ABSTRACT

of the dissertation for the philosophical doctor degree (PhD) on specialty 6D070100 - "Biotechnology"

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Obtaining and studying active strains of cyanobacteria and their application in biotechnology

General description of work

In the dissertation work, the results of studying the potential of isolated from various ecosystems and collection active strains of cyanobacteria in agrobiotechnology and biotechnology of hydrogen production are considered.

Relevance of the research topic

Cyanobacteria are a large group of microorganisms that are widespread in nature. They inhabit nature from freshwater to oceans and are among the most abundant organisms in terms of biodiversity. Cyanobacteria absorb sunlight through special photosynthetic pigments and use the resulting electrons to perform cellular functions. Cyanobacteria are involved in the production of oxygen, nitrogen fixation, the release of secondary metabolites, etc. is an important object in biotechnology.

Studies of the diversity and physiological characteristics of cyanobacteria have so far indicated that they are important objects in the field of biotechnology. In recent years, cyanobacteria are known as rich sources of biologically active compounds, and are recognized as major producers with the ability to produce many biological metabolites. They are used in biotechnological industries such as food, fuel, fertilizers, dyes and toxins, vitamins, enzymes and pharmaceuticals, secondary metabolites.

In recent years, interest in alternative energy sources has increased, as a result of which new terms have entered science: biodiesel, biohydrogen, biobutanol, bioethanol, bio-oil and biogas. In particular, the interest of scientists is reduced to inexhaustible sources of raw materials used in the production of biofuels, including the process of obtaining hydrogen based on biophotolysis. The production of hydrogen among all branches of bioenergy is technologically expensive, but the most environmentally friendly, due to which it can play an important role in solving many energy problems that arise in the future. 50% of hydrogen energy is currently extracted from coal reformation, while only 0.1% is biologically generated. The reason for this is that, despite intensive research, until now no active producer has been found capable of producing high amount of biohydrogen. To date, the ability of various biological objects to produce hydrogen has been studied. In this context, cyanobacteria have adapted to the release of molecular hydrogen by carrying out a biophotolysis process using energy sources derived from sunlight. The launch of biohydrogen production based on the activity of the nitrogenase and hydrogenase enzymes may be a solution to many problems that arise on a global scale. Nitrogen fixation and hydrogen production involve 2 different enzymes – nitrogen and hydrogen. Nitrogenase simultaneously absorbs free nitrogen from the air and releases energy in the form of hydrogen stored in reserve under stress. Accordingly, this enzyme requires a lot of energy to work. The enzyme of hydrogenesis is found

in vegetative cells, is active in cells under anaerobic conditions and catalyzes hydrogen molecules.

In addition, the enzyme nitrogenase, that producing hydrogen, fixes free nitrogen in the air and converts it into an accessible form for plants. The enzyme nitrogenase, contained in the cell, simultaneously performs the processes of absorption of free nitrogen in the air and its conversion into ammonia (NH_3), with the release of hydrogen into the external environment as a final product and the conversion of ATP energy into ADP. Nitrogenase is a multidisciplinary complex enzyme with multiple substrates, responsible for nitrogen fixation and found mainly in prokaryotic cells, which catalyzes all biochemical nitrogen fixation and also provides the global biogeochemical nitrogen cycle. Nitrogenase uses magnesium adenosine triphosphate and electrons to regenerate many substrates.

The hydrogenase enzyme is responsible for the release of hydrogen in vegetative cells, and the energy stored in the cell is used based on the activity of the nitrogenase enzyme. Hydrogenesis is another group of enzymes involved in the release of biohydrogen. This group of heterogeneous enzymes has a wide variety of structures, properties and functions. The enzyme catalyzes a simple chemical reaction, that is, the formation of hydrogen from protons and electrons.

In this regard, studies aimed at isolating active strains of cyanobacteria with high activity of nitrogenase and hydrogenase, as a result of which biohydrogen and biofertilizers are obtained, are one of the urgent problems of the biotechnological industry of the 21st century.

Purpose of research work

Study of the potential of collection and isolated active cyanobacterial strains from various ecosystems in agrobiotechnology and biotechnology of hydrogen production.

