MODERATE THERMOPHILIC PAENIBACILLI STRAIN ISOLATED FROM ARZAKAN (ARMENIA) GEOTHERMAL SPRING

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Introduction

Natural biotopes for the occurrence of thermophilic microorganisms are terrestrial or marine in origin and distributed worldwide [1]. Numerous geothermal springs of different geotectonic origin and with different physical-chemical properties are found in Armenia [2]. Recently microbiological investigations of some Armenian geothermal springs based on culture-independent and culture-dependent approaches were carry out. Various thermophilic representatives of *Bacillus* and related genera have been isolated and characterized from diverse hot springs in Armenia by using phenotypic and genotypic methods [3-5]. However, distribution of representatives of the genus *Paenibacillus* in geothermal springs located in Armenia is still poor investigated.

The genus *Paenibacillus* was originally established in 1993 [6] and several time was reassessed on the basis ofpolyphasic taxonomic results. There are 134 species and 4 subspecies with validly published names in the genus *Paenibacillus* (http://www.bacterio.cict.fr/p/paenibacillus.html). The members of the genus *Paenibacillus* exist in many kinds of habitats and the previous described species were mostly isolated from decomposing plant materials, soil samples and hot springs [6-11].

During a project to isolate thermophilic bacilli strains from geothermal spring in Armenia, a paenibacilli strain, designed as ArzA-5, was isolated and subjected to a polyphasic taxonomic study.

Material and Methods

The location of geothermal mineral spring in Arzakan was determined using GPS technology. Water temperature, pH and conductivity were measured *in situ* using a portative combined pH/EC/TDS/Temperature tester (HANNA HI98129/HI98130). Sediment samples of the studied springs were collected in sterile bottles and maintained on ice until processed.

To enrich bacilli the sediment (1g) samples were treated at 80^0C for 10 min, then were inoculated in Nutrient Broth (Difco) and incubated overnight at 50^0C with shaking at 240 rpm. Obtained culture was further purified by streaking samples on the same medium supplemented with agar (2%, w/v). The subcultures purity, cell morphology, sporulation and motility were determined by phase-contrast microscopy of freshly prepared wet mounts.

Morphological, physiological and biochemical characteristics of the isolate were tested using by commonly accepted methods [12].

DNA was extracted from pure isolates using GenEluteTM Bacterial Genomic DNA Kit (Sigma) according to the manufacturer's recommendations and used as a template in the PCR assays. 16S rRNA genes were amplified using universal primer pairs 27f (5'-GAGTTTGATCCTGGCTCA-3') and 1525r (5'-GAAAGGAGGAGATCCAGCC-3') (*Escherichia coli* numbering) [13]. PCR mixtures used for amplification of sequences contained 10 ng DNA, 5 μl 10×PCR buffer, 5 μl 10 mM dNTP (dATP, dGTP, dCTP and dTTP), 1 μl each primer (25 pmol/μl), 1,5mM $MgCl_2$, 0,2 μl *Taq* DNA polymerase, 2 μl0.1% bovine serum albumin, and sterile water up to the final volume of 50 μl. PCR amplification was completed using an DNA Engine thermocycler (BIO RAD). First, the templates were denaturized for 3 min at 96^0C, then 30 cycles of the following steps were completed: denaturation for 30 s at 96^0C for, annealing for 30 s at 55^0C, and extension at 2.5 min at 72^0C. The 30 cycles were followed by a final 10 min extension at 72^0C. PCR products were purified using GenEluteT PCR Clean-up Kit (Sigma) according to the manufacturer's recommendations, and were sequenced with 27f primer. Sequencing was performed on a ABI PRISM capillary sequencer according to the protocol of the ABI Prism BigDye Terminator kit (Perkin Elmer).

16S rRNA gene sequences were compared with those contained in the GenBank by using BLASTn search at the NCBI web site (http://www.ncbi.nlm.nih.gov/Blast.cgi)[14]. The assembled 16S rRNA gene sequences were aligned with a representative set of 16S rRNA gene sequences obtained from the GenBank database. The sequences were edited and aligned with EditSeq and MegAlign 5.1. Phylogenetic reconstructions were produced using the neighbor-joining tree-building algorithm. Confidence in branching points was determined by bootstrap analysis (1000 replicates) [15].

