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Collection of living diatom cultures of Limnological Institute: Trends and use potential

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ABSTRACT. Microalgae culture collections enable the practical use of the products of their metabolism and provide an overview of the morphological, genetic and physiological diversity known in nature. However, despite the fact that diatoms are a unique object, a little number of species is maintained in culture in world collections compared to their huge diversity. The Department of Cell Ultrastructure of the Limnological Institute of the Siberian Branch of the Russian Academy of Sciences (LIN SB RAS) has created a collection of monoclonal cultures of diatoms isolated from different regions of Lake Baikal and other water bodies. Currently, the collection is represented by 144 strains of planktonic and benthic diatoms belonging to 20 species. Regularly reseeded cultures are used to solve a number of problems related to ecology, taxonomy, cell biology, molecular genetic research, as well as for use in biotechnology.

Keywords: culture collection, diatom strains, monoclonal cultures, Lake Baikal

1. Introduction

Diatoms (Bacillariophyta) represent one of the most diverse groups of microalgae (Round et al., 1990). The results of diatom studies are used in paleolimnology (Smol, 1985; Bradbury et al., 1994; Rühland et al., 2003), biostratigraphy (Baldauf and Barron, 1991; Yanagisawa and Akiba, 1998; Khursevich et al., 2001), quality assessment surface water (The diatoms..., 2010) and archeology (Battarbee, 1988; Juggins and Cameron, 2010). The cultivation of diatoms and the study of their morphology underlies the creation of biomaterials and nanotechnologies (Kröger and Poulsen, 2008; Gordon et al., 2009). Diatoms are used in aquaculture to feed shellfish (Becker, 1994; Lebeau and Robert, 2003a). Many species are able to synthesize a wide range of biologically active substances, such as toxins, pigments, and antibiotics (Bozarth et al., 2009; Mimouni et al., 2012; Kuczynska et al., 2015). Some intracellular metabolites such as eicosapentaenoic acid (EPA), triacylglycerols and amino acids are extracted and used in the pharmaceutical and cosmetic industries (Lincoln et al., 1990; Lebeau and Robert, 2003b).

However, in the world collections of microalgae, an extremely small number of diatom species are maintained in a living state. According to the World Data Center for Microorganisms, Culture Collections Information Worldwide (WDCM CCINFO) database (Komaristaya et al., 2015; Wu et al., 2017), 61 species of diatoms are cultivated in the 12 largest collections of the world, while green algae - 1404 species. In Russia, representatives of Bacillariophyta are part of several collections. The largest number of diatom species is maintained in culture in the collection of the Laboratory of Algae and Microalgae of the Karadag Scientific Station named after T.I. Vyazemsky (KNS) (Davidovich et al., 2015a; 2017). Other Russian collections contain single cultures of diatoms (collection of algae strains of the Biological Institute of St. Petersburg University, CALU (Pinevich et al., 2004); collections of microalgae cultures of the Kovalevsky Institute of Marine Biological Research, Russian Academy of Sciences (Orlova et al., 2011), A.V. Zhirmunsky National Scientific Center of Marine Biology FEB RAS (Stel'mah, 2008)).

The problems of maintaining diatom cultures are associated with their sensitivity to the composition of the medium, the need for frequent reseeding, as well as the peculiarities of maintaining the temperature regime and lighting during cultivation (Wang and Seibert, 2017). It is also necessary to take into account the rate of cell division, the stages of their life cycle, and the features of crossing diatoms (Davidovich et al., 2015b; 2017). Since 2001, LIN SB RAS has begun cultivating planktonic diatoms from Lake Baikal under strictly

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controlled conditions (Bondarenko and Guselnikova, 2002; Safonova et al., 2007). At present, a collection of monoclonal cultures of diatoms isolated from different regions of Lake Baikal and other water bodies has been created. Regularly reseeded cultures of diatoms are kept in liquid media. The main task of the work is to replenish and maintain the collection fund with strains of diatoms for the purpose of further comprehensive research.

