State-of-Art and activities of the culture collection of experimental strains of microalgae and cyanobacteria isolated from cold environments @IBOT

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Abstract

Culture collections contain cyanobacterial and microalgal strains as type species taxonomical studies and future exploitation in basic and applied research. The Culture collection of experimental strains, isolated from cold environments, at the Institute of Botany CAS, Třeboň, Czech Republic, is a working non-public culture collection, *i.e.*, the strains are being isolated and used in experiments, but they are not available for sale. At present (June 2025), the culture collection contains 350 defined strains of cyanobacteria and microalgae isolated from Antarctica (168 strains), Arctic (148 strains), Europe (18 strains) and North America (14 strains). The origin of two strains is unknown. The strains originate from snow/glacier (17 strains), lacustrine (39 strains), hydro-terrestrial (24 strains), and terrestrial (72 strains) environments. It is planned to determine the original environment of the remaining 198 strains in the near future. From a taxonomical point of view, 153 strains belong to the Cyanophyceae class, 38 to the Trebouxiophyceae class, 32 to the Chlorophyceae class, 23 to the Klebsormidiophyceae class, 8 to the Zygnematophyceae class and 94 to the Xanthophyceae class. The Ulvophyceae and Bacillariophyceae classes are both represented by a single strain. In addition, methods of sampling, isolation, maintenance, and determination of strains are described.

DOI: 10.5817/CPR2025-S-9

Received July 4, 2025, *accepted* October 31, 2025.
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Acknowledgements: This study / work was supported by the long-term research development project No. RVO 67985939 of the Czech Academy of Sciences (all), the TAČR projects TN02000044 (JL, JK) and TS02030210 (JE, JK). JK, OB, JE, JŠ were also funded by the Czech Science foundation project 22–08680 L. OB was additionally supported by the Researchers at Risk Fellowship (Czech Academy of Sciences) and the IIE Scholar Rescue Fund (IIE-SRF) Fellowship.
AK, JE, JK, LN and OB are very grateful for the help and support from the staff of the Czech Arctic Research Infrastructure, University of South Bohemia in České Budějovice, Czech Republic. JK gives thanks for the additional support received from the Faculty of Education, University of West Bohemia in Plzeň, Czech Republic.

Key words: culture collections, polar cyanobacteria and algae, microorganisms, strains, cryopreservation

1. Introduction

Cyanobacteria and eukaryotic microalgae are essential components of the microbial communities in cold environments, such as the polar regions, high mountains, glaciers, tundra, lakes, and periglacial soils. These ecosystems are characterized by extreme and/or fluctuating conditions that include low temperatures, a short growing season, freeze – thaw cycles, desiccation, high UV radiation, and limited nutrient availability. Despite these harsh constraints, diverse phototrophic microorganisms persist in these habitats, being primary producers, contributing to soil stabilization, and nutrient cycling (Elster 2002, Elster and Benson 2004).

A wide range of physiological and metabolic adaptation-acclimatization mechanisms allows cyanobacteria and microalgae to survive in such environments. These include the production of antifreeze proteins, extracellular polysaccharides, specialized pigments, and modifications of membrane composition and photosynthetic machinery (Kvíderová et al. 2019). Studying these mechanisms not only enhances our understanding of microbial life in extreme conditions but also contributes to broader ecological insights into global biogeochemical processes in rapidly changing cold-climate ecosystems.

Beyond basic research, cold-adapted strains have potential applications in biotechnology and industry. Their ability to grow at low temperatures and produce bioactive compounds makes them promising for use in the bioremediation of disturbed areas, in cosmetics, as pharmaceuticals, as biofertilizers, and in the food industry (Kvíderová et al. 2017). Furthermore, insights into their stress resistance mechanisms may lead to innovations in synthetic biology and cryopreservation technologies.

Culture collections keep microalgal strains as type species for taxonomical studies and for future exploitation in basic and applied research. At present, the World Federation of Culture Collections has registered 768 culture collections from 76 countries with WDCM-CCINFO and 131 of them have been registered with the WFCC as affiliate members from 49 countries with a total of 966 registered users (WFCC 2025[4]). The first collections of laboratory-maintained cyanobacteria and microalgae were established at the end of the 19th and beginning of the 20th century. At that time scientific interest in the experimental study of microalgae performed using defined strains was on the increase (Day et al. 2004). The study of cyanobacteria and microalgae was closely connected with the developing algal culturing techniques at the Charles University in Prague (Czech Republic). In 1913, Václav Uhlíř founded a collection of cyanobacteria from lichens, but he died in World War I in 1915, and his collection disappeared. Between 1914 and 1916, Silvestr Prát (1895–1990) followed the idea for the study of strains in laboratory experiments and showed the importance of microalgal cultures for studies of plant physiology in general and for phycology and hydrobiology (algal bioassay) in particular. At same time a centre of cultivation for microalgae was established within the German section of the Charles University in Prague by the newly appointed Professor of Plant Physiology, Ernst Georg Pringsheim (1881–1970). Shortly before World War II, Pringsheim took refuge in the United Kingdom and took with him some strains of microalgae to establish a collection in England (the Culture Collection of Algae and Protozoa – CCAP). Several

strains were later used to help establish culture collections in Germany (the Sammlung von Algenkulturen at Göttingen – SAG), and in the USA (the University of Texas at Austin – UTEX). The majority of the Prague collection was moved to Třeboň in 1979 (Department of Phycology, Institute of Botany, Czechoslovak Academy of Science) and became part of what is now the CCALA. At present, there are about 438 different strains isolated by E.G. Pringsheim and his wife Olga available from the five culture collections (CCALA, CCAP, SAG, UTEX and CAUP) (Day et al. 2004).

