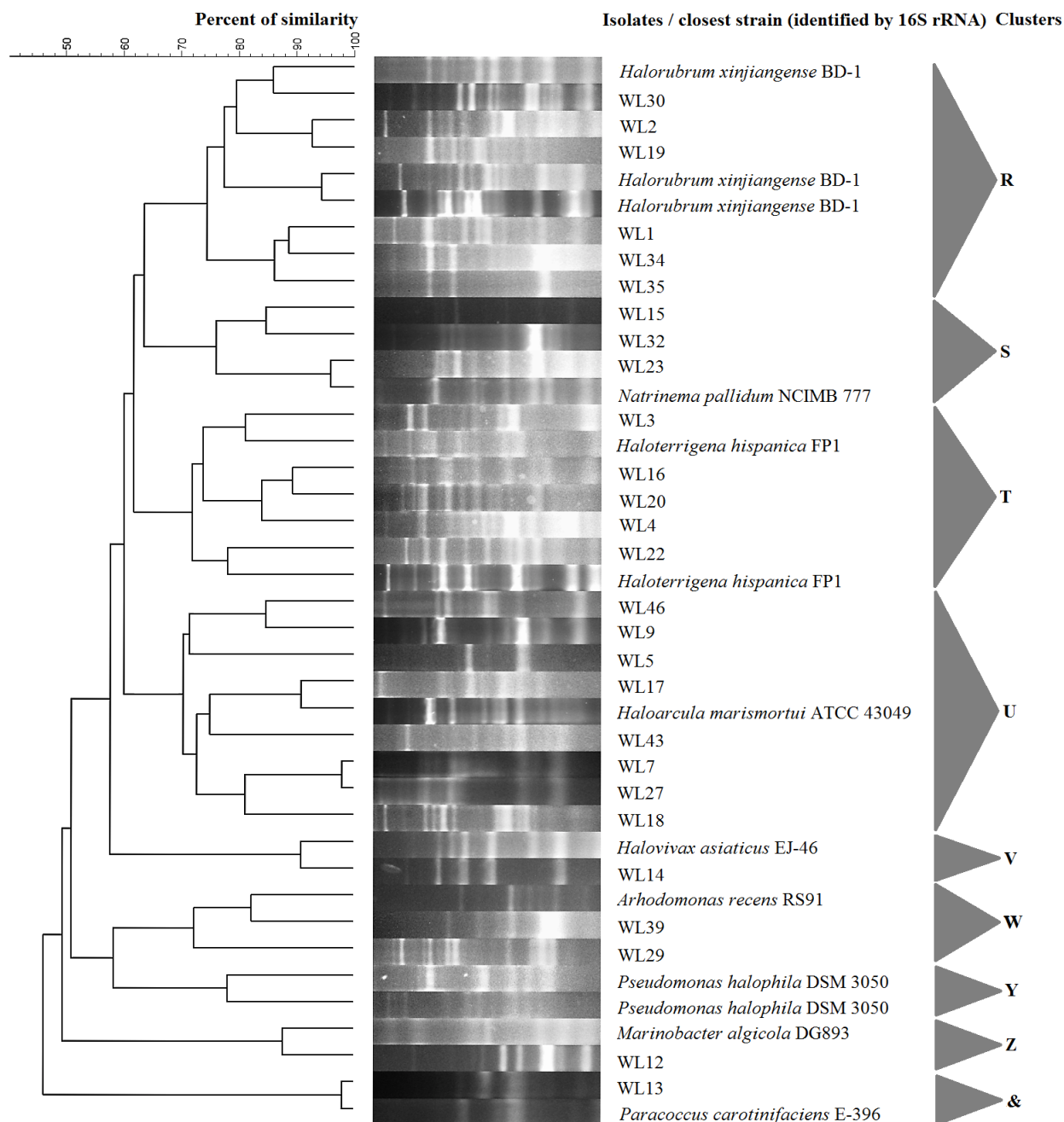

Figure 2. UPGMA dendrogram for the rep-PCR data calculated using the Jaccard coefficient from green region. The top bar indicates the percent of similarity. The letters on the right indicate the clusters formed in a 70% cut-off.
