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S.K. Sandybayeva, K. Bolatkhan, A.B. Kakimova, A.K. Toktybay, G.A. Akhmetova, D. Salauat, M.S. Amangeldin, B.K. Zayadan*

Al-Farabi Kazakh National University, Kazakhstan, Almaty
*e-mail: zbolathkan@gmail.com

ISOLATION AND STUDY OF MORPHOLOGICAL AND CULTURAL PROPERTIES OF CYANOBACTERAL COMMUNITY FROM HOT SPRINGS IN ALMATY REGION

The diversity of cyanobacteria from four hot springs in the Chundzha settlement, located in the Almaty region, was studied to determine the species composition and isolate axenic cultures of thermophilic cyanobacteria. The objective was to investigate the diversity, morphological and cultural properties of thermophilic cyanobacteria from the Chundzha hot springs, and to establish axenic cultures for potential utilization in biotechnology, as these organisms are scientifically interesting and valuable as a source of thermostable biomolecules. As a result of the study, eight axenic cyanobacteria cultures were isolated and identified as Oscillatoria subbrevis, Phormidium ambiguum, Nostoc commune, Synechococcus elongatus, Synechocystis sp., Tolypothrix tenuis, Anabaena cylindrica and Spirulina fusiformis. Optimal cultivation conditions were also determined: the isolated cyanobacteria cultures exhibited high growth rates when cultivated in BG-11, Zarruk, and Gromov nutrient media at an optimal temperature of 28-36°C, pH 7, and a light intensity of 50 μmol photons $m^{-2}s^{-1}$.

Key words: hot springs, thermophiles, cyanobacterial diversity, isolated axenic cultures, morphological and cultural properties, selection of cultivation conditions, dynamics of growth.

С.Қ. Сандыбаева, К. Болатхан, А.Б. Какимова, А.К. Тоқтыбай, Г.А. Ахметова, Д. Салауат, М.С. Амангельдин, Б.К. Заядан*

[1]Әл-Фараби атындағы Қазақ ұлттық университеті, Қазақстан, Алматы қ.
*e-mail: zbolathkan@gmail.com

Алматы облысының ыстық су көздерінен бөлініп алынған цианобактериялық қауымдастықтың морфологиялық және дақылдық қасиеттерін зерттеу

Термофильді цианобактериялардың түрлік құрамын анықтау және аксеникалық дақылдарын бөліп алу үшін Алматы облысы Шонжы елді мекенінде орналасқан төрт ыстық су көздерінен алынған цианобактериялардың әртүрлілігі зерттелді. Бұл зерттеудің мақсаты Шонжы ыстық су көздеріндегі термофильді цианобактерияларының алуан түрлілігін, морфологиялық және дақылдық қасиеттерін зерттеу және оларды өнеркәсіптік биотехнологияда қолдану үшін аксеникалық дақылдар қатарына енгізу болды, себебі бұл организмдер ғылыми тұрғыдан қызықты және термотұрақты биомолекулалардың көзі ретінде құнды. Зерттеу нәтижесінде цианобактериялардың 8 аксеникалық дақылдары бөлініп алынып, морфологиялық ерекшеліктеріне қарай олар Oscillatoria subbrevis, Phormidium ambiguum, Nostoc commune, Synechococcus elongatus, Synechocystis sp., Tolypothrix tenuis, Anabaena cylindrica және Spirulina fusiformis ретінде анықталды. Сонымен қатар, оларды дақылдаудың оптималды жағдайлары анықталды: бөлініп алынған цианобактерия дақылдарының жоғары өсуі BG-11, Заррук және Громов қоректік орталарында pH 7 кезінде 28-36°C оптималды температурада және 50 мкмоль $м^{-2}$ $с^{-1}$ жарық қарқындылығында байқалды.

Түйін сөздер: ыстық су көздері, термофилдер, цианобактериялардың алуан түрлілігі, оқшауланған аксеникалық дақылдар, морфологиялық және дақылдық қасиеттері, өсіру жағдайларын таңдау, өсу динамикасы.

С.К. Сандыбаева, К. Болатхан, А.Б. Какимова, А.К. Токтыбай, Г.А. Ахметова,
Д. Салауат, М.С. Амангельдин, Б.К. Заядан*
Казахский национальный университет им. аль-Фараби, Казахстан, г. Алматы
*e-mail: zbolathkan@gmail.com

Выделение и изучение морфологических и культурных свойств цианобактерального сообщества из горячих источников Алматинской области

Разнообразие цианобактерий из четырех источников горячей воды населенного пункта Чунджа, расположенного в Алматинской области, было изучено для определения видового состава и выделения аксеничных культур термофильных цианобактерий. Целью настоящего исследования было изучение разнообразия, морфологические и культуральные свойствами термофильных цианобактерий горячих источников Чунджи и введение их в аксеновые культуры для возможного использования в промышленной биотехнологии, поскольку эти организмы интересны с научной точки зрения и ценны как источник термостабильных биомолекул. В результате исследования были выделены 8 аксеничных культур цианобактерий и были определены как Oscillatoria subbrevis, Phormidium ambiguum, Nostoc commune, Synechococcus elongatus, Synechocystis sp., Tolypothrix tenuis, Anabaena cylindrica и Spirulina fusiformis. Также были установлены оптимальные условия культивирования: высокий рост выделенных культур цианобактерий наблюдался при выращивании в питательных средах BG-11, Заррука и Громова с оптимальной температурой 28-36°C при pH 7 и интенсивности света 50 мкмоль м$^{-2}$ с$^{-1}$.

Ключевые слова: горячие источники, термофилы, разнообразие цианобактерий, изолированные аксеновые культуры, морфо-культуральные свойства, подбор условий культивирования, динамика роста.

Introduction

Hot springs are isolated and unique habitats located in remote regions worldwide, where groundwater emerges from the Earth's surface at specific points [1]. These springs are widespread across the globe and are characterized by water temperatures significantly higher than the average annual air temperature in their respective regions, often reaching the boiling point [2]. Life has thrived in hot springs long before reaching the surface, thanks to the warm water that supports the survival of abundant thermophilic microorganisms, including algae and bacteria [3, 4]. These microorganisms have adapted to high temperatures and can thrive in environments vastly different from their dispersal pathways [5]. One notable group of organisms found in hot springs is cyanobacteria. Cyanobacteria are photosynthetic prokaryotes that produce oxygen and exist as single cells, colonies, or filaments [6]. They possess cellular mechanisms that allow them to adapt to environmental changes and proliferate rapidly, forming dense populations [7, 8].