Cyanobateria and microalgae have been studied at the Department of Phycology, the Institute of Botany at the Academy of Science of the Czech Republic in Třeboň since 1960. The research has been focused on taxonomy and ecology, using several approaches combined with molecular biology, biochemistry, and physiology. Recently, special attention is paid to organisms and communities from extreme habitats, such as polar and mountain ecosystems or hot springs. Since 1988, researchers at the Department of Phycology have participated in many projects that studied the ecology of cyanobacteria and microalgae in polar and alpine ecosystems. Field studies are followed by detailed autecological, taxonomical, and ecophysiological laboratory studies. The Phycology Department and the Culture Collection of Autotrophic Organisms (CCALA) participated in the EU project COBRA (The COnservation of a vital European scientific and Bacteriological Resource: microAlgae and cyanobacteria, No. QLRI-CT-2001-01645, 2001 – 2005) which helped to establish cryopreservation technology across European culture collections (Day et al. 2005).

At present, the CCALA is a part of the Experimental Garden and Gene Pool Collections of the Institute of Botany of the Czech Academy of Sciences in Třeboň (CCALA 2025[1], IBOT 2025[3]) that serves as a unique resource for taxonomic, physiological, and experimental research. It includes microalgal strains isolated from Arctic and Antarctic locations, high-altitude mountain habitats, and cryosoils in North America and Europe. The availability of these strains allows controlled studies of their growth, stress response, and biochemical properties (CCALA 2025[1]).

Besides CCALA, there are several non-public collections of experimental strains at the Department of Phycology at the Institute of Botany CAS in Třeboň. The information on strains is not usually available for public, since the research on these strains is continuing and some strains could become subject of intellectual property rights (patents, utility models *etc*.). In this paper, the structure, composition, and potential of the current non-public working culture collection of maintained cold-environment cyanobacteria and microalgae is presented.

2. Strain collection

2.1. Original locations

The strains were collected between 1988 and 2024 during field research at various cold environments around the world (Fig. 1). Various environmental samples were collected from the Arctic and Antarctic regions, as well as from some temperate mountains in Europe and North America. In the Arctic, samples were taken from northern Sweden (Abisko, Lapland), the Svalbard archipelago, Ellesmere Island (Canadian Arctic) and Russia (Petrozavodsk: White Sea, Karelia, Russian Arctic). Antarctic samples were taken from maritime Antarctica; James Ross and Vega

Islands (NE Antarctic Peninsula), King George and Deception Islands (South Shetlands Archipelago), South Georgia as well as from continental Antarctica; Queen Maud Land (East Antarctica) in the vicinity of the Princess Elisabeth Station (Belgium) and Showa/Syowa Station (Japan). In addition, samples were gathered from mountains; Slovakia (High Tatras), the Swiss and Austria Alps, and the USA (Arizona, Montana, New York State, Vermont). This broad geographic range provides insights into the diversity and adaptations of microalgae in extreme climatic conditions, as well as allowing comparisons with populations from polar and temperate mountain regions.

During sampling, the GPS coordinates were recorded using either a tourist-class GPS receiver or a cell phone (δ = 2-5 m depending on device and number of visible satellites) for repeated sampling. If necessary, the data were converted to decimal format. For sampling sites map drawing, geographical coordinates (longitude, latitude) were processed using R software (R 4.3.2, packages ggplot2 for data visualisation, ggrepel for site labels).

The habitat types where the samples and microalgal community were collected are crucial for the selection of proper isolation methods. Limnetic, hydro-terrestrial and terrestrial environments are classified according to temperature stability and water availability (Elster 2002, Elster and Benson 2004). Snow fields and glaciers are considered to be separate environments with specific habitat types and special microalgal communities (Table 1).

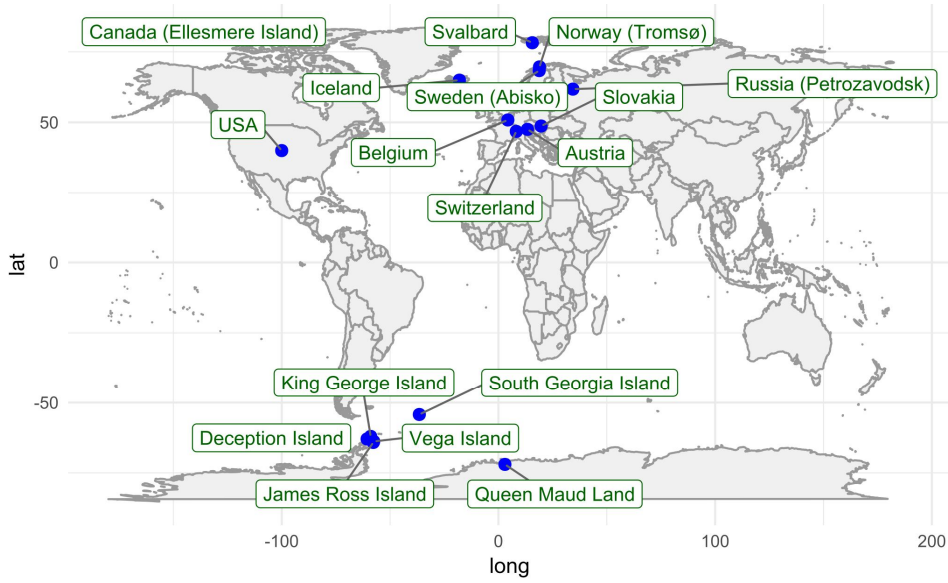

Fig. 1. Map of the original localities of the strains in the culture collection (R 4.3.2, packages ggplot2, ggrepel).