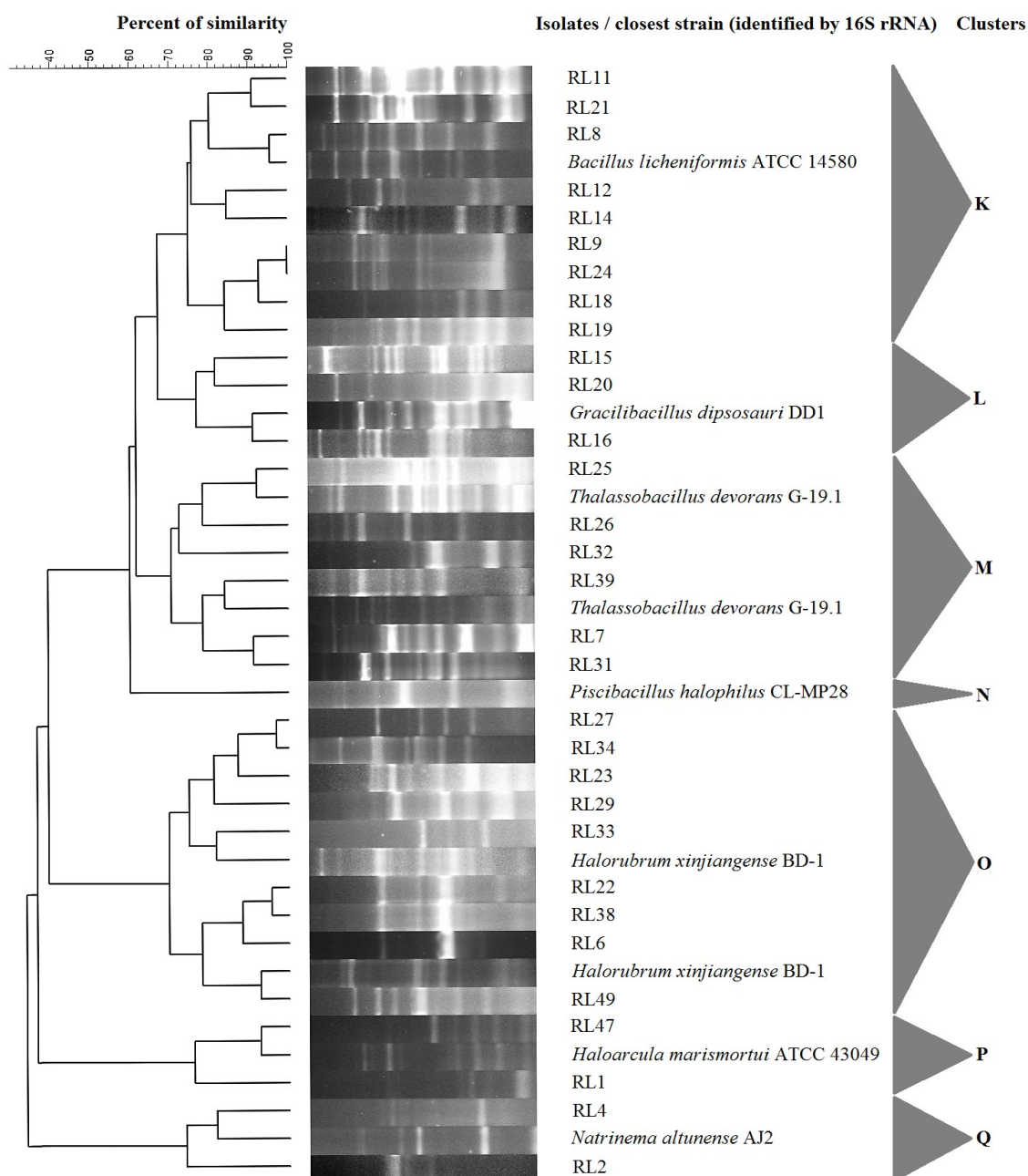
Figure 3. UPGMA dendrogram for the rep-PCR data calculated using the Jaccard coefficient from red region. The top bar indicates the percent of similarity. The letters on the right indicate the clusters formed in a 70% cut-off.