Thermophilic cyanobacteria are of scientific interest due to their resemblance to ancient forms of life on Earth, and they hold value as a source of thermostable biomolecules [9]. These microorganisms have developed various adaptations to thrive in extreme environmental conditions, exhibiting a high degree of heat resistance that enables them to survive and function at elevated temperatures. The lipid composition of their cell membranes is specifically adapted to withstand high temperatures, characterized by a higher proportion of saturated fatty acids, which enhances membrane rigidity and stability [10]. This adaptation prevents denaturation and promotes the re-coagulation of damaged proteins [11]. Furthermore, thermophilic cyanobacteria produce heat shock proteins (HSP) in response to high temperatures, which aid in stabilizing cellular components [12]. Moreover, thermophilic cyanobacteria often possess unique pigment compositions that protect them from intense light and ultraviolet radiation in hot environments. They can synthesize specific carotenoids and mycosporin-like amino acids (MAAs), which act as photoprotective compounds [13]. In recent decades, extensive research has been conducted on thermophilic cyanobacteria as promising candidates for various applications in agriculture, pharmaceuticals, nutraceuticals, and biofuels. However, fully exploring the industrial potential of thermophilic cyanobacteria requires fundamental research and technological innovations, focusing on the detailed study of each biological aspect of these organisms.

The Almaty region is located in the foothills of the Zailiysky Alatau at an altitude of 700-900 meters (2300-3000 feet) above sea level. The location of this region in the mountains, where a high level of volcanism is noted, provides it with geothermal hot springs. There are about 140 hot springs in the area, where the water temperature ranges from 30°C to 98°C [14]. One of the large artesian thermal springs with low-mineralized radon water is the springs of the village of Chundzha, located in the Uigur region between the Ketmen Mountains and the Ili river valley [15]. The diversity of cyanobacteria in the hot springs of the Almaty region remains insufficiently studied and requires immediate attention. Thermophilic cyanobacteria from the thermal springs of the city of Zharkent (43°97' 14.93"N, 79°66' 12.09"E) were the first and perhaps the most thoroughly studied organisms [16]. Although cyanobacteria from some other thermal springs have also been studied to some extent [17, 18], many places such as Gorelnik, Alma-Arasan, Turgen, Chundzha still remain unexplored. In this context, the isolation, purification, taxonomic characterization, and axenic cultivation of novel thermophilic strains of cyanobacteria can potentially serve as a valuable resource for the production of thermostable enzymes, proteins, and pigments. The objective of this paper was to investigate the diversity of thermophilic cyanobacteria from Chundzha thermal springs, along with their morphological and cultural properties, and to establish axenic cultures for potential utilization in industrial biotechnology.

Materials and methods

Research sites and sampling

Four hot water springs located in the Chundzha settlement, Uygur district of the Almaty region, approximately 250 km (155 miles) from the city of Almaty, were investigated. These hot springs are known for their highly mineralized water, which is heated to temperatures ranging from 37-50°C (95-122°F) due to active volcanic processes. It is believed that the water from the Chundzha hot springs possesses therapeutic properties [19]. To collect samples, water from the four springs was collected in pre-sterilized plastic bottles with a volume of 1 liter. The samples were obtained from wells numbered as № 1587, 3422, 964, and 1478. The collected water samples were filtered through membranes with a pore diameter of 0.45 microns and then stored in a refrigerator at a temperature of 40°C until further processing. Biological mats, concretions, and sediments were randomly selected from the sampling sites using sterile forceps and a spatula, and they were placed in sterile glass containers. Planktonic strains of cyanobacteria were also collected from the water samples using sterile glass vials and test tubes. The sampling period spanned from October 10 to 25, 2021. The water temperature at each collection point was measured using a thermometer, and the pH of the water was determined using a digital pH meter (HM Digital PH-80, USA).

Determination of species composition and morphological analysis of cyanobacteria cultures

The biological mats obtained from the selected hot springs were transferred to 250 ml flasks containing BG-11 medium after thorough washing with double-distilled water [20]. For subsequent isolation and purification of cyanobacteria strains, solid (agar) media were used. The species composition of the cyanobacteria was determined using available identification keys [21-28] and microscopic analysis performed with a MicroOptix series microscope connected to a monitor for image output. The growth patterns and morphological studies of cyanobacterial cultures were conducted at different growth stages using both liquid and solid Zarruk media [29]. Traditional microbiological methods were employed to obtain an enrichment culture and isolate algologically pure cyanobacterial cultures. The cyanobacterial suspensions were grown in 500 ml flasks with sterile conditions, using mineral media such as Zarruk, Gromov, and BG-11. Morphological identification of the cyanobacterial isolates was carried out using an optical light microscope (MicroOptix MX 300T, Austria) equipped with a digital camera and an imaging system.

Determination of the growth dynamics of cyanobacterial cultures

To conduct the experiment, the cells of the studied cyanobacteria were continuously cultured in a laboratory luminostat in 500 ml glass vessels, where they were aerated with a sterile gas-air mixture enriched with 1% CO_2 under artificial lighting with a light intensity of 50 μmol m^{-2} s^{-1}. The inoculate of the selected cyanobacterial cultures elected from the preliminary culture during the *log* phase [A_{750}=0.2], and cultured in Zarruk, Gromov and BG-11 media, where the pH was adjusted to 7.5. The culture temperature was maintained at 28±2°C. Samples from the cultures were aseptically collected in 5 ml every day, and analyzed by the spectrophotometric method (PD-303UV, Japan) at a wavelength of λ=750 nm (OD_{750}) to monitor the growth of cyanobacterial cultures. The growth rate coefficient of cyanobacteria was determined as the difference between the maximum and initial density of the cell suspension [30]. The biomass or dry mass was analyzed every 3 days. Cyanobacteria suspensions were collected in the stationary growth phase by centrifugation (Eppendorf 5804 R centrifuge) for 10 min at 4500 rpm. Then the separated cells were washed three times with distilled water and the number of cell granules in fresh form was determined. Dry weight was recorded after drying the pellets in an oven at 60°C until a constant weight was reached [31]. Content of chlorophyll *a* was measured spectrophotometrically following the protocols of Zavřel et al. [32].

Statistical analysis

The tables and figures show the results of two or three repetitions with four to six records in each. The number of repetitions required for a $P \leq 0.05$ significance level was determined using the degree of data scatter. The relative standard error was 2-5% for the main indicators and less than 10% for the minor components. Data were statistically analyzed and expressed as mean ± standard deviation.

