Cyanobacterial community structure and isolation of thermophilic cyanobacteria in the Zharkent geothermal spring

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Abstract. Cyanobacterial biodiversity represents a significant reservoir for the potential discovery of new promising microorganism species. The purpose of this research is to explore the diversity of cyanobacteria and identify thermotolerant species found in the Zharkent geothermal spring situated in the Zhetysu region. At the time of water sampling from the spring, the temperature reached 75-80 °C. Cyanobacterial mats in the form of microbial agglomerations were identified on the stream periphery. Nine cyanobacterial species were found in these cyanobacterial mats, including *Synechococcus, Phormidium, Gloeocapsa, Oscillatoria, Fischerella*, and *Nostoc*. Among the cyanobacteria inhabiting the hot spring, 44% were non-heterocystous forms, while the rest exhibited heterocystous characteristics or were present as single-celled organisms. The order *Oscillatoriales* was the most predominant, including four species, followed by the order *Chroococcales* with three species. Pure cultures of cyanobacteria, such as *Oscillatoria formasa*, *Nostoc commune, Anabaena cylindrica*, and *Fischerella thermalis*, were isolated from the geothermal spring. These cultures were subjected to thermotolext56rance assessment at different temperatures. All examined strains exhibited a high growth rate at 45-50°C, with a slowdown at 55 and 60°C. The optimal growth temperature was 45-50°C, except for the *Fischerella thermalis* strain, which showed active growth at 60°C. The obtained results emphasize the potential application of the isolated strains in biotechnology.

1 Introduction

Geothermal springs are distinct natural formations characterized by the emergence of hot water from the Earth's surface. These ecosystems possess stable abiotic characteristics such as temperature, pH, and ion composition, creating favorable conditions for diverse microorganisms [1]. Research indicates that this consortium of microorganisms not only has the ability to adapt to extreme conditions but also persists over extended periods, providing a unique perspective for studying

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microbial evolution [2]. Special attention should be given to thermal cyanobacteria, which dominate the microflora of Geothermal springs.

These microorganisms are not only adapted to high temperatures but also have the ability to produce diverse secondary metabolites with bioactive properties [3]. This opens up prospects for biotechnological research, including the isolation and enrichment of collections of thermotolerant cyanobacterial cultures for the study and utilization of their potential bioactive compounds [4]. Thus, hot springs are not only unique natural phenomena but also a valuable source of microorganisms that can be applied in biotechnology for obtaining biologically active substances. Despite the partial exploration of cyanobacteria from some thermal springs, many of these unique habitats remain insufficiently studied [5].

The Zharkent Geothermal spring, located in the mountainous terrain on the slopes of the Small Jungar Alatau in the Zhetysu region, is of scientific interest due to its unique characteristics. The elevation of this spring is 1100 meters above sea level, a significant factor in the context of its geographical location. The water mineralization of the Zharkent Geothermal spring covers a wide range of values, varying within the range of 0.2–1.5 g/L. This mineralization is attributed to various chemical compositions of the water, including mixed calcium-sodium and chloride-sulfate sodium compositions. Such diversity in chemical compounds underscores the chemical heterogeneity of this natural spring. The temperature regime of the water in the spring also exhibits interesting features. At depths ranging from 200 to 600 meters, the water temperature fluctuates between 20°C and 30°C, while at deeper levels, from 1000 to 2000 meters, there is a significant increase in temperature to 60°C–85°C. This phenomenon can be explained by the geothermal activity in this region [6].

The study of thermophilic cyanobacteria from diverse ecosystems is of fundamental importance in the context of interdisciplinary research aimed at a comprehensive understanding of their morphology, genetics, physiology, and biochemistry. It also holds significant value for identifying potential strains of cyanobacteria with promising biotechnological and industrial characteristics. The objective of the current research was to explore the biological diversity of thermophilic cyanobacteria in the Geothermal spring of Zharkent in the Zhetysu region and isolate thermotolerant strains of cyanobacteria.