Results and Discussion

The Arzakan geothermal spring is located at 40^0 27' 36.10'' N, 44^0 36' 17.76'' E, at 1490 m above sea level with a temperature of ˜44°C, pH 7.0-7.2, and a conductivity of 4378.3 μS/sm. Arzakan spring belongs to the category of hot springs from low-temperature fields and is characterized by neutral to alkaline pH and high concentration of dissolved minerals and gases. The studied spring is related to the hydrocarbonate sodium class of mineral springs (>20% is HCO_3^- and >20% is Na^+) [2].

Sediment samples were analyzed to evaluate the bacilli abundance. A moderate thermophilic paenibacilli strain designed as ArzA-5 with optimum growth temperature 45-50^0C were isolated and further identified.

Colonies grown for 48 h at 45^0C on NB agar are circular, convex, creamcolored, opaque and usually 1–3 mm in diameter (Fig. 1). Cells are Gram-positive sporeforming rods (1.2–1.6 by 3.2–4.1μm) (Fig. 2). Subterminal oval endospores are formed in slightly swollen sporangia.

Optimum growth pH is 7.5. Oxidase and catalase positive. Nitrate is reduced to nitrite, citrate or propionate is not utilized. Does not produce gas from glucose. Acid is produced from various carbohydrates. Voges-Proskauer reaction is negative, indole or hydrogen sulfide is not produced. Produces gelatinase, amylase, lipase but not caseinase, phenylalanine deaminase or dihydroxyacetone. The morphological, physiological and biochemical characteristics of strain ArzA-5 and some related type strains are presented in Table 1.

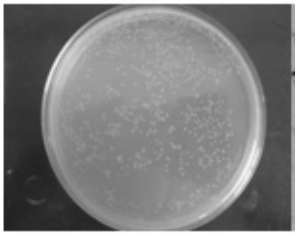

Fig. 1. The strain ArsA-5 grown on NB agar for 48 h at 50°C, showing circular, convex, creamcolored, opaque colonies.

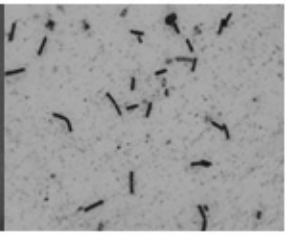

Fig. 2. Rod shaped cells of the strain ArzA-5 stained with methylene blue (×2000).

Table 1.

Differential characteristics comparison of strains ArzA-5 and the related type strains of *Paenibacillus* specieas.

Phenotypic characteristics	ArzA-5	XIL14	WP-I^T	DSM 24^T
Cell size [μm]				
Width	1.2–1.6	1.5-1.55	0.6-0.8	0.8-1.3
Length	3.2–4.1	3.9-4	2.5-3.5	2.7-4.2
Motility	+	+	+	+
Endospore				
Form	Oval	Oval	Ellipsoidal	Oval
Location	Subterminal	Subterminal	Terminal	Subterminal
Sporangium swell	+	+	+	+
Optimum temperature	45-50˚C	37 ˚C	42-45˚C	37˚C
Optimum pH	7.5	7	6.5-7.0	7.5
Oxidase	+	-	-	NA
Voges-Proskauer test	-	+	-	-
Nitrate reduction to nitrite	+	+	-	+
Acid from				
D-Glucose	+	+	+	+
L-Arabinose	+	+	-	+
D-Manitol	+	+	+	+
Gas from glucose	-	-	-	
Hydrolysis of				
Casein	-	-	+	-
Gelatin	+	+	+	+
Starch	+	+	NA	+
Tweens	+	+	NA	NA
Utilization of Citrate	-	-	-	
Formation of				
Indole	-	-	-	-
Dihydroxyacetone	-	-	NA	-

Species listed are as follows: ArzA-5, *P. xylanilyticus* XIL14, *P. thermophilus* WP-I^T and *P. macerans* DSM 24^T. Data of ArzA-5 were determined in the present study. Data for *P. xylanilyticus* XIL14, were obtained from Revas et al. [16], *P. thermophilus* WP-I^T and *P. macerans* DSM 24^T were obtained from Zhou et al. [11].
Symbols in the table: +, positive; -, negative; NA, not available.

16S rRNA gene from the extracted DNA of the isolate was successfully amplified by PCR and further sequenced. BLAST results based on 16S rRNA

gene sequences was shown the closest relative of the isolate was *P. xylanilyticus* (JQ649398) with the sequence similarity rates of 97%. Neighbour-joining phylogenetic analysis based on 16S rRNA gene sequences showed that strain ArzA-5 has the closest affinity to the type strain of *P. xylanilyticus* (JQ649398) with high level of bootstrap support (Fig. 3).