Environment	Habitat	*Note*	Community	*Note*
Glacier/snow	glacier surface		glacial algae	growing on the glacier surface
	snow field	persistent and non-persistent	snow algae	growing in melting snow
	cryoconite		sediment	
Limnetic	lake		plankton	growing in water column
			benthos	growing at bottom, not forming any visible biofilm or microbial mats
			periphyton	forming visible biofilm or microbial mats
			epipelon	growing on mud/fine sediment, not forming any visible biofilm or microbial mats
			epipsamnon	growing on sand, not forming any visible biofilm or microbial mats
Hydro-terrestrial	streams	glacier- and snow-fed streams	periphyton	
	coastal pool	freshwater to brackish	periphyton	
	small pools	strictly freshwater	periphyton	
	seepage	water seeps from ground	periphyton	
	wetland	no water seeps	periphyton	
Terrestrial	glacial soil	deglaciated soil with low nutrient and organic content	edaphon	growing in soil or on its surface, not forming any carpet-like structures
			soil crust	forming any carpet-like structures on soil surface and reaching several cm below
	soil	well-developed soil	edaphon	
			soil crust epipsamnon	
	sand		epipsamnon soil crust	
	wet rock	water sprayed rock near a stream, waterfall	epilithon	growing on the rock surface
	rock	Receive water from melting snow, precipitation or air	epilithon	
	skeleton		endolithon	growing inside a rock or other solid natural or artificial surface

Table 1. Types of polar environments and according to water availability and temperature stability and related habitats and microbial communities (Elster 2002, Elster and Benson 2004).

2.2. Sampling procedures

The sampling procedures were selected according to the local environment, habitat, and community types. The basic workflow is following: decision of sampling strategy and photo-documentation of the site » GPS measurement » measurement of physico-chemical parameters (temperature, PAR, pH, *etc*.) » sampling and *in situ* biological measurements if applicable (*e.g.* variable chlorophyll fluorescence). The *in situ* measurements depend on type of sample type, for instance, dissolved oxygen concentration is not measured in soil. The instrumentation changes in time as well, for instance if some measuring device is broken and is replaced by a new one, or the GPS receiver could be replaced by a cell phone. If novel methods are introduced, typically -omics, the new protocols are added. The sampling procedures were optimized for the below-specified environments:

Snow: Surface snow was harvested using a shovel and placed in sterile plastic containers. The samples were placed in a thermos bottle to avoid rapid thawing.

Plankton: Plankton samples were taken from the surface layer of lakes by simply filling a sterile plastic bottle or using a van Dorn sampler. Before proceeding to strain isolation, samples were concentrated using syringe and GF/C filters (Whatman, UK).

Periphyton: Periphyton samples were collected from both lentic and lotic environments. In lake systems, the samples were obtained from the littoral zone of different substrate types (rocks, sediments). In streams, the sampling focused on cobble and boulder surfaces with well-developed communities. The samples were collected using the standard scraping technique with a toothbrush and spatula or knife to remove the biofilm matrix. The dislodged material was immediately rinsed with the ambient water and placed in sterile collection containers.

Soil and biological soil crusts: Sampling either takes place along transects or within permanent plots. The transect-based approach addresses the effects of environmental gradients, such as elevation, pH, or moisture, on the qualitative and/or quantitative composition of microalgal communities. Transect length varies depending on the site characteristics but remains fixed throughout the study to maintain consistency. The second approach focuses on repeated sampling at fixed plots to monitor microalgal dynamics over time. Plot size and the number of replicates depend on the research objectives and typically range between 10 and 20 m^2. Sampling targets the layer of top-soil, up to 3-5 cm deep.

Sampling distinguishes between individual and composite soil samples. Individual samples help detect species that form localized “blooms” or patches on the soil surface. Each sample typically includes a very thin soil layer (usually 3-5 mm) and a small surface area (up to 10 cm^2). Multiple individual samples along a transect allow for an evaluation of microalgal distribution patterns in relation to environmental gradients.

Composite samples mainly support floristic assessments of particular locations. They consist of 10 to 20 individual subsamples and may originate from transects or permanent plots. To ensure representative coverage within plots, sampling points follow a stochastic selection approach (Hollerbach and Schtina 1969, Kostikov et al. 2001).

Biological soil crusts (BSCs) undergo separate collection. Sterile spatulas gently remove the crusts from the soil surface. The crusts display variability in colour (black, dark or light green, brown), density, and structure. When crusts are bound tightly to the underlying soil, sampling includes the uppermost layer of soil.

