



Biolog. Journal of Armenia, 3 (69), 2017

DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) PROFILES OF THE PARTIAL 16S rRNA GENES DEFINED BACTERIAL POPULATION INHABITING IN ARMENIAN GEOTHERMAL SPRINGS

H.H. PANOSYAN, A.A. MARGARYAN, A.A. TRCHOUNIAN

*YSU, Department of Biochemistry, Microbiology and Biotechnology
hpanosyan@ysu.am*

Denaturing gradient gel electrophoresis (DGGE) of the partial 16S rRNA gene PCR amplicons was used to profile bacterial populations inhabiting in Arzakan, Jermuk and Karvachar geothermal springs. The spring in Arzakan was colonized by chemolithotrophic and phototrophic primary producers related to phyla Betaproteobacteria, Gammaproteobacteria and Epsilonproteobacteria including. The spring in Jermuk was colonized by phylotypes related to sulfur and hydrogen oxidizing chemolithotrophs belonging to Epsilonproteobacteria, along with a diversity of Bacteroidetes, Spirochaetes, Ignavibacteriae and Firmicutes. Representatives of the phylum Firmicutes were the major component of the bacterial community of Karvachar geothermal spring. Most of the sequences derived from DGGE bands were closely related to uncultivated microorganisms and according to BLASTn analysis shared less than 97% similarity with their closest matches in GenBank, indicating that studied springs harbor a unique community including novel microbial species.

*Geothermal springs – bacterial community – 16S rRNA genes – DGGE –
sequence – BLASTn analysis*

16S ռՐՆԹ-ի գեների հատվածների ՊՇՌ-ամպլիկոնների դենատուրացնող գրադիենտային ժել էլեկտրաֆորեզով (ԴԳԺ) որոշվել է Արզականի, Ջերմուկի և Զարվաճառի երկրաջերմային աղբյուրների բակտերիական համակեցությունների կազմը: Արզականի երկրաջերմային աղբյուրում գերակշռում են Betaproteobacteria, Gammaproteobacteria և Epsilonproteobacteria ֆիլումներին պատկանող բեմոլիտոտրոֆ և ֆոտոտրոֆ առաջնային արտադրիչ բակտերիաները: Ջերմուկի երկրաջերմային աղբյուրում գերակշռող Epsilonproteobacteria ֆիլումին պատկանող ծծմբաջրածինը և ջրածինը օքսիդացնող բեմոլիտոտրոֆներից զատ հայտնաբերվել են Bacteroidetes, Spirochaetes, Ignavibacteriae և Firmicutes ֆիլումներին պատկանող ֆիլոտիպեր: Զարվաճառի երկրաջերմային աղբյուրի բակտերիական համակեցությունում գերակշռող են Firmicutes ֆիլումի ներկայացուցիչները: ԴԳԺ պրոֆիլներից ստացված սեքվենավորված հաջորդականությունների մեծամասնությունը համընկել են մանրէների չկուլտիվացվող ձևերի հետ, իսկ BLASTn վերլուծությամբ 97%-ից պակաս նմանություն են ցուցաբերել GenBank-ում առկա հաջորդականությունների հետ, ինչը վկայում է, որ ուսումնասիրված աղբյուրները բնակեցված են մանրէային նոր տեսակներ ներառող մենահատուկ համակեցություններով:

*Երկրաջերմային աղբյուրներ – բակտերիական համակեցություններ – 16S ռՐՆԹ-ի գեներ – ԴԳԺ –
սեքվենավորում – BLASTn վերլուծություն*