Results and discussion

Community structure of cyanobacteria in hot springs

Kazakhstan possesses abundant resort resources, including a diverse range of climate, mineral, and thermal waters, as well as therapeutic mud. According to the Department of Balneology of the Kazakh Research Institute of Cardiology, extensive studies have been conducted on approximately 500 medicinal mineral water outlets, 78 mud lakes, and 50 climatic areas in the country. Notably, various types of thermal waters have been discovered in different regions of Kazakhstan. On the northern slopes of the Zailiysky Alatau, thermal sulphate-hydrocarbonate sodium (Alma-Arasan) and iodine-bromine, chloride calcium-sodium waters (north of the city of Alma-Ata) have been identified. On the northern slopes of the Dzhungarsky Alatau, thermal radon chloride-sulfate sodium waters containing nitrogen (Jarkent-Arasan, Kapal-Arasan) have been found. In the eastern part of the republic, there are sulfate-hydrocarbonate sodium-magnesium springs (Rakhmanovskie Klyuchi) and thermal chloride-sulfate calcium-sodium springs (Barlyk-Arasan). In the south of Kazakhstan, thermal nitrogen hydrocarbonate sodium waters (Saryagach) and radon sulfate-hydrocarbonate-chloride sodium waters (Merke) have been discovered. In recent years, in the Kustanai region in the north of the country, medium-mineralized sulfate-chloride sodium water from the source in the Sosnovy Bor sanatorium has been widely utilized for medicinal purposes [33]. One well-known thermal artesian spring with low-mineralized radon water possessing healing properties is the Chundzha mineral waters. These springs are located in the Uigur region between the Ketmen mountains and the Ili river valley (Figure 1). The Chundzha area is home to approximately 140 hot springs, where the water is heated by igneous intrusions associated with areas of active volcanism [34]. Evidence of past volcanic activity can be observed on the way to Chundzha, with the Buguty massif remaining as a residual feature of an ancient volcano. Although the volcano became dormant over 200 million years ago, the magma channels have not completely closed, resulting in the heating of the water, which subsequently rises to the surface under immense pressure [14]. The thermal mineral springs, or hot springs, of Chundzha are categorized into warm (20–37°C), hot (37–50°C), and very hot (50–100°C) temperatures [16]. During the sampling period, the temperature of the hot springs ranged from 38 to 50 °C. The pH of the water was recorded within the alkaline range of 7 to 8. Based on the data, the water composition of the studied wells, such as well №1587 (recreation areas «Mirage,» «Derevushka») and well №3422 (recreation area «Ulan»), is characterized as weakly mineralized (up to 0.43 g/dm3), weakly alkaline (pH 8.1-8.4), rich in nitrogen compounds (up to 95%), and hyperthermal. The complex composition of these waters includes chloride, sulfate, and hydrocarbonate, along with elevated levels of silicic acid (39.0-40.0 mg/dm3). The mineral waters from wells №964 (recreation area «Navat») and №1478 (recreation area «Altyn-Su») in the Karadalinskoye deposit are classified as medical-table, low-mineralized, thermal waters. The mineral waters from wells №964 (recreation area «Navat») and №1478 (recreation area «Altyn-Su») in the Karadalinskoye deposit are classified as medical-table, low-mineralized thermal waters. These waters have a slightly alkaline pH and contain nitrogen. Well №964 has a complex composition of sulfate, chloride, and hydrocarbonate, with sodium as the dominant ion. On the other hand, well №1478 has a composition dominated by hydrocarbonate, sulfate, and chloride ions, also with sodium as the primary cation [35].

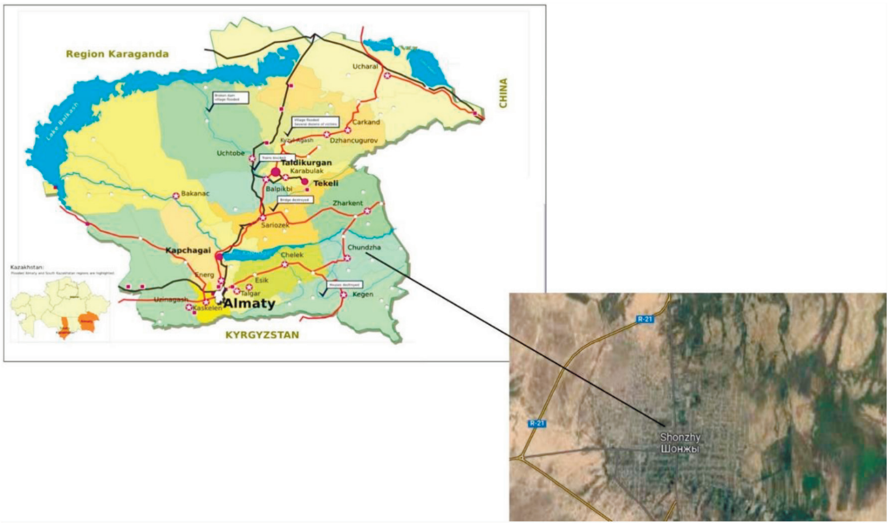

Figure 1 – The geographical location of sampling sites (Chundzha, 43°00'10.0"N 79°59'30.0"E)

Cyanobacteria exhibit remarkable adaptability to various environmental conditions, including exposure to gamma or ultraviolet radiation, high temperatures, salinity, desiccation, and heavy metals [36]. These environmental factors can induce changes in cyanobacterial morphology (such as alterations in cell wall structure), biochemical processes (e.g., production of screening pigments), or physiological responses (e.g., state transitions), as well as genetic changes resulting from acclimatization to extreme conditions [37]. In response to different stressors, cyanobacteria produce a diverse array of secondary metabolites, which contribute to their protection. These secondary metabolites serve various functions, including defense against herbivores and predators, antioxidant properties, chemosensory abilities, thermotolerance, and photoprotection [38]. Exploiting these qualities, cyanobacteria hold potential applications in cosmetology, the production of food additives, and the pharmaceutical industry within the field of industrial biotechnology.

During the study of water samples collected from the thermal area, a total of 71 species belonging to 7 taxonomic groups were identified. The dominant group was Chlorophyceae, accounting for 46.5% of the identified species, with a total of 33 species. Cyanophyceae (33.8%) represented the second largest group, comprising 24 species, while Bacillariophyceae (diatoms) accounted for 12.7% with 9 species (Fig. 2).