2 Materials and Methods

The object of the study was a Geothermal spring located in the Zhetysu region, village Kermegash, at coordinates (44.06908824440221, 79.83526310658152). Water samples were collected during the summer months of 2023. At the time of obtaining algal samples, the water temperature ranged within 75-80°C, water mineralization was 0.3-0.9 g/dm³, pH ranged from 8.8 to 9.3, transparency was 0.5-1 m, and the depth varied from 0.5 to 1.5-2 m. A total of 15 algal samples were collected, specifically from locations with prominently expressed cyanobacterial vegetation (Figure 1). All collected samples were thoroughly labeled, indicating the sample number, time and location of collection, and the collector's surname. Cyanobacteria were examined using "Premere" and "MicrosAustria" light microscopes with magnifications ranging from x40 to x100. Approximately 30–40 fields of view were observed for each water sample on no fewer than 5 preparations.

Identification of cyanobacterial species was carried out in both native and fixed states of cells, using formaldehyde and iodine solutions as fixatives. Species identification of cyanobacteria was performed with the help of taxonomic keys [7-8].

Fig. 1. Location of sampling sites (44.06908824440221, 79.83526310658152)

The acquisition of a cyanobacterial stock culture was conducted through conventional methods. Standard microbiological techniques were utilized to isolate pure cultures from the initial stock cultures [9]. Axenic algal cultures were obtained through multiple successive subcultures in appropriate nutrient media. Monocultures of cyanobacteria were established through streaking and micropipette inoculation. The algal purity of the isolated cultures was verified through microscopic examination [10]. Cyanobacteria were cultivated in 500 ml flasks under sterile conditions, using Gromov and Zarruk mineral media.

To assess the thermotolerance of cyanobacteria and evaluate their viability at higher temperatures, they were incubated at OD 750 ~0.3 in special media in 250 ml flasks. The process was conducted under 24-hour artificial lighting with Flora Led 35 D120 lamps (80 µmol pGeothermalons · m-2 · s-1) at temperatures of 35°C, 40°C, 45°C, 50°C, 55°C, and 60°C for a duration of 10 days. All experiments were carried out in triplicate.

3 Results and Discussion

At the time of water sampling from the Geothermal spring, the temperature reached 75-80 °C. Cyanobacterial mats were identified at the stream's periphery, forming microbial aggregations (Figure 2). Describing them, it can be noted that the microbial mat possesses a dense structure, with a greenish tint, and has a thickness of approximately 0.5–0.8 cm. Microscopic examination of water samples from the geothermal spring indicated the prevalence of filamentous cyanobacteria.It is important to highlight that this ecological feature, specifically the presence of

filamentous cyanobacteria, may have significant implications for understanding microorganisms' adaptations to the extreme conditions of high temperatures in Geothermal springs [11].

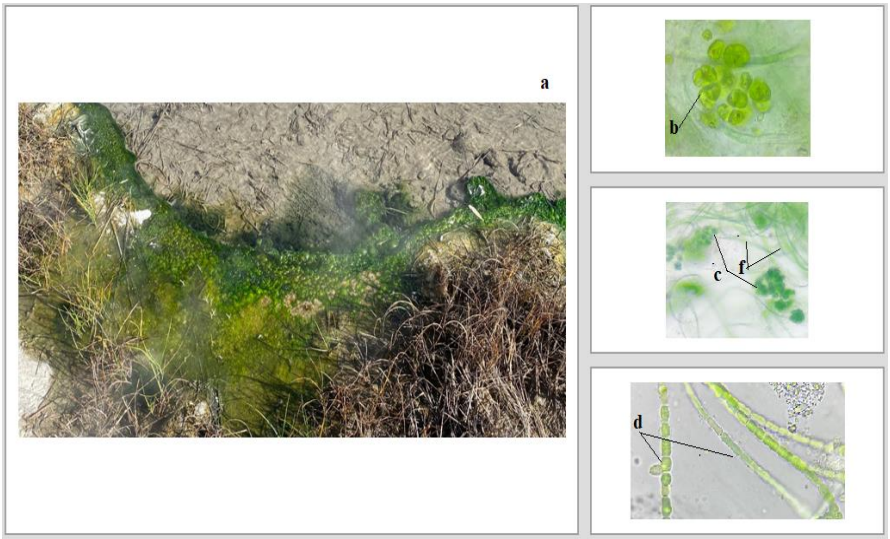

Fig. 2. Microphotographs of cyanobacteria from the Zharkent Geothermal springa– Microbial Mat, b - *Gloeocapsa gelatinosa,* c -*Gloeocapsopsis thermalis*, d -*Fischerella thermalis*, f - *Phormidium sp*