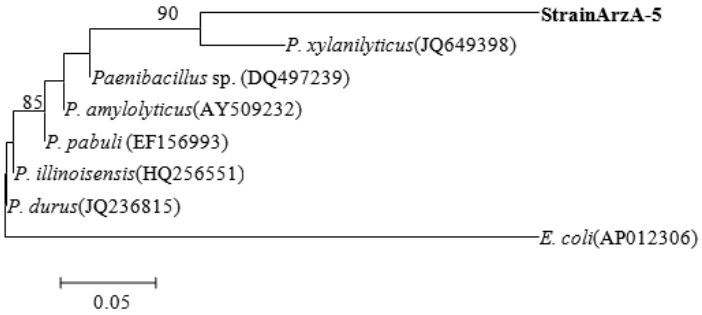

Fig. 3. Phylogenetic tree based on 16S rRNA gene sequence analysis, constructed using the neighbor-joining method showing the position of strain ArzA-5. Numbers on branch modes are bootstrap values (1000 resamplings, only values above 50% are shown). Bar 0.05 substitution per nucleotide position.

The polyphasic taxonomic study results indicated that the moderate thermophilic strain ArzA-5 probably represents a novel species or subspecies within genus *Paenibacillus*. Obtained results show the importance of further investigation to determine exact taxonomic position of the isolate.

Key words: *Paenibacillus, geothermal springs, thermophiles*

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ՉԱՓԱՎՈՐ ԹԵՐՄՈՖԻԼ ՊԱԵՆԻԲԱՑԻԼԱՅԻՆ ՇՏԱՄ ՄԵԿՈՒՍԱՑՎԱԾ ԱՐԶԱՔԱՆԻ (ՀԱՅԱՍՏԱՆ) ԵՐԿՐԱՋԵՐՄԱՅԻՆ ՀԱՆՔԱՅԻՆ ԱՂԲՅՈՒՐԻՑ

Հ. Հ. ՓԱՆՈՍՅԱՆ
Կենսաբանական գիտությունների թեկնածու,
դոցենտ, ԳՊՀ պրոֆեսոր,
ԳՊՀ կենսաբանության, էկոլոգիայի և
առողջ ապրելակերպի ամբիոն,
ԵՊՀ մանրէաբանության, մանրէների և բույսերի կենսատեխնոլոգիայի
ամբիոն

Արզաքանի երկրաջերմային հանքային աղբյուրից մեկուսացվել է ArzA-5 անվանակոչված չափավոր թերմոֆիլ պաենիբացիլային կուլտուրա: Մեկուսացված կուլտուրայի 16S ռՌՆԹ-ի գենի նուկլեոտիդային հաջորդականությունների համեմատումը GenBank-ի տվյալների բազայում առկա հաջորդականությունների հետ թույլ է տվել դրանց 97% նմանությամբ նույնականացնել Paenibacillus xylanilyticus տեսակին: Մեկուսացված կուլտուրայի պոլիֆազային տաքսոնոմիական վերլուծությունը վկայում է դրա Paenibacillus ցեղի նոր տեսակի կամ ենթատեսակի պատկանելիության մասին:

Բանալի բառեր - *Paenibacilles, երկրաջերմային աղբյուրներ, թերմոֆիլներ*

УМЕРЕННЫЙ ТЕРМОФИЛЬНЫЙ ПАЕНИБАЦИЛЯРНЫЙ ШТАММ, ИЗОЛИРОВАННЫЙ ИЗ ГЕОТЕРМАЛЬНОГО МИНЕРАЛЬНОГО ИСТОЧНИКА АРЗАКАНА (АРМЕНИЯ)

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Из геотермального минерального источника Арзакан изолирована умеренная термофильная паенибацилярная культура обозначенная как ArzA-5. Сравнение последовательностей 16S рДНК изолятас нуклеотидными последовательностями базы данных GenBank'а указывает на его 99% сходство с *Paenibacillus xylanilyticus*. Полифазный таксономический анализ изолята свидетелствует его принадлежность к новому виду или подвиду рода *Paenibacillus*.

Ключевые слова: *Paenibacillus, геотермальные источники, термофилы*