Research tasks

1. Isolation and identification of axenic cultures of cyanobacteria from various ecosystems;
2. Identification of isolated cyanobacterial strains;
3. Determination of nitrogenase activity of isolated cultures and collection strains of cyanobacteria;
4. Study of the influence of the biomass of the isolated strains of cyanobacteria on the productivity of rice crops;
5. Study of the effect of biomass of collection strains of cyanobacteria on the yield of *Sunrise* T-4 strawberry;
6. Determination of nitrogenase activity of strains of collection cultures of cyanobacteria;
7. Investigation of the ways of hydrogen evolution by cultures of cinobacteria that do not form heterocysts;
8. Study of the process of hydrogen evolution by heterocyst strains of cyanobacteria.

Research objects

Isolated cultures from various ecosystems and collection strains of cyanobacteria were used as an object of research work. Collection strains –

Synechocystis sp. PCC 6803, *Desertifilum* sp. IPPAS B-1220, *Synechococcus* sp. I12 и *Phormidiumcorium* B-26, *Anabaena* sp. 7912, *Anabaena* sp. Z-1, *Anabaena variabilis* R-I-5, *Nostoc calsicola* RI-3, *Nostoc* sp. S-2, *Synechocystis* sp. PCC 6803. Selected Crops – *Anabaena* sp. Bl-4, *Nostoc* sp. J-14, *Cylindrospermum* sp. J-8, *Anabaena variabilis* K-31 and *Tolypothrix tenuis* J-1, *Oscillatioria* Sh-11. High-stemmed *Sunrise* T-4 strawberry and Akmarzhan rice.

Research methods

In the course of the work, microbiological, algological, biotechnological, molecular genetic, agrotechnical, physical and chemical methods were used.

Scientific novelty of research

For the first time, the composition of the algal flora and the percentage of various phototrophic microorganisms of rice fields in the Zhanakorgan region of the Kyzylorda region was studied. As a result of the study, 6 algologically pure and 5 axenic cultures of cyanobacteria were isolated.

The identification and phylogenetic analysis of the isolated strains of cyanobacteria *Anabaena* sp. Bl-4, *Nostoc* sp. J-14 and *Tolypothrix tenuis* J-1.

For the first time, the nitrogenase and hydrogenase activity of the studied strains of cyanobacteria was investigated, and the high ability to release hydrogen was determined in the strain *Desertifilum* sp. IPPAS B-1220.

The growth-stimulating effect of strains of nitrogen-fixing cyanobacteria *Anabaena variabilis* R-I-5 was also determined in relation to strawberries (variety *Sunrise* T-4) and rice crops (variety Akmarzhan).

Scientific and practical significance of the work

Isolated new strains of cyanobacteria *Anabaena* sp. Bl-4, *Nostoc* sp. J-14, *Cylindrospermum* sp. J-8, *Anabaena variabilis* K-31, *Oscillatoria* Sh-11, *Tolypothrix tenuis* J-1 are included in the collection of microalgae «CCMKazNU» of the laboratory of photobiotechnology of Al-Farabi Kazakh National University for further study and use in various scientific research.

A technological scheme of a five-section photobioreactor for the cultivation of phototrophic microorganisms has been developed and patented (Patent for a useful model «Photobioreactor for the cultivation and selection of phototrophic microorganisms», №38863 at 05.06.2019). The photobioreactor made according to this technological scheme is recommended for mass cultivation of strains of cyanobacteria and microalgae and for selection work with crops.

The active strains of nitrogen-fixing strains *Anabaena variabilis* R-I-5 and *Anabaena* sp. B1-4, selected in the course of research work, are currently used in the greenhouse of Al-Farabi Kazakh National University as a biofertilizer to increase the productivity of crops.

