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ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2021, vol. 6, no. 4, pp. 3–18
https://doi.org/10.21072/mbj.2021.06.4.01

SCIENTIFIC COMMUNICATIONS

UDC 582.26/.27-113:547.979.8-3

WORKING COLLECTION OF CAROTENOGENIC MICROALGAE LIVING CULTURES OF A. O. KOVALEVSKY INSTITUTE OF BIOLOGY OF THE SOUTHERN SEAS

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Received by the Editor 15.10.2020; after reviewing 02.02.2021;
accepted for publication 29.09.2021; published online 30.11.2021.

The article contains information on the specialized working collection of carotenogenic microalgae maintained by the staff of the animal physiology and biochemistry department of A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS (IBSS). The collection was established within the framework of IBSS scientific and applied research to study the mechanisms of stress tolerance in eurybiontic and extremophilic single-celled phototrophs and to identify commercially significant sources of highly valuable ketocarotenoids of astaxanthin group used for medicine and food production. The collection contains 44 microalgal strains of various taxonomic and ecological specialization with a pronounced ability to hypersynthesize secondary carotenoids and lipids under extreme conditions (drying, nutrient starvation, high-intensity illumination, extreme temperature and salinity, effect of toxicants, *etc.*). The main ways to replenish the fund are direct exchange of carotenogenic species with leading Russian and foreign collections of microalgae and own field sampling in the Black Sea areas of Crimea and Caucasus. The majority of strains in the collection represent two orders of the class Chlorophyceae: Chlamydomonadales (25 strains) and Sphaeropleales (15 strains), since the phenomenon of secondary carotenogenesis is widespread in these orders. Out of them, inhabitants of ephemeral freshwater ponds predominate, as well as aerophilic and soil microalgae. All strains are maintained under controlled conditions on agarized mineral media as pure cultures. Description of the collection accession includes the following data: a) current taxonomic status of the species verified according to updated information from corresponding collections and algological databases, namely AlgaeBase and NCBI Taxonomy Browser; b) species basionym and known synonyms; c) date and source of the strain deposition; d) author's surname, geographic location, and biotope, from which the strain was isolated; e) accession number of sequences associated with the strain in NCBI (if any); and f) nutrient medium, on which the strain is maintained in the IBSS collection. The significance of the collection for morphological, biological, physiological, and biochemical studies of growth, secondary carotenogenesis, and biotechnological potential in green microalgae is discussed.

Keywords: carotenogenic microalgae, collection storage, Chlorophyta, carotenoids, astaxanthin

The concept of carotenogenic microalgae implies a group of eukaryotic algae, taxonomically and ecologically heterogeneous ones, with a characteristic stress response – massive accumulation of specific secondary (extraplastid) carotenoids, which are structurally and functionally unrelated to photosynthesis and perform a protective function. In terms of chemical structure, such carotenoids in most microalgae

are products of the enzymatic oxidation of β-carotene to astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione) occurring on the surface of cytoplasmic lipid globules (oleosomes). The main functional significance of this stress reaction – secondary carotenogenesis – is to reduce the intensity of oxidative stress, which inevitably develops under acute negative effects, to a level adequate to possibility of formation of resting phases by vegetative cells, ensuring preservation of their viability under extreme conditions for a long time (Shah et al., 2016 ; Solovchenko, 2015).

The discovery of extremely high antioxidant and regulatory activity in astaxanthin and its closest precursors (Capelli et al., 2019 ; Han et al., 2013) caused a high interest in the late 1990s in the problem of secondary carotenogenesis in microalgae and a boom in growth and metabolic research of the most prominent astaxanthin producer – the planktonic green microalga *Haematococcus pluvialis* Flotow (Chlorophyceae, Chlamydomonadales) – which became a classic model object in corresponding investigations and the first species cultivated commercially as an algal astaxanthin source. Over the last 20 years, numerous studies on various aspects of *H. pluvialis* viability and mass cultivation were carried out in different countries; their main results are summarized in several widely known reviews serving as a reference point in the continuously growing information flow on this problem (Lemoine & Schoefs, 2010 ; Li et al., 2011 ; Shah et al., 2016 ; Solovchenko, 2015 ; Zhang et al., 2020).

The patterns of secondary carotenogenesis revealed on the example of *H. pluvialis* are extrapolated by many authors to all groups of carotenogenic microalgae without considering their origin and features of biology; this can be true only partially due to lack of reliable factual data on other species. In reviews, the lists of carotenogenic microalgae usually contain no more than 10–15 names of single representatives of 5–7 genera of the class Chlorophyceae (*Chlorella*, *Coelastrella*, *Scenedesmus*, *Ankistrodesmus*, *Chlorococcum*, *etc.*). Moreover, the experimental data on the specificity of secondary carotenogenesis in such species and their potential as astaxanthin sources are still few and episodic (references can be found in the reviews listed above).

In fact, the phenomenon of secondary carotenogenesis in microalgae is much more widespread in nature and is characteristic of representatives of two algae phyla (Chlorophyta and Euglenophyta) and five classes (Chlorophyceae, Trebouxiophyceae, Eustigmatophyceae, Ulvophyceae, and Euglenophyceae). In the overwhelming majority of cases, these are aerophilic and soil species, phycobionts of epilithic lichens, planktonic inhabitants of drying eutrophic ponds, cryophilic inhabitants of snow and ice, *etc.*, which repeatedly experience during their annual life cycles acute-extreme conditions: starvation, dehydration, high UV exposure and temperature, and so on (Minyuk, 2020). In most of such species, growth characteristics in culture and peculiarities of physiological, biochemical, and molecular genetic mechanisms of induction and regulation of astaxanthin biosynthesis are still poorly studied, although, considering high ecological plasticity of terrestrial species, among them there may be highly productive and unpretentious in mass production sources of astaxanthin and ketocarotenoids similar in structure and biological activity.

A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS (hereinafter IBSS) is one of the pioneers of research on physiology and metabolism of astaxanthin producers in the post-Soviet space. The basic condition and tool for the development of this direction was the establishment of the own experimental fund of carotenogenic microalgae living cultures (the working collection). The main principles are as follows: algae ought to represent the most abundant taxa and ecological groups of astaxanthin producers, have a reliably established taxonomic status, and be stored under identical, strictly

controlled conditions. The collection began to form in 2002: from the Komarov Botanical Institute of RAS, the first strain of *Haematococcus pluvialis* Flotow (LABIK 927-1) was obtained (from V. M. Andreeva) and cultivated in the laboratory. To date, the collection includes 44 strains of carotenogenic Chlorophyceae, is a structural part of the IBSS general collection of microalgae living cultures (acronym is IBSS; registration number in the World Data Center for Microorganisms is 1201) (World Data Centre for Microorganisms, 2021), and has an internal identifier IBSSca. Since 2017, the IBSS collection is included in the national depository bank of living systems "Noah's Ark" – Lomonosov Moscow State University project aimed at creating a multifunctional network storage of biological material. The collection ID in the "Noah's Ark" database is IBSS-ALGAE (Mikroorganizmy i griby, 2020).

The main ways to replenish the IBSSca fund are direct exchange of carotenogenic species with leading Russian and foreign collections (their list is given in Material and Methods section) and own field sampling in the Black Sea areas of Crimea and Caucasus (Dantsyuk et al., 2015 ; Chelebieva et al., 2018). Most of the strains represent two orders of the class Chlorophyceae [Chlamydomonadales (25 strains) and Sphaeropleales (15 strains)], in which the phenomenon of secondary carotenogenesis is most widely represented. Out of them, inhabitants of ephemeral freshwater ponds prevail, as well as aerophilic and soil species. The collection includes 4 strains of the halophilic microalga *Dunaliella salina* (Dunal) Teodoresco, 1905 isolated from Crimean hypersaline lakes by IBSS staff. The species is distinguished by a unique form of secondary carotenogenesis: its final product is β-carotene (Ben-Amotz & Avron, 1990).