Soil microalgae are typically studied using different approaches, including direct microscopic observation, examination of soil enrichment cultures, the use of contact glass slides, and the inoculation of soil material into liquid and solidified (agar) media. An important step in sample processing is the isolation of strains in monoculture. Obtaining pure cultures enables accurate taxonomic identification, detailed morphological and molecular analyses, and the use of these strains in experimental studies.

2.3. Storage and transport

After collection, all types of samples were placed in sterile containers or sealed plastic bags. To prevent material degradation and preserve the native microbial composition, samples are either transported cooled (at approximately +4°C) or frozen (at −20°C), depending on the expected transport duration and logistical conditions. Upon arrival at the laboratory, the samples were stored under conditions consistent with those in the field at the time of collection until microbiological analyses and the isolation of pure cultures were initiated. The time between sampling and initiation of isolation ranges from several days to several weeks. If the samples cannot be used for isolated immediately after arrival, they are kept in a freezer at −20°C.

3. Strain isolation, cultivation, and maintenance

3.1. Strain isolation

The strains were isolated by spreading on agar plates. During isolation, the cultures were kept at 10°C and 30 μmol m^{-2} s^{-1} in a cultivation box. The strains were grown on solid ½ BG-11 medium (Table 2) in Petri dishes (Fig. 2A). The molar N:P ratio of 100.7 indicates strong P limitation. Since the pH of the medium without any adjustment is ca 7.4 and the nutrient content is relatively low, the ½ BG-11 medium (Allen 1968, Allen and Stainer 1968, Rippka et al. 1979, Andersen 2005; Table 2) is suitable for the majority of strains that originate from oligotrophic conditions. A low nutrient content and P limitation also allows the selection of slow-growing strains that could be out-competed by faster growing strains in nutrient rich conditions (Table 2).

For a limited number of Antarctic strains (25%), isolation was started at the field station to avoid delays due to the long interval between sampling and arrival at the laboratory in the Czech Republic.

Snow microalgae are kept in BBM medium (Bold 1949, Bischoff and Bold 1963, Andersen 2005; Table 3). This medium is slightly acidic with a pH of 6.6. The N:P molar ratio of 1.71 indicates extreme N limitation (Table 3).

3.2. Cultivation and maintenance

Similarly to isolation, strains are kept at 10°C and 30 μmol m^{-2} s^{-1} in a cultivation box. The strains were grown on solid ½ BG-11 (Table 2) or BBM (Table 3) in Petri dishes or agar slopes in test tubes (Fig. 2). For experimental work, the strains are transferred into liquid BG-11 medium to make a strong inoculum and then transferred to experimental media that could be different to BG-11 or BBM. For instance,

½ Simmer-Šetlík medium (SŠ medium; Zachleder and Šetlík 1982; Table 4) is used for large volume thin-layer pilot plant cultivation (150/250 L; *e.g.* Řezanka et al. 2025). The final pH of ½ SŠ is neutral (pH 7) and the N:P molar ratio of 8 may indicate N limitation (Table 4).

Component	**Stock Solution**	**Quantity used per L of media**		**Final concentration in medium**	
				Original BG-11	**½ BG-11**
Fe-citrate solution		1 mL		*see* below	
$NaNO_3$	-	1.5 g		1.5 g L^{-1}	0.75 g L^{-1}
$K_2HPO_4.3H_2O$	40 g L^{-1}	1 mL		0.04 g L^{-1}	20 mg L^{-1}
$MgSO_4.7H_2O$	75 g L^{-1}	1 mL		0.075 g L^{-1}	37.5 mg L^{-1}
$CaCl_2.2H_2O$	35 g L^{-1}	1 mL		0.035 g L^{-1}	17.5 mg L^{-1}
Na_2CO_3	20 g L^{-1}	1 mL		0.02 g L^{-1}	10 mg L^{-1}
Na-EDTA	1 g L^{-1}	1 mL		0.001 g L^{-1}	0.5 mg L^{-1}
Trace metals solution		1 mL		*see* below	
	Stock Solution	**Quantity used per L of solution**	**Concentration in solution**		
Fe-citrate solution					
Citric acid	6 g L^{-1}	1 mL	0.006 g L^{-1}	6 µg L^{-1}	3 µg L^{-1}
Ferric ammonium citrate	6 g L^{-1}	1 mL	0.006 g L^{-1}	6 µg L^{-1}	3 µg L^{-1}
Trace metals solution					
H_3BO_3	-	2.86 g	2.86 g L^{-1}	2.86 mg L^{-1}	1.43 mg L^{-1}
$MnCl_2.4H_2O$	-	1.81 g	1.81 g L^{-1}	1.81 mg L^{-1}	0.905 mg L^{-1}
$ZnSO_4.7H_2O$	-	0.22g	0.22 g L^{-1}	0.22 mg L^{-1}	0.11 mg L^{-1}
$CuSO_4.5H_2O$	79 g L^{-1}	1 mL	0.079 g L^{-1}	79 µg L^{-1}	39.5 µg L^{-1}
$Na_2MoO_4.2H_2O$	-	0.391 g	0.391 g L^{-1}	391 µg L^{-1}	195.5 µg L^{-1}
$Co(NO_3)_2.6H_2O$	49.4 g L^{-1}	1 mL	0.0494 g L^{-1}	49.4 µg L^{-1}	24.7 µg L^{-1}

Table 2. The composition of ½ BG-11 medium recalculated from Allen 1968, Allen and Stainer 1968, Rippka et al. 1979, and Andersen 2005, and resulting nutrient concentrations.