2. Materials and methods

Phytoplankton sampling is carried out using the Apstein plankton net (27 μm mesh size) during annual expeditions during open water and ice periods. Samples are placed in sterile plastic flasks with sterile Diatom Media (DM) (Culture collection..., 1988) and transported to the laboratory. To obtain monoclonal strains, single cells are taken from the samples using a micropipette and placed on a sterile slide, controlling the process with an inverted light microscope Axiovert 200 (Zeiss, Germany). Each cell is washed sequentially on a slide in three drops of sterile DM, then transferred to 96-well flat bottom plate containing 200 μl of DM. Cells are grown in a mini-incubator at 8 ˚C and 16 μmol/m^2·s light intensity with a 12:12 light-dark cycle (Safonova et al., 2007). After the number of cells per well reaches 10^3, the culture is transferred into 24-well plates with 2 ml of medium and then into 100 ml Erlenmeyer flasks for further growth. The cultivation of the obtained strains is carried out in refrigerated cabinets equipped with lamps with controlled lighting and temperature conditions of 4 ˚C, 8 ˚C and 12 ˚C. The scheme of work is shown in Fig.1. Cultures were reinoculated once a month. The duration of keeping clonal cultures alive varies from 3 to 10 years.

All strains are examined by microscopy. The chloroplasts were analyzed by phase contrast and epifluorescence microscopy (Axiostar plus, Zeiss, Germany) at 1000× magnification using an HBO 50W/AC ASRAM ultraviolet lamp with an excitation spectrum of 365 nm. Microphotographs are taken with a PIXERA Penguin 600CL camera with AXIOSET software. The study of diatom valves is carried out with a scanning electron microscope (SEM) FEI Company Quanta 200 (FEI Company, USA). To prepare samples for SEM, cells are treated with mixture of conc. nitric and hydrochloric acids (Kaluzhnaya and Likhoshway, 2007) or 30% hydrogen peroxide at 80 ˚C for 5 h, followed by washing with distilled water. The resulting sample is applied to a SEM stub, dried in air, and coated with colloidal gold in an SDC 004 vacuum evaporator (Balzers, Liechtenstein). Permanent light microscopy (LM) patterns are fixed on glass slides in Diatom Mountant resin (Naphrax®, UK). Processing of the results of LM is carried out using the programs Video Test 5.0, xT microscope Control, GIMP 2.99.2.

To create a DNA bank from obtained strains, DNA was extracted from the biomass of diatoms, as described earlier (Marchenkov et al., 2018).

3. Results and discussion

To date, we have formed a collection of living cultures of diatoms, which is represented by 144 strains of planktonic and benthic diatoms belonging to 20 species: *Asterionella formosa* Hassall; *Aulacoseira islandica* (O. Müller) Simonsen; *Achnanthidium sibiricum* M. Kulikovskiy, Lange-Bertalot, A. Witkowski & G. Khursevich; *Achnanthidium affine* (Grunow) Czarnecki; *Amphipleura* sp.; *Ellerbeckia arenaria* f. *teres* (Brun) Crawford; *Encyonema minutum* (Hilse) D.G. Mann; *Encyonema silesiacum* (Bleisch) D.G. Mann; *Encyonema ventricosum* (C. Agardh) Grunow; *Fragilaria capucina* Desmazières; *Fragilaria crotonensis* Kitton; *Fragilaria radians* (Kützing) D.M. Williams & Round; *Hannaea baicalensis* Genkal, Popovskaya & Kulikovskiy; *Nitzschia graciliformis* Lange-Bertalot & Simonsen; *Nitzschia dissipata* (Kützing) Rabenhorst; *Staurosirella* sp., *Staurosira elliptica* (Schumann) Cleve & Möller; *Stephanodiscus meyeri* Genkal & Popovskaya; *Ulnaria acus* (Kützing) Aboal and *Ulnaria danica* (Kützing) Compère & Bukhtiyarova (Table S1). The strains were isolated from different regions of the pelagic zone of Lake Baikal, in Chivyrkuisky Bay, Barguzinsky Bay and Maloye More Strait, as well as from the Selenga and Buguldeyka rivers. Also in the collection are strains from Lake Labynkyr (Sakha Republic (Yakutia)) and the Yenisey River (Krasnoyarskiy Region).

