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(կենսաբանություն և քիմիա)

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STUDY OF BACTERIAL DIVERSITY OF KARVACHAR GEOTHERMAL SPRING USING CULTURE-INDEPENDENT METHODS

Introduction. Thermophiles are organisms growing at elevated temperatures. They are generally and somewhat arbitrarily separated into two general categories based on their cardinal growth temperatures: thermophiles (T_{opt}>45^{o}C), and hyperthermophiles (T_{opt}>80^{o}C) [Madigan et al., 2012].

The habitats of thermophilic microbes are high temperature environments including terrestrial geothermal springs. The mostwell-known and biologically most studied terrestrial geothermal springs are in North America (Yellowstone National Park) [Meyer-Dombard et al., 2005], the Kamchatka Peninsula [Bonch-Osmolovskaya et al., 1999], and Iceland [Marteinsson et al., 2001]. Extremophiles inhabiting hot springs are considered to be the closest living descendants of the earliest life forms on Earth [Stan-Lotterand Fendrihan, 2012]. In addition, thermophiles and hyperthermophiles produce a variety of hydrolytic enzymes such as lipases, glycosidase, peptidase and other biomolecules, which are of industrial interest [Li et al., 2005; Antranikian and Egorova, 2007].

It is difficult to detect most of microorganisms in extreme environments by cultivation-based methods. Therefore, culture-independent metagenomic strategies allow approaches to assess the phylogenetic composition and functional potential of microbial communities living in such environments [Amann et al, 1995].

Numerous hot springs have been discovered and studied in Armenia and Nagorno-Karabakh, microbiota of which can provide a valuable source for biotechnology. However, our knowledge of microbial community structure of many of them is still scarce [Mkrtchyan, 1969; Panosyan, 2006]. The present study regards the diversity of the bacteria of Karvachar (Nagorno-Karabakh) geothermal spring based on culture-independent methods.

Materials and methods. The primary methods used by us are *sampling, physical-chemical measurements and isolation of total environmental DNA.* The location of Karvachar geothermal spring was determined using GPS. The water temperature and pH were measured *in situ* using portative combined pH/EC/TDS/Temperature tester (HANNA HI98129/HI98130). Sediment samples were aseptically collected and transferred in sterile flasks keeping on ice until being processed. Sampling and physical-chemical measurements were performed in May 2013. The PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc.) was used to extract microbial total DNA from sediment samples according to the manufacture's recommendations. Extracted environmental DNA was used as a template for polymerase chain reaction (PCR).

Amplification of DNA. The 16S rRNA genes were amplified with 16SF 5'-GAGTTTGATCCTGGCTCAG-3' and 16SR 5'-GAAAGGAGGTGATCCAGCC-3' primers using DNA Engine Peltier Thermal Cycler (Bio-Rad). Amplification was performed under the following conditions: initial denaturation at 95ºC for 5 min, followed by30 cycles of denaturation at 94ºC for 1 min, annealing at 55ºC for 30 s and extension at 72ºC for 30 s, with final extension at 72ºC for 10 min [Kowalchuk et al., 2004]. The reactions were subsequently cooled at 4^{o}C.The PCR products were visualized by gel-electrophoresis. PCR products were purified using GeneElute^{TM} PCR Clean-Up Kit (Sigma) according to the manufacture's recommendations**.**

Clone library construction and sequencing. PCR products were cloned into *E. coli* chemically competent cells using CloneJET PCR Cloning Kit (Ferments) according to the manufacture's recommendations. The plasmids were purified using GenElute Plasmid Miniprep Kit (Sigma). The presence of inserted genes was observed by 0.8% agarose gel-electrophoresis. Sequencing of rDNA clones has been performed on ABI PRISM capillary sequencer according to the protocol of the ABI Prism BigDye Terminator Kit (Perkin Elmer). Closest matches of 16SrDNA sequences were identified by basic local alignment tool (BLAST) [Altschul et al., 1997]. Obtained sequences were manually grouped into operational taxonomic units (OTU).

Results and discussion. Karvachar geothermal spring is located at N 40.17417° E 46.27500°, with temperature of 70 ºC, pH 7.3 and conductivity of 4600 μSsm^{-1}. The environmental DNA was successfully isolated and 16S rRNA genes were amplified (Fig. 1). Then the clone library was constructed.

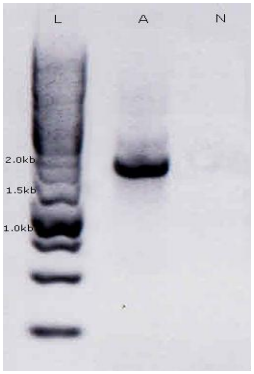

Figure 1. *Gel-electrophoresis of PCR product of 16SrRNA genes: L- DNA size marker, A- PCR product, N- negative control*

More than 100 colonies were picked and the plasmids were extracted. The plasmids with correct inserts (Fig. 2) were analyzed further. A total of 36 clones were initially sequenced with forward directions.