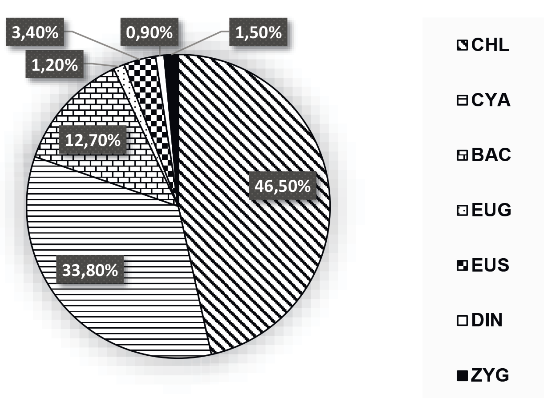

Figure 2 – Abundance of various groups of algoflora in Chunzha thermal springs. CHL: Chlorophyceae, CYA: Cyanophyceae, BAC: Bacillariophyceae, EUG: Euglenophyceae, EUS: Eustigmatophyceae, DIN: Dinophyceae, ZYG: Zygnematophyceae

According to the results of the study of the selected samples, algocenoses of hot springs in the Uygur district are characterized by the predominance of green algae by taxa, but the occurrence of cyanobacteria is much more common than the first. Microalgae and cyanobacteria from the source are assigned to 57 species and subspecies belonging to 3 divisions (Cyanophyta, Chlorophyta, Bacillariophyta), 7 classes, 7 orders, 15 families, 22 genera. The following dominant genera of microalgae were recorded at all the studied points: *Chlorella, Dunaliella, Euglena, Scenedesmus,* etc. As well as genera of cyanobacteria – *Synechoccocus, Synechocystis, Phormidium, Oscilatoria, Nostoc, Trichormus, Anabaena, Spirulina,* etc. Cyanobacteria unite 24 species represented by 13 intraspecific taxa and belong to 5 orders (*Chroococcacales, Spirulinales, Nostocales, Oscillatoriales, Synechococcales*). Among the 5 orders of species diversity, *Oscillatoriales* predominate – 9 or 37.5%, *Nostocales* – 6 or 25% and *Synechococcales* – 4 or 16.6%. The dominant species were planted in nutrient media for further isolation and purification from the accompanying microflora.

Search and isolation of axenic cultures of cyanobacteria

To obtain axenic cultures from the enrichment culture, conventional microbiological techniques were employed, including dilution, reseeding, and cell division using a micromanipulator (Fig. 3). The isolated cultures were transferred to both liquid and solid media, specifically Tamiya, BG11, Zarruk, and Gromov, in flasks and Petri dishes, which were then exposed to light. Isolation of cyanobacteria on agar plates is a commonly used method. A small volume of the suspension was aseptically streaked onto a Petri dish containing agar-based culture medium using the "strike" method, and the dishes were subsequently incubated until colonies developed. In some cases, antibiotics such as ampicillin and chloramphenicol were added at concentrations of 2000 units/ml to obtain bacteriologically pure strains of microalgae. Thus, 8 algological and bacteriologically pure cyanobacteria cultures belonging mainly to the genera *Oscillatoria, Phormidium, Nostoc, Synechococcus, Synechocystis, Tolypothrix, Anabaena* and *Spirulina* were isolated from samples of water from a hot spring in the Uygur district.

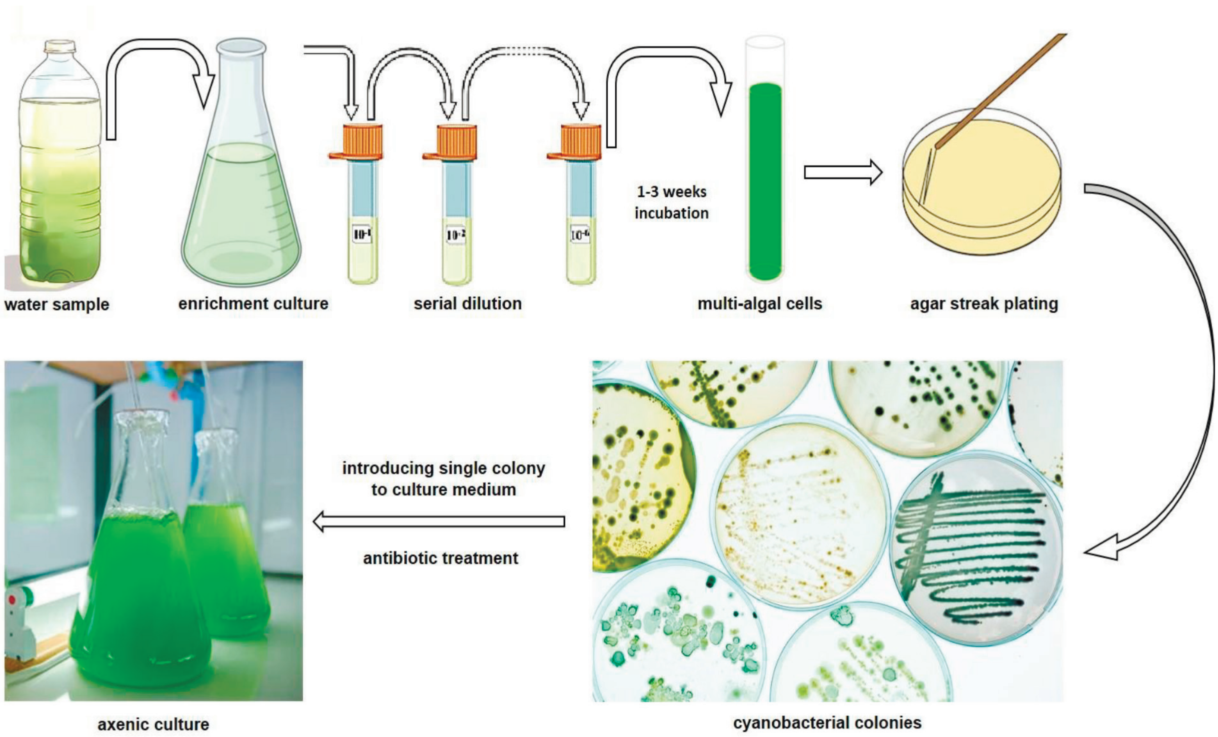

Figure 3 – Isolation of axenic culture by microbiological methods

Morphological evaluation of isolated cyanobacterial cultures

Cyanobacteria are morphologically diverse Gram-negative prokaryotes, encompassing a range of forms including unicellular, colonial, and multicellular structures. Multicellular cyanobacteria exhibit a filamentous structure, wherein cells can be spherical, rod-shaped, or curved. These cells may exist as single entities or cluster together, held in place by a shared sheath or envelope resulting from consecutive divisions. Cyanobacteria exhibit a wide array of adaptive mechanisms that contribute to their successful proliferation in diverse environmental conditions [39].