3.3. Cryopreservation of strains

Cryopreservation in liquid nitrogen (-196°C) is used for long-term maintenance of strains (Mori et al. 2002, Day et al. 2005, Day and Brand 2005, Day et al. 2007, Elster et al. 2008, Lukešová et al. 2008). The cryopreservation facility at the Department of Phycology, the Institute of Botany CAS, was established in 2001 as part of the COBRA project (Day et al. 2005). The laboratory houses a Kryo 10 Series III planner for controlled freezing (BioMed, USA; Fig. 3A) and two MVE XLC 230 auto-fill cryostores (MVE Biological Solutions, USA; Fig. 3B).

Component	Stock Solution		Quantity used per L of media	Final concentration in medium
$NaNO_3$	25 g L^{-1}		10 mL	0.25 g L^{-1}
$CaCl_2.2H_2O$	2.5 g L^{-1}		10 mL	0.025 g L^{-1}
$MgSO_4.7H_2O$	7.5 g L^{-1}		10 mL	75 mg L^{-1}
K_2HPO_4	7.5 g L^{-1}		10 mL	75 mg L^{-1}
KH_2PO_4	17.5 g L^{-1}		10 mL	0.175 g L^{-1}
NaCl	2.5 g L^{-1}		10 mL	25 mg L^{-1}
EDTA solution			1 mL	*see* below
Acidifed iron solution			1 mL	*see* below
H_3BO_3	11.42 g L^{-1}		1 mL	11.42 mg L^{-1}
Trace metals solution			1 mL	*see* below
EDTA solution				
EDTA	50 g L^{-1}			50 mg L^{-1}
KOH	31 g L^{-1}			31 mg L^{-1}
Acidified iron solution				
$FeSO_4.7H_2O$	0.498 g 100 mL^{-1}	= 4.98 g L^{-1}		4.98 mg L^{-1}
H_2SO_4 (96%)	0.1 ml 100 mL^{-1}	= 1 ml L^{-1}		1 µl L^{-1}
Trace metals solution				
$ZnSO_4.7H_2O$	8.82 g L^{-1}			8.82 mg L^{-1}
$MnCl_2.4H_2O$	1.44 g L^{-1}			1.44 mg L^{-1}
MoO_3	0.71 g L^{-1}			0.71 mg L^{-1}

Table 3. The composition of BBM medium (Bold 1949, Bischoff and Bold 1963, Andersen 2005).

During the COBRA project (Day et al. 2005), strains from CCALA were cryopreserved to elucidate the stages at which critical damage during cryopreservation occurs and this information was used to optimize the protocols for each group of microalgae. The following cryopreservation protocols were tested (Day et al. 2005):

- One step protocol: direct to liquid N_2 (l N_2) with DMSO (dimethyl sulfoxide, 5% v/v) or no cryoprotectant;
- Two step protocol: cooling at –30°C for 15 min. » l N_2 with DMSO (5% v/v) or methanol (10% v/v) as cryoprotectants;
- Two step protocol: controlled cooling at the rate of 1°C min^{-1} to –40°C » cooling at –40°C for 15 min. » l N_2 with DMSO (5% v/v) or methanol (10% v/v) as cryoprotectants.

The post preservation stability tests and normal and abnormal post-storage recovery responses in a wide range of organisms were evaluated and the most effective method includes pre-treatment with DMSO (5% v/v), followed by a two-step protocol with controlled cooling at a rate of 1°C min^{-1} to –40°C » –40°C for 15 min. » l N_2 (Day et al. 2005).

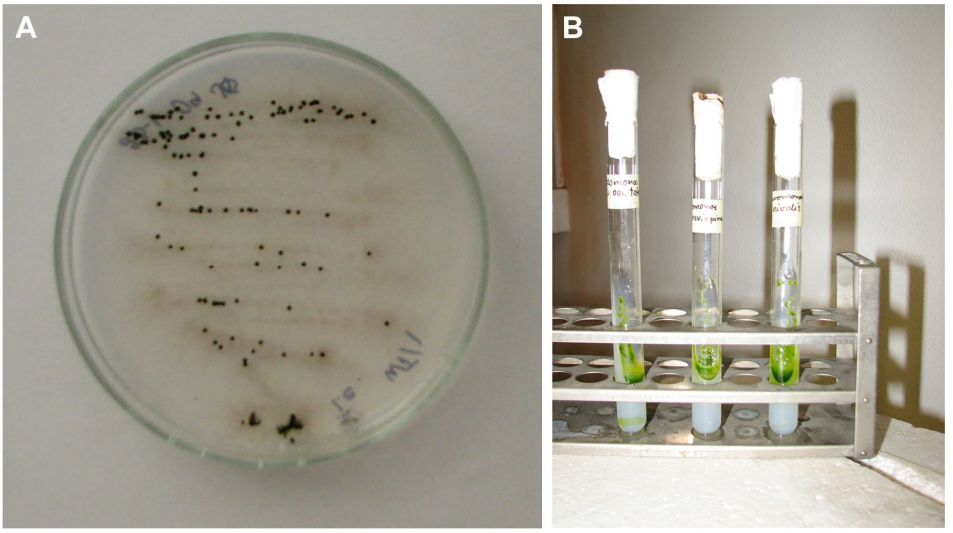

Fig. 2. (**A**) Petri dish with microalgal colonies during strain isolation, and (**B**) the agar slope with an isolated strain.