Денатурирующий градиентный гель-электрофорез (ДГГЭ) ПЦР-ампликонов фрагментов гена 16S рРНК использован для профилирования бактериальных популяций, обитающих в геотермальных источниках Арзакана, Джермука и Карвачара. Геотермальный источник Арзакан колонизирован представителями фил Betaproteobacteria, Gammaproteobac-

teria и Epsilonproteobacteria, включая хемолитотрофные и фототрофные первичные продуценты. Источник Джермук колонизирован филотипами, принадлежащими к серу и водород окисляющим хемолитотрофам, принадлежащими к филе Epsilonproteobacteria, а также с разнообразными Bacteroidetes, Spirochaetes, Ignavibacteriae и Firmicutes. Представители филы Firmicutes были основными компонентами бактериального сообщества геотермального источника Карвачар. Большинство секвенированных последовательностей, полученных из ДГГЭ профиля были тесно связаны с некультивируемыми микроорганизмами и по BLASTn анализу совпадали с ближайшими филогенетически сходными в GenBank менее 97%, что указывает на уникальное сообщество, включающее новые бактериальные виды в изучаемых источниках.

Геотермальные источники – бактериальное сообщество – гены 16S rPHK – ДГГЭ – секвенирование – BLASTn анализ

Among natural thermophilic environments, terrestrial geothermal springs are the most common and accessible biotopes. Terrestrial hot springs are primarily associated with tectonically active zones and widely distributed in various regions of Earth. These habitats have attracted broad interest since they are analogs for primitive Earth [25] and serve as source to isolate new thermophiles with unique properties. Thermostable enzymes synthesized by thermophilic microorganisms are active at harsh conditions and applied in many industrial processes [4, 20]. Phylogenetic characterization of microbiota has been extensively studied for geothermal springs located in Iceland [12], Azores [21], the United States [2], Bulgaria [26], Russia [13], China [8], India [22], Malaysia [3] and other parts of world. Between not well known ecological zones of the Earth, springs located in geothermal systems in the Minor Caucasus, still represent a challenge for searching of new phylotypes and unrevealed biotechnological resources. In Armenia, where traces of recently active volcanic processes are still noticeable, many geothermal springs with different geotectonic origins and physicochemical properties are found [1]. Armenian geothermal springs have been well characterized in terms of their geological and geochemical properties. Although some reports on the microbial diversity in hot springs located at different geographic areas of Armenia are available, its microbial community structure is still needs to be studied [7, 18, 19].

Culture-independent methods are at present considered the best tool to reflect the greater part of microbial community composition because of the existence of high numbers of as-yet-uncultured microorganisms. Molecular methods based on 16S rDNA such as PCR–DGGE fingerprinting method have been widely used to reveal dominant bacterial and archaeal populations of hot springs [6, 15, 23]. The clone library construction and sequencing are very time-consuming, while DGGE offers a more rapid method to evaluate microbial dominant populations in environmental samples.

The aim of this study was to examine the microbial community thriving at geothermal springs located on the territory of Armenia and Nagorno Kharabakh. PCR–DGGE fingerprinting method was applied to water/sediments mixer samples to obtain information about the occurrence of the dominant bacterial population. DGGE banding patterns were evaluated, and bacterial populations were identified by sequencing of the individual bands.

Materials and methods. Study sites and sample collection. Water/sediment samples were collected from three moderate temperature (42–70 °C) terrestrial geothermal (mesothermal) springs located on the territory of Armenia (Arzakan and Jermuk) and Nagorno Kharabakh (Karvachar). Water temperature, pH and conductivity were measured *in situ* during the sampling using a portable combined pH/EC/TDS/Temperature tester (HANNA HI98129/HI98130). Geographical locations and elevations of these springs were determined using a portable GPS (GERMIN 64s). The geothermal spring in Arzakan is located at 40°27'36.10" N, 44°36'17.76" E,

at 1490 m above sea level, with a temperature of 44 °C, pH 7.0-7.2, and conductivity of 4378.3 $\mu\text{S cm}^{-1}$. The spring in Jermuk is located at 39°50'47.90" N, 45°40'06.70" E, at 2100 m above sea level, with a temperature of 53 °C, pH 7.5, and conductivity of 4340 $\mu\text{S cm}^{-1}$. The spring in Karvachar is located in north Nagorno-Karabakh at 40°17'41.7" N, 46°27'50" E, at 1584 m above sea level, with a temperature 70 °C, pH 7.3 and conductivity 4600 $\mu\text{S cm}^{-1}$. Sediment samples were collected from a shallow part in the outlet of the spring using sterile flasks and were maintained on ice until processing.