The features of some strains obtained from Russian and Ukrainian collections are as follows: their European and American origin, long-term collection storage (up to 100 years in some cases), and a multistage path of transfer from depositing collections to the storages in the Commonwealth of Independent States. Some strains were isolated in China and obtained directly from the Institute of Hydrobiology of the Chinese Academy of Sciences; those are absent in other Russian collections. Several strains were isolated by us from field samples.

Every strain has an electronic passport including the following information: a) current taxonomic status of the species verified according to updated data of the depositing collections and algological databases – AlgaeBase (https://www.algaebase.org/) and NCBI Taxonomy Browser (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/); b) basionym and known synonyms of the species; c) date and source of the strain deposition into the IBSS collection; d) author's surname, as well as date, geographic location, and biotope, from which the natural sample was isolated; e) identifiers (ID) of nucleotide sequences in the NCBI database (if any); and f) nutrient medium, on which the strain is maintained in the IBSS collection.

It should be noted that in carotenogenic microalgae, astaxanthin biosynthesis is always associated with massive accumulation of neutral lipids suitable for obtaining high-quality biofuel; therefore, the species can serve as sources of two products highly demanded by the market (Minyuk et al., 2017, 2020 ; Solovchenko, 2015). This fact significantly expands the borders of research and practical use of varieties of the IBSSca collection.

The aim of this work was to distribute information about IBSS freshwater and terrestrial strains of microalgae, which are active producers of astaxanthin and lipids, among specialists of the corresponding profile to expand scientific contacts and cooperation in the sphere of fundamental and applied problems of algology.

MATERIAL AND METHODS

Data on the Collections, That Provided IBSS With Strains of Carotenogenic Microalgae. Acronym of every collection, its affiliation, registration number in the World Data Centre for Microorganisms (WDCM), and link to a website or catalogue are given in Table 1.

Table 1. Data on the collections of living cultures, that provided strains of carotenogenic microalgae

Collection acronym	Collection name, scientific organization, and country	No. in WDCM	Website or collection catalogue	Number of strains transferred to IBSS
ACKU	The Algae Culture Collection at the Kyiv University, Ukraine	994	https://biology.univ.kiev.ua/ (Kostikov et al., 2009)	14
CALU	The Collection of the Living Cultures of Cyanobacteria, Algae, and Algal Parasites at the Saint-Petersburg State University, Russia	461	https://researchpark.spbu.ru/collection-ccem-rus/1628-ccem-kollekciya-calu-rus	5
IPPAS	The Collection of Microalgae and Cyanobacteria at the Timiryazev Institute of Plant Physiology of RAS, Russia	596	http://cellreg.org/catalog/	4
FACHB	The Freshwater Algae Culture Collection at the Institute of Hydrobiology, Wuhan, China	873	http://algae.ihb.ac.cn/english/Cultrues.aspx	4
UTEX	The Culture Collection of Algae at the University of Texas, Austin, the USA	606	http://www.utex.org	2
PLY	The Marine Biological Association Culture Collection, Plymouth, the UK	128	https://www.mba.ac.uk/facilities/culture-collection	1

Field Sampling Areas. Own microalgae strains were isolated from field samples collected in three local climatic zones of the Black Sea area (humid subtropics, marine temperate continental climate, and highlands of the Central Caucasus), as well as in the Arctic climate zone (Svalbard archipelago). Detailed information on biotopes and geographic locations of the spots, where the strains were isolated from the natural environment, is given in Strain Systematic List section.

Obtaining Pure Cultures and Identification of Microalgae. Most of our own strains were isolated from dry orange-red or yellow-brown deposits on stones or from the walls of various water tanks and were mature resting stages of microalgae (aplanospores or cysts). This circumstance made it much easier to obtain microalgae pure cultures since dry samples exposed to open solar irradiation practically did not contain associated species. A particle of the sample was placed in a well of a glass slide and moistened with a drop of distilled water; then, under an MBS-10 microscope or XS-6320 binocular (Ningbo Shengheng, China), single cells were isolated with a dissecting needle and a micropipette into a glass tube with 0.5–1.0 mL of 2–3-times diluted sterile BBM nutrient medium. The tube was closed with a cotton-gauze plug and left for several days at room temperature and twilight light for spore germination and vegetative cell accumulation. Further sample purification was carried out by standard streaking technique on agarized media (1.5–2.0 %) in Petri dishes (Temraleeva et al., 2014 ; Brand et al., 2013).

Algae taxonomic identification was carried out by morphometric and biological features using the species guides (Andreeva, 1998 ; Anisimova & Gololobova, 2006 ; Dedusenko-Shchegoleva et al., 1959), as well as the results of molecular genetic analysis of fragments of nucleotide sequences of the nuclear 18S rRNA gene and internal transcribed spacer ITS2. Genetic analysis included procedures

for the isolation of total DNA; amplification of target fragments; their electrophoretic detection and purification followed by sequencing; preliminary analysis and search for homologues by the BLAST algorithm in the GenBank database (NCBI); construction of multiple alignments; selection of an evolutionary model; and building of a phylogenetic tree (Chelebieva et al., 2013, 2018 ; Minyuk et al., 2017).

Storage Conditions for Microalgae Cultures. Pure microalgae strains are maintained in the collection in an active vegetative state by subculture method on slant agarized (1.5–2.0 %) nutrient media (Gaisina et al., 2008 ; Temraleeva et al., 2014 ; Brand et al., 2013 ; Lourenço, 2020), depending on the biological peculiarities of the species (Ben-Amotz & Avron, 1990 ; Bischoff & Bold, 1963 ; Fučíková & Lewis, 2012) (Table 2).

Table 2. Nutrient media composition for carotenogenic microalgae collection storage

Component	Nutrient medium		
	OHM (Fábregas et al., 2000)	BBM (Bischoff & Bold, 1963)	Ben-Amotz (Ben-Amotz & Avron, 1990)
KNO_3	410 mg·mL^{-3}		505 mg·mL^{-3}
$NaNO_3$		250 mg·mL^{-3}	
$CaCl_2 \cdot 2H_2O$	110.9 mg·mL^{-3}	25 mg·mL^{-3}	
$CaCl_2$			22.2 mg·mL^{-3}
$FeC_6H_5O_7 \cdot 5H_2O$	2.62 mg·mL^{-3}		
$FeCl_3$			0.325 mg·mL^{-3}
$FeSO_4 \cdot 7H_2O$		4.98 mg·mL^{-3}	
$MgSO_4 \cdot 7H_2O$	246.5 mg·mL^{-3}	75 mg·mL^{-3}	
$MgSO_4$			600 mg·mL^{-3}
Na_2EDTA			1.86 mg·mL^{-3}
Na_2HPO_4	30 mg·mL^{-3}		
$NaHCO_3$			4.2 mg·mL^{-3}
K_2HPO_4		75 mg·mL^{-3}	
KH_2PO_4		175 mg·mL^{-3}	27.2 mg·mL^{-3}
$MnSO_4 \cdot H_2O$	0.85 mg·mL^{-3}		
$MnCl_2$			0.882 mg·mL^{-3}
$ZnSO_4 \cdot 7H_2O$		8.82 mg·mL^{-3}	
$ZnCl_2$			0.136 mg·mL^{-3}
$CuCl_2$			0.945 mg·mL^{-3}
$CuSO_4 \cdot 5H_2O$	0.012 mg·mL^{-3}	1.57 mg·mL^{-3}	
$Na_2MoO_4 \cdot 2H_2O$	0.12 mg·mL^{-3}		
$(NH_4)_6 \cdot Mo_7O_{24}$			1.164 mg·mL^{-3}
MoO_3		0.71 mg·mL^{-3}	
$CoCl_2 \cdot 6H_2O$	0.011 mg·mL^{-3}		
$CoCl_2$			0.130 mg·mL^{-3}
$Co(NO_3)_2 \cdot 6H_2O$		0.49 mg·mL^{-3}	
Cr_2O_3	0.076 mg·mL^{-3}		
SeO_2	0.005 mg·mL^{-3}		
H_3BO_3		11.42 mg·mL^{-3}	
KOH		31 mg·mL^{-3}	
NaCl		25 mg·mL^{-3}	58.5 mg·mL^{-3}
Biotin	25 μg·mL^{-3}		
Vitamin B_1	17.5 μg·mL^{-3}		
Vitamin B_{12}	15 μg·mL^{-3}		