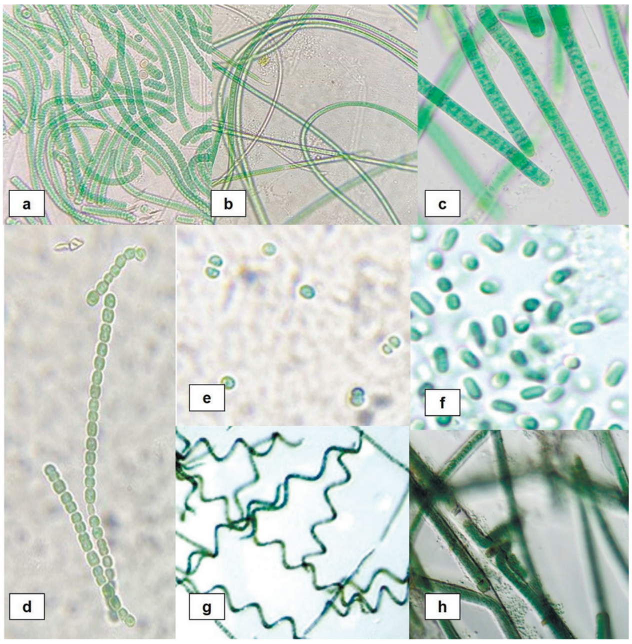

Figure 4 – Cells images (100x) of new isolated cyanobacterial strains:
a- *Anabaena cylindrical, b- Oscillatoria limnetica, c- Phormidium sp., d- Nostoc commune, e- Synechocystis sp., f- Synechococcus elongates, g- Spirulina fusiformis, h- Tolypothrix tenuis*

According to the main morphological characteristics, *Phormidium* sp. is a filamentous type of cyanobacteria (Fig. 4c) that does not fix nitrogen, since it does not possess heterocytes (specific atmospheric N_2-binding cells). The filaments (trichomes) were unbranched, usually straight, very long, not strongly twisted, and slightly curved. The end parts of the threads (ends) were not sharp, but slightly curved. The filaments consisted of cylindrical cells, the length of which somewhat exceeded their thickness (1.84 µm). The next species from the family *Oscillatoriaceae*, identified as *Oscillatoria limnetica*, is also a filamentous form without heterocysts (Fig. 4b). The trichomes were single, almost straight, not attenuated at the apices, not constricted near the transverse walls; the cells are not granulated on the transverse walls, the length of the cells varied between 1.5-3.1 µm and the width from 6.5 to 7.1 µm; the terminal cage was rounded, calyptras were absent. *Nostoc* commune, belonging to the family *Nostocaceae*, consists of straight blue-green spherical vegetative cells with trichomes (6-8 µm long, 3-5 µm wide), the ends of which are not narrowed and clearly branched at the division points. In most cases, heterocysts are intercalary, solitary, light brown in color. Akinetes are rare, oval, and differ in size from vegetative cells (Fig. 4d). Another species from the *Nostocaceae* family that is a filamentous cyanobacterium with heterocysts was *Anabaena cylindrica* (Fig. 4a). Trichomes are often randomly arranged, while solitary trichomes are composed of spherical cells. Among them, heterocysts are rare, and akinetes are ubiquitous. Trichomes have small deep narrowings, the ends of which are not narrowed, and the walls are covered with mucous membranes. Cells are cylindrical, barrel-shaped or spherical, whitish or light blue-green, and heterocysts are oval, sometimes spherical, more than vegetative cells. The isolated cyanobacterial culture *Synechococcus elongatus* has round or ellipsoidal binary cells with rounded ends; the sizes of mature cells varied from 0.5 to 1.6 microns in width and from 2.4 to 3.7 microns in length (Fig. 4f). *Synechocystis sp.* – a unicellular, spherical, nitrogen-free cyanobacterium with a diameter of 0.7-8 microns without a layer of mucus or thin and colorless. Small (2 microns in diameter) spherical photosynthetic unicells without a mucous membrane, the cell is longer than the width (Fig. 4e). *Spirulina fusiformis* is characterized by its regularly coiled trichomes of isopolar and cylindrical shapes forming a spiral, the distance between the spirals is 2.7-5 µm. Trichome cells are shorter than wide; 6-8 µm wide, 2.6-5.6 µm long. The trichomes are motile, without or with a thin, inconspicuous sheath, and with rounded apical cells (Fig. 4g). *Tolypothrix tenuis* are filamentous cyanobacteria with basal heterocytes and free apical ends, with false branches arising directly below the heterocytes. False branching solitary, less often with double false branching. The filaments are flexible with monocomponent trichomes, the cells are cylindrical blue-green or olive-green in color, the terminal cells are rounded (Fig. 4h). As a result of morphological studies, the isolated new axenic cultures of cyanobacteria were identified as *Oscillatoria subbrevis, Phormidium ambiguum, Nostoc commune, Synechococcus elongatus, Synechocystis sp., Tolypothrix tenuis, Anabaena cylindrica,* and *Spirulina fusiformis.*

Determination of cultivation conditions and study of growth dynamics of cyanobacterial cultures

As mentioned earlier, the characterization and utilization of newly discovered cyanobacteria species play a crucial role in biotechnology for the production of bioactive compounds. To achieve this, several key challenges need to be addressed, including the selection of an appropriate nutrient medium, particularly a nitrogen source, which significantly influences the biomass yield and composition of phototrophic microorganisms [40]. In this study, all the newly isolated cyanobacteria cultures were cultivated for a duration of 12 days under laboratory conditions using three different nutrient media: BG11, Zarruk, and Gromov. As a result of the experiment, a high growth of the cultures of *Nostoc commune, Anabaena cylindrica* and *Synechococcus elongatus* was observed when grown in a BG-11 nutrient medium, while the growth of these cultures in the Zarruk and Gromov nutrient medium was insignificant. A suitable medium for growing cultures of *Phormidium sp., Oscillatoria limnetica* and *Spirulina fusiformis* was Zarruk's medium. And the cultures of *Tolypothrix tenuis* and *Synechocystis sp.* showed a higher growth dynamics in Gromov nutrient medium.

In addition to the nutrient medium composition, various environmental factors such as pH, temperature, light intensity, oxygen, and carbon dioxide concentrations play significant roles in the stability and growth dynamics of cyanobacteria. Therefore, it is important to carefully control these conditions. Temperature is a crucial factor as many cellular processes in cyanobacteria are temperature-dependent, with their rates typically peaking between 25°C and 40°C. Light availability is also a limiting factor in microalgae biotechnology, as it serves as the primary energy source for photoautotrophic organisms [41]. To investigate the influence of these factors on the growth dynamics of selected cyanobacteria strains and determine the optimal conditions for biomass yield, we conducted experiments.