The main provisions for the defense

Six axenic cultures of cyanobacteria isolated from a rice field, 3 of which were identified as *Anabaena* sp. Bl-4, *Nostoc* sp. J-14 and *Tolypothrix tenuis* J-1;

The positive effect of molybdenum (Mo^{+6}) at a concentration of 1 μmol on the nitrogenase activity in the *Anabaena* sp. Bl-4;

Growth-stimulating effect of nitrogen-fixing cyanobacteria *Anabaena variabilis* R-I-5 and *Anabaena* sp. Bl-4 in relation to strawberry (variety Sunrise T-4) and rice crop (variety Akmarzhan);

The ability of the *Synechocystis* sp. PCC 6803 for the evolution of hydrogen in dark conditions and the strain of *Desertifilum* sp. IPPAS B-1220 in lighting conditions;

Adding 10 μmol of diuron to the suspension of the *Desertifilum* sp. IPPAS B-1220 increases hydrogen evolution by 1.5 times.

When determining the ratio of the reaction centers of the pigment-protein complexes PS1 and PS2 in the studied strains of cyanobacteria in accordance with 77 K of the obtained spectrum of fluorescent radiation, *Synechocystis* sp. PCC 6803 and *Desertifilum* sp. IPPAS B-1220 had high PS1/PS2 ratios close to each other.

Influence of lighting conditions on the release of hydrogen by heterocyst strains of cyanobacteria, which consists in the intensive release of hydrogen by the *Anabaena variabilis* R-I-5 strain in the light and by the *Nostoc calsicola* RI-3 strain in the dark.

Personal contribution of the author

Analysis of literature data related to the problem under study, determination of the goals and objectives of the work, conducting experimental research, statistical processing of results and analysis, writing a dissertation, preparation was carried out with the personal participation of the author. In addition, the author contributed to the publication of all scientific papers (CCSES articles, impact factor articles, patent, abstracts): manuscript preparation, graphing, analysis of results, correspondence and responses to reviewers.

Connection of work with the plan of state programs

The dissertation work was performed within the framework of projects: AP05131743 «Development of scientific and methodological foundations of biomonitoring technology and forecasting the state of contaminated aquatic ecosystems using phototrophic microorganisms» (2018-2020), AP05131218 «Development of a waste-free technology for biological wastewater treatment and the use of carbon dioxide based on cyanobacteria for the potential production of biodiesel» (2018-2020), AP08052481 «Development of a technology for the production of biodiesel based on active strains of microalgae» (2020 -2022) and AP08052402 «Development of technology for the production of fertilizers based on nitrogen-fixing cyanobacteria» (2020-2022).

Approbation of work

The results of the research and the main provisions of the dissertation were reported and discussed at the following international scientific conferences and symposia:

1. International scientific conference of students and young scientists «Farabi Alemi», April 10-11, 2018, Almaty, Kazakhstan;

2. International Pushchino School - Conference of Young Scientists – «Biology - Science of the 21-century», April 23-27, 2018, Pushchino, RF;

3. 4th International Conference on Bioscience and Biotechnology (BioTech 2019), February 2-22, 2019, Kuala Lumpur, Malaysia;

4. 10th International Conference on the Study of Photosynthesis and Hydrogen Energy Devoted to Resilience, June 23-28, 2019, St. Petersburg, RF;

5. International Conference on Artificial Photosynthesis, March 2-5, 2019, Kyoto, Japan;

6. International Training Course on Industrial Synthetic Biotechnology, October 14 - November 2, 2019, Tianjin, China;

7. International Conference on Metabolic Engineering (MES 2019), October 20-22, 2019, Tianjin, China.

8. 11th European Workshop on the Biology of Cyanobacteria, 7-9 September 2020, Rua Alfredo Allen, Portugal.

Publications

The main composition of the thesis is presented in 12 printed works, including 3 articles in republican scientific journals from the list of the Committee for Control in Education and Science of the Republic of Kazakhstan, 3 scientific articles in the 1st quartile and 5 theses at international conferences. Based on the results of the study, a patent was obtained for a useful model «Photobioreactor for the cultivation and sorting of phototrophic microorganisms», №38863, 09/27/2019.

The structure and volume of the thesis

The dissertation work consists of 117 computer texts and abbreviated words, an introduction, a literature review, materials and research methods, research results and their discussion, conclusions and 243 used literature. The scope of work includes 8 tables, 52 figures and 1 annexes.