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Research article/Araştırma makalesi

Alkaliphilic bacterial diversity of Lake Van/Turkey

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Abstract

Lake Van, situated in Turkey's eastern Anatolian region, is a saline soda lake and it covers the lowest part of a vast basin. The importance of Lake Van comes from being one of the largest soda lakes and third largest endorheic lake on Earth with a volume of 607 km^3. Although several biological studies have been done for Lake Van, research on microbial diversity of the lake is still missing. This paper contributes to scientific knowledge by research on alkaliphilic bacterial diversity of Lake Van. In this regard, culture-dependent and culture-independent methods were applied to the samples, which were taken from different locations close to the shores of surrounding cities. Isolation was performed on 10 different media. Bacterial diversity was analyzed by 16S rRNA gene PCR amplification, sequencing and blast analysis against NCBI database. Results showed that members of Proteobacteria (especially Gammaproteobacteria) and Actinobacteria phylums were represented dominantly. Culture-independent DGGE (Denaturing Gradient Gel Electrophoresis) and FISH (Fluorescence In-Situ Hybridization) methods were also used to detect the microbial populations in Lake Van. The results showed that the community was dominated by Bacteria.

Key words: Lake Van, alkaliphilic bacteria, ARDRA, DGGE, FISH

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Van Gölü'nün alkalifilik bakteriyel çeşitliliği

Özet

Van Gölü Türkiye Doğu Anadolu Bölgesi'nde yer alan, büyük bir havzanın alçak kısmını kaplayan tuzlu bir soda gölüdür. Van Gölü'nün önemi Dünya'nın en büyük soda göllerinden bir olmasından ve 607 km^3'lük hacmi ile Dünya'nın en büyük 3. kapalı havza gölü olmasından kaynaklanmaktadır. Van Gölü ile ilgili bazı biyolojik çalışmalar yapılmasına rağmen mikrobiyal çeşitliliği üzerine araştırmalar yeterli değildir. Bu yüzden bu çalışma Van Gölü'ndeki alkalifilik bakteri çeşitliliği üzerine yapılan araştırmalara bilimsel katkılar sağlamaktadır. Bu amaçla, gölün çevresindeki şehirlerin kıyılara yakın, farklı noktalardan alınan su örneklerine kültür bağımlı ve kültürden bağımsız yöntemler uygulanmıştır. Kültür çalışmaları için 10 farklı besi yeri kullanılmıştır. Bakteriyel çeşitlilik, 16 S rRNA geninin PCR ile çoğaltılması, dizileme ve NCBI veri tabanı kullanılarak incelenmiştir. Sonuçlar, Proteobacteria (özellikle Gammaproteobacteria) ve Actinobacteria filumlarının baskın bir şekilde bulunduğunu göstermiştir. Mikrobiyal populasyonları belirlemek için kültür bağımsız yöntemler olan DGGE (Denatüre Edici Gradiyent Jel Elektroforezi) ve FISH (Fluoresan In Situ Hibridizasyon) kullanılmıştır. Sonuçlar kommunitede bakterilerin yoğun olduğunu göstermiştir.

Anahtar kelimeler: Van Gölü, alkalifilik bakteri, ARDRA, DGGE, FISH

1. Introduction

Lake Van, or Van Gölü in Turkish, is situated at about 43ºE longitude and 38.5ºN latitude in the eastern part of Anatolian peninsula, Turkey. The lake has a surface area of 3574 km^2, a volume of 650 km^3, a maximum depth of 450 m, and a maximum length of 130 km (Cukur, 2014). It is located at an altitude of 1649 m and it covers the lowest part of a large basin surrounded by high mountains in the south, highlands and mountains in the east and volcanic cones in the

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west. The lake does not have any natural outlet and, hence, it gathers surface water from all around the lake (Kadioglu et al., 1997). Lake Van, besides being closed basin, is a saline soda lake and is distinguished from other lakes in the world with the importance it owns. Considering its properties and volume, it is one of the largest soda lake and third largest closed lake on Earth (Reimer et al., 2009) as well as being the largest lake of Turkey.

There is basic information about the ecosystem of the lake and it has not been studied in detail due to difficulties of access. Fruit and grain-growing agricultural areas surround Lake Van region. Lake Van basin also lies along the flyways of many migratory birds and this closed basin hosts 213 species of the bird fauna found in Turkey (Yılmaz and Aslan, 2004). The only fish, known to live in Lake Van is *Chalcalburnus tarichi* or the Pearl Mullet, which is endemic to the Lake Van basin (Danulat and Kempe, 1992) and is rated in "Near Threatened" species (Freyhof, 2014). It feeds on phyto and zooplanktons (Sarı, 2008) and there are 103 phyto and 36 zooplanktons species recorded in Lake Van (Danulat and Kempe, 1992).

Rains and streams that feed the lake are among the water sources of Lake Van. At the north shore Delicay and Zilan, at the east shore Karasu, and at the south shore Engil and Gevas streams pour into the lake. Lake Van, as a saline lake, has different soda chemistry. This is due to the fact that alkali cations maintain the charge balance of bicarbonate and carbonate ions in addition to alkaline earth ions (Reimer et al., 2009). One of the reasons for Lake Van being salty is the increase of salt concentration as a result of evaporation, and the salinity ratio of the lake is 0.224% (Ciftci et al., 2008). Soda lakes are naturally occurring alkaline environments and they exhibit good examples of extreme conditions. These kinds of lakes are the representatives of the highly stable pH environments in the world (Jones et al., 1998). The chemistry of soda lakes is quite unique because of the presence of large amounts of carbonate/bicarbonate concentration in the form of Na_2CO_3 and $NaHCO_3$ (Jones et al., 1998). Soda lakes host a substantial number of microbial communities and studies on the microbial diversity of soda lakes help to improve the understanding of the biology of extreme environments. The studies also have the potential for the discovery of novel microorganisms and enzymes, which may be used, in the scope of biotechnological applications. Alkaliphilic microorganisms are quite common among the microbial communities and they show optimal growth at least 2 pH units above neutrality. However, some alkaliphilic microorganisms exhibit more than one pH optima (Horikoshi, 2008). As a result, stability at high pH makes these microorganisms important. Furthermore the enzymes of alkaliphilic microorganism show activity at a high pH and salinity values. Selected examples for the industrial applications of alkaliphilic microorganisms are enzymes such as proteases, lipases, and cellulases have been used for the production of biotechnologically advanced laundry detergents (Horikoshi, 2008). Alkaline protease is also used in the food industry, pharmaceuticals, and medical diagnosis (Kanekar et al., 2002). Apart from enzymes, alkaliphilic microorganisms can be used for the biodegradation of organic and inorganic pollutants and hydrocarbons (Sorokin et al., 2012).