Accounting for isolated strains is kept in a working electronic journal and is in the public domain. The journal contains the number and name of the strain, genus and species; dates of sampling, obtaining a monoclone and subsequent transfers of culture; permanent pattern code for LM, table number for SEM analysis and presence of DNA sample. For each strain, a database of microphotographs of LM and SEM is created (Fig. 2, Fig. 3). The name of the strains includes the first letters of the sampling point and the serial

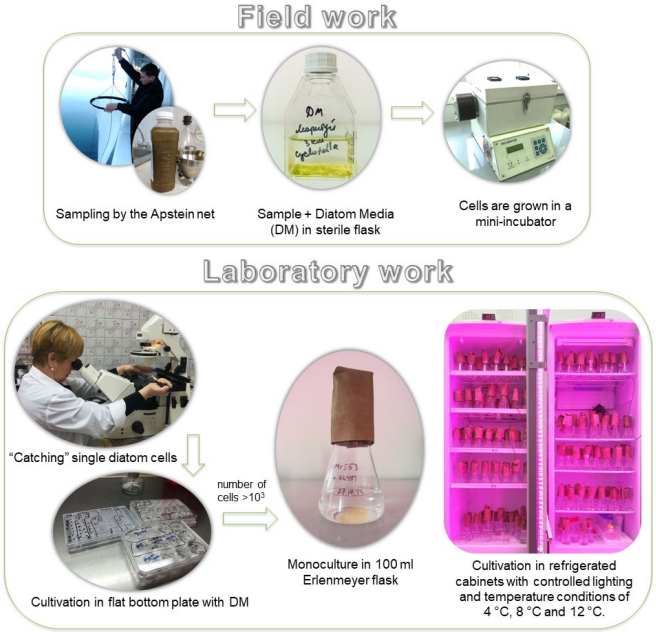

Fig.1. Scheme for obtaining monoclonal cultures of diatoms.

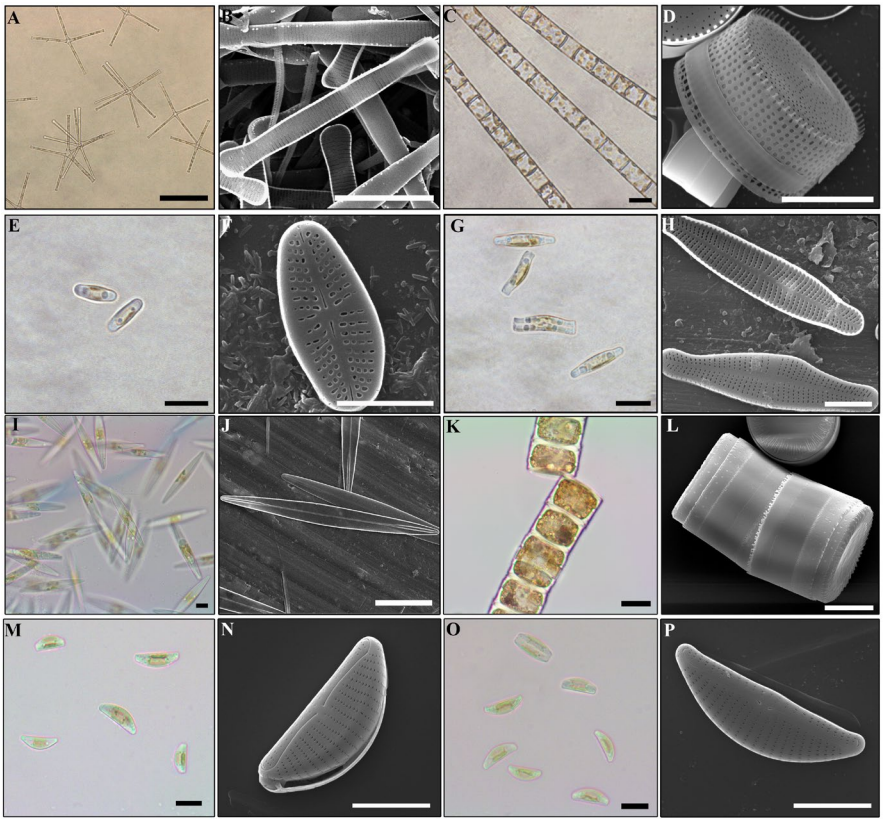

Fig.2. Microphotographs of monoclonal cultures of diatoms from the collection of LIN SB RAS. *Asterionella formosa* (A, B); *Aulacoseira islandica* (C, D); *Achnanthidium sibiricum* (E, F); *Achnanthidium affine* (G, H); *Amphipleura* sp. (I, J); *Ellerbeckia arenaria* f. *teres* (K, L); *Encyonema minutum* (M, N); *Encyonema silesiacum* (O, P); LM (A, C, E, G, I, K, M, O), SEM (B, D, F, H, J, L, N, P). Scale bar: 100 μm – A; 20 μm – C, J, K, L; 10 μm – B, E, G, I, M, O; 5 μm – D, N, P; 3 μm – F, H.