DNA extraction. PCR amplification and DGGE analysis. DNA was extracted from water/sediments (0.5 g) within 12 h of using enzymatic digestion [14] and a sodium dodecyl sulfate (SDS) lysis procedure modified from the protocol of Dempster et al. [5]. The samples were suspended and incubated at 65 °C for 30 min in 9.5 ml TE buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA) containing 10 ml RNAase (10 mg ml⁻¹) prior to addition of 0.5 ml 10% SDS and 50 ml proteinase K (20 mg ml⁻¹) and incubation at 37 °C for 1 h. Subsequently, 0.1 times the volume of 3 M sodium acetate (pH 4.6) and cetyltrimethylammoniumbromide (CTAB) extraction buffer (10 % CTAB in 0.7 M NaCl) were added, and the mixture was incubated at 65 °C for 20 min. DNA was extracted from the suspension with an equal volume of chloroform:isoamyl alcohol (24:1 v/v). To the aqueous phase, an equal volume of isopropanol was added. The tube was inverted a few times and centrifuged. The DNA pellets were washed twice with 70 % ethanol, air-dried, and re-suspended in TE buffer. Resolution of extracts on a 0.7 % agarose gel containing 0.01 % GelRed was used to estimate DNA quantity and quality.

The bacterial community structure in the samples was studied using PCR-DGGE as described by [17]. The extracted DNA was used as templates for amplification of the V3 region of bacterial 16S rRNA gene sequences using primers L340F with CG clamp and K517R (Table 1).

Table 1. Name and sequences of oligonucleotide primers used in PCR amplification

GC^a – clamp: GCGCCCGCCGCGCGGGCGGGCGGGGCGGGGCACGGGGGG

Primer name	Sequence (5' - 3')	Target ^b
L340 F	CCTACGGGAGGCAGCAG	Bacterial 16S rRNA, pos. 340-356
L340 GC F	GC ^a CCTACGGGAGGCAGCA	
K517 R	ATTACCGCGGCTGCTGG	Bacterial 16S rRNA, pos. 535-517

^b – Position (pos.) according to the *E. coli* numbering.

Amplification mixtures were used with a final volume of 50 μl and contained 1 μl DNA (≥ 100 ng), 10 μl 5 × OneTaq Standard Reaction Buffer, 1 μl 10 mM dNTPs, 0.5 μM of each primer, 2 μl MgCl₂ (25 mM), 0.25 μl OneTaq DNA Polymerase (1.0 U; BioLabs, New England). Amplification of the V3 region of bacterial 16S rRNA gene was performed corresponded to an initial denaturation 94 °C for 3 min follow by 30 cycles of three steps: 94 °C for 1 min, 55 °C for 30 s and 68 °C for 1 min and final extension at 68 °C for 10 min. PCR products were viewed under UV light after standard gel electrophoresis and ethidium bromide staining.

The DGGE analysis of PCR products was performed using TV-400-DGGE System (Topac Inc., USA) with 8 % (w/v) polyacrylamide gel (37.5:1 acrylamide/bisacrylamide) in 0.5 × TAE (20 mM Tris-HCl, 10 mM Acetat, 0.5 mM EDTA) buffer and denaturants (100% denaturant contains 7 M urea and 40% deionized formamide). A denaturant gradient was 30-70 %. Electrophoresis was performed at a constant voltage of 20 V for 10 min, following by 200 V for 4 hours. DGGE gels were stained with SYBR[®]Gold (Invitrogen, USA) for 60 min and photographed on Gel DocXR system (Bio-Rad Laboratories). Most of the bands were excised from the gel. The DNA in the excised gel slices were incubated in 20 ml of MiliQ water at 4°C for 24 h and re-amplified by PCR with the mentioned above primer set. Before being sequenced, PCR products were purified with GenElute[™] PCR Clean-up Kit (Sigma) as specified by the manufacturer.