The effect of different temperatures and pH levels on the growth of the isolated strains was examined in 250 ml flasks, with each experiment performed in triplicate. Temperature-controlled chambers were used to observe the growth dynamics at various temperatures: 20°C, 24°C, 28°C, 32°C, 36°C, and 40°C, over a period of 12 days in nutrient media (BG11, Zarruk, and Gromov) at pH 7.5. The initial optical density (OD_{750}) of all cultures in each treatment was set to 0.2±0.01. Similarly, the growth of isolates was assessed at different pH levels (pH 4, 7, and 9). The pH of the medium in each experimental treatment was adjusted using 1M HCl or NaOH. During the experiments, all cultures were shaken three times a day and subjected to a light-dark cycle of 16:8 hours, with a light intensity of approximately 50 µmol m^{-2} s^{-1}. Cultures were sampled every 3 days to monitor their growth at different temperatures, and the pH of the medium was measured at the end of the experiment using a C864P pH meter. By systematically evaluating the growth responses of cyanobacteria to these varying conditions, we aimed to identify the optimal light and temperature conditions, as well as pH values, for achieving maximum biomass yield.

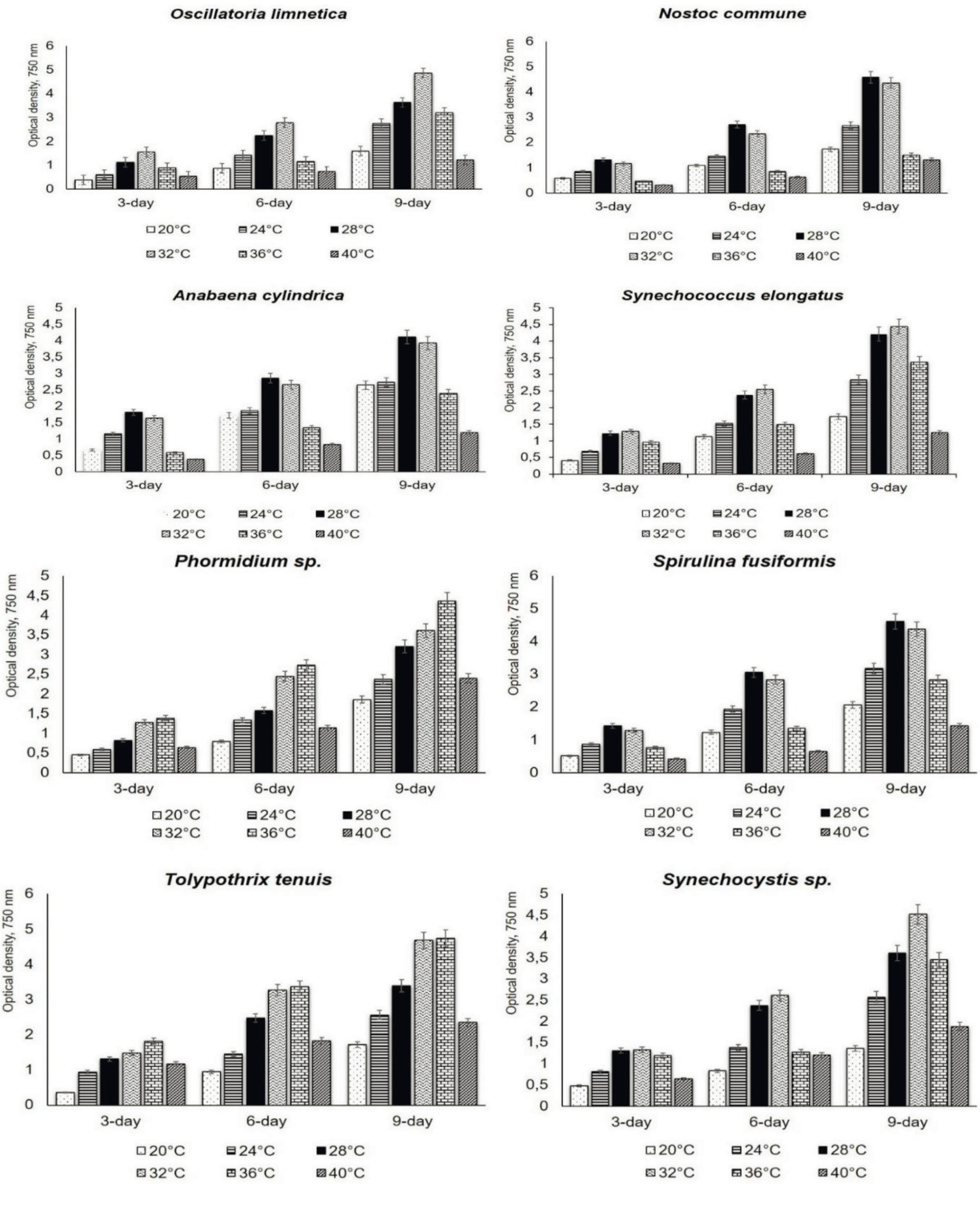

Figure 5 – Growth rate of cyanobacterial cultures under different temperatures

As the results showed (Fig. 5), for the cultures of *Oscillatoria limnetica, Synechococcus elongates,* and *Synechocystis sp.* the optimum temperature was 32°C. From the first day of cultivation, the cultures of *Nostoc commune, Anabaena cylindrica* and *Spirulina fusiformis* showed rapid growth at a temperature of 28°C. Growth dynamics of the remaining two cultures of *Phormidium sp.* and *Tolypothrix tenuis* showed high results at a temperature of 36°C. Thus, optimal growth temperatures of newly isolated cyanobacteria isolates were determined for further research.