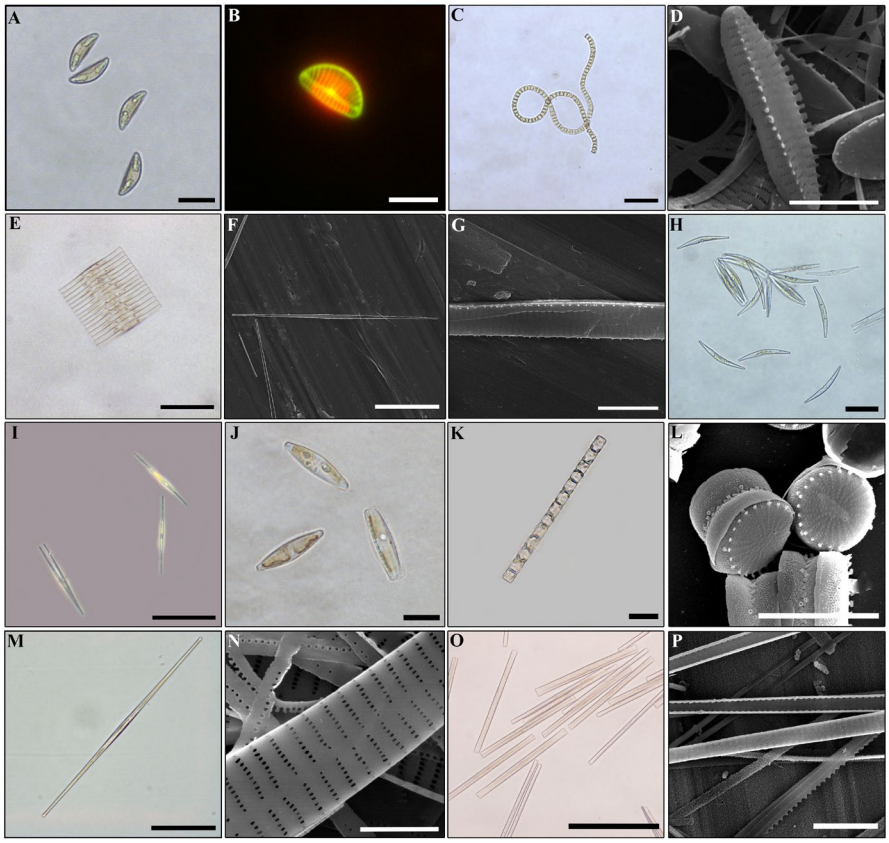

Fig.3. Microphotographs of monoclonal cultures of diatoms from the collection of LIN SB RAS. *Encyonema ventricosum* (A, B); *Fragilaria capucina* (C, D); *Fragilaria crotonensis* (E); *Fragilaria radians* (F, G); *Hannaea baicalensis* (H); *Nitzschia graciliformis* (I); *Nitzschia dissipata* (J); *Stephanodiscus meyeri* (K, L); *Ulnaria acus* (M, N); *Ulnaria danica* (O, P); LM (A, C, E, F, H-K, M, O), epifluorescence microscopy (B), SEM (D, G, L, N, P). Scale bar: 50 μm – C, F, H, I, M, O; 20 μm – A, B, E, K; 10 μm – D, J, L, P; 5 μm – G, N.

number corresponding to the order in which the clone has isolated. As monoclonal cultures age, some of them gradually disappear from the collection, but at the same time, all information about the morphometric features of strains and their DNA is preserved for subsequent use as new tasks become available.