Sequencing and basic local alignment search tool (BLAST) analysis. Sequencing of amplicons of the DNA extracted from the DGGE gel was performed on ABI PRISM capillary sequencer according to the protocol of the ABI Prism BigDye Terminator kit (Perkin Elmer). The presence of chimeric sequences was determined using the DECIPHER web tool (<http://decipher.cee.wisc.edu/FindChimeras.html>) [28]. Raw data of DNA sequences were analyzed with program Chromas and BioEdit. Closest matches for partial 16S rDNA sequences

were identified by BLAST with nucleotide database in National Center for Biotechnology Information (NCBI; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Results and Discussion. PCR-DGGE based analysis of the extracted environmental DNA was done to provide a snapshot of the microbial communities' structure in the studied geothermal springs. PCR-DGGE allowed a rapid evaluation of the distributions of amplifiable sequence types. Individual DGGE bands were sequenced, and the sequences were compared with those in GenBank. DGGE patterns have shown the occurrence of complex bacterial communities in all analyzed samples (Fig. 1). BLASTn results of the partial bacterial 16S rRNA gene sequences extracted from the excised DGGE bands are listed in Table 2.

The largest number of the bands was observed in sample collected from Jermuk geothermal spring. Eight bacterial sequences obtained from the sediment/water samples of Jermuk geothermal spring were affiliated with the following bacterial taxonomical groups: Epsilonproteobacteria, Bacteroidetes, Spirochaetes, Ignavibacteriae and Firmicutes. The sequences from bands J-1, J-2, J-5, J-6 and A-2 showed similarity with those of the phylum Epsilonproteobacteria, retrieved from different environments, including deep-sea hydrothermal systems. Fragment J-3 showed almost 98 % similarity with an uncultured bacterium of the phylum Spirochaetes, and shared 90% similarity with *Exilispira thermophile*, an anaerobic, thermophilic spirochaete isolated from a deep-sea hydrothermal vent chimney [9]. The sequence of fragment J-4 showed similarity (98%) with *Melioribacter roseus*, a moderately thermophilic facultatively anaerobic organotrophic bacterium representing a novel deep branch within Bacteroidetes/Chlorobi group [10]. Another fragment, J-6, was affiliated with *Sulfurospirillum alkalitolerans*, an obligate haloalkalitolerant anaerobe formate and H₂ utilizing and thiosulfate/sulfur reducing epsilonproteobacterium [24]. The sequences of J-7 and J-8 bands were moderately (<95%) and closely (97%) similar to members of Firmicutes and Bacteroidetes, respectively. The sequence of J-8 band was affiliated with strictly anaerobic, mesophilic, carbohydrate-fermenting, hydrogen-producing bacterium *Acetobacteroides hydrogenigenes*, isolated from reed swamp [27].

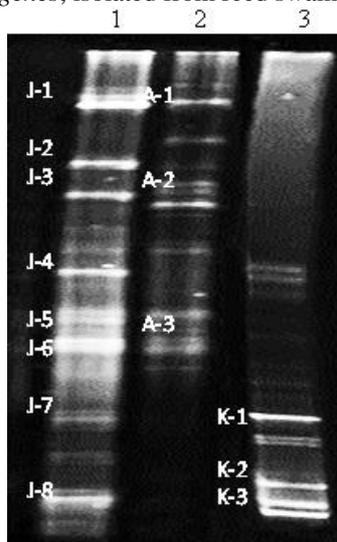


Fig. 1. Bacterial community profile determined with PCR-DGGE of partial 16S rRNA genes of the environmental DNA (1- Jermuk, 2- Arzakan, 3-Karvachar).