Agar stocks (two replicates for each strain) are stored under controlled conditions in a Snaige modified refrigerated display case, which is equipped with Feron DL 20W T4 6400K fluorescent lamps (Russia) and two additional thermostats (F/2000 type, FTWOF PRODIGY manufacturer), at a temperature of +12…+14 °C and continuous illumination of 2000 Lx.

Culture reseedings are performed according to the schedule, every 2–3 months (depending on the algae growth rate), observing all necessary aseptic techniques (Temraleeva et al., 2014 ; Brand et al., 2013). Those include two steps: 1) transferring reddened palmelloid cells from agar stock into liquid culture medium to obtain actively dividing vegetative cells; 2) sowing young vegetative cells on agar stock. The best algae growth on solid media was observed when using agar-agar for microbiological purposes (manufactured by Laboratorios Conda, Spain).

Current control of functional state of liquid cultures at the subculture stage (purity, cell division rate, cell shape and size, chlorophyll content, ratio of viable cells in culture, *etc.*) is carried out by the same methods as in our experimental studies using a Goryaev chamber (MiniMed, Russia), light microscope Leica DM1000 (Germany), digital camera Leica Microsystem AG (Germany), computer program ImageJ, and flow cytometer Cytomics FC 500 Beckman Coulter (USA) (Chelebieva et al., 2013, 2018 ; Chubchikova et al., 2011 ; Minyuk et al., 2014, 2016, 2017).

RESULTS AND DISCUSSION

Strain Systematic List and Description of the IBSS Working Collection of Carotenogenic Microalgae.

Class Chlorophyceae.

1. ***Deasonia granata*** (Starr) Ettl & Komárek, 1982. **Strain IBSS-11.** Chlamydomonadales, Actinochloridaceae. Obtained from the Saint-Petersburg State University collection in 2006 as *Chlorococcum granatum* CALU-859 = CCAP-213-1a. Isolated by E. G. Pringsheim in 1928 from soil in the vicinity of Prague (Czech Republic). Basionym: *Chlorococcum humicolum*. Subcultures: SAG 213-1a; UTEX 116 (as *Neospongiococcum granatum* Deason, 1971). GenBank nucleotide sequence identifiers (GenBank IDs): KM020105; MK541716. Medium: BBM + agar.
2. ***Deasonia granata*** (Starr) Ettl & Komárek, 1982. **Strain IBSS-94.** Chlamydomonadales, Actinochloridaceae. Obtained from the Kyiv University collection in 2009 as strain ACKU 566-06. Isolated by E. G. Pringsheim from soil in the vicinity of Prague, presumably before 1928. Heterotypic synonyms: *Chlorococcum multinucleatum* Starr, 1955; *Neospongiococcum granatum* Deason, 1971. Deposited into SAG (213-1a). Subcultures: CCAP 213/1A; UTEX 116 (as *Neospongiococcum granatum* Deason, 1971). GenBank ID: KM020105. Medium: BBM + agar.
3. ***Tetracystis*** **sp. /** (***Macrochloris*** **sp.**?). **Strain IBSS-95.** Chlamydomonadales, Chlorococcaceae/Actinochloridaceae. Obtained from the Kyiv University collection in 2009 as strain ACKU 170-02. Isolated by I. Yu. Kostikov from soil of a coniferous forest in the Ardennes, Wiltz, near the city of Kanndorf, Geisht (Luxembourg). Medium: BBM + agar.
4. ***Bracteacoccus giganteus*** Bischoff et Bold, 1963. **Strain IBSS-96.** Sphaeropleales, Bracteacoccaceae. Obtained from the Kyiv University collection in 2009 as strain ACKU 461-06. Isolated by I. Yu. Kostikov (B-145) in 1996 from acid brown soil in Belgium (High Ardennes, Waroneu experimental polygon, spruce forest). Deposited by I. Yu. Kostikov. Medium: BBM + agar.
5. ***Bracteacoccus minor*** (Chodat) Petrová, 1931. **Strain IBSS-97.** Sphaeropleales, Bracteacoccaceae. Obtained from the Kyiv University collection in 2009 as strain ACKU 506-06. Deposited

into SAG (221-1). Isolated by R. Chodat in 1913 from soil. Subcultures: CCAP 221/1; UTEX 66. Basionym: *Botrydiopsis anglica* Fritsch et John, 1942; *Botrydiopsis minor* Schmidle ex Chodat, 1913; *Dictyococcus minor* (Schmidle) Pascher, 1937. GenBank IDs: KF673367; KT199253.1. Medium: BBM + agar.