Figure 4. UPGMA dendrogram for the rep-PCR data calculated using the Jaccard coefficient from white region. The top bar indicates the percent of similarity. The letters on the right indicate the clusters formed in a 70% cut-off.

The combination of the rep-PCR along with 16S rRNA gene sequencing allowed identifying 120 strains. In the white region more isolated strains (77.5 %) belonged to the family *Halobacteriacae* (*Halorubrum* 22.5 %, *Halorcula* 22.5 %, *Haloterrigena* 17.5 % , *Natrinema* 10 % and *Halovivax* 5 % of isolates obtained) and the remaining 22.5 % of isolates belonged to bacteria, *Arhodomonas*, *Pseudomonas*, *Marinobacter* and *Paracoccus*. In red region isolated strains (57.5 %) belonged to *Bacillaceae* (*Bacillus* 25 % of isolates obtained, *Thalassobacillus* 20 % of isolates obtained, *Gracilibacillus* 10% of isolates obtained, and *Piscibacillus* 2.5% of isolates obtained) and the remaining 42.5% of isolated strains belonged to archaea, *Halorubrum* 27.5 %, and *Haloarcula* 15 %. In green region isolated strains belonged to the following genera: *Pseudoalteromonas* (32.5 %), *Salinivibrio* (10 %), *Aliidiomarina* (10 %), *Haloterigena* (10%), *Kocuria* (7.5%), *Streptomyces* (7.5%), *Algoriphagus* (7.5%), *Vibrio* (7.5%), *Halomonas* (5%) in domain of bacteria, and *Natrinema* (2.5%) in archaea.