In recent years, studies on alkaliphilic microorganisms of soda lakes have attracted increasing research interest and various methods were employed in order to investigate microbial diversity. Among these studies, researchers used culture-dependent methods, culture-independent methods or a combination of those (Foti et al., 2008; Deshmukh et al., 2011; Mwirichia et al., 2011). Diversity of specific alkaliphilic microorganisms was also investigated using 16S rRNA and functional genes (Sorokin and Kuenen, 2005; Antony et al., 2012; Tourova et al., 2014). Apart from these, high-throughput sequencing of the 16S rRNA amplicon techniques were utilized in order to examine the structure and diversity of various microbial communities (Simachew et al., 2005; Matyugina and Belkova, 2015). Although many of isolates have already been identified and studied from different soda lakes worldwide, alkaliphilic diversity of the Lake Van has not been studied in detail until now. Assorted studies have been conducted for Lake Van and Lake Van region such as observation of environmental impacts (Ciftci et al., 2008), influence of climatic change (Kadioglu et al., 1997), examination of lake sediments, determination of lake water quality and the levels of heavy metals (Bilgili et al., 1995; Öztürk et al., 2005), plant ecology and diversity (İlcim et al., 2013). On the other hand, the knowledge is quite limited on the microbial diversity of the lake. Among the small number of example microbial studies on Lake Van, investigation of bacterial diversity of microbiolite samples (López-García et al., 2005) and determination of enzymes and protein characteristics of alkaliphilic *Bacillus* species (Berber and Yenidünya, 2005) can be quoted.

The goal of this study is to evaluate the diversity and structure of alkaliphilic bacteria in the world's largest soda lake, Lake Van, in order to contribute to scientific knowledge and to form a baseline for prospective research. In the scope of this study, both culture-dependent and culture-independent techniques were used to provide an efficient investigation for alkaliphilic bacteria. In this regard, employed techniques are Polymerase Chain Reaction (PCR), Denaturing Gradient Gel Electrophoresis (DGGE) and Fluorescence In-Situ Hybridization (FISH). The results of this study reveal the presence of Proteobacteria species (especially Gammaproteobacteria) and Actinobacteria phylums in Lake Van.

2. Materials and methods

2.1. Sample collection and analysis

Lake Van consists of two sections; the main body of water is separated from its much shallower northern arm by a narrow passage. This fact raises various risks such as influence of environmental conditions on sample content

depending on time, temperature and water level decrease. Therefore, the sampling areas in this study, which was conducted under financial constraints, were only selected from the main body of water. Water samples were obtained from four different locations and these locations are the shores of Lake Van being close to the end points in four different directions as east, west, north and south. The names of these shores to be used as sampling locations are Van (approx. 38.6ºN, 43.3ºE), Tatvan (approx. 38.5ºN, 42.3ºE), Ahlat (approx. 38.8ºN, 42.6ºE) and Edremit (approx. 38.3ºN, 43.1ºE) (Figure 1). Water samples (5L) were taken approximately 1-2 m away from the shore and at 1 m depth. Samples were immediately transported with sterile bottles and stored at room temperature. Salinity (Eclipse Hand Refractometer), chemistry (Perkin Elmer Optical Emission Spectrophotometer Optima 4300 DV) and pH (Mettler Toledo) of the samples were determined.

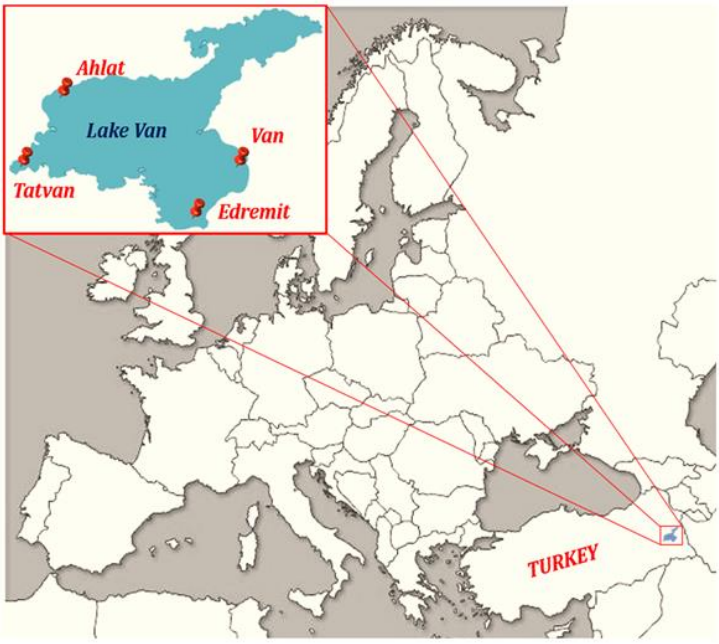

Figure 1. Lake Van sampling regions

2.2. Isolation and cultivation of alkaliphilic bacteria

In order to isolate alkaliphilic bacteria from water samples, enrichment culture technique was applied. For this purpose, 100 µl water sample from each location was inoculated to plates containing 10 different media. These media are Modified Growth Medium (MGM) in different NaCI concentrations such as 2-3-5-10-12% (Dyall-Smith, 2009), Horikoshi I and II medium (Horikoshi, 2008), Gauze Agar (Selyanin et al., 2005), Actinomycetes Isolation Agar (Sigma) and Glycerol Yeast Extract Agar. All plates were incubated at 37°C for 4-15 days. Different colony morphotypes were isolated by replication and the obtained pure cultures stocked in glycerol at -85ºC.