The biodiversity of cyanobacteria identified in the Geothermal spring samples include nine species from different genera, including *Fischerella*, *Synechococcus, Phormidium, Oscillatoria,* and *Gloeocapsa, Nostoc,* all classified under the *Cyanophyceae* class. These observations indicate the diversity of cyanobacteria that have successfully adapted to the conditions of this thermal biotope. Previous research findings also demonstrate extensive cyanobacterial diversity in Geothermal springs across different geographical regions. Studies by Debnath, Mandal & Ray [12] on the Bakreswar Geothermal water spring in India revealed 18 species belonging to 12 genera of cyanobacteria. Additionally, research by Sompong et al. [13] documented 19 genera and 36 species of cyanobacteria thriving in nine thermal springs (temperature range: 30-80 °C) in northern Thailand.

Cyanobacterial mats at temperatures ranging from 40 to 50 °C are typically represented by species such as *Phormidium, Oscillatoria, Pseudanabaena, Calothrix*, and *Fischerella* [14]. Typically, there is an evident pattern of species distribution across different thermal springs, influenced by the capacity of organisms to acclimate to elevated temperatures and the physicochemical characteristics of their surroundings.Consequently, there is an uneven distribution of thermophilic cyanobacteria in Geothermal springs worldwide [5]. From the overall species diversity of cyanobacteria inhabiting the Zharkent Geothermal spring, 44% constituted non-heterocystous forms, while the remaining expressed heterocystous forms and were also present in the form of single-celled organisms. Considering the number of species and abundance, the order *Oscillatoriales* was the most predominant, including four species, followed by the order *Chroococcales*

with three species. Similar studies in various regions of the world have confirmed the dominance of representatives of the order *Oscillatoriales* in microbial mats of Geothermal springs [5]. These results indicate that certain cyanobacterial species have successfully adapted to high-temperature conditions and demonstrate photosynthetic capabilities.

Four cyanobacterial isolates were obtained from the Geothermal spring, as illustrated in Figure 3. All isolates underwent detailed microscopic analysis, followed by identification based on cell morphology, following the descriptions provided in the works of Gollerbakh [7], A.M. Muzafarov [8], and contemporary taxonomic studies.

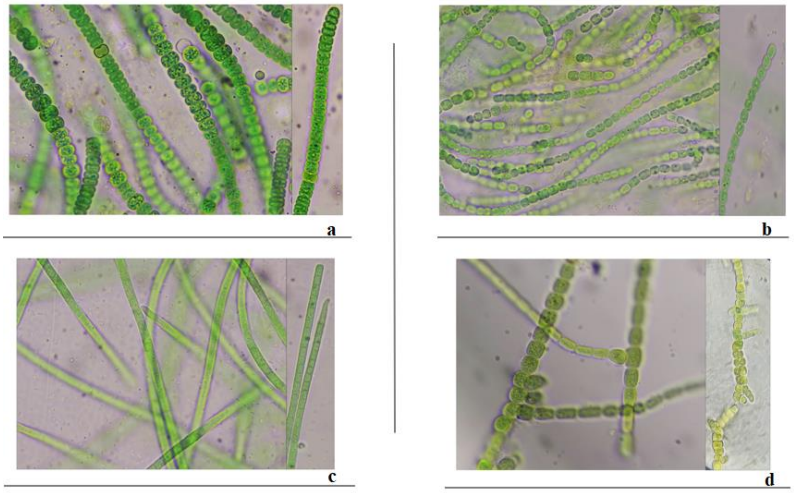

Fig. 3. Microphotographs of the isolated cyanobacterial cultures from the Zharkent Geothermal spring, (a- *Nostoc commune*, b- *Anabaena cylindrica*, c- *Oscillatoria formasa*, d- *Fischerella thermalis*)

Considering morphological traits, the isolated cyanobacterial cultures belong to the order *Oscillatoriales (Oscillatoria formasa), Nostocales (Nostoc commune, Anabaena cylindrica, Fischerella thermalis*). According to literature sources, the isolated cultures obtained from the Zharkent thermal springs containing hot water are present in diverse locations around the globe [15, 16].