All bacterial sequences derived from Arzakan geothermal spring were close to Proteobacteria (affiliated with the Beta-, Epsilon- and Gammaproteobacteria). The sequence of A-1 band was shown 94 % similarity to *Rhodofera* sp., a phototrophic, purple nonsulfur betaproteobacterium. The sequence of A-2 band, affiliated with the phylum Epsilonproteobacteria, was shown 98 % similarity to *Sulfurimonas* sp., a hydrogen-oxidizing chemolithoautotrophic bacteria isolated from a rearing tank with dissolved hydrogen. The band A-3 showed 96 % similarity with *Pseudomonas* sp. As-33 (Gammaproteobacteria) isolated from wheat rhizosphere.

Representatives of the phylum Firmicutes were the major components in bacterial community of Karvachar geothermal spring. The sequences of K2 and K3 bands were 100 % identical to representatives of genus *Geobacillus* (*Geobacillus* sp. strain N7 and *G. kaustophilus* strain PS9). The sequence of K1 band, affiliated with the Bacteroidetes, was shown 94% similarity to *Flexibacter* sp., isolated from water environment.

Table 1. Blast results of bacterial 16S rRNA gene sequences derived from excised DGGE bands

Band	Seq. length (bp)	Closest match /Accession no./	Closest cultivated match /Accession no./	Identity, % Closest match/ Closest cultivated match	Phylogenetic affiliation
Jermuk					
J-1	157	Uncultured clone 3-2 /GQ324229/	<i>Arcobacter pacificus</i> /NR148627/	96/100	Epsilonproteobacteria
J-2	152	Uncultured clone 3-2 /GQ324229/	<i>Arcobacter</i> sp. /L42994/	95/98	Epsilonproteobacteria
J-3	122	Uncultured <i>Exilispira</i> sp. clone /KT757670/	<i>Exilispira thermophila</i> /NR041644/	98/90	Spirochaetes
J-4	154	Uncultured clone 88_C6 /KT985576/	<i>Melioribacter roseus</i> /NR074796/	100/98	Ignavibacteriae
J-5	138	Uncultured <i>Wolinella</i> sp. clone /GQ324220/	<i>Wolinella succinogenes</i> /KM462073/	95/94	Epsilonproteobacteria
J-6	172	Uncultured clone 1-16S-5C /JQ172713/	<i>Sulfurospirillum alkalitolerans</i> /NR108632/	93/93	Epsilonproteobacteria
J-7	148	Uncultured clone PS1B_0085 /KU830682/	<i>Fastidiosipila sanguinis</i> /KJ419955/	95/91	Firmicutes
J-8	173	Uncultured clone 3-12 /GQ324232/	<i>Acetobacteroides hydrogigenes</i> /NR133950/	97/97	Bacteroidetes
Arzakan					
A-1	186	Uncultured DGGE band EA-5 /FJ406515/	<i>Rhodofera</i> sp. /KU569902/	95/98	Betaproteobacteria
A-2	132	Uncultured clone 3-2 /GQ324229/	<i>Sulfurimonas</i> sp. /AB930173/	98/100	Epsilonproteobacteria
A-3	153	Uncultured isolate 10-2 /LN875311/	<i>Pseudomonas</i> sp. /KC906191/	98/96	Gammaproteobacteria
Karvachar					
K-1	134	Uncultured clone B_OTU_1064 /KX031046/	<i>Flexibacter</i> sp. /EU273858/	100/94	Bacteroidetes
K-2	133	<i>Geobacillus</i> sp. N7 /KU291217/	<i>Geobacillus</i> sp. /KU291217/	100	Firmicutes
K-3	131	<i>Geobacillus kaustophilus</i> /KY883609/	<i>Geobacillus kaustophilus</i> /KY883609/	100	Firmicutes

Although most of the retrieved sequences are similar to uncultured Bacteria, some of them are phylogenetically associated with environmental clones obtained from similar thermal habitats. Most of the detected bacteria seem to be mesophilic or moderately thermophilic. The optimum growth temperature of the closest cultivated relatives to the microorganisms detected in DGGE profile suggested that they are likely able to grow at reservoir temperature and, therefore, should not be regarded as contaminants.