2.3. PCR amplification and ARDRA analysis

For PCR amplification of the 16S rRNA gene, boiling method was applied to isolates from different media. Briefly, a loop full of cells from pure culture was transferred to tube containing 200 µl of sterile distilled water and was suspended. After waiting for 5-10 minutes in the water bath set to 100°C, cells were lysed and then samples were centrifuged at 10000 rpm. Finally, the supernatant was transferred to sterile tube as DNA template for PCR reaction and was kept at -20°C for use. Each of isolates' DNA extracts and Bacteria specific universal primer set 27F (5'-AGAGTTTGATCATGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT3') (Lane et al., 1985) were combined for PCR reaction of 16 S rRNA genes. To control of the quality and signals of PCR products, 1% agarose gels were prepared and products were visualized under UV light after ethidium bromide staining. Then, Amplified ribosomal DNA restriction analysis (ARDRA) with the enzyme *Hinf I* (New England Biolabs) was applied to PCR products to group and characterize of isolates (Vaneechoutte et al., 1992). Reaction mixtures were incubated at 37°C overnight. Banding patterns were analyzed by using 2% agarose gel, which was run for 4 hours in 1X TBE (Tris-Boric acid-EDTA) buffer at 50 volts. Finally, restriction patterns were compared and grouped for sequencing.

2.4. Nucleic acid extraction

Total DNA was extracted from water samples combining previously described protocols (Nogales et al., 1999; Cifuentes et al., 2000; Mutlu et al., 2008). For collection of microorganisms by filtration, 200 ml water sample were passed through GV filter (Millipore, Isopore GTTP04700) with a pore diameter of 0.22 mm, using vacuum membrane

filter system. After cutting the one filter into small pieces, small pieces of membrane filters were transferred to RNase- and DNAse-free 2 ml tube, Extraction buffer (100 mM Tris-HCI pH: 8, 100 mM EDTA), lysozyme (3 mg/ml) were added and incubated at 37°C for 15 minutes in shaking incubator at 150 rpm. Then, proteinase K (150 mg/ml) and 10% sodium dodecyl sulfate (SDS) were added and incubated at 37°C for 40 minutes in shaking incubator. Following the incubation, 5 M NaCl and CTAB (10% CTAB - Cetil Trimethyl Ammonium Bromide, 0.7 M NaCl) was added and mixed by inverting the tube. Later, samples were immersed into liquid nitrogen for 1-2 minutes and were incubated at 65°C. This step was repeated for 3 times. After these steps phenol: chloroform: isoamyl alcohol (25:24:1) was added and centrifuged at 13000 rpm at 4˚C for 5 minutes for extraction and purification of nucleic acids. Then, sodium acetate (3 M, pH 4.8), isopropanol and $MgCI_2$ (1 M) were added and centrifuged at 13000 rpm for 30 minutes at 4˚C. 70 % alcohol was added on pellet and centrifuged again at 13000 rpm for 15 minutes at 4˚C. After ethanol precipitation, the pellet was dried at room temperature and suspended with sterile water. To control the quality of nucleic acids, 1% agarose gels were prepared and products were visualized under UV light after ethidium bromide staining (Lane et al., 1985). Finally, samples were stored at -85 °C.

2.5. DGGE analysis

Total nucleic acid extraction products from environmental water samples were used for amplification of the 16 S rRNA gene fragment for DGGE analysis. Towards this aim, 341F-GC (5'-GCclamp-CCTACGGGAGGCAGCAG-3') and 907R (5'-CCGTCAATTCCTTTRAGTTT-3') (Muyzer et al., 1993) bacteria primer combinations were used. DGGE was performed using a denaturing gradient of 30 - 60% denaturants in polyacrylamide gel with modifications of Muyzer et al.'s procedure in Ingeny System, (Leiden, NL) (Muyzer et al., 1993). Individual bands were excised and re-amplified with 341F and 907R primer combination. At last, PCR products were sequenced.

2.6. Sequence analysis and nucleotide sequence accession number

Sequence analysis was performed using 27F, 907R and 1492R primers on Beckman CEQ 8000 DNA analyzer. (Beckman Coulter, Fullerton, CA). Then 16S rRNA gene sequences were compared to reference sequences at NCBI (http://www.ncbi.nlm.nih.gov) by using BLAST programme (Altschul et al., 1990). Finally, representative sequences were determined and stored in GenBank with the accession numbers KM248755 to KM248759, KM362990 to KM363002 and KM388972 to KM388984. Phylogenetic analysis was performed with these sequences and sequences stored in GenBank were used to build tree by the "phylogeny.fr" tool. (Dereeper et al., 2008; Dereeper et al., 2010).

2.7. FISH analysis

Fixation of water samples was applied according to the previous protocol of Anton et al., (1999). Hybridization, DAPI staining, and microscopic examination were applied as decribed in protocol of Snaidr et al., (1997). 16S rRNA-targeted oligonucleotide probe EUB338, which is specific for members of the Bacteria domain, was used for hybridization (Amann et al., 1990). The filters were dried, DAPI staining was applied and microscopic examination was performed by fluorescence microscopy (Leica DM6000B).

3. Results

3.1. Chemical characterization of water samples

The salinities of water samples varied from 1.2% to 1.8% and pH varied from 9.5 to 10.2. The pH of the samples was alkaline. Na^+ and K^+ were the most abundant ions followed by the Mg^{2+} (**Hata! Başvuru kaynağı bulunamadı.**).

