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COLLECTION OF PURE CULTURES OF ALGAE AND CYANOBACTERIA FOR RESEARCH, TEACHING AND BIOTECHNOLOGICAL APPLICATIONS (NATURE RESEARCH CENTRE, LITHUANIA)

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Abstract

Koreivienė J., Kasperovičienė J., Savadova K., Karosienė J., Vitonytė I., 2016: Collection of pure cultures of algae and cyanobacteria for research, teaching and biotechnological applications (Nature Research Centre, Lithuania) [Dumblių ir melsvabakterių kolekcija mokslui, mokymui ir biotechnologiniams tyrimams (Gamtos tyrimų centras, Lietuva)]. – Bot. Lith., 22(1): 87–92.

The collection of pure cultures of algae and cyanobacteria deposited at the Nature Research Centre is a unique and diverse culture collection in Lithuania. It was initiated on the basis of a few national projects in 2010 as an outcome of ecological and molecular studies on invasive and harmful bloom-forming algae and cyanobacteria. To date, the collection holds more than 500 strains, which belong to ten classes, over 70 genera and 140 species. Strains were isolated from Lithuanian freshwaters and the Curonian Lagoon. The collection serves for various research topics including species interactions, molecular analysis, biotechnology (remediation of wastewaters, cell wall disruption, high- and low-value bioproducts), as well as for teaching purposes.

Keywords: cyanobacteria, culture, freshwaters, Lithuania, microalgae.

The European Union has recently adopted an ambitious strategy for the development of bioeconomy and advised to cover the growing demand of the food, energy and industrial sectors from the innovative use of sustainable biological resources (Enzing et al., 2014). In this approach, algae have great importance for the future application in industry and other fields. Algal culture collections are irreplaceable and unique source providing consistency and standardized quality material for many types of investigations such as comparative taxonomic, physiological, ecotoxicological and *ex situ* ecological studies (Day et al., 2004). Ecological investigations of algal and cyanobacteria community in the ecosystems nowadays are often combined with the experimental studies in which pure cultures are included. Also, the development of sustainable and friendly technologies that involve new trends of applied sciences has started in the last two decades, therefore, algae and cyanobacteria serve as natural ecological resource for human being. Based on cultured species, a number of environmental and biotechnological studies that include wastewater treatment, ecological friendly biofuel, aquaculture, production of various types of high-value products such as fatty acids, pigments or antitumor agents useful for cosmetic and drug industries have been developed (Gachon et al., 2007; Mata et al., 2010; Brennan et al., 2012).

Increasing demand for novel industrial products requires algal isolates and simultaneously promotes the establishment of culture collections. Recently, 45 collections in European countries have been registered in the database of the World Data Centre for Microorganisms (http://www.wfcc.info). Day et al. (2004) noted that about 10 thousand algae and cyanobacteria strains are deposited at about 90 governmental and private microalgae culture collections in Europe. France, Germany, Russia and the United Kingdom

are the leaders among countries that have the largest number of collections (Table 1). Several thousands of strains originated from different climate regions and various types of environments (freshwater, marine, hypersaline or terrestrial) are maintained in the largest culture collections. Nevertheless, the authentic strains isolated from the original localities in the particular region or country is an invaluable resource of species richness for the biodiversity conservation. Moreover, local strains are very important for the sustainable human life development as they can be safely and successfully introduced into regional technologies that prevent a threat of alien species invasion (Blackburn & Volkman, 2012; Koreivienė & Staniulis, 2014).