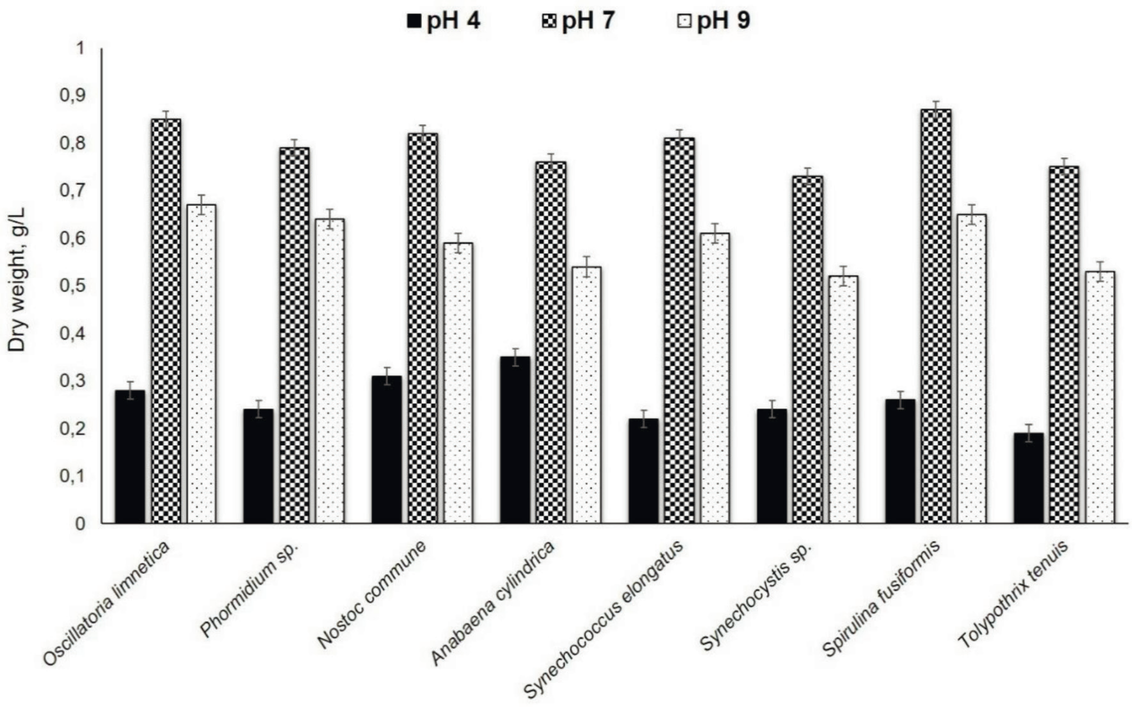

Figure 6 – Biomass production of cyanobacterial cultures at different pH levels

Most cyanobacteria are characterized by good growth in a neutral or slightly alkaline environment, usually for them the optimal pH of the medium is 7.2-7.5. The acidity of the medium affects the stability of the components of the nutrient medium, their availability, especially the digestibility of growth factors and vitamins [40-41]. The studied cultures of cyanobacteria showed enhanced growth over time at various pH levels of 4, 7, and 9 (Fig. 6). All isolates showed the maximum production of dry biomass at neutral pH 7. The maximum dry biomass yield of *Spirulina fusiformis, Oscillatoria limnetica* and *Nostoc commune* was 0.87, 0.85 and 0.81 g/L at pH 7, respectively ($p<0.05$).

Light is a crucial factor that influences the growth and physiology of cyanobacteria, and changes in illumination can have a significant impact on their growth patterns. Cyanobacteria exhibit morphological and physiological changes in response to different light intensities [42]. Therefore, it is important to conduct a detailed study of these changes to develop methods and strategies for controlling the yields of various metabolites for commercial purposes. In our study, we aimed to assess the comparative effect of different light intensities on the growth of newly discovered cyanobacteria species. The cultures were grown under four different lighting intensities: 30, 50, 100, and 200 μmol photons m^{-2} s^{-1}. The growth of the cyanobacteria species was monitored every three days. Measurements of optical density (OD_{750}) and chlorophyll a (Chl a) content were taken separately for each species. At the beginning of the experiment, the initial optical density index was set to 0.2±0.01 for all eight experimental variants. By monitoring the growth parameters and assessing the optical density and chlorophyll a content at regular intervals, we aimed to determine the specific effects of different light intensities on the growth dynamics of the cyanobacteria species under investigation. This information will provide valuable insights into optimizing light conditions for maximizing the growth and productivity of these cyanobacteria strains.

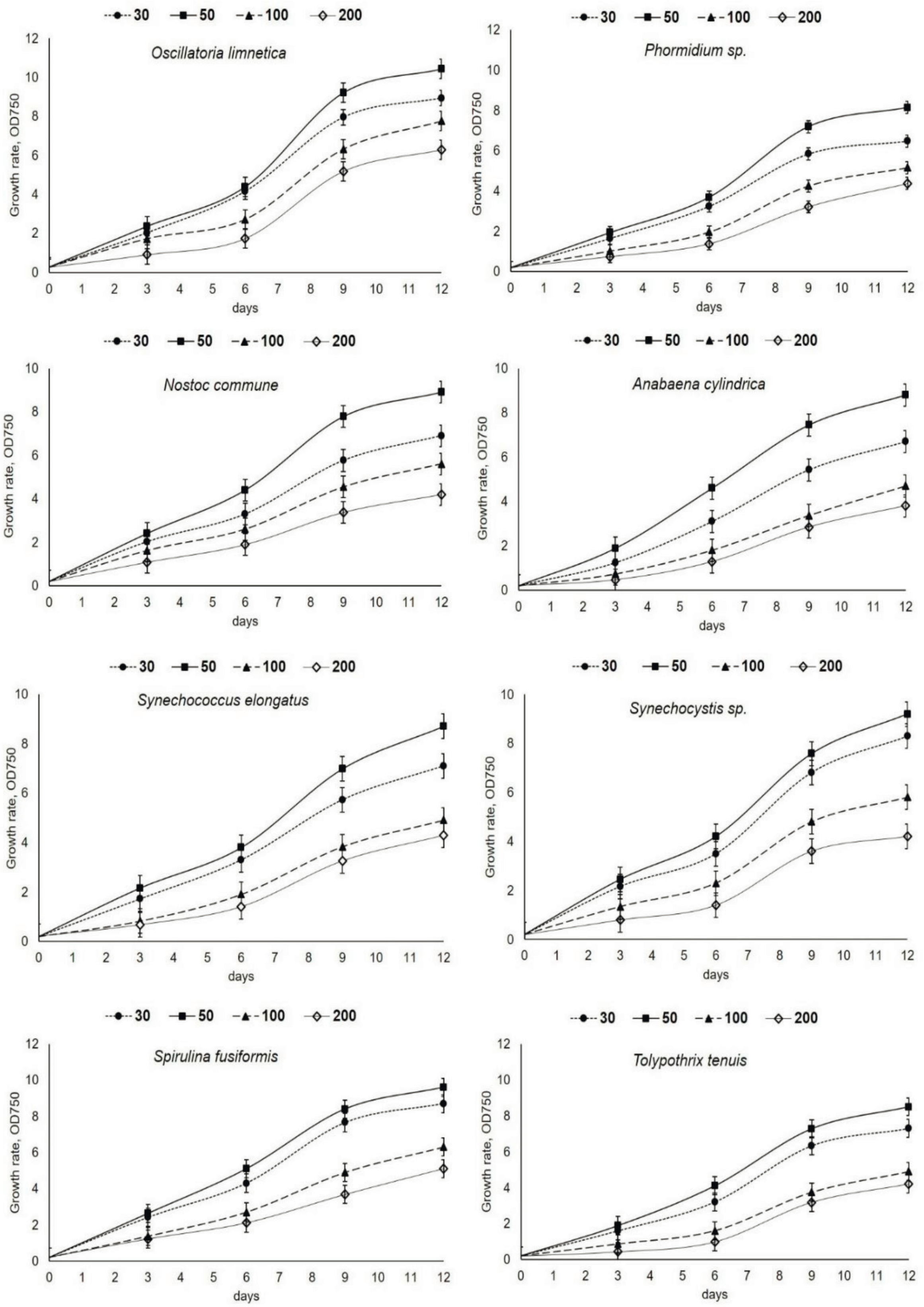

Figure 7 – Changes in growth rate of cyanobacterial cultures with respect to light intensity