Table 1. Sampling regions, salinity and ionic features of water sample

Water Sample	Name	pH	Salinity (%)	Na^+ (ppm)	K^+ (ppm)	Ca^{2+} (ppm)	Mg^{2+} (ppm)	Cd (ppm)
M	Central	9.5	1.7	7003	430.4	<	86.25	169.5
T	Tatvan	9.8	1.8	8323	493.1	0.313	86.81	0.2354
A	Ahlat	9.6	1.8	11870	721.2	0.095	87.26	0.214
E	Edremit	10.2	2.0	9221	647.1	0.380	87.24	0.2564

3.2. Isolation and characterization of alkaliphilic bacteria

One hundred and twenty-four isolates were determined in different colors and morphology using low percentage of salt containing modified growth medium (MGM), alkaline nutrient agar, Horikoshi I and II medium, starch casein agar, actinomycetes isolation medium, Gauze agar and glycerol yeast extract agar medium. Approximately 1500 base pairs of bacterial 16S rRNA fragments were amplified with PCR analysis for 93 isolates from the shores of Van, Edremit, Ahlat and Tatvan. For these 93 isolates enzymatic restriction analysis were applied. When ARDRA results comprise for each gel individually; 6 different profiles from 18 isolates which was isolated from Van (Central), 7 different profiles from 17 isolates which was isolated from Ahlat, 12 different profiles from 34 isolates which was isolated from Edremit (**Hata! Başvuru kaynağı bulunamadı.**), 7 different profiles from 24 isolates which was isolated from Tatvan.

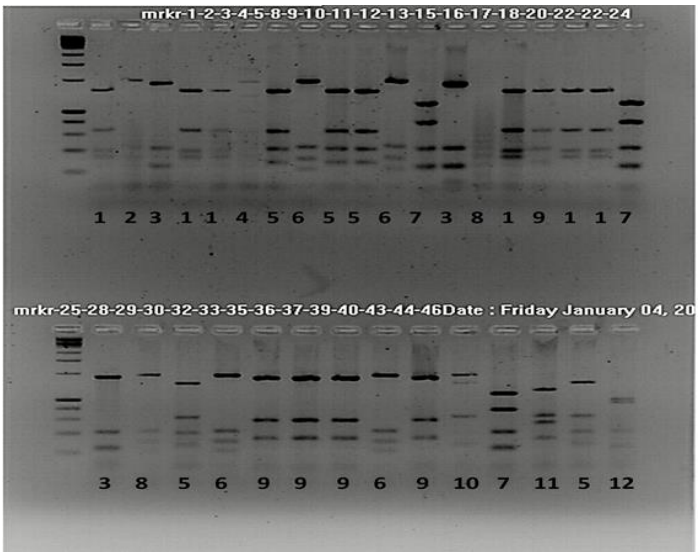

Figure 1. Gel image of ARDRA profiles from Edremit sampling region

The ARDRA profiles were found to be highly different when analyzing the samples. The nucleotide sequences of the purified PCR products were identified. 16S rRNA gene sequences were compared with reference sequences at NCBI using BLAST. BLAST results showed that, members of Proteobacteria and Actinobacteria were represented dominantly (Table 2). *Halomonas*, *Alkalimonas*, *Marinobacter*, *Rhodococcus* and *Vibrio* in Bacteria domain were detected as abundant species (**Hata! Başvuru kaynağı bulunamadı.**).

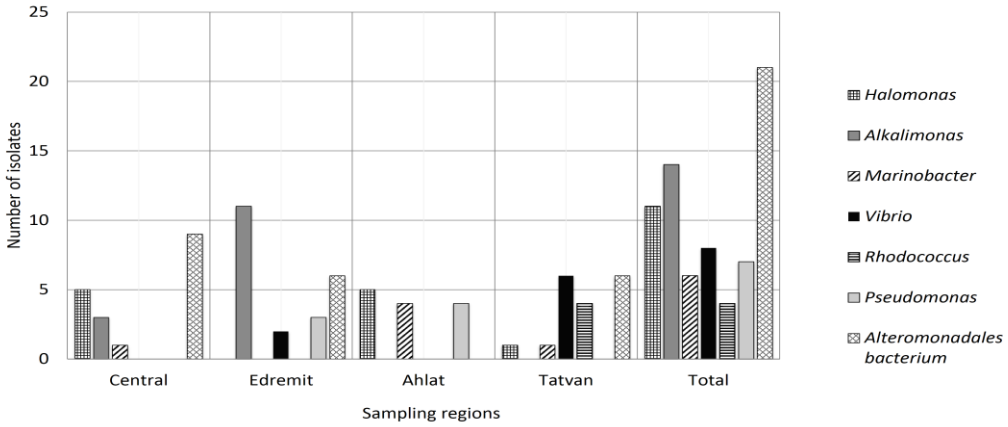

Figure 2. Bar chart of dominant isolates in Lake Van

In addition to these, *Rhodococcus sp.*, *Pseudomonas sp.*, *Rheinheimera sp.*, *Agrococcus sp.*, *Planococcus sp.*, *Belliella sp.*, and *Bacillus sp.* were obtained from results. Phylogenetic analysis was performed with sequences, and

partial sequences from analysis were used to build tree (Figure 4). Many of the isolates were classified under Proteobacteria phylum. (Table 2)