In Lithuania, the first attempts to maintain cultures were initiated by S. Budrienė at the Nature Research Centre (NRC, formerly the Institute of Botany) about fifty years ago. Strains of green algae (*Chlorella vulgaris*, *Chlorococcum humicolum*, *Scenedesmus quadricauda*, *S. obliquus*, *Pediastrum boryanum*) and cyanobacteria (*Microcystis* sp., *Phormidium bijugatum*, *P. uncinatum*) were used in experimental studies to test the influence of temperature and nutrients on algal growth or performed the analysis of free amino acids in algae biomass and their environment (Jankevichius et al., 1972, 1983, 1988). However, the strains were lost and the establishment of a new culture collection had to be started. The development of a new collection began in 2010, when a few national projects were implemented, and ecological, molecular studies on invasive and harmful bloom-forming algae and cyanobacteria were started. To date, the collection holds more than 500 strains, which belong to ten classes of prokaryotes and eukaryotes, over 70 genera and 140 species (Fig. 1; http://www.gamtostyrimai.lt). Green algae represent the largest part of the species in the collection (Figs 1−2). The largest number of the strains belong to cyanobacteria (~44% of all strains in the collection), green algae (~32%) and raphidophytes (16%) (Fig. 1). However, the number of strains in the collection is not steady as new strains are isolated constantly or some of these are lost or destroyed. Strains are isolated using micropipette and are grown in various types of liquid media depending on the species characteristics and requirements (after Andersen, 2005).

To date, the collection is generally used for research and teaching purposes. Biological and ecological peculiarities, genetic diversity and adaptations of *Gonyostomum semen* in new areas have been studied using isolated strains of the raphidophyte (Karosienė et al., 2012, 2014; Koreivienė et al., 2012, 2013; Kasperovičienė et al., 2015a). Also, the investigations have been focused on cyanobacteria and their role in algal blooms in Lithuanian freshwaters. Therefore, for providing more precise information about cyanotoxin structure, their quantity and producers, the strains from *Aphanizomenon* (s. l.), *Dolichospermum*, *Microcystis*, *Planktothrix* and other genera have been isolated for cyanotoxins and molecular analysis

Table 1. Collections of pure cultures of algae and/or cyanobacteria in the countries of Europe registered by Day et al. (2004) and in the World Data Centre for Microorganisms (http://www.wfcc.info)

Country	Day et al. (2004)	World Data Centre	Country	Day et al. (2004)	World Data Centre
Armenia	–	1	Lithuania	1	–
Austria	2	1	Luxembourg	1	–
Belarus	–	1	Netherlands	1	–
Belgium	4	3	Norway	2	1
Bulgaria	1	1	Poland	–	1
Czech Republic	5	2	Portugal	5	2
Denmark	1	1	Romania	1	–
Finland	2	1	Russia	7	7
France	12	8	Slovakia	2	1
Germany	11	4	Spain	5	1
Greece	3	–	Sweden	2	–
Hungary	2	–	Turkey	–	2
Ireland	1	–	Ukraine	2	2
Israel	2	–	United Kingdom	10	3
Italy	5	3			

(Savadova et al., 2015). Draft genome sequencing of the most important and heavy bloom-forming cyanobacterium *Aphanizomenon flos-aquae* in the Curonian Lagoon has been performed (Šulčius et al., 2015).

Furthermore, the collection appliance deals with the study of the properties of non-native, invasive algae and cyanobacteria species. Several alien cyanobacteria such as *Raphidiopsis mediterranea*,

