ISOLATION AND IDENTIFICATION OF A THERMOPHILIC CYANOBACTERIUM FROM BALCOVA, IZMIR, TURKEY

B.H. Cadirci [1] *, I. Tuney [2], I. Yasa [1], A. Sukatar [2], G. Gokcen [3]

[1] Ege University Faculty of Science Department of Biology Basic and Industrial Microbiology Section 35100 Bornova Izmir - bilge.hilal.cadirci@ege.edu.tr

[2] Ege University Faculty of Science Department of Biology Hydrobiology Section 35100 Bornova Izmir

[3] IYTE Engineering Faculty Department of Mechanical Engineering Urla 35430 Izmir

Abstract

Hydrothermal systems have prevailed throughout geological history on earth, and ancient Archaean hydrothermal deposits could provide clues to understanding earth's earliest biosphere. A cyanobacterium living at 50°C was isolated from Balcova geothermal field. 16s rDNA was extracted and amplified for sequence analysis.

Keywords : Cyanobacteria, Biodiversity, Genetics.

Introduction

Thermophiles are microorganisms that live and grow in extremely hot environments that would kill most other microorganisms. They can be isolated from a number of marine and terrestrial geothermally-heated habitats including shallow terrestrial hot springs, hydrothermal vent systems, sediment from volcanic islands, and deep sea hydrothermal vents. They grow best at temperatures that are between 50-70°C. They will not grow if the temperature reaches 20°C [5].

Microorganisms thriving in high-temperature terrestrial and deep-sea hydrothermal systems have investigated by several authors. A thermophilic cyanobacteria *Synechococcus elongatus* in the temperature range 60-80°C was found [1]. Papke and colleagues [4] studied two groups of thermophilic cyanobacteria: the unicellular *Synechococcus* and the colonial, filamentous *Oscillatoria*. The thermophilic cyanobacteria *Thermosynechococcus elongatus* and *T. vulcanus*, which were isolated from a Japanese hot spring, grow optimally at ca. 57°C (3). In these extreme environments, the microbial and geochemical interactions are tightly interwoven, providing many of the basic constituents for the primordial synthesis of organic molecules and for the evolution of fundamental metabolic processes (5). Whether life originated at vents in the archaean age (3.8-2.5 Ga) and whether chemolithoautotrophic thermophiles are ancestors of life is unresolved; however, hydrothermal environments probably did support the early evolution of chemolithoautotrophs and thermophiles (5).

Turkey is the seventh-richest country in the world in geothermal potential. Balçova geothermal district, called Agamemnon Spas in ancient times, is one of the geothermal districts in Izmir City. There are 8 production wells in Balçova with a temperature interval between 86-138°C. In this research, a thermophilic cyanobacterium was isolated from Balcova geothermal field and 16s rDNA was extracted and amplified for sequence analysis.

Materials and Methods

Balçova is located at the western tip of Anatolia. Samples were collected from outflows of the hot spring pipes, 38°23'21.29" N and 27°01'58.97"E of Balçova. Water sample was transported to the laboratory by hot line in 30-60 min. Two different cyanobacteria mediums were prepared for cultivation (7). Samples from hot spring were inoculated into the mediums and incubated at 65°C and at 45°C for two weeks. The isolate was purified by serial dilution. The material was refrigerated (4°C) until analysis.

DNA was extracted by Bio Basic commercial kit (BS423-50) according to manufacture's instructions. DNAs' quality was checked by agarose gel electrophoresis and spectrophotometer.

PCR amplifications were performed with PE Applied Biosystem, GeneAmp PCR System 9700 using universal bacteria primers. Fifty picomols of each primer, 25 nmol of each deoxynucleosidetriphosphate, 25 μl of 10x PCR buffer, 0.5 U of Taq polymerase and 20 ng template DNA mixed in volume of 50 μl in a 0.5-ml test tubes. Two different primer pairs used for amplification of 16S rDNA of *Schizothrix sp*. DNA amplified under following conditions: 5 min at 95°C for initial denaturation, 94°C for 30 s, 50°C for 30 s, 72°C for 2 min for 10 cycle. 92°C for 30 s, 50°C for 30 s, 72°C for 2.5 min for 20 cycle and 72°C for 5 min for final extension step. Amplified DNA checked by agarose gel electrophoresis and photographed.

Results and Discussion

An organism that thrives in an extreme environment is an extremophile; in more than one extreme it is a polyextremophile. Examples of the latter would include *Sulfolobus acidocaldarius*, an archaea that flourishes at pH 3 and 80°C. 'Extremes' include physical extremes (for example, temperature, radiation or pressure) and geochemical extremes (for example, desiccation, salinity, pH, oxygen species or redox potential)[6]. In this study the cyanobacteria was isolated from a 96°C geothermal fluid leakage near the hydrothermal well. The fluid was formed a pond at 45° insisting cyanobacterial formations. Microscopic identification revealed that only one genus existed in the sample identified as *Schizothrix sp*. The isolate was cultivated in two media at 50°C. It has been suggested that the photosynthetic apparatus itself is the most temperature-sensitive component of the cyanobacteria with the highest temperature tolerances (i.e., 73 to 74 °C). The photosynthetic apparatus of thermophilic cyanobacteria is similar to that of higher plants; however, it is resistant to high temperatures at the subcellular level [1]. In this study 16S rRNA region of the isolated cyanobacteria was amplified by universal cyanobacteria primers successfully [2]. The sequence of 16S rRNA region of the cyanobacterium will be used to constract phylogenetic trees between related taxa in further studies. The discovery of extreme environments and the organisms that inhabit them has made more plausible the search for life outside the Earth, and even the possibility of panspermia (the transport of life from one planet to another).

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