Table 2. Isolates and their closest matches in GenBank

Isolates	**Nearest neighbour**	**Similarity (%)**	**Accession**	**Source**	**Phylum**
M 7	*Halomonas sp.* 15-7 16S	99 (852/858)	HM598402.1	soda meadow saline soil	Proteobacteria(Gammaproteobacteria)
M 4	*Halomonas sp.* IB-559	99 (1416/1437)	AJ309560.1	high concentration of sodium nitrite	Proteobacteria(Gammaproteobacteria)
M 3	*Halomonas sp.* 10043	98 (1172/1190)	EU432575.1	mud volcano	Proteobacteria(Gammaproteobacteria)
M 5	*Marinobacter excellens* strain KMM	99 (1419/1432)	NR_025690.1	sediments of the sea	Proteobacteria(Gammaproteobacteria)
M 49	*Alkalimonas collagenimarina* strain AC40	98 (1424/1456)	NR_041515.1	deep-sea sediment	Proteobacteria(Gammaproteobacteria)
T 32	*Halomonas sp.* AMP-12	98 (1145/1178)	HM104378.1	activated sludge	Proteobacteria(Gammaproteobacteria)
T 35	Uncultured bacterium	99 (722/726)	FJ152919.1	alkaline saline soils	
T 52	*Marinobacter mobilis* strain B17	98 (724/744)	GQ214550.1	mangrove sediment	Proteobacteria(Gammaproteobacteria)
T 58	*Vibrio metschnikovii*	99 (764/772)	AB681962.1	culture collection	Proteobacteria(Gammaproteobacteria)
AT 28	*Rheinheimera sp. UDC526*	99 (723/725)	JQ895023.1	sea water	Proteobacteria(Gammaproteobacteria)
AT 33	*Rhodococcus sp.* BS-15	99 (690/698)	AB808578.1	sediment of the deep sea	Actinobacteria
AT 46	*Agrococcus jenensis* strain 1RN-3D2	99 (725/734)	EU379252.1	commercial airline cabin air	Actinobacteria
AT 49	*Rhodococcus sp.* A105-53B	98 (702/716)	KC422659.1	high arsenic groundwater sediment	Actinobacteria
AT 59	*Rhodococcus sp.* Dza15	99 (693/695)	JQ977264.1	rhizoplane	Actinobacteria
AT 63	*Rhodococcus sp.* D48	99 (684/689)	AY582940.1	deep-sea sediment	Actinobacteria
A 21	*Marinobacter sp.* LNM-5	99 (672/676)	AB758589.1	*Porphyra yezoensis*, red alga	Proteobacteria(Gammaproteobacteria)
A 23	*Gamma proteobacterium* E-116	97 (1154/1184)	FJ764790.1	haloalkaline lake	Proteobacteria(Gammaproteobacteria)
E 20	*Alkalimonas delamerensis* strain:1E1	99 (684/690)	NR_044879.1	soda lakes	Proteobacteria(Gammaproteobacteria)
E 36	*Gamma proteobacterium* O-010	99 (1029/1934)	DQ812540.1	seawater	Proteobacteria(Gammaproteobacteria)
E 40	*Nitrincola sp.* LAR05R9	99 (728/730)	JX945779.1	soda lakes	Proteobacteria(Gammaproteobacteria)
E 43	*Vibrio metschnikovii* strain SU1	98 (658/671)	HQ658055.1	textile effluent	Proteobacteria(Gammaproteobacteria)
2E 21	*Vibrio metschnikovii* strain SU1	99 (892/901)	HQ658055.1	textile effluent	Proteobacteria(Gammaproteobacteria)
2E 32	*Planococcus sp.* PP_B2A.1	99 (753/756)	KC250890.1	marine diatom	Firmicutes (Bacilli)
2T 30	*Bacillus okhensis* strain Kh10-101	98 (892/910)	NR_043484.1	saltpan	Firmicutes(Bacilli)
E 11	*Alkalimonas delamerensis* strain 1E1	98 (1019/1038)	NR_044879.1	soda lakes	Proteobacteria(Gammaproteobacteria)
AE 24	*Rheinheimera tuosuensis* strain TS-T4	97 (952/979)	NR_133840.1	saline lake water	Proteobacteria(Gammaproteobacteria)
AE 28	*Pseudomonas sp.* Ri83	98 (987/1005)	AM905940.1	groundwater from well	Proteobacteria(Gammaproteobacteria)
E 4	*Belliella sp.* LW3	99 (908/916)	FM956478.1	soda lake	Bacteroidetes/Chlorobi group (Bacteroidetes)
A 9	*Pseudomonas sp.* H-107	99 (954/959)	KF021789.1	marine bacteria	Proteobacteria(Gammaproteobacteria)