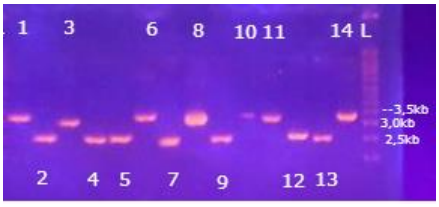

Figure 2. *Gel-electrophoresis of some plasmids: L- DNA size marker; 1,3,6,8,10,11,14 - plasmids with correct insert; 2,4,5,7,9,12,13 - plasmids without insert or containing incorrect insert*

Obtained phylotypes share 83-98 % similarity with known cultivated species and 89-99 % similarity with uncultured phylotypes represented in GenBank database (Table 1). Most of OTUs are represented only by one clone. The level of similarity of obtained phylotypes with cultivated species and uncultured phylotypes are mainly less than 94% and 96 % respectively. This is an evidence of unique composition of Karvachar geothermal spring microbiota.

Analyzed sequences originate from phyla Proteobacteria (53%), Cyanobacteria (28%), Bacteroidetes (5%), Planctomycetes (3%), Verrucomicrobia (3%), Chloroflexi (5%) and unclassified phylotypes (3%) (Fig. 3). The higher presence of Gram negative bacteria was observed. The dominating bacterial group was the phylum Proteobacteria. Earlier, it was

shown that Proteobacteria dominate in similar aquatic systems [Cole et al., 2013]. A few phylotypes belonging to the phylum Bacteroidetes were obtained, while representatives of some phyla such as phylum Firmicutes, were not detected. One of the dominating groups was Cyanobacteria, representatives of which dominate especially on top layer of microbial mats and are the most important primary producers in hot spring ecosystems [Roeselers et al., 2007].

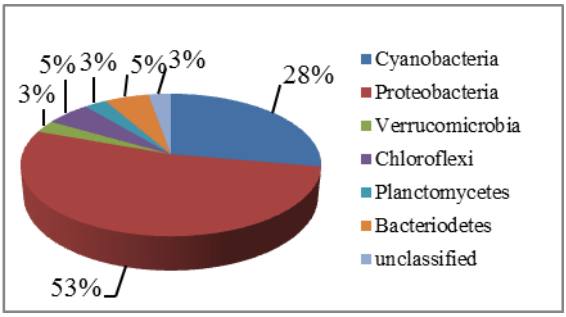

Figure 3. *Pie chart showing phylum level distribution of bacterial phylotypes In Karvachar geothermal spring*

Table 1.

BLAST results of closest relatives of clones obtained from the bacterial 16S rRNA genes

Phylum	OTU	Number of clones in OTU	Most closest match	Most closest species or genus	Similarity with closest match/species (%)
Cyanobacteria	Karv_33	4	Uncultured cyanobacterium clone 9B-12 JX298772	*Oscillatoriales cyanobacterium* KC463177	96/93
	Karv_65	3	Uncultured bacterium clone JW56-B011 HQ287189	*Leptolyngbya* sp. DQ431002	99/88
Verrucomicrobia	Karv_05	1	Uncultured bacterium clone FGL7S_B115 FJ438007	*Luteolibacter* strain A5J-40 AB331894	96/94
Cyanobacteria	Karv_42	3	Uncultured cyanobacterium clone 9B-12 JX298772	*Leptolyngbya scottii* KC463191	92/90
Chloroflexi	Karv_60	2	Uncultured Chloroflexi bacterium clone A379 EU283567	Anaerolineae bacterium SW7 AB669272	91/83
Proteobacteria	Karv_59	2	Uncultured bacterium clone sh-xj96 JQ327992	*Rehaibacterium terrae* KC999854	99/95
	Karv_34	2	Uncultured bacterium clone INDI_S_SPR_05A KF836161	*Pelobacter propionicus* NR074975	94/88
Planctomycetes	Karv_23	1	Uncultured Planctomycetales bacterium clone TUM-Mbac-B1-K2-116 EU812984	*Planctomycete* sp. JF443763	98/95
Proteobacteria	Karv_25	1	Uncultured Azoarcus sp. clone A1230 EU283470	*Betaproteobacterium* DQ168643	99/98
	Karv_26	1	Uncultured bacterium clone SSPF_8F_a04 KC713071	*Geothermobacter ehrlichii* NR042754	99/81
	Karv_29	1	Uncultured bacterium clone sh-xj96	*Stenotrophomonas maltophila*	96/92