Overall, 34 representative isolated strains for all regions and each clusters were subjected to 16S rRNA genes sequencing analysis. These data revealed the presence of *Gammaproteobacteria*, *Alphaproteobacteria*, *Actinobacteria*, *Bacilli*, *Bacteroidetes* and *Halobacteria* in Lake Meyghan (Fig. 5 and 6). Sequences belonging to archaea especially *Halobacteria* were found in white region more than red and green regions with 8, 4, and 2 strains, respectively. The *Halobacteria* was most diverse with ten OTUs, followed by the *Gammaproteobacteria* with ten OTUs and the *Bacilli* with five OTUs (Fig. 5 and 6).
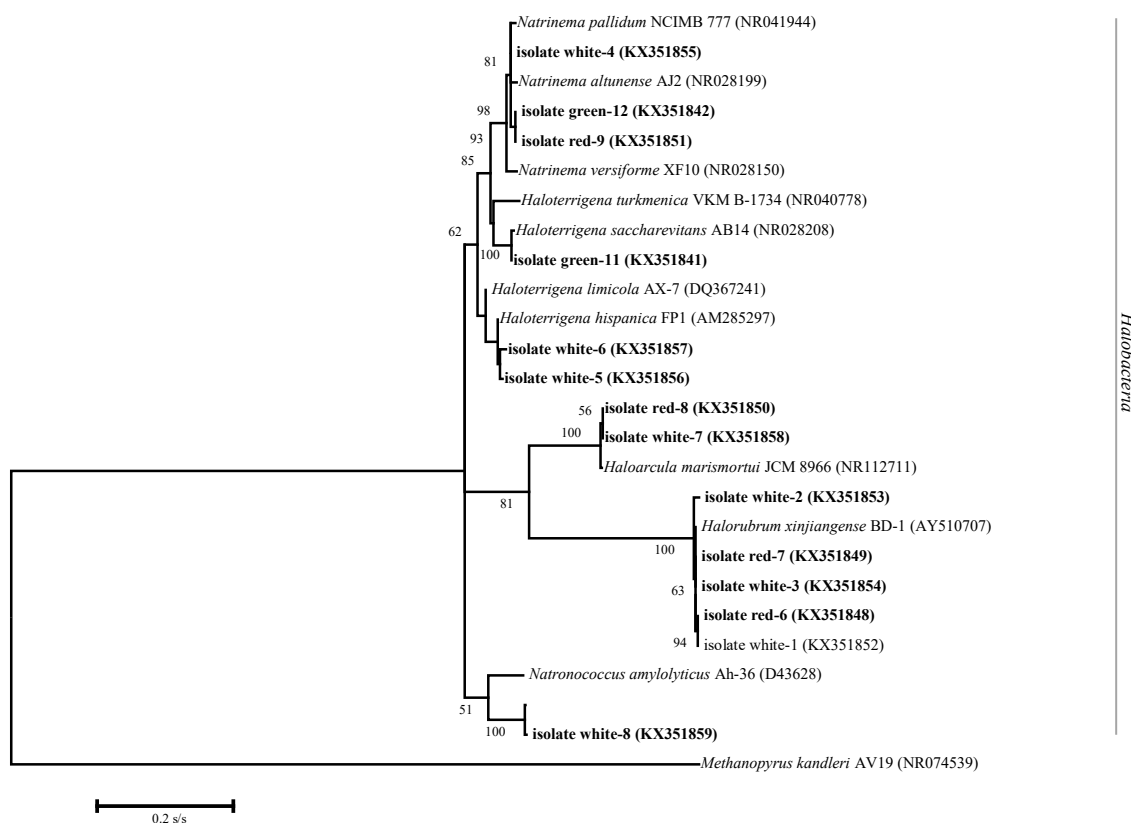
Figure 5. Phylogenetic reconstruction of 16S rRNA of archaeal sequences recovered from Lake Meyghan. The most likely topology shown here was