6. ***Bracteacoccus* sp. Strain IBSS-104.** Sphaeropleales, Bracteacoccaceae. Obtained from the Kyiv University collection in 2011 as strain ACKU 65-02. Isolated by I. Yu. Kostikov in 1996 from soil of an oak forest in Belgium (High Ardennes, Waroneu experimental polygon, sampling spot QL-120, sample B-25). Deposited by I. Yu. Kostikov. Medium: BBM + agar.
7. ***Chromochloris zofingiensis*** (Dönz) Fučíková & L. A. Lewis, 2012. **Strain IBSS-20.** Sphaeropleales, Chromochloridaceae. Obtained from the Saint-Petersburg State University collection in 2006 as *Chlorella zofingiensis* Dönz, 1933, strain CALU-190. Isolated from soil in the vicinity of Zofingen (Switzerland). Taxonomic status was changed based on molecular genetic analysis (Fučíková & Lewis, 2012). Subcultures: CCAP-211/14 = CAUP H 1905 = UTEX 32 = SAG 211-14 = ATCC 30412. Homotypic synonyms: *Chlorella zofingiensis* Dönz, 1934; *Muriella zofingiensis* (Dönz) Hindák, 1982; *Mychonastes zofingiensis* (Dönz) Kalina & Puncochárová, 1987. GenBank IDs: GU827478.1; HQ902940; KR904902; KP645230; HQ902932; HQ902929. Medium: BBM + agar.
8. ***Chlamydomonas* cf. *debaryana*** Goroschankin, 1981. **Strain IBSS-105.** Chlamydomonadales, Chlamydomonadaceae. Obtained from the Kyiv University collection in 2011 as strain ACKU 45-02. Isolated by E. N. Demchenko in the Cherkasy Region (Pekari village, Kaniv District, Ukraine) from a puddle on a dirt road. Deposited by E. N. Demchenko. In AlgaeBase and NCBI Taxonomy Browser, *Chlamydomonas debaryana* Goroschankin, 1891 is currently regarded as *Edaphochlamys debaryana* (Goroschankin) Pröschold & Darienko, 2018 (Pröschold et al., 2018). Medium: BBM + agar.
9. ***Chlamydomonas montana*** Romanenko, 1999. **Strain IBSS-106.** Chlamydomonadales, Chlamydomonadaceae. Obtained from the Kyiv University collection in 2011 as strain ACKU 167-03. Isolated by E. N. Demchenko in 2002 from cracks of granite outcrops in the regional landscape park Granite-steppe lands of Buh, Gard tract (Yuzhnoukrainsk, Mykolaiv Region, Ukraine). Deposited by E. N. Demchenko. Medium: BBM + agar.
10. ***Chlamydomonas* sp. Strain IBSS-88.** Chlamydomonadales, Chlamydomonadaceae. Isolated by I. N. Chubchikova and N. V. Dantsyuk in 2006 from a freshwater spring pond in the vicinity of Sevastopol (Sakharnaya Golovka village). Isolated into pure culture and identified by N. V. Dantsyuk. Medium: BBM + agar.
11. ***Ettlia carotinosa*** Komárek, 1989. **Strain IBSS-98.** Chlamydomonadales, Chlamydomonadales incertae sedis. Obtained from the Kyiv University collection in 2009 as strain ACKU 573-06. Isolated by F. Mainx, presumably before 1954, from soil in the Czech Republic. Deposited into SAG (213-4) by E. G. Pringsheim in 1954. Heterotypic synonyms: *Chlorococcum wimmeri* Rabenhorst; *Neochloris wimmeri* (Hilse) P. A. Archibald & Bold; *Protococcus wimmeri* Hilse. Subcultures: CCAP 213/4; UTEX 113. GenBank IDs: KR181935; KR181934; GU292342. Medium: BBM + agar.
12. ***Neospongiococcum gelatinosum*** (Archibald & Bold) Ettl & Gärtner, 1987. **Strain IBSS-99.** Chlamydomonadales, Chlorococcaceae. Obtained from the Kyiv University collection in May 2009 as strain ACKU 631-06 (15 K1A). Isolated by P. A. Archibald, presumably before 1970, from soil of a peat bog in the Elkhart County (Indiana, the USA). Deposited into SAG (64.80) by P. A. Archibald, presumably before 1980. According to molecular data of SAG and (Kawasaki et al., 2015),

it is identified as *Chlorococcum oleofaciens* Trainor & Bold, 1954. Basionym: *Chlorococcum gelatinosum* Archibald et Bold, 1970. Subculture: UTEX 1773. GenBank IDs: KX147356; KM020103; JN968584; KX782323; AB983631; AB983613. Medium: BBM + agar.

13. ***Pseudospongiococcum protococcoides*** Gromov & Mamkaeva, 1974. **Strain IBSS-10.** Chlamydomonadales, Chlorococcaceae. Obtained from the Saint-Petersburg State University collection in March 2006 as strain CALU-221. Isolated by B. V. Gromov in 1962 from surface soil layer in the vicinity of Sevastopol. GenBank ID: KU057947. Medium: BBM + agar.
14. ***Spongiochloris spongiosa*** (Vischer) Starr, 1955. **Strain IBSS-100.** Chlamydomonadales, Chlorococcaceae. Basionym: *Asterococcus spongiosus* Vischer, 1945. Obtained from the Kyiv University collection in 2009 as strain ACKU 649-06, Vischer 318. Isolated by W. Vischer in 1942 from soil in Unterengadin (Switzerland). Deposited into SAG (280-2b) by E. G. Pringsheim in 1954. Subcultures: CCAP 3/1; UTEX 1. GenBank IDs: KR607497; MK541715; AF395511; U34776.1. Medium: BBM + agar.
15. ***Chlorosarcinopsis* sf *dissociata*** Herndon, 1958. **Strain IBSS-107.** Chlamydomonadales, Chlorosarcinaceae. Obtained from the Kyiv University collection in 2011 as strain ACKU 309-04. There is no information on the strain origin in the ACKU catalogues. Medium: BBM + agar.
16. ***Dunaliella salina*** (Dunal) Teodoresco, 1905. **Strain IBSS-79.** Chlamydomonadales, Dunaliellaceae. Isolated by N. V. Shadrin in 2006 from a salt lake at Khersones Cape near Sevastopol. Isolated into pure culture and identified by N. V. Dantsyuk. Medium: Ben-Amotz + agar.
17. ***Dunaliella salina*** (Dunal) Teodoresco, 1905. **Strain IBSS-92.** Chlamydomonadales, Dunaliellaceae. Isolated by A. B. Borovkov in 2008 from the Donuzlav Salt Lake (Crimea, Russia). Isolated into pure culture and identified by N. V. Dantsyuk. Medium: Ben-Amotz + agar.
18. ***Dunaliella salina*** (Dunal) Teodoresco, 1905. **Strain IBSS-86.** Chlamydomonadales, Dunaliellaceae. Isolated by N. V. Shadrin in 2008 from the Sivash Salt Lake (western shore) (Krasnoperekopsky District, Crimea). Isolated into pure culture and identified by N. V. Dantsyuk. Medium: Ben-Amotz + agar.
19. ***Dunaliella salina*** (Dunal) Teodoresco, 1905. **Strain IBSS-78.** Chlamydomonadales, Dunaliellaceae. Isolated by T. A. Kukhareva in May 2017 from the Koyashskoye Salt Lake (southern Kerch Peninsula, Crimea). Isolated into pure culture and identified by N. V. Dantsyuk. Medium: Ben-Amotz + agar.
20. ***Dunaliella tertiolecta*** Butcher, 1959. **Strain IBSS-87.** Chlamydomonadales, Dunaliellaceae. Obtained in January 2003 from the collection of algae of the Turkish Institute of Marine Sciences (Erdemli) as PLY-83. The authentic strain was deposited in the Marine Biological Association Culture Collection (Plymouth, the UK). Isolated by B. Føyn in 1928 in the Oslofjord inlet (Atlantic Ocean) off the coast of southeastern Norway. Subcultures: CCAP19/6B; UTEX LB999; CCMP364. GenBank IDs: AY572957; HM243579; HQ828109; JF260981; KJ094612; KJ756820. Medium: Ben-Amotz + agar.
21. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-16.** Chlamydomonadales, Haematococcaceae. Obtained from the collection of green algae cultures of the algology laboratory of the Komarov Botanical Institute of RAS (from V. M. Andreeva) in 2002 as strain LABIK 92-1 (Mainx). Isolated in the Czech Republic. The exact date and spot of isolation are unknown. Is identical to the strain CALU-79 *Chlorococcum wimmeri* Rabenhorst by Mainx = *Haematococcus pluvialis* Flotow emend. Wille; Coll. Pringsheim, Praha, A-93. Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875 (Nakada & Ota, 2016). Medium: OHM + agar.

22. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-18.** Chlamydomonadales, Haematococcaceae. Isolated by G. S. Minyuk in 2003 in the vicinity of Adler from a reddish-brown deposit on the walls of an empty pool for *Arthrospira platensis* cultivation at the AgroViktoriya enterprise (the Imereti Lowlands, Veseloe-Psou village, Adler Region, Krasnodar Territory). Spot geographic coordinates are 43°25′07″N, 40°00′09″E; height above sea level is 7 m. Isolated into pure culture by O. A. Galatonova. Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875. GenBank ID: KU193764.1. Medium: OHM + agar.
23. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-17.** Chlamydomonadales, Haematococcaceae. Obtained from the Timiryazev Institute of Plant Physiology collection in 2004 as strain IPPAS H-239. It was transferred to the IPPAS collection from the Czechoslovak Academy of Sciences collection in 1958 as strain A-63, Prague, Prát. Isolated by W. Vischer in 1923 in Switzerland (Botanical Garden of the University of Basel). Deposited into SAG (3-1d) by E. G. Pringsheim in 1954. Subcultures: CCAP 34/1D = CALU-333 = JSBG BS-2. Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875. GenBank IDs: KC153467; MG022681. Medium: OHM + agar.
24. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-73.** Chlamydomonadales, Haematococcaceae. Obtained from the collection of the Institute of Hydrobiology of the Chinese Academy of Sciences in 2007 as strain FACHB-712. Isolated in 2007 from the Donghu Lake (Wuhan, Hubei Province, China). Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875. Medium: OHM + agar.
25. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-74.** Chlamydomonadales, Haematococcaceae. Isolated by N. V. Shadrin in 2008 in the Central Caucasus area from a puddle of melted snow on the left glacial slope of the Bezengi Gorge (Misses-Kosh, Kabardino-Balkaria). Spot geographic coordinates are 43°03′25″N, 43°05′49″E; height above sea level is 2200 m. Isolated into pure culture by N. V. Dantsyuk. Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875. GenBank ID: KU193763.1. Medium: OHM + agar.
26. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-75.** Chlamydomonadales, Haematococcaceae. Isolated by I. N. Drobetskaya in 2008 in Sevastopol from red-brown sediment at the bottom of a water tank (balcony of a multi-storey residential building). Spot geographic coordinates are 44°36′00″N, 33°32′00″E; height above sea level is 232 m. Isolated into pure culture by N. V. Dantsyuk. Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875. GenBank ID: KU193762.1. Medium: OHM + agar.
27. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-108.** Chlamydomonadales, Haematococcaceae. Isolated by D. A. Davydov in 2011 on Nordaustlandet (Svalbard archipelago, Norway) from a pond with red deposit on pebbles (0.1-m depth). The material was obtained in 2015. Isolated into pure culture by N. V. Dantsyuk in 2017. Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875. Medium: OHM + agar.
28. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-111.** Chlamydomonadales, Haematococcaceae. Isolated by I. N. Chubchikova in 2018 in Sevastopol (Maksimova dacha) from a red-brown deposit on the walls of a plastic water tank. Isolated into pure culture by N. V. Dantsyuk. Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875. Medium: OHM + agar.
29. ***Neochloris oleoabundans*** S. Chantanachat & H. C. Bold, 1962. **Strain IBSS-101.** Sphaeropleales, Neochloridaceae. Obtained from Ikhlyas-Agro-Energiya enterprise (Saki, Crimea) in 2009 as a strain from the University of Texas collection; the number is unknown. Isolated

by S. Chantanachat in 1958–1962 in Saudi Arabia in sand dunes of the Rub' al Khali desert. Deposited into UTEX by H. C. Bold in 1962. Synonym: *Ettlia oleoabundans* (S. Chantanachat & H. C. Bold) J. Komárek, 1989 (Chlorophyceae; Chlamydomonadales; Chlamydomonadales incertae sedis) (Komárek, 1989). GenBank IDs: KX350066; JX978410. Genome and transcriptome assembly data in GenBank: PRJNA412701; PRJNA354501; PRJNA305197; PRJNA297494; PRJNA79207. Medium: BBM + agar.

30. ***Coelastrella rubescens*** Kaufnerová & Eliás, 2013. **Strain IBSS-12.** Sphaeropleales, Scenedesmaceae, Coelastroideae. Obtained from the Timiryazev Institute of Plant Physiology collection in 2006 as *Scotiellopsis rubescens* Vinatzer, 1975, strain IPPAS H-350. It was transferred to the IPPAS collection from the Institute of Botany at the University of Innsbruck (from J. Lukavský) in 1988 as strain Vinatzer/Insbruck V195 (CCALA 475). Isolated by G. Vinatzer in 1988 from soil in the Dolomites (South Tyrol, Italy). GenBank ID: KT962984.1. Medium: BBM + agar.
31. ***Coelastrella*** **sp. Strain IBSS-112.** Sphaeropleales, Scenedesmaceae, Coelastroideae. Obtained from the University of Tehran branch in 2020 as strain KNUA037. Isolated on the Caspian Sea coast (Nur-Sultan, Kazakhstan). GenBank ID: KT883911. Medium: BBM + agar.
32. **sf *Scotiellopsis* sp. Strain IBSS-109.** Sphaeropleales, Scenedesmaceae, Coelastroideae. Obtained from the Kyiv University collection in 2011 as strain ACKU 14-02. There is no information on the strain origin in the ACKU catalogues. Medium: BBM + agar.
33. ***Acutodesmus dimorphus*** (Turpin) P. M. Tsarenko, 2001. **Strain IBSS-89.** Sphaeropleales, Scenedesmaceae. Isolated by N. V. Dantsyuk and I. N. Chubchikova in 2006 from a freshwater pond in the vicinity of Sevastopol (Sakharnaya Golovka village). Isolated into pure culture by N. V. Dantsyuk. Identified by P. M. Tsarenko in 2008. Medium: BBM + agar.
34. ***Desmodesmus communis*** (E. Hegewald) E. Hegewald, 2000. **Strain IBSS-82.** Sphaeropleales, Scenedesmaceae, Desmodesmoideae. Obtained from the Timiryazev Institute of Plant Physiology collection in May 2007 as *Scenedesmus quadricauda* (Turpin) Brébisson, 1835, strain IPPAS S-313, Greifswald/15. Isolated in the vicinity of Greifswald (Germany). Subcultures: CAUP H-522 = CCALA-463. GenBank ID: MN178487. Medium: BBM + agar.
35. ***Scenedesmus obliquus*** (Turpin) Kützing, 1833. **Strain IBSS-9.** Sphaeropleales, Scenedesmaceae, Scenedesmoideae. Obtained from the Saint-Petersburg State University collection in March 2006 as strain CALU-13. It was transferred to the CALU collection from the Czechoslovak Academy of Sciences collection in 1960 as strain Pringsheim, Praha, A-125. Subcultures: CCALA 45; IPPAS S-305. Synonyms: *Tetradesmus obliquus* (Turpin) M. J. Wynne, 2016; *Acutodesmus obliquus* (Turpin) Hegewald & Hanagata, 2000 (Wynne & Hallan, 2015). Medium: BBM + agar.
36. ***Scenedesmus obliquus*** (Turpin) Kützing, 1833. **Strain IBSS-81.** Sphaeropleales, Scenedesmaceae, Scenedesmoideae. Obtained from the collection of the Institute of Hydrobiology of the Chinese Academy of Sciences in 2007 as strain FACHB-12. Isolated in Hebei Province (China) in 1960. Medium: BBM + agar.
37. ***Scenedesmus rubescens*** (Dangeard) Kessler et al., 1997. **Strain IBSS-91.** Sphaeropleales, Scenedesmaceae, Scenedesmoideae. Synonym: *Halochlorella rubescens* P. J. L. Dangeard, 1966. Obtained from the Timiryazev Institute of Plant Physiology collection in 2007 as strain IPPAS D-292. It was transferred to the IPPAS collection from the Institute of Botany of the Academy of Sciences of the Uzbek SSR in 1989. Isolated on the Kamchatka Peninsula from a deposit

on a lake shore. Subculture: CALU-449. Last identification: E. S. Chelebieva and S. V. Skrebovskaya in 2013 (Chelebieva et al., 2013). Currently regarded as *Halochlorella rubescens* (Wynne & Furnari, 2014). GenBank ID: KU057946. Medium: BBM + agar.