Phylum	OTU	Number of clones in OTU	Most closest match	Most closest species or genus	Similarity with closest match/species (%)
			JQ327992	KF724885	
	Karv_31	1	Uncultured bacterium clone SINP624 HM127593	Gammaproteobacterium JX981925	98/96
	Karv_32	1	*Methylococcus capsulatus* NR074213	*Methylococcus capsulatus* NR074213	91/91
	Karv_38	1	Uncultured bacterium clone SLE33F GU390196	*Thauera mechernichensis* NR026473	97/97
	Karv_43	1	Uncultured bacterium clone AN1C1CE03 JQ426750	*Brevundimonas kwangchunensis* HF570075	94/93
Bacteroidetes	Karv_46	1	Uncultured soil bacterium clone 3-4 JN417538	*Adhaeribacter terreus* NR044551	93/93
	Karv_45	1	Uncultured Bacteroidetes bacterium cloneZac7152 FJ485124	Bacteroidetes bacterium AY162091	93/84
Proteobacteria	Karv_53	1	Gammaproteobacterium DQ812540	Gammaproteobacterium DQ812540	92/92
	Karv_55	2	Uncultured bacterium clone Bac_SB_78 JQ739128	*Roseinatronobacter monicus* DQ659237	98/97
	Karv_56	1	Uncultured bacterium clone A36 KC442847	*Phyllobacteriumendophyticum* NR109517	90/89
	Karv_62	1	*Roseinatronobactermonicus* NR043914	*Roseinatronobacter monicus* NR043914	98/98
	Karv_75	1	Uncultured bacterium clone: HDBW-WB09 AB237672	*Caldimonas hydrothermale* AM283038	89/87
	Karv_80	1	Uncultured bacterium clone AN1C2BD04 JQ428170	*Saccharophagus* sp. KF022099	92/93
	Karv_81	1	Uncultured sludge bacterium S10 AF234762	*Azoarcus* sp. AF011329	90/89
unclassified	Karv_76	1	Uncultured bacterium clone ncd2327c12c1 JF199240	Bacterium LY17 KC921146	93/87

Thus, the taxonomic groups detected in Karvachar geothermal spring are quite diverse. The microbiota of this geothermal spring plays an important role in biochemical cycles of biogenic elements and probably has a huge impact on physical-chemical characteristics of spring water. Therefore, detailed investigations of bacterial community structure using both culture-independent and culture-based methods, could allow isolating new thermophilic bacteria with biotechnological potential.

References

1. **Altschul S., Madden T., Schaffer A., Zhang J., Zhang Z., Miller W., Lipman D.,** Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research, (1997) 25, 3389-3402.
2. **Amann R., Ludwig W., Schleifer K.,** Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiol. Rev., (1995) 59, 143-169.
3. **Antranikian G., Egorova K.,** Extremophiles, a Unique Resource of Biocatalysts for Industrial Biotechnlology: Physiology and Biochemistry of Extremophiles. Ed. by Ch. Gerday and N. Glansdorff, ASM Press, Washington D.C., (2007), 361-406.

4. **Bonch-Osmolovskaya E., Miroshnichenko M., Slobodkin A., Sokolova T., Karpov G., Kostrikina N., Zavarzina D., Prokof'eva M., Rusanov I., Pimenov N.,** Biodiversity of anaerobic lithotrophic prokaryotes in terrestrial hot springs of Kamchatka. Microbiology, (1999) 68, 343-351.
5. **Cole J., Peacock J., Dodsworth J., Williams A., Thompson D., Dong H., Wu G., Hedlund B.,** Sediment microbial communities in Great Boiling Spring are controlled by temperature and distinct from water communities. The ISME Journal, (2013) 7, 718-729.
6. **Kowalchuk G., de Bruijn F., Head I., Akkermans A., van Elsas D.,** Molecular Microbial Ecology Manual, Second Edition. Kluwer Academic, (2004) V.1, 517-519.
7. **Li W., Zhou X., Lu P.,** Structural features of thermozymes. Biotechnol. Adv., (2005) 23, 271-281.
8. **Madigan M., Martino J., Clark D.,** Brock Biology of Microorganisms (13th Ed.) Pearson, (2006) 136.
9. **Marteinsson V., Hauksdottir S., Hobel C., Kristmannsdottir H., Hreggvidsson G., Kristjansson J.,** Phylogenetic diversity analysis of subterranean hot springs in Iceland. Appl. Environ. Microbiol, (2001) 67, 4242-4248.
10. **Meyer-Dombard D., Shock E., Amend J.,** Archaeal and bacterial communities in geochemically diverse hot springs of Yellowstone National Park, USA, Geobiology, (2005) 3, 211-227.
11. **Mkrtchyan S.,** (Ed.) Geology of Armenian SSR, Publishing house of AS of ASSR, Yerevan, (1969) V. IX, 521 (in Russian).
12. **Panosyan H.,** Diversity of Extremophilic Bacilli Isolated From Terrain Sites of Geothermal Anomalies and Hot Springs of Armenia**.** Book of Abstract: The 6th International Congress on Extremophiles. Brest, Brittany, France, (2006), 154.
13. **Roeselers G., Norris T., Castenholz R., Rysgaard S., Glud R., Kühl M., Muyzer G.,** Diversity of phototrophic bacteria in microbial mats from Arctic hot springs (Greenland). Environmental Microbiology, (2007) 9 (1), 26-38.
14. **Stan-Lotter H., Fendrihan S.,** Adaption of Microbial Life to Environmental Extremes: Novel Research Results and Application. Springer, (2012) 296.