obtained under the General-Time-Reversible substitution model with gamma distributed rate heterogeneity and a proportion of invariable sites (GTR + Γ + I). Bootstrap analysis was performed with 100 replicates and values greater than 50% are shown. Scale represents the expected number of substitutions per site.
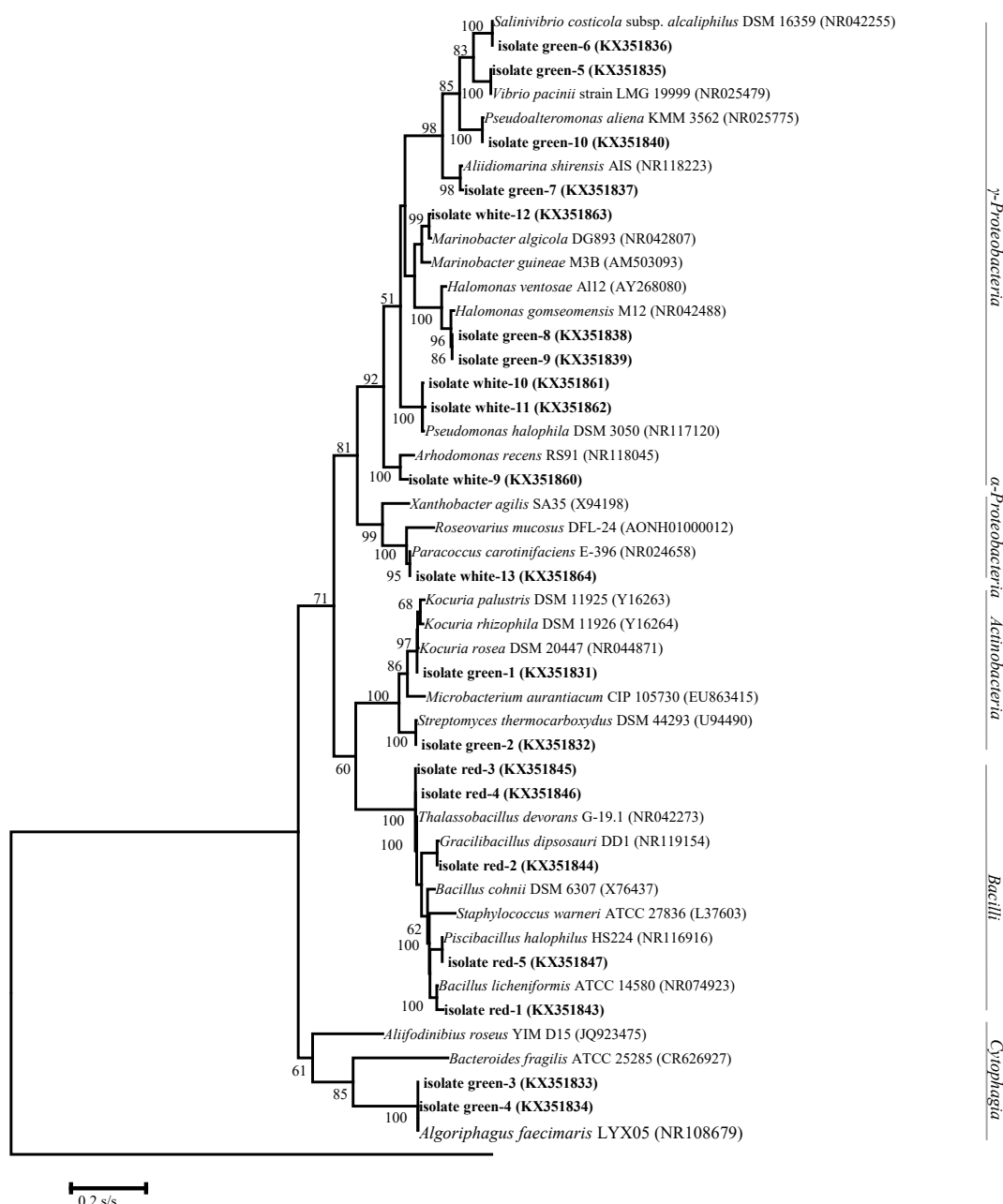
Figure 6. Phylogenetic reconstruction of 16S rRNA of bacterial sequences recovered from Lake Meyghan. The most likely topology shown here was obtained under the General-Time-Reversible substitution model with gamma distributed rate heterogeneity and a proportion of invariable sites (GTR + Γ + I). Bootstrap analysis was performed with 100 replicates and values greater than 50% are shown. Scale represents the expected number of substitutions per site.