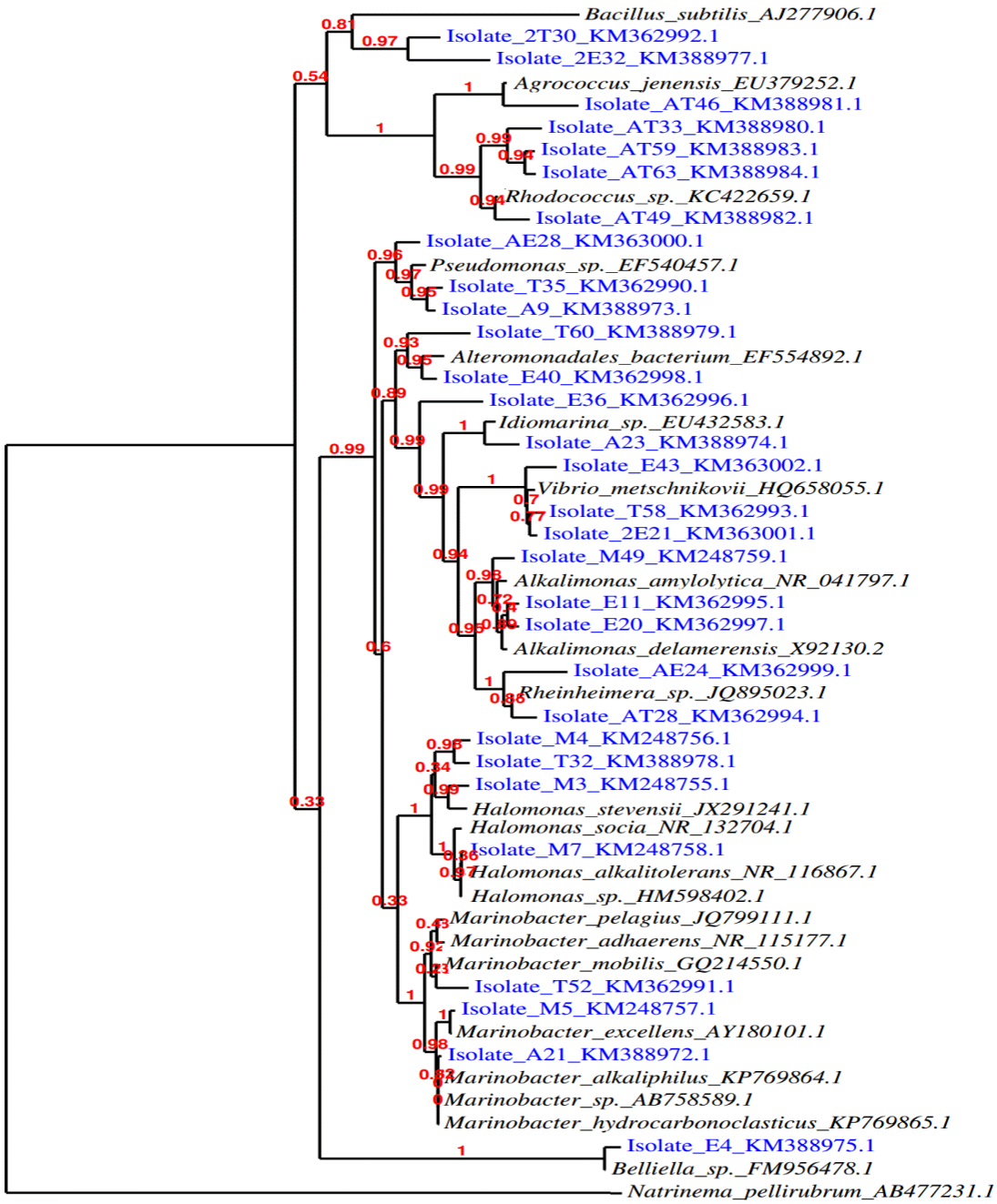

Figure 3. Phylogenetic tree based on 16S rRNA gene sequences from isolates belonging to Bacteria (indicated by blue colour). The sequence of *Natrinema pellirubrum* was selected as the outgroup. The horizontal scale bar shows the number of substitutions per site.

3.3. DGGE analysis

Amplicons obtained from environmental genomic DNA (water samples from Van Lake) were then analyzed by DGGE. Selected 18 bands were excised from the gel, re-amplified and 7 of these bands were sequenced. Denaturing gradient gel electrophoresis (DGGE) was performed to study population dynamics (**Hata! Başvuru kaynağı bulunamadı.**). As result of this gel in sequence analysis *Citrobacter sp.* and uncultured bacterium clones were identified (As shown in Table 3).When gel was evaluated some of bands were common in all samplings and some

patterns were represent dominantly, but all of bands were not able to sequence in detailed. For reason that was troubles in re-amplification of DGGE bands.

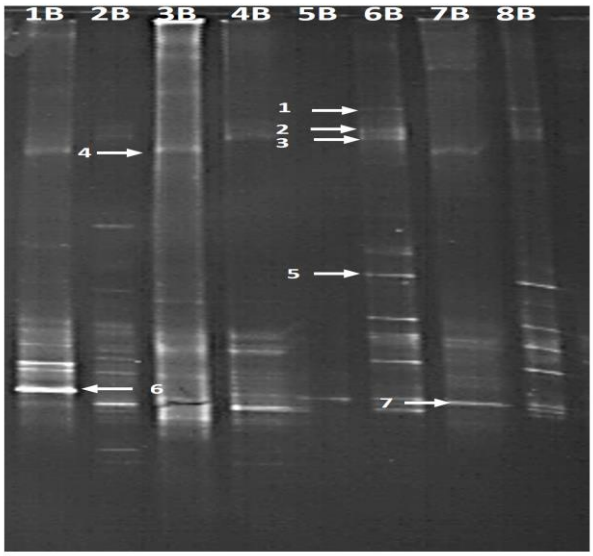

Figure 4. DGGE analysis of bacterial diversity in Lake Van samples (1B and 3B: Tatvan, 2B and 4B: Ahlat, 5B and 7B Van Central, 6B and 8B Edremit)

Table 3. DGGE bands and their closest similar sequences in GenBank

Bands(Bacteria)	Closest Relative in Gene Bank	Similarity %
1	*Citrobacter* sp. P074 16S ribosomal RNA gene, partial sequence (KC252814.1)	%95
2	*Citrobacter* sp. ChDC B352 16S ribosomal RNA gene, partial sequence (KF733678.1)	%99
3	*Kluyvera* sp. UIWRF0577 16S ribosomal RNA gene, partial sequence (KR190084.1)	%92
4	Uncultured bacterium clone SINP1135 16S ribosomal RNA gene, partial sequence (HM127404.1)	%94
	Loktanella vestfoldensis strain HME9315 16S ribosomal RNA gene, partial sequence (KF911340.1)	%93
5	Uncultured bacterium clone 5o46 16S ribosomal RNA gene, partial sequence (EU644798.1)	%95
	Flavobacteriaceae bacterium X15M-6 16S ribosomal RNA gene, partial sequence (KJ782428.2)	%93
6	Uncultured bacterium clone D11 16S ribosomal RNA gene, partial sequence (JF683447.1)	%74
	Polaribacter sp. NBRC 110213 16S ribosomal RNA gene, partial sequence (KM502317.1)	%73
7	Uncultured bacterium DGGE gel band C-2_Bac_a 16S ribosomal RNA gene, partial sequence (EF622424.1)	%95
	Bacteroidetes bacterium Omega 16S ribosomal RNA gene, partial sequence (KF830693.1)	%87

3.4. FISH analysis

After the fluorescence microscopy examination for FISH, DAPI stained cells and EUB338 bacteria probe hybridized cells were compared. EUB338 probe's positive signals obtained for Bacteria. Presence of the long and short rods, curved rods, spiral-shaped cells, coccus-shaped cells and spindle-shaped cells were detected in the samples (**Hata! Başvuru kaynağı bulunamadı.**- 11).