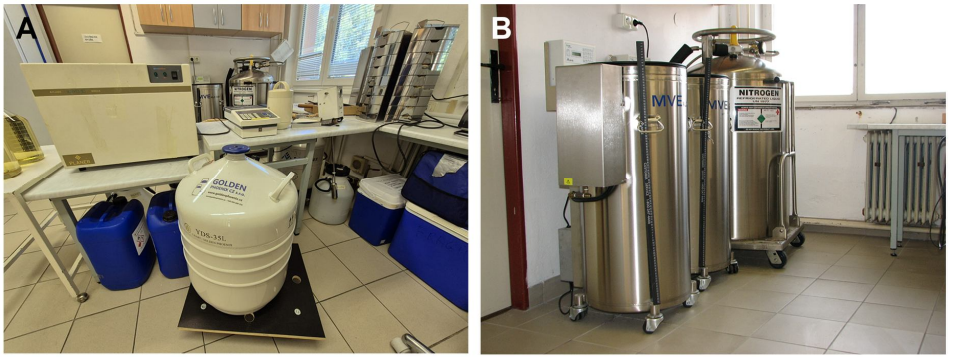

Fig. 3. The laboratory of cryopreservation at the Department of Phycology, Institute of Botany CAS, Třeboň, (A) Kryo 10 Series III planner for controlled freezing (BioMed, USA) and (B) MVE XLC 230 auto-fill cryostorages (MVE Biological Solutions, USA).

This two-step protocol with DMSO and pre-treatment continuous cooling is currently applied for cryopreservation for CCALA and the working collection of cyanobacterial and microalgal strains from cold environments. For cryopreservation, the cultures do not need to be axenic, however, a reduction of contamination load is recommended for better recovery. Both cultures growing in liquid or on solidified medium (agar) may undergo successful cryopreservation. The majority of strains of polar and alpine microalgae have been successfully cryopreserved without any special pre-adaption conditions (*e.g.* nitrogen deficiency). From our experience confirmed by other authors (refer to Day and Fleck 2015 for overview), actively growing cultures in late exponential, linear or early stationary growth phases generally

survive cryopreservation with greater viability than those in late stationary or declining growth phases, or those growing under stressful conditions. Better resistance of cultures growing on agar are suspended and homogenised in a liquid medium. Before cryopreservation, the cultures are centrifugated, and no significant decrease in the vitality of centrifuged strains was reported. Cultures are cryopreserved in the medium used for cultivation with the addition of DMSO (5% v/v).

At present, all strains isolated from polar and alpine environments within the microalgal collections at the Institute of Botany have now been cryopreserved.

4. Strain identification

4.1. Morphotypes

During the isolation and maintenance of experimental stock cultures, the diversity of morphotypes, contamination and strain viability is checked using light and fluorescence microscopy. The Olympus BX-51 microscope (Olympus, Japan) is equipped with Nomarski contrast (differential interference contrast), and fluorescence filter cube sets for DAPI, SYTOX® Green and CTC staining (all Olympus, Japan; Tashyreva et al. 2013). The microphotographs are taken using a DP-74 digital camera, and image analysis and further image processing are performed using cellSens Entry software (Olympus, Japan).

For morphotype identification, the following keys are used:

- All groups: Hindák 1978, Kaštovský et al. 2018a, 2018b;
- For soil microalgae in general: Ettl and Gärtner 2014;
- for Cyanobacteria: Hindák 2008, Komárek and Anagnostidis 1999, 2005; Komárek 2013;
- for Chlorophyta: Ettl and Gärtner 1986, Andreieva 1998;
- for Bacillariophyta: Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b;
- For Xanthophyceae: Ettl 1978.

After identification, the scientific names are verified using the AlgaeBase online database (Guiry and Guiry 2025[2]).

4.2. Molecular taxonomy

Molecular taxonomy is used to confirm the morphological identification of isolated strains and to clarify their phylogenetic affiliations. Total genomic DNA is extracted from pure cultures using standard protocols (CTAB-based chloroform/ isoamyl extraction method or commercial kits) adapted for cyanobacteria and microalgae.

For cyanobacteria, most commonly fragments of the 16S rRNA gene and 16S-23S ITS region is used. For eukaryotic microalgae, commonly applied markers include the 18S rRNA gene, the ITS1-5.8S-ITS2 cluster, and plastid genes such as *rbcL* and *tufA* (Leliaert et al. 2012, Darienko et al. 2015, Saraf et al. 2022).

Following DNA isolation, amplification is performed using universal or group-specific primers. PCR products are sequenced using the 3-step Sanger method. The resulting sequences are compared

against reference databases (*e.g.*, NCBI GenBank, SILVA) using BLAST to obtain a preliminary taxonomic classification. To resolve the phylogenetic relationships more accurately, phylogenetic trees are constructed, primarily using maximum likelihood and neighbour-joining methods.