Անի Սաղաթելյան, Հովիկ Փանոսյան

ՔԱՐՎԱՃԱՌԻ (ԼԵՌՆԱՅԻՆ ՂԱՐԱԲԱՂ) ԵՐԿՐԱՋԵՐՄԱՅԻՆ ԱՂԲՅՈՒՐԻ ՄԱՆՐԷՆԵՐԻ ԿԵՆՍԱԲԱԶՄԱԶԱՆՈՒԹՅԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ ՄՈԼԵԿՈՒԼԱՅԻՆ ՄԵԹՈԴՆԵՐՈՎ

Բանալի բառեր՝ Քարվաճառի երկրաջերմային աղբյուր, 16Տռ-ՌՆԹ-ի գեն, կլոնային գենադարան, հաջորդականությունների վերլուծություն, BLAST վերլուծություն

Մոլեկուլային մեթոդների կիրառմամբ ուսումնասիրվել է Քարվաճառի (Լեռնային Ղարաբաղ) երկրաջերմային աղբյուրի մանրէների կենսաբազմազանությունը: Բակտերիական համընդհանուր օլիգոնուկլեոտիդային փորձարկումների կիրառմամբ ստացվել է 16Տռ-ՌՆԹ-ի գենադարան: Վերլուծված հաջորդականությունները ցույց են տվել դրանց պատկանելությունը Proteobacteria, Cyanobacteria, Bacteroidetes, Planctomycetes, Verrucomicrobia, Chloroflexi ֆիլումներին և դեռևս չդասակարգված ֆիլոտիպերին: Գերակշռող են Proteobacteria ֆիլումի ներկայացուցիչները: Ստացված հաջորդականությունների մեծամասնության վերլուծությունը վկայում է մանրէների չկուլտիվացվող ձևերի առկայության մասին, իսկ դրանց 89-96 % նմանությունը GenBank-ի հաջորդականություններին հաստատում է ուսումնասիրված համակեցության կառուցվածքի մենահատկությունը:

Ани Сагателяан, Овик Паносян

ИССЛЕДОВАНИЕ БАКТЕРИАЛЬНОГО РАЗНООБРАЗИЯ ГЕОТЕРМАЛЬНОГО ИСТОЧНИКА КАРВАЧАР (НАГОРНЫЙ КАРАБАХ) МОЛЕКУЛЯРНЫМИ МЕТОДАМИ

Ключевые слова: геотермальный источник Карвачар, ген 16SpРНК, библиотека генов, секвенирование, BLAST анализ

В статье описано исследование бактериального биоразнообразия геотермального источника Карвачар (Нагорный Карабах) молекулярными методами. Использование бактериальных универсальных праймеров дало возможность получить библиотеку генов 16S рРНК. проанализированные последовательности принадлежали к филумам Proteobacteria, Cyanobacteria, Bacteroidetes, Planctomycetes, Verrucomicrobia, Chloroflexi и К неклассифицырованным филотипам с доминированием представителей филума Proteobacteria. Большинство полученных последовательностей свидетельствует о наличии некультивируемых форм микроорганизмов, а 89…96 % их имеют сходство с последовательностями базы данных GenBank, что подтверждает уникальность исследованного сообщества.

Ani Saghatelyan, Hovik Panosyan

STUDY OF BACTERIAL DIVERSITY OF KARVACHAR GEOTHERMAL SPRING USING CULTURE-INDEPENDENT METHODS

Keywords: Karvachar geothermal spring, 16 rRNA gene, clone library, sequence, BLAST analysis

The bacterial diversity of Karvachar (Nagorno-Karabakh) geothermal spring was investigated using culture-independent methods. Bacterial universal oligonucleotide primer set was used to generate 16S rRNA gene libraries. Analyzed sequencesare originated from phyla Proteobacteria, Cyanobacteria, Bacteroidetes, Planctomycetes, Verrucomicrobia, Chloroflexi and yet unclassified pylotypes with domination of phylum Proteobacteria. Most of the obtained sequences were closely related to uncultivated microbes and shared 89-96 % similarity with their closest matches of GenBank database confirming unique composition of the studied community.