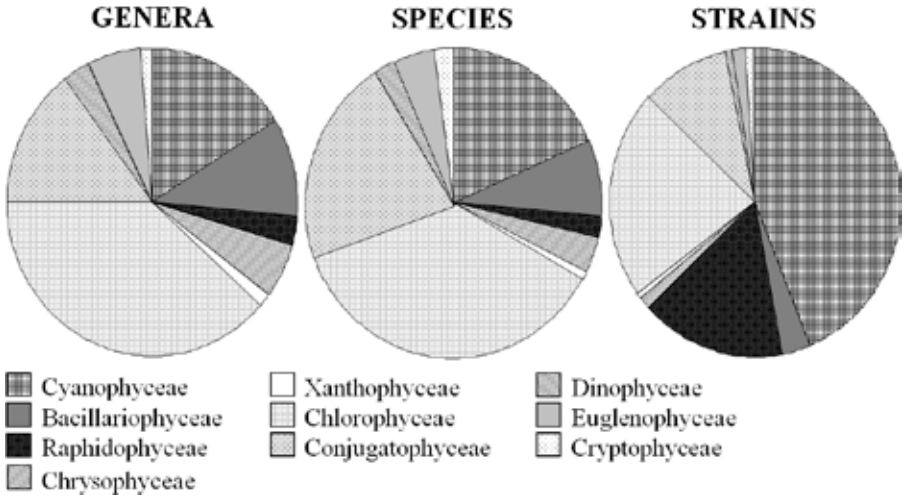

Fig. 1. Diversity of algae and cyanobacteria in the collection of pure cultures deposited at the Nature Research Centre, Lithuania