Discussion

Iran has a great diversity of saline and hypersaline environments that prokaryotes population needs to be elucidated. These include permanent hypersaline lakes such as Lake Urmia in the North West and seasonal hypersaline lakes such as Aran-Bidgol and Howz-e Soltan in the center of Iran. Jookar Kashi et al. [47] studied cultivable microorganisms in Urmia Lake and found that their isolates belonged to Proteobacteria (21.4%), Firmicutes (78.6%), and Actinobacteria (1.8%). Lake Meyghan is one of the most important hypersaline playa in Iran, because of its mineable sodium sulfate deposit that is the largest in the Middle East. According to its physicochemical properties, this lake was classified as thalassohaline [46]. The ion composition of Lake Meyghan reflected that

of seawater: Na^+ was the dominant cation, Cl^- was the dominant anion. The pH was about between 7.7 to 8.8. We used qPCR to calculate microbial total cells and number of bacteria and archaea in different regions of the lake. This technique is one of the best methods, used by researchers to estimate the number of cells in the environment because with this technique number of culturable and non-culturable bacteria and archaea cells determined. Total prokaryote cell counts in the lake in green, red, and white regions were 8.1×10^6 (cells mL^{-1}), 5.5×10^6 (cells mL^{-1}), and 3.6×10^6 (cells mL^{-1}), respectively, and in the range of the microbial populations found in other similar environments studied (10^6-10^7 cells mL^{-1}), but lower than some other environmental investigations, perhaps the reason for this due to the fact that toxic sulfur compounds exist in this environment [46, 48]. Salterns in Santa Pola (Spain) contained 1×10^7 to 5×10^7 cells mL^{-1} (49, 50). Mutlu et al. (2008) found that the total cell counts per milliliter were 1.38×10^7 in the Tuz Lake, a hypersaline environment in Inland Turkey [48]. And also, Makhdoumi-Kakhki et al. [46] calculated that the total cells in the Aran-Bidgol Salt Lake in Iran around 3–4×10^7 cells mL^{-1} and 50–75 % and 18–37% of detected cells related to archaea and bacteria, respectively.

The objective of performing rep-PCR in this study was to estimate genetic similarity among the bacterial and archaeal isolates. Screening of isolates using rep-PCR technique is inexpensive, easy, and rapid. Despite the fact that the majority of the rep-PCR DNA studies have been carried out for epidemiological and strain tracking purposes, this technique also enjoys great versatility and can be adopted to study microbial community, ecology, and evolution. Comparative studies conducted suggest that rep-PCR is a rapid method for characterising taxonomic diversity and phylogenetic structure [33]. Aanniz et al. (2015) investigated the diversity of thermophilic bacteria in hot springs, salt marshes, and desert soils in Moroccan by using rep-PCR along with 16S rRNA gene sequencing techniques [25]. The combination of these two methods allowed the researchers to identified 219 isolates (91.25%) to the species level. The researchers indicate that these techniques were a powerful tool for identification of bacteria to the species level [25]. Kathleen et al. (2014) used rep-PCR analysis and 16S rRNA sequencing to investigate the diversity of bacteria in Sarawak. Researchers stated that rep-PCR method had a high discrimination power when compared to other DNA fingerprinting techniques and can be reduce the cost for DNA sequencing [26]. Also, De Bellis et al. (2015) used rep-PCR along with 16S rRNA gene sequencing techniques to survey the biodiversity of bacterial strains isolated from vineyard soils [27].

After the dendrograms were generated based on the rep-PCR analysis, the representative isolates for each region and each cluster were subjected to 16S rRNA genes sequencing analysis. Among the 120 screened bacteria and archaea isolated strains from these three regions, 34 representative strains were selected for identification using 16S rRNA PCR analysis and DNA sequencing. The combination of the rep-PCR along with 16S rRNA gene sequencing allowed identifying 120 isolates, the cost of which was equal to the amount needed for just sequencing of 34 isolates and this method can be useful when we want excellent information about the diversity of a region in a very rapid and inexpensive way.

In the present study, the rep-PCR technique similarly revealed considerable diversity between isolates, generating a dendrogram with 10, 7, and 9 clusters for a 70 % similarity cut-off for the green, red, and white regions, respectively. The bacteria distribution in green region is more diverse than red and white regions, while the white region is the least diverse region due to high concentration of saline in these regions. In the white region more isolated strains (77.5 %) belonged to *Halobacteriacae* and many isolates were related to the genera *Halorubrum* and *Haloarcula* as could be expected because of the high salt concentration existed in this region and our qPCR result showed that archaea were the dominant microbes in this region. This is consistent with previous reports, Makhdoumi-Kakhki et al. (2012), who studied 101 archaeal isolates from Aran-Bidgol Salt Lake in Iran and found that their isolates belonged to *Halobacteriacae* family, with *Halorubrum* and *Haloarcula* being the most abundant [46]. In surveys performed in IncheBoroun wetland in Iran the archaeal isolates were belonged to *Haloarcula* (30%) and *Halorubrum* (27.5%) [51]. Results of other studies conducted in other hypersaline habitats of the world shows that members of *Halorubrum* constitute 70% of the isolates of solar slatterns in Australia and in Ayakekumu salt