38. ***Scenedesmus rubescens*** (Dangeard) Kessler et al., 1997. **Strain IBSS-102.** Sphaeropleales, Scenedesmaceae, Scenedesmoideae. Obtained from the Kyiv University collection in 2009 as strain ACKU 64-06. Isolated by F. Dangeard in 1965 near Bordeaux (France) from a culture of brown algae. Deposited into SAG by E. Kessler. Subcultures: SAG 595 = CCAP 232/1. Synonyms: *Halochlorella rubescens* P. J. L. Dangeard, 1966; *Chlorella fusca* var. *rubescens* Kessler et al., 1968. GenBank IDs: X74002; MK975491; HG514422; HG514373; HG514402. Medium: BBM + agar.
39. ***Ankistrodesmus*** **sp.** Corda, 1838. **Strain IBSS-85.** Sphaeropleales, Selenastraceae. Obtained from the collection of the Institute of Hydrobiology of the Chinese Academy of Sciences in 2007 as strain FACHB-49. Isolated by K. Lin in 1979 in Wuhan (China). Identified by L. Li. Medium: BBM + agar.
40. ***Ankistrodesmus*** **sp.** Corda, 1838. **Strain IBSS-93.** Sphaeropleales, Selenastraceae. Isolated by N. V. Dantsyuk in 2008 from a freshwater spring pond in the vicinity of Sevastopol (Sakharnaya Golovka village). Isolated into pure culture and identified by N. V. Dantsyuk. Medium: BBM + agar.

Class Trebouxiophyceae.

41. ***Chlorella fusca*** Shihira et Krauss, 1965. **Strain IBSS-110.** Chlorellales, Chlorellaceae. Obtained from the Kyiv University collection in 2011 as strain ACKU 38-04. Isolated by E. N. Demchenko in 2009 in the regional landscape park Granite-steppe lands of Buh (Ukraine). Currently regarded as *Desmodesmus abundans* (Kirchner) E. H. Hegewald, 2000 (AlgaeBase, 2021). Medium: BBM + agar.
42. ***Chlorella*** **sp. Strain IBSS-103.** Chlorellales, Chlorellaceae. Obtained from Ikhlyas-Agro-Energiya enterprise (Saki, Crimea) in 2009 as a strain from the University of Texas collection. There is no detailed information on the strain. Medium: BBM + agar.
43. ***Botryococcus braunii*** Kützing, 1849. **Strain IBSS-76.** Trebouxiales, Botryococcaceae. Obtained from the collection of the Institute of Hydrobiology of the Chinese Academy of Sciences in 2007 as strain FACHB-759. Isolated by C.-H. Xu from a lake in Kunming (Yunnan Province, China). Isolated into pure culture by Q. Lin; identified by R. Li. Medium: BBM + agar.

Class Eustigmatophyceae.

44. ***Chlorobotrys neglectus*** Pascher & Geitler, 1925. **Strain IBSS-90.** Eustigmatales, Eustigmataceae. Isolated by G. S. Minyuk in 2006 from a deposit on the walls of an empty freshwater tank (balcony of a multi-storey building) in Sevastopol. Identified by P. M. Tsarenko. Synonym: *Chloridella neglecta* (Pascher & Geitler) Pascher. Medium: BBM + agar.

Conclusion. The specialized working collection of microalgae IBSSca contains 44 strains of eurybiontic and extremophilic Chlorophyceae species with a pronounced ability to hypersynthesize secondary carotenoids and lipids under extreme external effects. The establishment and replenishment of the collection is the basic condition for carrying out comparative research on the peculiarities of growth and secondary carotenogenesis in microalgae of various taxonomic and ecological specialization, aimed at identifying physiological and biochemical mechanisms of stress tolerance in extremophilic species and searching for new commercially promising astaxanthin producers and technically valuable lipids. Using the material of research carried out on the basis of the collection in 2005–2020, more than 30 articles were published in national and foreign scientific journals indexed in the RSCI, Scopus,

and WoS (the key works are cited above), 23 reports were made at international and regional conferences, and 3 patents were registered for the invention of methods for cultivating three microalgae species to obtain carotenoids and lipids (Patent 2541455, 2015 ; Patent 2661086, 2018 ; Patent 2715039, 2020).

This work was carried out within the framework of IBSS state research assignment "Investigation of mechanisms of controlling production processes in biotechnological complexes with the aim of developing scientific foundations for production of biologically active substances and technical products of marine genesis" (No. 121030300149-0).

Acknowledgement. The authors express their sincere gratitude to all colleagues, who contributed to the establishment of the experimental fund of carotenogenic microalgae at IBSS, first of all to I. Yu. Kostikov, D. Sc., Prof.; P. M. Tsarenko, D. Sc., Prof.; D. A. Los, D. Sc., Prof.; and A. V. Pinevich, D. Sc., Prof., for providing microalgae strains from reference collections and for assistance in taxonomic identification of field isolates. Special thanks are expressed to N. V. Shadrin, senior researcher, PhD (IBSS), for his help in replenishing the collection with the strains from the Institute of Hydrobiology of the Chinese Academy of Sciences and with field samples from Crimean hypersaline lakes.

REFERENCES

1. Andreeva V. M. *Pochvennye i aerofil'nye zelenye vodorosli (Chlorophyta: Tetrasporales, Chlorococcales, Chlorosarcinales).* Saint Petersburg : Nauka, 1998, 351 p. (in Russ.)
2. Anisimova O. V., Gololobova M. A. *Kratkii opredelitel' rodov vodoroslei. Uchebnoe posobie.* Moscow : MGU, 2006, 159 p. (in Russ.)
3. Gaisina L. A., Fazlutdinova A. I., Kabirov R. R. *Sovremennye metody vydeleniya i kul'tivirovaniya vodoroslei* : uchebnoe posobie. Ufa : Izd-vo BGPU, 2008, 152 p. (in Russ.)
4. Dantsyuk N. V., Chelebieva E. S., Chekanov K. A. Novye izolyaty zelenoi mikrovodorosli *Haematococcus pluvialis* Flotow (Chlorophyceae) iz razlichnykh raionov Prichernomor'ya. *Vodorosli i tsianobakterii v prirodnykh i sel'skokhozyaistvennykh ekosistemakh* : materialy II Mezhdunar. nauch.-prakt. konf., posvyashch. 150-letiyu so dnya rozhd. prof. Emilii Andrianovny Shtinoi, 19–23 Oct., 2015. Kirov, 2015, pp. 98–102. (in Russ.)
5. Dedusenko-Shchegoleva N. T., Matvienko A. M., Shkorbatov L. A. *Opredelitel' presnovodnykh vodoroslei SSSR.* Iss. 8. *Zelenye vodorosli. Klass vol'voksovye.* Leningrad : Izd-vo AN SSSR, 1959, 230 p. (in Russ.)
6. Mikroorganizmy i griby. *Depozitarii zhivykh sistem "Noev kovcheg"* : [site]. (in Russ.). URL: http://depository.msu.ru/category-project/mikroorganizmy-i-griby#fung_infosys [accessed: 14.10.2020].
7. Kostikov I. Yu., Demchenko E. M., Berezovska M. A. Kolektsiia kultur vodorostei Kyivskoho natsionalnoho universytetu imeni Tarasa Hryhorovycha Shevchenka. Kataloh shtamiv (2008 r.). *Chornomorskyi botanichnyi zhurnal*, 2009, vol. 5, no. 1, pp. 37–79. (in Ukr.)
8. Minyuk G. S. *Karotinogennye mikrovodorosli. Baza dannykh.* A. s. No. 2020621092. Zayavka No. 2020620921. 10.06.2020, opubl. 30.06.2020. Bull. no. 7. (in Russ.)
9. Minyuk G. S., Chelebieva E. S., Chubchikova I. N., Dantsyuk N. V., Drobetskaya I. V., Sakhon E. G., Chivkunova O. B., Chekanov K. A., Lobakova E. S., Sidorov R. A., Solovchenko A. E. pH and CO_2 effects on *Coelastrella (Scotiellopsis) rubescens* growth and metabolism. *Fiziologiya rastenii*, 2016, vol. 63, iss. 4, pp. 601–610. (in Russ.). http://doi.org/10.7868/S0015330316040102