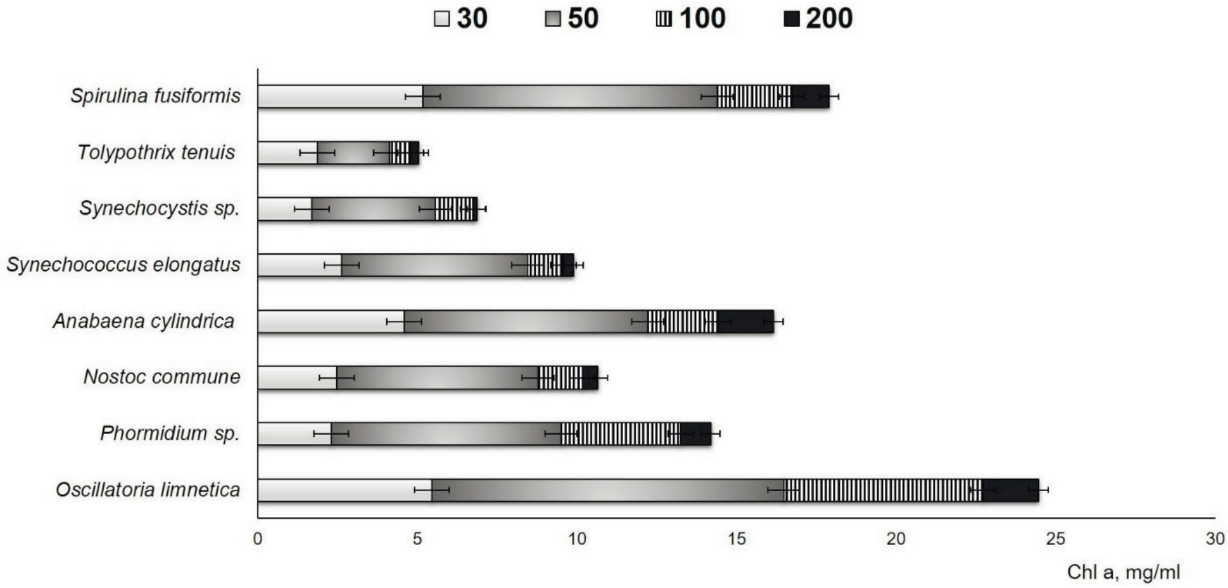

Figure 8 – Changes in content of Chl a obtained after 12 days of cultivation for selected cyanobacterial cultures under different irradiance (µmol photons m^{-2} s^{-1})

According to the results of the growth profile, when the light intensity varied from 30 to 200 µmol per square meter per day, significant changes in the density of the suspension of cyanobacteria cultures were observed. As shown in Figure 7a, b, it was found that the maximum values of the growth rate coefficient of all isolated cultures of cyanobacteria were observed when grown with illumination of 50 µmol m^{-2} s^{-1} and had growth rates from 0,20 to 10,43 for *Oscillatoria limnetica*.

The maximum cell-specific concentration of Chl a (about 11,04 mg/ml) was noted for *Oscillatoria limnetica* at 50 µmol photons m^{-2} s^{-1} light intensity and it was about 6,3 times higher than the minimum at 200 µmol photons m^{-2} s^{-1}(Fig. 8). For *Spirulina fusiformis* and *Anabaena cylindrica* the maximum cell-specific Chl a concentrations (9,23 mg/ml and 7,64 mg/ml, respectively) were also recorded at 50 µmol photons m^{-2} s^{-1}. On the other hand, the minimum values for all phenotypes of cyanobacteria were obtained at 200 µmol photons m^{-2} s^{-1}.

Optimal values indicated that these cyanobacterial cultures preferred higher water temperatures and alkaline environments, but did not require higher light levels or higher nitrogen and phosphorus concentrations. That is, these new isolated strains of cyanobacteria had high adaptive abilities to the environment. This result is consistent with some reports of cyanobacteria [43].

Conclusion

The present article focuses on studying the structure of the cyanobacteria community in four hot springs located in Chundzha, Almaty region. These hot springs vary in temperature, pH, and nutrient levels, leading to different cyanobacteria preferences. The aim of the study was to investigate the influence of environmental factors such as temperature, light intensity, and mineral nutrition on the physiology of cyanobacteria. Understanding these factors is important for modeling primary production in aquatic ecosystems and addressing various biotechnological challenges. The study identified the species composition of the algal community in the four thermal springs, with a total of 24 cyanobacteria species belonging to 13 intraspecific taxa and five orders (*Chroococcacales, Spirulinales, Nostocales, Oscillatoriales, Synechococcales*). Among these, *Oscillatoriales* was the most dominant order, comprising 9 species (37.5%), followed by *Nostocales* with 6 species (25%) and *Synechococcales* with 4 species (16.6%). Eight axenic cyanobacterial cultures were isolated and identified, including *Oscillatoria subbrevis, Phormidium ambiguum, Nostoc commune, Synechococcus elongatus, Synechocystis* sp., *Tolypothrix tenuis, Anabaena cylindrica*, and *Spirulina fusiformis*. The optimal cultivation conditions for these cyanobacterial cultures were determined. *Nostoc commune, Anabaena cylindrica*, and *Synechococcus elongatus* showed high growth rates when cultivated in the BG-11 nutrient medium. *Phormidium* sp., *Oscillatoria limnetica*, and *Spirulina fusiformis* exhibited optimal growth in the Zarruk medium, while the Gromov nutrient medium was chosen for cultures of *Tolypothrix tenuis* and *Synechocystis* sp. Furthermore, the study identified the optimal growth temperatures for different cultures. *Oscillatoria limnetica, Synechococcus elongatus*, and *Synechocystis* sp. showed optimal growth at 32°C, while *Nostoc commune, Anabaena cylindrica*, and *Spirulina fusiformis* exhibited rapid growth at 28°C. *Phormidium* sp. and *Tolypothrix tenuis* showed enhanced growth at 36°C. The studied cyanobacterial cultures demonstrated favorable growth at pH 7 and a light intensity of 50 μmol photons m^{-2} s^{-1}.

This knowledge of the specific growth conditions for the newly isolated cyanobacteria strains enables the production of biomass with a defined composition. By leveraging the combined effects of pH, light, and temperature, it becomes possible to explore the prospects of these cyanobacteria strains in biotechnology applications.

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Conflict of interest

All authors have read and are familiar with the content of the article and have no conflict of interest.

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