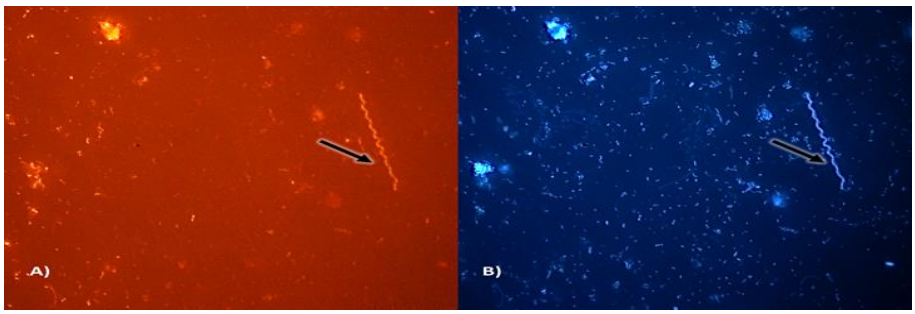

Figure 5.Visualization of microorganisms in Tatvan hybridizing with EUB338 (A) and DAPI (B)

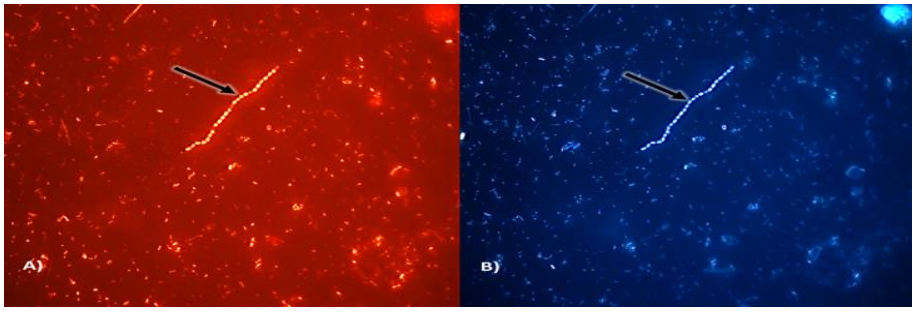

Figure 6.Visualization of microorganisms in Tatvan hybridizing with EUB338 (A) and DAPI (B)

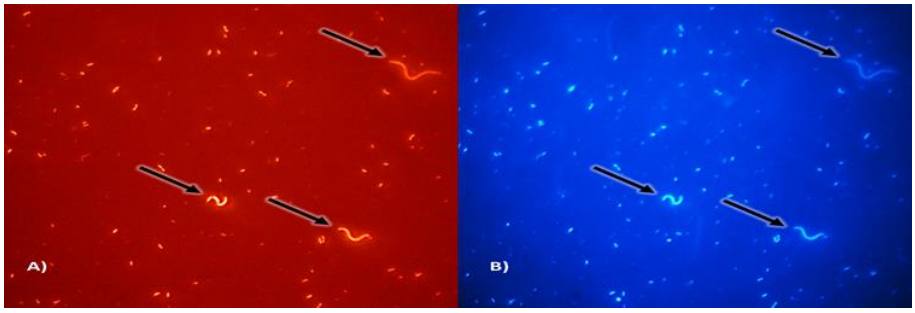

Figure 7. Visualization of microorganisms in Edremit hybridizing with EUB338 (A) and DAPI (B)

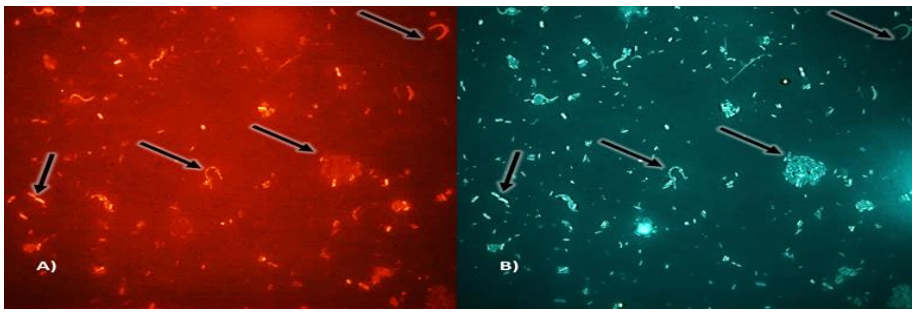

Figure 8. Visualization of microorganisms hybridizing in Edremit with EUB338 (A) and DAPI (B)

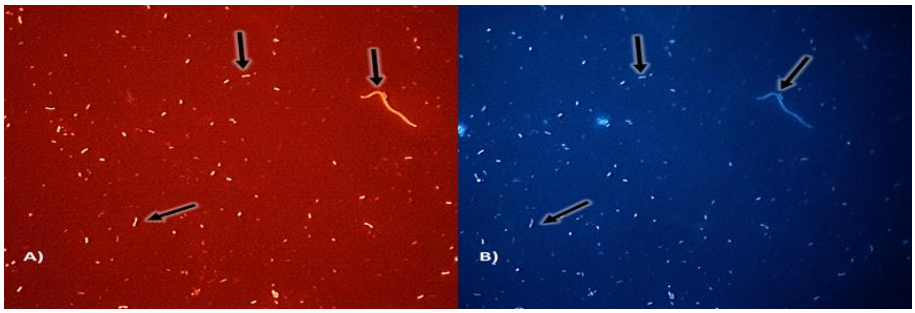

Figure 10. Visualization of microorganisms hybridizing in Ahlat with EUB338 (A) and DAPI (B)

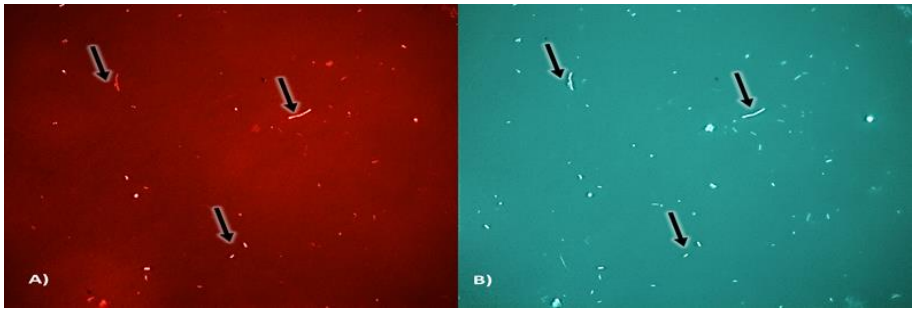

Figure 11. Visualization of microorganisms hybridizing in Van (Central) with EUB338 (A) and DAPI (B)

4. Conclusions and discussion

Soda lakes, particularly Eastern Rift Valley lakes (Kenya and Tanzania), Texcoco Lake (Mexico), Lonar Lake (India) and Kulunda Steppe soda lakes (Altai, Russia) have been the subjects of relatively detailed microbial diversity analysis (Kanekar et al., 2002; Jan-Roblero et al., 2004; Foti et al., 2008; Deshmukh et al., 2011; Mwirichia et al., 2011; Antony et al., 2012). However, until recently, the microbial diversity of the Lake Van has been limited. Early preliminary study from microbiolites of the Lake Van indicated some details for alkaliphilic bacterial diversity (López-García et al., 2005).