The collection is based on diatoms of the genera *Ulnaria* (Kützing) Compère (102 strains) and *Fragilaria* Lyngbye (14 strains). They serve as model objects for cytological and molecular biological studies, such as studying the genes encoding silicic acid transport proteins (SIT) (Grachev et al., 2002; Marchenkov et al., 2016; 2018), identifying the causes inhibition of diatom development under the influence of various growth inhibitors (Sherbakova et al., 2005; Safonova et al., 2007). The model strain *F. radians* was used to develop a method for pilot cultivation of diatoms in a 100-liter glass photobioreactor, which resulted in the production of 10–20 grams of wet biomass of diatoms per week in a semi-continuous mode (Vereshchagin et al., 2008). Lipids, proteins, and biogenic silica were isolated and characterized from the obtained biomass and the macro- and microelement composition of siliceous valves was determined. To study the physical factors affecting the growth and reproduction of diatoms, a complex technique for cultivating microalgae and an incubator with automatic control of temperature and lighting, and a program for express automatic cell counting have been developed (Aslamov and Jewson, 2009; Aslamov and Makarov, 2010).

Cultivation of diatoms isolated from natural communities is accompanied by the development of microorganisms associated with them. Under laboratory conditions, the natural community of Lake Baikal, including diatoms and associated microorganisms, was reproduced, and their influence on the growth of diatoms was also characterized (Zakharova et al., 2010; 2013a; 2013b; Mikhailov et al., 2018; Bedoshvili et al., 2021a).

Obtaining axenic cultures of diatoms is important, since these widespread organisms are the objects of various studies, and are also promising as producers of biologically active substances and other organic molecules. Cultivated strains of *F. radians*, *U. acus*, and *U. danica* isolated from Lake Baikal were used to develop protocols for obtaining axenic cultures (Shishlyannikov et al., 2011; Zakharova et al., 2020a). Axenic cultures of these species of diatoms, which are one of the dominant species of phytoplankton of Lake Baikal, serve as objects of genomic research. Within the framework of the genome project, the complete mitochondrial (Ravin et al., 2010) and chloroplast (Galachyants et al., 2012) genomes were sequenced, and preliminary data on the structure of the complete genome of the freshwater araphid pennate diatom *U. acus* (= *S. acus*) were obtained (Galachyants et al., 2015) and conducted transcriptomic studies (Galachyants et al., 2019).

Species *Achnanthes minutissima* Kützing, *U. acus*, *Stephanodiscus meyeri*, *Aulacoseira islandica*, *Ulnaria danica*, *Ellerbeckia arenaria* and *Achnanthidium sibiricum* are model objects for numerous studies of the structure of silica valves and the processes underlying the morphogenesis of the silica valves of diatoms (Kharitonenko et al., 2015; Bedoshvili et al., 2017a; 2017b; 2018a; 2018b; 2019; 2021b; Petrova et al., 2020; Cvjetinovic et al., 2021a; 2021b).

A study of the morphology and delimitation of representatives of the genera *Fragilaria* and *Ulnaria*, isolated from Lake Baikal and Lake Labynkyr, was carried out in comparison with species from freshwater reservoirs in Europe and Asia, using phylogenetic methods and methods for determining species boundaries, scanning electron microscopy, and interclonal crossing experiments (Zakharova et al., 2020b). Research in this direction is necessary for the development of modern monitoring methods based on barcoding.

Thus, the working collection of living diatoms of the Department of Cell Ultrastructure of LIN SB RAS is the basis for numerous ecological, cytological, molecular biological and genetic studies. New valuable strains and the development of methods for their cultivation are in demand in further research. Therefore, we also plan to study the features of the cultivation of individual taxa of diatoms, identifying the most promising taxa for long-term cultivation and obtaining substances that may be of interest for biotechnological purposes and fundamental research.

4. Conclusions

Thus, despite the difficulty in maintaining diatom cultures in a living state, a collection of living cultures of diatoms, which is represented by 144 strains belonging to 20 species, has been created in the Department of Cell Ultrastructure of the LIN SB RAS. Due to the presence of constantly reseeded strains and axenic cultures, the genome and transcriptome of freshwater diatoms were deciphered for the first time, and new data on the genes of silicic acid transport were obtained. Diatoms from the collection are the objects of research on the formation of siliceous valves, as well as in experiments on interaction with bacteria. New valuable strains and the development of methods for their cultivation are in demand in further research. Therefore, we also plan to study the features of the cultivation of individual taxa of diatoms, identifying the most promising taxa for long-term cultivation and obtaining substances that may be of interest for biotechnological purposes and basic research.

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Conflict of interest

The authors declare no conflict of interest.

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