Bacterial richness was relatively higher in the samples of warmer geothermal springs (Jermuk and Arzakan). Probably high temperature of Karvachar geothermal spring (70 °C) was the limiting factor of bacterial population. Besides thermal conditions, the limiting factor for microbial diversity and biomass may be a combination of abiotic factors including pH and high mineralization. The Jermuk spring is circumneutral (pH 7.5), classified as a $\text{HCO}_3^-/\text{SO}_4^{2-}/\text{Na}^+$ -type spring, and has a relatively high dissolved mineral content ($4340 \mu\text{S cm}^{-1}$) [1]. That is why it is not surprising detection of obligate haloalkalitolerant anaerobic thiosulfate/sulfur reducing Epsilonproteobacteria in Jermuk geothermal spring. Also, a sequence related to *Arcobacter* sp., a sulphide-oxidiser epsilonproteobacterium, was recovered. These microbes involved in the chemosynthetic primary production and in the turnover of the reduced sulphur compounds. In contrast to Jermuk, in Arzakan geothermal spring the primary production is supported by representatives of purple nonsulfur anoxygenic phototrophs (like *Rhodospirillum rubrum* sp.) and hydrogen- and sulfide-oxidizing chemolithoautotrophs (like *Sulfurimonas* sp.). It was shown earlier that moderate-temperature geothermal systems cool enough to permit phototrophy at the source with neutral or alkaline pH are often colonized by visible microbial growth that forms laminated mats or streamers dominated by phototrophic bacteria [11]. Relatively low temperature (44°C) and neutral pH in Arzakan spring can support growth of phototrophic bacteria due to obvious light effect in the outlet of the spring.

According to the DGGE profile, the Karvachar geothermal spring was colonized mainly by geobacilli. Representatives of genus *Geobacillus* were the most abundant obligate thermophiles most frequently isolated from hot springs [16]. Having large metabolic potential, thermophilic, aerobic, endospore-forming bacteria represent the major components of bacterial saprotrophic complexes and are involved in the aerobic degradation of autochthonous and allochthonous organic matters.

Heterotrophic microorganisms detected in DGGE profile were clustered among the fermentative microorganisms, which are actively involved in C-cycle.

The molecular phylogenetic approach applied in this study has several potential biases (e.g., preferential PCR amplification, different susceptibility to the cell lysis procedures, contamination by non-indigenous strains), which need to be acknowledged during data interpretation. In addition, the number of bands is not large enough to fully represent the microbial community. However, although the results do not provide a complete quantitative picture of microbial diversities, they do provide a reliable first estimate of the microbial community structures.

Most of the obtained sequences share less than 97% similarity with uncultured bacterial clones. The sequences recovered in these studies revealed the presence of unknown microorganisms only distantly related to known isolates that suggests the existence of as-yet-undetected microbial assemblages in these ecosystems. Such studies are required not only to understand the microbial communities that are native to the hot springs, but also to further identify novel taxa.

This study is one of few published studies focusing on Bacteria in mesothermal systems located in the Minor Caucasus. Based on PCR-DGGE profile it was shown that studied springs were colonized by microorganisms belonging to the phyla Betaproteobacteria, Gammaproteobacteria, Epsilonproteobacteria, Bacteroidetes, Spirochaetes, Ignavibacteriae and Firmicutes. Most of the obtained bacterial sequences shared less than 97% identity with their closest match in GenBank indicating a unique community of studied environments. Bacterial communities described here include a high diversity and inferred abundance of yet uncultivated microorganisms and justify continued synergy of cultivation-dependent and -independent studies to better understand the microbiology of moderate-temperature geothermal systems.

ACKNOWLEDGMENT

The work was supported by Research Grant from State Committee of Science, Ministry of Education and Science of Armenia, to HP (15T-1F399), Armenian National Science and Education Fund based in New York, USA, to HP (ANSEF-NS-microbio 3362 and 4676) and grant from the Norwegian Cooperation Program in Higher Education with Eurasia (CPEA-2011/10081).

