THERMOPHILES HARBORDED IN ARMENIAN GEOTHERMAL SPRINGS AND THEIR BIOTECHNOLOGICAL POTENTIAL

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Geothermal springs create unique niches for formation specific biocenosis of thermophiles and serve as source to isolate new microbes with biotechnological potentials. Natural geothermal springs are generally associated with tectonically active zones. On the territory of Armenia and Nagorno Karabakh, where the traces of the recently active tectonic processes are still noticeable, numerous geothermal springs with temperatures in the range from 27°C to 70°C and with neutral pH are found.

In this context the prokaryotic diversity thriving in previously uninvestigated Armenian geothermal springs were studied using both culture-independent molecular and culture-dependent approaches. Near full-length 16S rRNA genes were amplified from total community DNA using universal bacterial and archaeal oligonucleotide primer sets and clone libraries were constructed. The PCR-DGGE fingerprinting method and pyrosequencing were also applied to obtain information about the occurrence of the dominant prokaryotic populations. Amplification of small-subunit rRNA genes using “universal” primers followed by pyrosequencing (pyrotags) revealed highly diverse microbial communities in springs, with >99% of pyrosequences corresponding to members of the Domain Bacteria. Analyses of archaeal clone libraries revealed that springs Arzakan and Jermuk are inhabited by methanogenic Euryarchaeota (Methanomicrobiales, Methanosarcinales and relatives of Methanomassiliicoccioluminysinis), close relatives of the ammonia-oxidizing archaean (AOA) “Candidatus Nitrososphaerargensins”, and the yet-uncultivated Miscellaneous Crenarchaeotal Group and Deep Hydrothermal Vent Crenarchaeota group 1. Methanogenic enrichments confirmed the predicted physiological diversity, revealing methylotrophic, acetoclastic, and hydrogenotrophic methanogenesis.

BLAST results and phylogenetic analysis of obtained bacterial sequences indicated that they originated mainly from Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Epsilonproteobacteria, Bacteroidetes, Chloroflexi, Verrucomicrobia, Firmicutes, Cyanobacteria and Planctomycetes. The majority of the phylotypes identified in the gene libraries were most closely related to uncultivated organisms detected only by molecular methods and shared less than 95% identity with their closest match in GenBank, indicating a unique community structure of novel species with undiscribed biotechnological potentials.

New gammaproteobacterial methanotrophic strains affiliated to family Methylcoccaceae was isolated. A successful enrichments contained organisms with high 16S rRNA gene sequence identity (97%) with Nitrosipiracidulau and N.moscoviensis were also obtained.

Draft genome Thermuscotoductus strain K1 isolated from Karvachargothermal spring was sequenced (>2.38Mbp, 54 contigs, 65.1% G+C content). Strain K1 shares about 80% genome sequence similarity with T. scotoductus strain SA-01, recovered from a deep gold mine in South Africa. Physiological and genome-related differences between the newly isolated strain and strain SA-01 are discussed in a biogeographical perspective.

Our interest was oriented also to screening of thermophilic aerobic endospore-forming bacteria due to their industrial interests. More than 130 strains were isolated and identified as representatives of the genera Anoxybacillus, Aeribacillus, Anaerobacillus, Bacillus, Brevibacillus, Geobacillus, Paenibacillus, Sporosarcina, Ureibacillus and Thermoactinomyces. Some isolates shared less than 96-97% 16S rRNA sequence identity with their closest match in GenBank, indicating that the studied geothermal springs harbour novel species. Bacilli isolates were also screened for their amylolytic, proteolytic and lipolytic activities, and active producers of thermostable enzymes were selected. Draft genome of amylase producer Anoxibacillusflavithermus strain K103 was sequenced (>2.72Mbp, 49 contings, 41.6% G+C content). Isolated thermophilic bacilli were analyzed also for exopolysaccharide (EPS) production and active producers of EPS were selected too. The composition and chemical-physical properties of EPSs produced by Geobacillus thermodenitrificans ArzA-6 and G. toebii ArzA-8 were studied.
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