For comparison of closely related taxa, additional bioinformatic approaches may be employed. In particular, hypothetical secondary structures of the ITS rRNA region are predicted for the studied strains and compared with secondary structures of previously characterized and identified taxa. This approach is especially useful when dealing with cryptic species or in cases where a high degree of similarity of the primary nucleotide sequences does not allow for reliable taxonomic resolution (Coleman and Mai 1997, Coleman 2007, Caisová et al. 2013).

5. Polar and alpine strains overview

At present (June 20, 2025), the culture collection of experimental strains contains 357 strains of cyanobacteria and microalgae. Identification to at least the level of the genus of seven strains remains to be completed using molecular taxonomy methods. Therefore, these strains were not used in further analyses. Two possible duplicates were discovered, *Oscillatoria* sp. (JR08) – *Oscillatoria* sp. (CCALA 879) and *Neocystis mucosa* (BSC - 18 – 11a) – *Neocystis mucosa* (CCALA 1141) and for the purpose of this review they were treated as separate strains. One additional strain included a moss, *Drepanocladus longifolius* (Amblystegiaceae, Hypnales; CCALA 869) isolated from Esmeralda Lake, Vega Island, Antarctic.

The polar strains, isolated from the Arctic and Antarctic, including northern parts of Europe (Abisko, Sweden) and North America (Ellesmere Island – Canadian Arctic), form major part, 92% (322 strains), of the culture collection. Alpine strains that originate from mountain regions outside the Arctic and Antarctic were represented by 19 strains from Europe and North America. Five of them were obtained from the Culture Collection of Cryophilic Algae, Branch of Bioanalytics and Bioprocesses (IZI-BB), the Fraunhofer Institute for Cell Therapy and Immunology, Potsdam-Golm, Germany (CCCryo), and two of them from the Culture Collection of Algae, the College of Natural Sciences, University of Texas at Austin, Austin, Texas, USA (UTEX). Seven strains originated from continental Europe (Belgium) and North America (Montana, USA) from outside the Arctic and as they are not alpine were considered to be temperate.

5.1. Origin of strains

Most of the strains were isolated from the Antarctic (168 strains, 48.0% of all strains) and the Arctic (148 strains, 42.3% of all strains). In Antarctica, the strains predominantly originated from three regions: Queen Maud Land (79 strains, 22.6% of the total), James Ross Island (44 strains, 12.6% of the total) and Deception Island (33 strains, 9.4% of the total). Eight strains were isolated from King George Island (2.3%). A single strain was isolated from each of South Georgia and the Vega Islands (0.3% of the total; *see* Fig. 4).

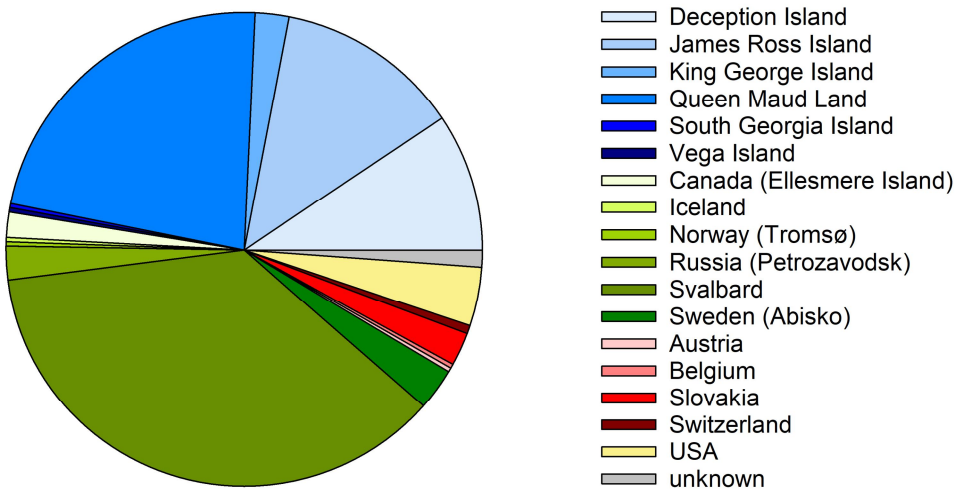

Fig. 4. Origin of strains in the experimental strain culture collection from individual main regions and sub-regions (n = 350).

As for the Arctic, Svalbard was the main source of strains (128 strains, 36.6% of the total), followed by Abisko, Sweden (10 strains, 2.9% of the total), Karelia – White Sea vicinity, of the total Russian Arctic (8 strains, 2.3% of the total) and Ellesmere Island, Canada (6 strains, 1.7% of all strains). A single strain (0.3% of all strains) was obtained from snow near Tromsø, Norway, and another from a whale skeleton in Iceland (Fig. 4).

The other regions predominantly provided alpine microalgae; from the High Tatra Mts., Slovakia (8 strains, 2.3% of the total) and temperate strains from Montana, USA (10 strains, 2.9% of the total). Two strains (0.6% of the total) of snow algae came from the Furka Pass, Switzerland, and New York State, USA. A single strain (0.3% of the total) of alpine snow microalgae came from the Austrian Alps, and Arizona and Vermont, USA, and a temperate strain from Belgium. Finally, the original location of four strains (1.1% of the total) was undetermined (Fig. 4).