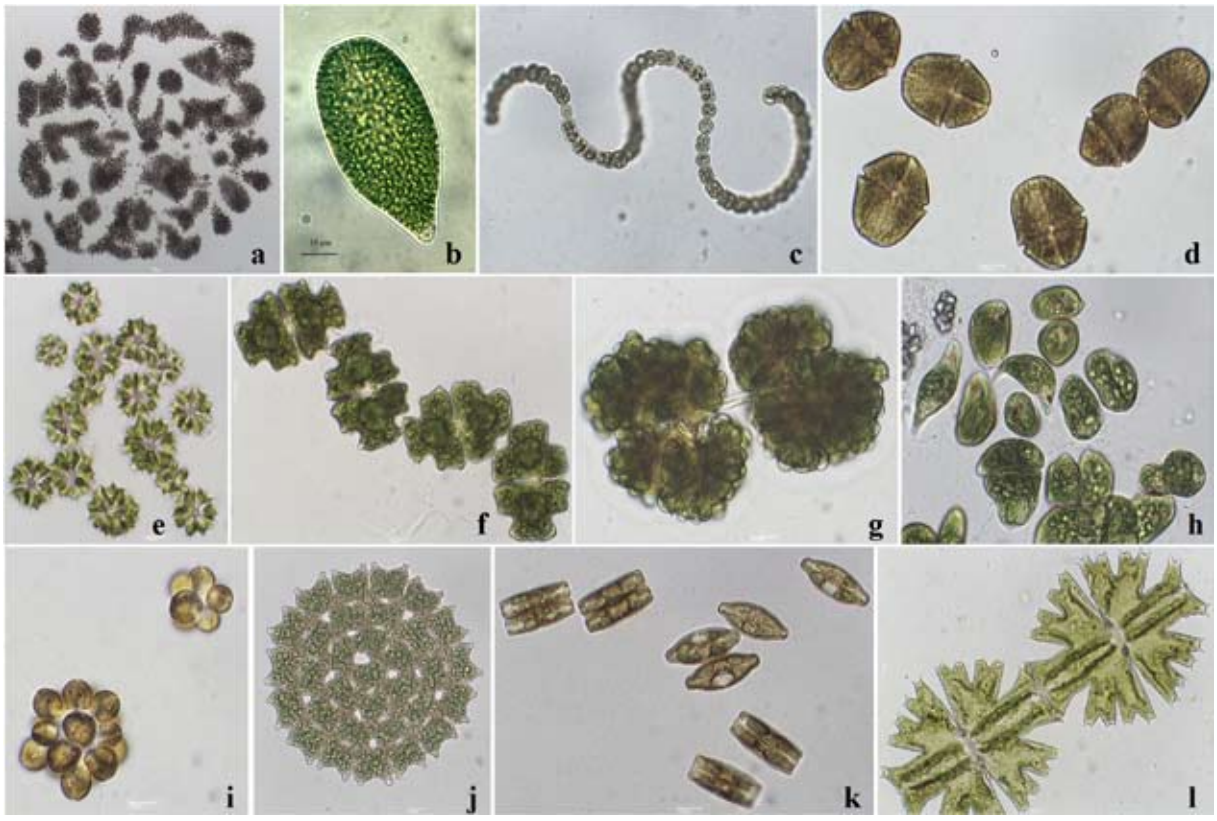

Fig. 2. Some algae and cyanobacteria strains from the collection of pure cultures deposited at the Nature Research Centre: a – *Microcystis*, b – *Gonyostomum*, c – *Dolichospermum*, d – *Gymnodinium*, e – *Sorastrum*, f – *Euastrum*, g – *Botryococcus*, h – *Euglena*, i – *Synura*, j – *Pediastrum*, k – *Achnanthes*, l – *Micrasterias*

Fig. 3. Collection of pure cultures (a) and the experiment of nutrients elimination from wastewater by using microalgae (b)

Chrysosporum bergii and *Sphaerospermopsis aphanizomenoides* have been isolated and their biology, ecology and toxicity analysed (Koreivienė & Kasperovičienė, 2011; Kasperovičienė et al., 2012).

The application of microalgae in remediation of wastewaters for mitigation of eutrophication and harmful algae blooms in the country is one of the research topics at the NRC. The strains from coccoid green algae (*Scenedesmus*, *Chlorella*, *Ankistrodesmus*, *Pediastrum*, *Coelastrum*, etc.) have been tested as an eco-friendly tool to remove nutrients from the various types of wastewater. The growth rate, abundance, biomass yield variations and the efficiency of nutrient elimination from wastewater have been assessed in the series of laboratory experiments under conditions characteristic of Lithuanian climate during the summer period (Fig. 3) (Koreivienė et al., 2014a, b, 2016).

The screening and optimization of strains for low- and high- value bioproducts have been started on the basis of collaborations with the other research institutions and international partners. The cultures were involved to test oil accumulation, fatty acids, pigments, agents with oxidative, anticancer and antibacterial properties. The efficiency of product extraction from algae depends on the composition and architecture of their cell wall. Therefore, the efficiency of cell wall rupture using compound piezomechanical systems has been tested with several strains of coccoid green algae (Vasiljev et al., 2013; Kasperovičienė et al., 2015b).

In conclusion, the collection of pure cultures of algae and cyanobacteria serves for training of students and researchers in algal identification and culturing techniques, provides information about algal diversity. More importantly, the strains have already been used in various scientific activities and the team of phycologists at the NRC is constantly getting involved in the new research fields and broaden the geography of collaboration.

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DUMBLIŲ IR MELSVABAKTERIŲ KOLEKCIJA MOKSLUI, MOKYMUI IR BIOTECHNOLOGINIAMS TYRIMAMS (GAMTOS TYRIMŲ CENTRAS, LIETUVA)

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Santrauka

Gamtos tyrimų centro dumblių ir melsvabakterių kolekcijoje palaikoma didelė iš Lietuvos gėlų vandens telkinių ir Kuršių marių izoliuotų rūšių įvairovė. Kolekcija įkurta 2010 m., vykdant invazinių ir potencialiai toksinių, vandens „žydėjimus“ sukeliančių dumblių ir melsvabakterių ekologijos ir genetinius tyrimus Lietuvos mokslo tarybos finansuojamų projektų laikotarpiu. Šiuo metu kolekcijoje yra daugiau nei 500 kamienų, priklausančių 10 klasių, apie 70 genčių ir 140 rūšių. Algologiškai gryni kamienai naudojami moksliniams eksperimentiniams tyrimams ir edukaciniams tikslams. Gamtos tyrimų centro tyrėjų grupė, vykdanti algologinius tyrimus, toliau aktyviai plėtoja biotechnologinius taikomojo pobūdžio darbus, susijusius su nuotekų vandenvala, ląstelių sienelių ardymo technologijomis, vykdo komerciškai vertingų bioproduktų paiešką.