10. Patent 2541455 Rossiiskaya Federatsiya. MPK6 C12N 1/12. *Sposob kul'tivirovaniya odnokletochnoi zelenoi vodorosli Haematococcus pluvialis* / Minyuk G. S., Terent'eva N. V., Drobetskaya I. V., Chubchikova I. N. ; FGBUN "Institut morskikh biologicheskikh issledovanii imeni A. O. Kovalevskogo RAN". No. GR 2014149886/93; zayavl. 03.10.2014; prior. 12.05.2008; opubl. 10.02.2015. Bull. no. 4. (in Russ.)
11. Patent 2661086 Rossiiskaya Federatsiya. PK C12N 1/12; C12P 23/00; C12R 1/89. *Sposob kul'tivirovaniya mikrovodorosli Coelastrella rubescens dlya polucheniya karotinoidov i lipidov* / Minyuk G. S., Chubchikova I. N., Drobetskaya I. V., Dantsyuk N. V., Chelebieva E. S. ; FGBUN "Institut morskikh biologicheskikh issledovanii imeni A. O. Kovalevskogo RAN". No. GR 2017110990; zayavl. 31.03.2017; opubl. 11.07.2018. Bull. no. 20. (in Russ.)
12. Patent 2715039 Rossiiskaya Federatsiya MPK C12N 1/12; C12P 23/00; C12R 1/89. *Sposob kul'tivirovaniya mikrovodorosli Chromochloris zofingiensis dlya polucheniya lipidov i karotinoidov* / Minyuk G. S., Chubchikova I. N., Dantsyuk N. V., Drobetskaya I. V., Chelebieva E. S., Sidorov R. A., Solovchenko A. E. ; FITs Institut biologii yuzhnykh morei imeni A. O. Kovalevskogo. No. GR 2715039; zayavl. 01.07.2019; opubl. 21.02.2020. Bull. no. 6. (in Russ.)
13. Temraleeva A. D., Mincheva E. V., Bukin Yu. S., Andreeva A. M. *Sovremennye metody vydeleniya, kul'tivirovaniya i identifikatsii zelenykh vodoroslei (Chlorophyta).* Kostroma : Kostromskoi pechatnyi dom, 2014, 215 p. (in Russ.)
14. Chelebieva E. S., Minyuk G. S., Drobetskaya I. V., Chubchikova I. N. Dynamics of chemical composition of *Ettlia carotinosa* Komárek, 1989 (Chlorophyceae) under experimental induction of secondary carotenogenesis. *Morskoj ekologicheskij zhurnal*, 2013, vol. 12, no. 2. pp. 78–87. (in Russ.)
15. Chelebiieva E. S., Skrebovska S. V. Unicellular spore-forming alga *Pseudospongiococcum protococcoides* position detection in the system Chlorophyta. *Visnyk Lvivskoho universytetu. Seriia biolohichna*, 2013, iss. 62, pp. 75–81. (in Ukr.)
16. Chubchikova I. N., Drobetskaya I. V., Minyuk G. S., Dantsyuk N. V., Chelebiyeva E. S. Screening of green microalgae as a potential source of natural ketocarotenoids. 2. Features of growth and secondary carotenogenesis in the representatives of the genus *Bracteacoccus* (Chlorophyceae). *Morskoj ekologicheskij zhurnal*, 2011, vol. 10, no. 1, pp. 91–97. (in Russ.)
17. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway : [site]. URL: https://www.algaebase.org/search/species/detail/?species_id=27671 [accessed: 30.01.2021].
18. Ben-Amotz A., Avron M. The biotechnology of cultivating of the halotolerant alga *Dunaliella*. *Trends in Biotechnology*, 1990, vol. 8, pp. 121–126. https://doi.org/10.1016/0167-7799(90)90152-N
19. Ben-Amotz A., Katz A., Avron M. Accumulation of β-carotene in halotolerant algae: Purification and characterization of β-carotene-rich globules from *Dunaliella bardawil* (Chlorophyceae). *Journal of Phycology*, 1982, vol. 18, iss. 4, pp. 529–537. https://doi.org/10.1111/j.1529-8817.1982.tb03219.x
20. Bischoff H. W., Bold H. C. *Phycological Studies.* IV. *Some Soil Algae From Enchanted Rock and Related Algal Species.* Austin, TX : University of Texas, 1963, vol. 6318, 95 p.
21. Brand J. J., Andersen R. A., Nobles D. R. Jr. Maintenance of microalgae in culture collections. In: *Handbook of Microalgal Culture:*

Applied Phycology and Biotechnology. 2nd ed. / A. Richmond, Q. Hu (Eds). Chichester, UK : John Wiley and Sons, 2013, pp. 80–89. https://doi.org/10.1002/9781118567166.ch5

