ANNOTATION

of PhD thesis for the degree of Doctor of Philosophy (PhD) on speciality “8D05105 - Biotechnology”

Sandybayeva Sandugash Kalzhankyzy

Isolation and screening of potential strains of cyanobacteria-producers of valuable pigments for obtaining biologically active additives

General description of work: The work is devoted to isolation and study of active strains of cyanobacteria-producers of valuable pigments from various thermal springs for obtaining biologically active additives.

Relevance of the research topic:

According to the latest data from the World Food Programme, a record 349 million people in 79 countries are facing acute food shortages. Their numbers continue to grow at such a rate that existing resources are unlikely to meet their needs. This issue is particularly relevant in the context of record increases in the cost of delivering food aid, driven by rising food and fuel prices. In addition, global challenges such as the energy crisis, food security issues, increasing disease prevalence, global warming and other environmental factors are exacerbating the situation. Tackling these issues requires an economically viable and long-term sustainable approach.

In response, the food industry has intensified efforts to develop synthetic food additives, which are favoured for their high stability, strong sensory properties and relatively low production costs. However, growing concerns about the potential adverse effects of synthetic components on human health and the environment, as well as changing consumer preferences towards natural products, are driving demand for biologically safe and environmentally friendly food ingredients.

In this context, phototrophic microorganisms represent a significant scientific and biotechnological interest as a source of commercially valuable bioactive compounds. These microorganisms exhibit a remarkable biochemical diversity that is still insufficiently explored. Their cells contain easily digestible proteins, lipids and polysaccharides as well as a unique spectrum of bioactive metabolites, including polyunsaturated fatty acids (such as alpha- and gamma-linolenic acids), carotenoids, chlorophyll, phycocyanin and essential macro- and microelements.

Of particular interest are cyanobacteria, widely distributed prokaryotes that inhabit diverse ecosystems and exhibit high metabolic plasticity. They synthesise a wide range of biologically active compounds with antibacterial, antifungal, antiviral and antioxidant properties, making them promising candidates for applications in the pharmaceutical and agricultural industries. Among these compounds, naturally occurring pigments are of particular value, including chlorophylls, carotenoids (carotenes and xanthophylls) and phycobiliproteins (C-phycocyanin, allophycocyanin and C-phycoerythrin). These pigments are increasingly used in the cosmetics, food and textile industries due to their unique biochemical properties and natural origin.

Chlorophyll is the primary photosynthetic pigment responsible for oxygenic photosynthesis and is found in all phototrophic microorganisms. Carotenoids

perform both primary and secondary functions and their composition varies according to species and cultivation conditions. Among the commercially important carotenoids, astaxanthin, lutein and β-carotene are the best known. Phycobiliproteins are a unique class of light-harvesting proteins found exclusively in cyanobacteria and red algae. The most studied members of this group are phycocyanin and phycoerythrin. Together, these three classes of pigments enable cyanobacteria to efficiently absorb light over a broad range of the visible spectrum. Because of their physicochemical properties and biological functions, these compounds have great potential for biotechnological applications.

Thermophilic cyanobacteria are of particular scientific interest as analogs of ancient life forms on Earth and as valuable sources of thermally stable biomolecules. Their unique adaptations to extreme conditions, including a modified lipid composition of membranes with a high content of saturated fatty acids and the production of heat shock proteins, enable survival at elevated temperatures and stabilization of cellular components. Additionally, thermophilic cyanobacteria possess unique pigment compositions that protect them from high-intensity light and ultraviolet radiation, making them promising candidates for applications in agriculture, pharmaceuticals, nutraceuticals, and biofuel industries.

However, not all cyanobacterial strains exhibit equal efficiency in pigment production. Their productivity is significantly influenced by cultivation conditions. Therefore, identifying and selecting potential cyanobacterial strains with high pigment production capacity, as well as optimizing their cultivation conditions, is essential.

This study aims to expand the arsenal of microbial strains with high potential for application in the food industry and the production of bioactive compounds. The research topic is highly relevant, as it addresses an important scientific and social challenge while contributing to the acquisition of new fundamental knowledge with subsequent practical applications.

Aim of the research: Isolation and identification of active strains of cyanobacteria from thermal springs, study of their morphological-physiological and biochemical properties to obtain valuable pigments from their biomass.

Objectives of the study:

1. Isolation and identification of axenic cultures of cyanobacteria from thermal springs.
2. Screening of active cyanobacterial strains based on biomass productivity and the accumulation of bioactive compounds in cells.
3. Study of pigment composition of cells of isolated strains of cyanobacteria and improvement of parameters of their cultivation to increase their productivity and accumulation of pigments.
4. Comprehensive evaluation of biological activity of extracts obtained from cyanobacteria biomass.
5. Development of regulations for obtaining active pigments on the basis of cyanobacteria biomass in laboratory conditions.

Objects of the study:

The objects of the study were new strains of cyanobacteria designated as *Oscillatoria subbrevis* CZS 2201, *Phormidium ambiguum* CZS 2205, *Nostoc calcicola* TSZ 2203, *Trichormus variabilis* BK-1 and *Synechococcus* sp. CZS 2204, isolated from thermal springs of Chundzha thermal springs of Almaty region.

Research Methods:

Microbiological, algological, biotechnological, molecular-genetic, physical-chemical and statistical methods of research were used in the work.