REFERENCES

1. Геология Армянской ССР, IX. Минеральные воды. Ереван, Изд. АН Арм. ССР, 1969.
2. Bowen De León K., Gerlach R., Peyton B.M., Fields M.W. Archaeal and bacterial communities in three alkaline hot springs in Heart Lake Geysir Basin, Yellowstone National Park. *Front. Microbiol.*, 4, 330, 2013.
3. Chan C.S., Chan K.-G., Tay Y.-L., Chua Y.-H., Goh K.M. Diversity of thermophiles in a Malaysian hot spring determined using 16S rRNA and shotgun metagenome sequencing. *Front. Microbiol.*, 6, p. 177, 2015.
4. DeCastro M.E., Rodríguez-Belmonte E., González-Siso M.I. Metagenomics of thermophiles with a focus on discovery of novel thermozymes. *Front. Microbiol.*, 7, p. 1521, 2016.
5. Dempster E.L., Pryor K.V., Francis D., Young J.E., et al. Rapid DNA extraction from ferns for PCR-based analyses. *Biotechniques*, 27, p. 66-68, 1999.
6. Ferris M.J., Muyzer G., Ward D.M. Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined populations inhabiting a hot spring microbial mat community. *Appl. Environ. Microbiol.*, 62, 2, p. 340-346, 1996.
7. Hedlund B.P., Dodsworth J.A., Cole J.K., Panosyan H.H. An integrated study reveals diverse methanogens, Thaumarchaeota, and yet-uncultivated archaeal lineages in Armenian hot springs. *Antonie van Leeuwenhoek*, 104, 1, p. 71-82, 2013.
8. Hou W., Wang S., Dong H., Jiang H., Briggs B.R., Peacock J.P., Huang Q., Huang L., Wu G., Zhi X., Li W., Dodsworth J.A., Hedlund B.P., Zhang C., Hartnett H.E., Dijkstra P., Hungate B.A. A Comprehensive census of microbial diversity in hot springs of Tengchong, Yunnan province China Using 16S rRNA Gene Pyrosequencing. *PLoS ONE*, 8, 1, e53350, 2013.
9. Imachi H., Sakai S., Hirayama H., Nakagawa S., Nunoura T., Takai K., Horikoshi K. *Exilispira thermophila* gen. nov., sp. nov., an anaerobic, thermophilic spirochaete isolated from a deep-sea hydrothermal vent chimney. *Int. J. Syst. Evol. Microbiol.*, 58, 10, p. 2258-65, 2008.
10. Kadnikov V.V., Mardanov A.V., Podosokorskaya O.A., Gavrillov S.N., Kublanov I.V., Beletsky A.V., Bonch-Osmolovskaya E.A., Ravin N.V. Genomic analysis of *Melioribacter roseus*, facultatively anaerobic organotrophic bacterium representing a novel deep lineage within Bacteroidetes/Chlorobi group. *PLoS One.*, 8 (1):e53047, 2013.
11. Klatt C.G., Wood J.M., Rusch D.B., Bateson M.M., Hamamura N., Heidelberg J.F., Grossman A.R., Bhaya D., Cohan F.M., Kuhl M., Bryant D.A., Ward D.M. Community ecology of hot spring cyanobacterial mats: predominant populations and their functional potential. *ISME J.*, 5, p. 1262-1278, 2011.
12. Krebs J.E., Vaishampayan P., Probst A.J., Tom L.M., Marteinsson V.T., Andersen G.L., Venkateswaran K. Microbial community structures of novel Icelandic hot spring systems revealed by PhyloChip G3 analysis. *Astrobiology*, 14, 3, p. 229-40, 2014.
13. Kublanov I.V., Perevalova A.A., Slobodkina G.B., Lebedinsky A.V., Bidzhieva S.K., Kolganova T.V., Kaliberda E.N., Rumsh L.D., Haertle T., Bonch-Osmolovskaya E.A. Biodiversity of thermophilic prokaryotes with hydrolytic activities in hot springs of Uzon caldera, Kamchatka (Russia). *Appl. Environ. Microbiol.*, 75, 1, p. 286-291, 2009.
14. Marmur J. Procedure for isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.*, 3, p. 208-218, 1961.