The results presented here indicated considerable microbial diversity of Lake Van. For these results, we used both culture dependent and culture independent studies. Culture-dependent techniques state of the sampling methods and culture conditions and these types of factors effect the range of species in the laboratory. 10 different media and incubation aerobically at 37°C were applied. The Gram-negative bacterial species were observed in Lake Van. The highest actively bacterial species were observed in Lake Van belonged to members of the Proteobacteria especially Gammaproteobacteria and the majority are related to *Halomonas* genus. Members of the genus *Halomonas* show growth different salt concentration and pH range so isolation from different environments can be easily. The other Gram-negative bacteria were *Alkalimonas, Marinobacter, Vibrio* and *Pseudomonas sp.* Previous studies have documented Proteobacteria isolates from other soda lakes (Jan-Roblero et al., 2004; López-García et al., 2005; Foti et al., 2008; Deshmukh et al., 2011; Mwirichia et al., 2011; Simachew et al., 2015; Matyugina and Belkova, 2015).

Gram-positive isolates were less diverse, a few Lake Van isolates related to the Actinobacteria, or high GC Gram positives. Most of them were related to environmental sequences and isolates from deep-sea sediment, high arsenic groundwater sediment, rainwater sample and sea water. Most abundant *Actinobacteria* genus was *Rhodococcus.* Many species can degrade most of environmental pollutants and transform or synthesize compounds for useful applications (Bell et al., 1998). *Rhodococcus* in soda lakes reported before in Lake Magadi, Kenya (Ronoh et al., 2013). Another species of the genus *Dietzia* isolated from Lake Van. *Dietzia* reported previously from soda lakes (Duckworth et al., 1996; Kanekar et al., 2002). The other Gram-positive bacteria were *Rheinheimera sp., Agrococcus sp., Planococcus sp.* and *Bacillus sp.*

These bacteria play important roles in biogeochemical cycles. The heterotrophic bacteria, can degrade organic matter which are produced by the autotrophic bacteria. *Bacillus sp., Dietzia sp.,* and *Alkalimonas* species were isolated from Lake Van. These bacteria are aerobic hydrolytics. Haloalkaliphiles were the most abundant groups isolated from soda lakes (Sorokin et al., 2014). *Halomonas* and *Actinobacteria* were abundant in Lake Van. They utilize monomeric organic compounds. Another important biogeochemical cycle is denitrification. Mostly salt-tolerant alkaliphilic *Halomonas* play key role in denitrification process in soda lakes (Sorokin et al., 2014). Our results showed that *Halomonas* was the most abundant genus and play key role in Lake Van's biogeochemical cycles. In addition Actinobacteria with role in nutrient cycling in their environment has ecological significance. For example, its role in bacterial nitrate reduction is important for the nitrogen cycle in the lake ecosystem. Organotrophic *Actinobacteria* form an important step between nitrogen and carbon cycle by using nitrate (Foti et al., 2008).

We used culture-independent techniques such as DGGE and FISH. An advantage of DGGE method is that DNA can be recovered from the gels and allow sequencing and identification of the population (Rees et al., 2004). DGGE method could be carried out only for the domain Bacteria. Some of bands were selected and recovered from the gel, re-amplified, and sequenced. Bacterial bands belong to *Citrobacter sp*. and uncultured DGGE bands. DGGE bands showed similarity some sequences obtained from meromictic soda lake (Soap Lake) in Washington State, soda lakes of the Kulunda Steppe (Altai, Russia) and Tibetian Lake (Qinghai Lake). *Citrobacter* isolation sources' are raw water and activated sludge. *Citrobacter* species are thought to be environmental contaminants and these species may be live intestinal tracts of man and animals. These bacteria can be isolated from soil, sewage, water and food (Nayar et al., 2014). As a similar to our results, *Citrobacter freundii* has been reported before in a study about phylogenetic diversity of soda lake alkaliphiles (Duckworth et al., 1996). Although these results, DGGE method in our study still needs optimizations for detection of alkaliphilic population dynamics. Also, fluorescently labeled rRNA-targeted oligonucleotide probes have become useful materials for culture-independent identification of bacteria. FISH can be used without the need to cultivation and define the microorganisms in an environment (Wagner et al., 2003). FISH analysis of samples from Lake Van indicated that Bacteria domain was abundant. After signal examination with Bacteria specific probe, the curved bacillus cells, long rods, cocci, vibrio and spirillum cells were observed in our samples.

In conclusion, with this work, we investigated alkaliphilic bacteria in Lake Van, Turkey and this study indicated industrial potential of cultures that could be used as efficient pigment, antibiotic producers and hydrocarbon degraders. Alkaliphilic bacterial group were detected based on comparative 16S rDNA analyses, culture-independent techniques DGGE and FISH. Although there are many limitations, culture-dependent approaches are still a prefered method of studying microbial ecology in natural environments. A conclusion based on culture-dependent methods can lead to the result that Proteobacteria and Actinobacteria members in Van Lake are active and play an important role in

the cycling of nutrients. These are seems to be dominant in Lake Van. Hybridization with Bacteria specific 16S rRNA-targeted oligonucleotides and DGGE methods help to demonstrate population dynamics. These results show that the diversity of alkaliphilic microorganisms in Lake Van is quite high and diverse. Future studies should aim to isolate members of the uncultured bacteria. In addition such as detection of functional genes and metagenomics can be applied to obtain detailed phylogenetic and physiological diversity of Lake Van.

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