The scientific novelty of the study:

For the first time, the algological composition and diversity of phototrophic microorganisms from the hot springs of Chundzha in the Uyghur District of the Almaty region were studied. As a result, eight axenic cultures of cyanobacteria were isolated, and their morphological and cultural properties were analyzed. A screening of the isolated cultures was conducted to evaluate their productivity and the accumulation of bioactive compounds (proteins, lipids, fatty acids, etc.). Qualitative and quantitative pigment analyses were performed on the cells of selected active strains, including *Oscillatoria subbrevis* CZS 2201, *Phormidium ambiguum* CZS 2205, *Nostoc calcicola* TSZ 2203, and *Synechococcus* sp. CZS 2204. Based on laboratory studies, a glycosylated carotenoid, myxoxanthophyll, with a purity of at least 93%, was obtained from the biomass of *Oscillatoria subbrevis* CZS 2201 for the first time.

The immunomodulatory effect of extracts from active complexes derived from cyanobacterial strains was evaluated in vitro on the growth and proliferation of tumor cell lines MiaPaCa2 (pancreatic carcinoma), HepG2 (liver carcinoma), and K562 (human myelogenous leukemia), as well as on the proliferation and growth of immune-competent bone marrow cells in mice.

Scientific and practical significance of the work:

New pigment-producing cyanobacterial strains were isolated from thermal spring waters, including *Oscillatoria subbrevis* CZS 2201, *Phormidium ambiguum* CZS 2205, *Nostoc calcicola* TSZ 2203, and *Synechococcus* sp. CZS 2204. These strains exhibit unique biochemical and physiological properties and demonstrate high productivity.

The isolated cyanobacterial strains have been added to the collection of phototrophic microorganisms at Al-Farabi Kazakh National University for further use in biotechnology.

The aforementioned strains of cyanobacteria were deposited in the 'Republican Collection of Microorganisms' LLP (RKM) at JSC “National Holding “QazBioPharm”, assigned the following numbers: RKM1063, RKM1060, RKM1061 and RKM1062, on 19/10/2023, respectively.

A patent for utility model No. 9607, dated 27/09/2024, entitled “Cyanobacterium *Oscillatoria subbrevis* CZS 2201 strain used as a raw material for production of glycosylated carotenoid – myxoxanthophyll”, was obtained.

Main points to be defended:

1. Axenic cultures of cyanobacteria belonging to the genera *Oscillatoria, Phormidium, Nostoc, Anabaena, Synechocystis, Spirulina, Trichormus* and

Synechococcus were isolated from thermal springs of Chunja, Uygur district, Almaty region.

2. Molecular genetic identification of the isolated strains was carried out and their taxonomic affiliation was confirmed as *Oscillatoria subbrevis CZS 2201, Phormidium ambiguum CZS 2205, Nostoc calcicola TSZ 2203, Trichormus variabilis BK-1* and *Synechococcus* sp. CZS 2204. The sequences of their 16S rRNA genes have been entered into the NCBI database with registration numbers OQ627016, OQ646791, OQ627023, OL780782 and OQ627024, respectively.

3. *Oscillatoria subbrevis* CZS 2201 and *Phormidium ambiguum* CZS 2205 were characterised by maximum accumulation of dry biomass and protein. The strain *Synechococcus* sp. CZS 2204 is characterised by the highest content of α-linolenic acid and *Nostoc calcicola* TSZ 2203 is identified as the most productive synthetic source of eicosapentaenoic acid.

4. *Oscillatoria subbrevis* CZS 2201 strain was characterised by maximum content of chlorophyll a, total carotenoids, and C-phycocyanin, C-allophycocyanin and C-phycoerythrin among the studied samples.

5. Extracts of active complexes obtained from the biomass of cyanobacteria *Oscillatoria subbrevis* CZS 2201 and *Phormidium ambiguum* CZS 2205 have an immunomodulatory effect, promoting the proliferation of immunocompetent cells and the formation of new colonies of lymphocytes in the bone marrow of mice.

6. Laboratory regulations for the production of glycosylated carotenoid myxoxanthophyll from the biomass of *Oscillatoria subbrevis* CZS 2201 were developed.

Personal contribution of the author:

The analysis of literary data devoted to the problem under study, setting the aim and objectives of the research, conducting experimental research, analysing the obtained results and statistical processing, and outlining the thesis work were carried out by the author independently.

Relation of the work to the plan of state programmes:

The dissertation work was carried out within the framework of the project AP14870171 ‘Creation of new domestic biologics based on biologically active substances of phototrophic microorganisms’ (2022-2024).

Approbation of scientific work:

The results of the research and the main provisions of the thesis work were presented and discussed at the following international scientific conferences and symposia:

1. International scientific-practical conference ‘Aspects and innovations of environmental biotechnology and bioenergy’, 12-13 February 2021, Almaty, Kazakhstan;

2. The 5th Symposium on EuroAsian Biodiversity (SEAB-2021)”. 1-3 July 2021, Almaty, Kazakhstan, Mugla, Turkey;

3. International Scientific Conference of Students and Young Scientists “Farabi World”, 6-8 April 2022/2023, Almaty, Kazakhstan;

4. “The Symposium on EuroAsian Biodiversity (SEAB-2023)”, 6-8 September 2023, Baku, Azerbaijan;

5. VII International Scientific and Practical Conference ‘Abu Ali Ibn Sino and Innovations in Modern Pharmaceutics’, 16-18 March 2024, Tashkent, Republic of Uzbekistan.

6. International Scientific Conference Global Energy Meet, 4-8 March 2024, Los Angeles, California, USA.

The main results of the thesis are presented in 19 published works: 5 articles in republican scientific journals from the list of the Committee for Control in the Sphere of Education and Science of RK, 2 scientific articles in Q1 journals and 1 review in Q2 journal, peer-reviewed in the databases Web of Science and Scopus, as well as 1 article and 10 abstracts in the proceedings of international conferences.

Dissertation structure:

The thesis consists 117 pages of text, including symbols and abbreviations, introduction, literature review, materials and methods, research results and their discussion, and conclusion, 270 sources of literature. Additionally, the work includes 6 tables, 31 figures, and 2 appendices.