lake in China 47% of isolates belonged to genus *Halorubrum* [52], also in Maras lake in Peru 50% of the isolates belonged to genus *Haloarcula* [53]. In the red region of Lake Meyghan more isolates (57.5 %) belonged to *Bacillaceae* and the remaining 42.5 % isolates belonged to archaea; *Halorubrum*, and *Haloarcula* but the result of qPCR showed that this region had more archaea cells than bacteria. Perhaps the reason for this is the different approaches with these two techniques. We found that *Bacillaceae* was the most cultarable prokaryotes in this region but in qPCR because of counting cultarable and non-cultarable prokaryotes we saw that archaea were the dominant prokaryotes in this region. So this is the reason for unsuccessful isolation *Haloquadratum walsbyi* in red and white regions that was dominated in other similar hypersaline environments. Of course, usually the cultivation of these microorganisms is difficult but, we can show it by culture independent techniques. Makhdoumi-Kakhki et al. (2012), stated that 40 % of the archaeal sequences from Aran-Bidgol Salt Lake were related to *Haloquadratum walsbyi* by using denaturing gradient gel electrophoresis (DGGE) analysis [46]. Mutlu et al. (2008) found that *Haloquadratum* spp. were the main components of the microbiota in Tuz Lake in Turkey [48]. So we can conclude that the dominant prokaryotes in this region were archaea but the most culturable prokaryotes were bacteria belonging to *Bacillaceae*. Also, isolation of members of the *Bacillaceae*, were previously reported by Dhiraj Paul et al. from Lonar Lake [54]. Our data demonstrated that the contributions of bacteria versus archaea also changed with increasing salinity with bacteria being the dominant group at low salinity (green region with 5% salinity) and archaea becoming more dominant at the highest salinity (white region with 30% salinity), we expected to find more diversity in green region than two other regions, and the result is a testimony to this claim. We recovered members of *Gammaproteobacteria*, found to constitute an important component in both saline and alkaline lakes [18, 19, 46]. Green region of Lake Meyghan is dominant with *Pseudoalteromonas*, *Salinivibrio*, and *Aliidiomarina*. Benlloch et al. (2002) investigated prokaryotic diversity of three salt ponds (8, 22, and 32% total salts, respectively) by using DGGE analysis and 16S rRNA gene sequencing from Santa Pola saltern in Spain. Most bacterial sequences in the 8% salt pond were belonged to *Proteobacteria* [55]. And also, Dhiraj Paul et al. (2016) found that *Proteobacteria* were the most predominant phyla, and Within the *Proteobacteria* members of *Gammaproteobacteria* were the most abundant [54].

Conclusion

In this study prokaryotic biodiversity from three sites of Lake Meyghan was investigated by using rep-PCR along with 16S rRNA gene sequencing. Based on rep-PCR and 16S rRNA gene sequencing, the recovered isolates were dominated by (77.5 %) *Halobacteriaceae* and many isolates were related to the genera *Halorubrum*, *Haloarcula*, *Haloterrigena*, *Natrinema*, and *Halovivax* in the white region with 30% salinity. In the red region with 20% salinity more isolated strains (57.5 %) belonged to *Bacillaceae* and the remaining 42.5 % of isolates belonged to archaea domain, *Halorubrum*, and *Haloarcula*. In the green region with 5% salinity we found most isolates belonged to *Gammaproteobacteria*. Our data demonstrated that the contributions of bacteria versus archaea also changed with increasing salinity with bacteria being the dominant group at low salinity (green region with 5% salinity) and archaea becoming more dominant at the highest salinity (white region with 30% salinity). The rep-PCR technique along with 16S rRNA gene sequencing technique can be a good choice when we want to obtain quick and basic information about biodiversity from environmental samples in very cost effective, simple, and rapid way. In this study we found these two techniques useful to investigate biodiversity in Lake Meyghan. Of course, for more information about this lake we need further study using other techniques and approaches like using metagenomic to obtain more completely information about this unique environment, but these two techniques show us basic information about this unique environment and its prokaryotes diversity.

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