15. *Maugeri T.L., Lentini V., Gugliandolo C., Italiano F., Cousin S., Stackebrandt E.* Bacterial and archaeal populations at two shallow hydrothermal vents off Panarea Island (Eolian Islands, Italy). *Extremophiles*, 13, 1, p. 199-212, 2009.
16. *McMullan G., Christie J.M., Rahman T.J., Banat I.M., Ternan N.G. and Marchant R.* Habitat, applications and genomics of the aerobic, thermophilic genus *Geobacillus*. *Biochemical Society Transactions*, 32, 2, 2004.
17. *Muyzer G., De Waal E.C., Uitterlinden, A.G.* Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.*, 59, p. 695-700, 1993.
18. *Panosyan H., Birkeland N.K.* Microbial diversity in an Armenian geothermal spring assessed by molecular and culture-based methods. *J. Basic Microbiol.*, 54, p. 1240-1250, 2014.
19. *Panosyan H.H.* Phylogenetic diversity based on 16S rRNA gene sequence analysis of aerobic thermophilic endospore-forming bacteria isolated from geothermal springs in Armenia. *Biolog. J. Armenia*, 62, 4, p. 73-80, 2010.
20. *Raddadi N., Cherif A., Daffonchio D., Neifar M., Fava F.* Biotechnological applications of extremophiles, extremozymes and extremolytes. *Appl. Microbiol. Biotechnol.*, 99, 19, p. 7907-13, 2015.
21. *Sahm, K., John, P., Nacke, H., Wemheuer, B., Grote, R., Daniel, R., et al.* High abundance of heterotrophic prokaryotes in hydrothermal springs of the Azores as revealed by a network of 16S rRNA gene-based methods. *Extremophiles*, 17, p. 649-662, 2013.
22. *Singh A., Subudhi E.* Profiling of microbial community of Odisha hot spring based on metagenomic sequencing. *Genomics Data*, 7, p. 187-188, 2016.
23. *Sharma A., Jani K., Shouche Y. S., Pandey A.* Microbial diversity of the Soldhar hot spring, India, assessed by analyzing 16S rRNA and protein-coding genes. *Annals of Microbiology*, 65, p. 1323-1332, 2014.
24. *Sorokin D.Y., Tourova T.P., Muyzer G.* Isolation and characterization of two novel alkalitolerant sulfidogens from a Thiopaq bioreactor, *Desulfonatronum alkalitolerans* sp. nov., and *Sulfurospirillum alkalitolerans* sp. nov. *Extremophiles*, 17, 3, p. 535-43, 2013.
25. *Stan-Lotter H., Fendrihan S.* *Adaption of Microbial Life to Environmental Extremes.* Wien, Springer-Verlag, 2012.
26. *Stefanova K., Tomova I., Tomova A., Radchenkova N., Atanassov I., Kambourova M.* Archaeal and bacterial diversity in two hot springs from geothermal regions in Bulgaria as demonstrated by 16S rRNA and GH-57 genes. *International Microbiology*, 18, p. 217-223, 2015.
27. *Su X.L., Tian Q., Zhang J., Yuan X.Z., Shi X.S., Guo R.B., Qiu Y.L.* *Acetobacteroides hydrogenigenes* gen. nov., sp. nov., an anaerobic hydrogen-producing bacterium in the family Rikenellaceae isolated from a reed swamp. *Int. J. Syst. Evol. Microbiol.*, 64, 9, p. 2986-91, 2014.
28. *Wright E.S., Yilmaz L.S., Noguera D.R.* DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences. *Appl. Environ. Microbiol.*, 78, 3, p. 717-725, 2012.

Received on 11.04.2017