22. Capelli B., Talbott S., Ding L. Astaxanthin sources: Suitability for human health and nutrition. *Functional Foods in Health and Disease*, 2019, vol. 9, no. 6, pp. 430–445. https://doi.org/10.31989/ffhd.v9i6.584
23. Chelebieva E. S., Dantsyuk N. V., Chekanov K. A., Chubchikova I. N., Drobetskaya I. V., Minyuk G. S., Lobakova E. S., Solovchenko A. E. Identification and morphological-physiological characterization of astaxanthin producer strains of *Haematococcus pluvialis* from the Black Sea region. *Applied Biochemistry and Microbiology*, 2018, vol. 54, no. 6, pp. 639–648. https://doi.org/10.1134/S0003683818060078
24. Fábregas J., Domínguez A., Regueiro M., Maseda A., Otero A. Optimization of culture medium for the continuous cultivation of the microalga *Haematococcus pluvialis*. *Applied Microbiology and Biotechnology*, 2000, vol. 53, pp. 530–535. https://doi.org/10.1007/s002530051652
25. Fučíková K., Lewis L. A. Intersection of *Chlorella*, *Muriella* and *Bracteacoccus*: Resurrecting the genus *Chromochloris* Kol et Chodat (Chlorophyceae, Chlorophyta). *Fottea*, 2012, vol. 12, iss. 1, pp. 83–93. https://doi.org/10.5507/fot.2012.007
26. Han D., Li Y., Hu Q. Astaxanthin in microalgae: Pathways, functions and biotechnological implications. *Algae*, 2013, vol. 28, iss. 2, pp. 131–147. https://doi.org/10.4490/algae.2013.28.2.131
27. Kawasaki Y., Nakada T., Tomita M. Taxonomic revision of oil-producing green algae, *Chlorococcum oleofaciens* (Volvocales, Chlorophyceae), and its relatives. *Journal of Phycology*, 2015, vol. 51, iss. 5, pp. 1000–1016. https://doi.org/10.1111/jpy.12343
28. Komárek R. Polynuclearity of vegetative cells in coccal green algae from the family Neochloridaceae. *Archiv für Protistenkunde*, 1989, vol. 137, iss. 3, pp. 255–273. https://doi.org/10.1016/S0003-9365(89)80033-8
29. Lemoine Y., Schoefs B. Secondary ketocarotenoid astaxanthin biosynthesis in algae: A multifunctional response to stress. *Photosynthesis Research*, 2010, vol. 106, iss. 1–2, pp. 155–177. https://doi.org/10.1007/s11120-010-9583-3
30. Li J., Zhu D., Niu J., Shen S., Wang G. An economic assessment of astaxanthin production by large scale cultivation of *Haematococcus pluvialis*. *Biotechnology Advances*, 2011, vol. 29, iss. 6, pp. 568–574. https://doi.org/10.1016/j.biotechadv.2011.04.001
31. Lourenço S. Microalgae culture collections, strain maintenance, and propagation. In: *Handbook of Microalgae-based Processes and Product* / E. Jacob-Lopez, M. J. Queroz, M. M. Maroneze, L. Q. Zepka (Eds). Cambridge, MA : Academic Press, 2020, pp. 49–84. https://doi.org/10.1016/b978-0-12-818536-0.00003-8
32. Minyuk G. S., Chelebieva E. S., Chubchikova I. N. Secondary carotenogenesis of the green microalga *Bracteacoccus minor* (Chodat) Petrova (Chlorophyta) in a two-stage culture. *International Journal on Algae*, 2014, vol. 16, iss. 4, pp. 354–368. http://dx.doi.org/10.1615/InterJAlgae.v16.i4.50
33. Minyuk G., Chelebieva E., Chubchikova I., Dantsyuk N., Drobetskaya I., Sakhon E., Chekanov K., Solovchenko A. Stress-induced secondary carotenogenesis in *Coelastrella rubescens* (Scenedesmaceae, Chlorophyta), a producer of value-added keto-carotenoids.

Algae, 2017, vol. 32, iss. 3, pp. 245–259. https://doi.org/10.4490/algae.2017.32.8.6

34. Minyuk G., Sidorov R., Solovchenko A. Effect of nitrogen source on the growth, lipid, and valuable carotenoid production in the green microalga *Chromochloris zofingiensis*. *Journal of Applied Phycology*, 2020, vol. 32, iss. 2, pp. 923–935. https://doi.org/10.1007/s10811-020-02060-0
35. Nakada T., Ota S. What is the correct name for the type of *Haematococcus* Flot. (Volvocales, Chlorophyceae)? *Taxon*, 2016, vol. 65, iss. 2, pp. 343–348. https://doi.org/10.12705/652.11
36. Pröschold T., Darienko T., Krienitz L., Coleman A. W. *Chlamydomonas schloesseri* sp. nov. (Chlamydophyceae, Chlorophyta) revealed by morphology, autolysin cross experiments, and multiple gene analyses. *Phytotaxa*, 2018, vol. 362, iss. 1, pp. 021–038. https://doi.org/10.11646/phytotaxa.362.1.2
37. Shah M. R., Liang Y., Cheng J. J., Daroch M. Astaxanthin-producing green microalga *Haematococcus pluvialis*: From single cell to high value commercial products. *Frontiers in Plant Science*, 2016, vol. 7, art. 531 (28 p.). https://doi.org/10.3389/fpls.2016.00531
38. Solovchenko A. E. Recent breakthroughs in the biology of astaxanthin accumulation by microalgal cell. *Photosynthesis Research*, 2015, vol. 125, pp. 437–449. https://doi.org/10.1007/s11120-015-0156-3
39. *World Data Centre for Microorganisms. Culture Collections Information Worldwide* : [site]. URL: http://ccinfo.wdcm.org/ [accessed: 30.01.2021].
40. Wynne M. J., Hallan J. K. Reinstatement of *Tetradesmus* G. M. Smith (Sphaeropleales, Chlorophyta). *Feddes Repertorium*, 2015, vol. 126, iss. 3–4, pp. 83–86. https://doi.org/10.1002/fedr.201500021
41. Zhang C., Chen X., Too H. Microbial astaxanthin biosynthesis: Recent achievements, challenges, and commercialization outlook. *Applied Microbiology and Biotechnology*, 2020, vol. 104, pp. 5725–5737. https://doi.org/10.1007/s00253-020-10648-2

РАБОЧАЯ КОЛЛЕКЦИЯ ЖИВЫХ КУЛЬТУР КАРОТИНОГЕННЫХ МИКРОВОДОРОСЛЕЙ ИНСТИТУТА БИОЛОГИИ ЮЖНЫХ МОРЕЙ ИМЕНИ А. О. КОВАЛЕВСКОГО

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В статье приведены сведения о специализированной рабочей коллекции каротиногенных микроводорослей отдела физиологии животных и биохимии Федерального исследовательского центра «Институт биологии южных морей имени А. О. Ковалевского РАН» (ФИЦ ИнБЮМ), созданной в рамках научной и прикладной тематик института для исследования механизмов стресс-толерантности у эврибионтных и экстремофильных одноклеточных фототрофов, а также для выявления коммерчески значимых источников высокоценных в медицинском и пищевом отношении кетокаротиноидов группы астаксантина. Коллекция насчитывает 44 штамма микроводорослей различной таксономической и экологической специализации с выраженной

способностью к гиперсинтезу вторичных каротиноидов и липидов при экстремальных внешних воздействиях (высыхание, острое голодание, высокая освещённость, температура и солёность, действие токсикантов и др.). Основными способами пополнения фонда являются направленный обмен каротиногенными видами с ведущими российскими и зарубежными коллекциями микроводорослей и собственные полевые сборы в причерноморских зонах Крыма и Кавказа. Большинство штаммов в коллекции — представители двух порядков класса Chlorophyceae [Chlamydomonadales (25 штаммов) и Sphaeropleales (15 штаммов)], так как именно в этих порядках явление вторичного каротиногенеза распространено наиболее широко. Среди них преобладают обитатели эфемерных пресноводных водоёмов, аэрофильные и почвенные микроводоросли. Все штаммы поддерживаются в состоянии альгологически чистых культур при контролируемых условиях на агаризованных минеральных средах. Описания вариететов коллекции включают следующие сведения: а) современный таксономический статус вида, верифицированный с учётом обновлённых данных депонирующих коллекций и альгологических баз AlgaeBase и NCBI Taxonomy Browser; б) базионим и известные синонимы вида; в) время и источник поступления штамма в коллекцию; г) фамилию автора, географическое место и биотоп, из которого штамм был изолирован; д) номер штамма в NCBI (если есть); е) питательную среду, на которой штамм поддерживается в коллекции ФИЦ ИнБЮМ. Проанализировано значение коллекции для проведения морфобиологических и физиолого-биохимических исследований особенностей роста, вторичного каротиногенеза и биотехнологического потенциала зелёных микроводорослей.

Ключевые слова: каротиногенные микроводоросли, коллекционное хранение, Chlorophyta, каротиноиды, астаксантин