To confirm the thermotolerance of the isolated strains and assess their viability at higher temperatures, incubation was carried out at temperatures of 35°C, 40°C, 45°C, 50°C, 55°C, and 60°C for 10 days. During the experiment, it was noted that all studied strains exhibited a high growth rate up to 45-50°C; however, with further temperature increase to 55°C and 60°C, a slowdown in the rate of cell growth was observed (Figure 4).

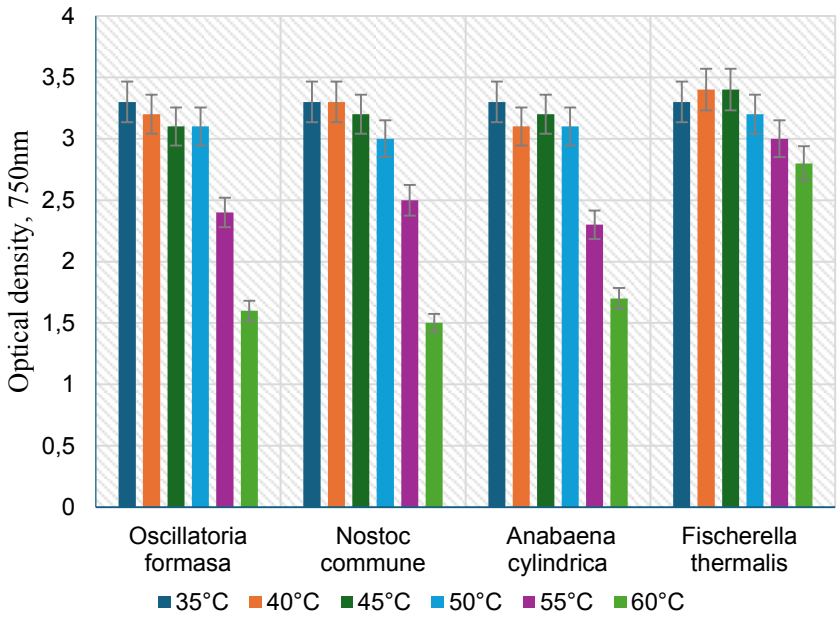

Fig. 4. Assessment of thermotolerance of cyanobacterial isolates obtained from thermal springs.

Despite the variability in environmental temperatures where these strains were originally isolated (ranging from 40 to 80°C), the optimum growth temperature for all investigated cyanobacterial strains was approximately 45-50°C. The research revealed that at a temperature of 60°C, active cell growth of cyanobacteria was observed exclusively in the *Fischerella thermalis* strain compared to other examined strains.

4 Conclusion

Studying biodiversity and isolating potential strains of cyanobacteria from extreme habitats represent crucial steps in contemporary scientific research. These efforts are not only aimed at documenting diversity but also provide potential subjects for biotechnological investigations. Cyanobacteria, due to their easy adaptability, are integral components of various ecosystems, including thermal springs. This article conducted a study on the biodiversity of a geothermal spring in the Zhetysu region near Zharkent. The overall diversity of cyanobacteria was identified, encompassing nine species belonging to various genera such as *Synechococcus, Phormidium, Gloeocapsa, Oscillatoria, Fischerella*, and *Nostoc*, all classified under the *Cyanophyaceae* class. During the research, four cyanobacterial isolates were obtained from the Geothermal spring, morphologically belonging to the *Oscillatoria* and *Nostocales* orders. To confirm thermotolerance and assess the viability of the isolated strains, incubation was performed at different temperatures. The experiment revealed that all studied strains exhibited high growth rates up to 45-50°C. However, with further temperature increase to 55 and 60°C, a slowdown in the pace

of cell growth was observed. Despite the variability in environmental temperatures, the optimum growth temperature for all examined cyanobacterial strains was approximately 45-50°C. A significant outcome of the study is the identification of active cell growth of cyanobacteria at a temperature of 60°C, exclusively observed in the *Fischerella thermalis* strain compared to other studied strains. These results underscore the significance and potential applicability of the isolated strains in various biotechnological processes.

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Authors' contribution

Conceptualization, N.R.A, B.K.Z, and M.A.; Methodology, S.N.S., N.E.B.; Software, S.N.S.; Formal Analysis, N.R.A.; Investigation, S.N.S., N.E.B; Resources, N.R.A.; Writing – Original Draft Preparation, N.R.A.; Writing – Review & Editing, S.N.S.; Visualization, Z.B.K.; Supervision, M.A.; Project Administration, N.R.A.

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