The data on original habitats and communities is less consistent since this information is missing for 198 strains (56.6% of the total), since some data from handwritten field notes have not been digitalized yet. In the known original habitats, a terrestrial environment dominates (47.4% of the known environments), followed by a lacustrine environment (25.7% of the known environments). Hydro-terrestrial and snow/glacier environments are less common, representing 15.8 and 11.2% of the known environments, respectively. In terms of habitats lake, soil, and glacial soil prevailed, making up 25.3%, 23.0% and 17.1% of the known habitats, respectively. The individual proportions of all the other habitats made up less than 10% of the known habitats (Fig. 5A). In terms of the known original communities, most strains were isolated from periphyton (34.9% of the known communities), soil crust (20.4% of the known communities), and edaphon (19.1% of the known communities), while the proportions of the other individual communities make up less than 10% of the total (Fig. 5B).

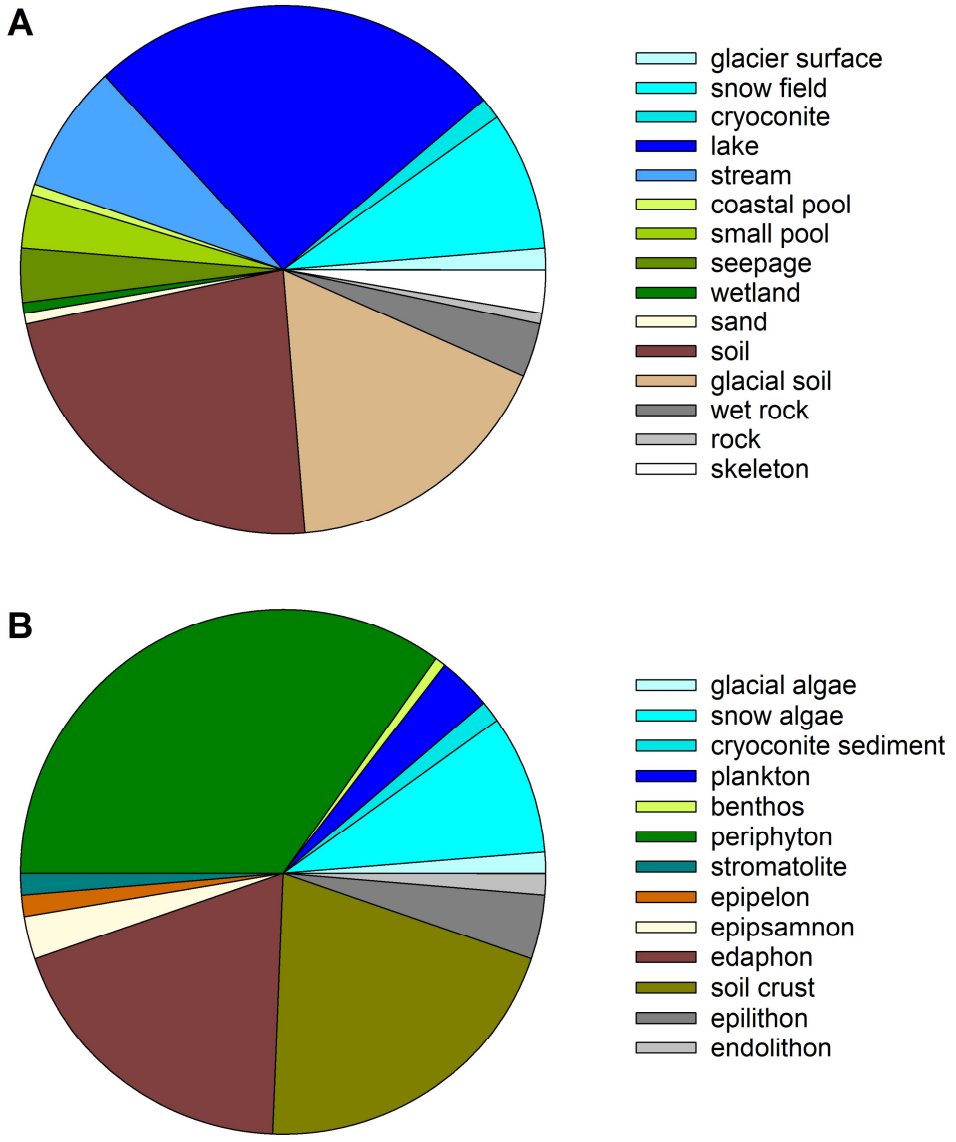

Fig. 5. Proportions of strains originating from (**A**) individual habitat and (**B**) community types (n = 152). The strains of unknown original habitat and community are not included.

5.2. Biological diversity of strains

The taxonomic composition of the culture collection strains is strongly driven by the isolation methods used and capability of a strain to grow in artificial conditions. Therefore, the taxonomic composition determined by *in situ* metagenomic will differ from the taxonomic composition of strains in the culture collection, this could also reflect particular research interests. In our culture collection, the majority of

strains belong to cyanobacteria (Cyanophyceae; 43.7% of the total) and yellow-green algae (Xanthophyceae; 26.9% of the total). Although green algae, *i.e.* Chlorophyta and Streptophyta, represent a total of 29.1% of all strains, they are split into five classes: Chlorophyta » Trebouxiophyceae (10.9%), Chlorophyceae (9.1%) and Ulvophyceae (0.3%); and Streptophyta » Klebsormidiophyceae (6.6%) and Zygnematophyceae (2.3%). Although diatoms are dominant in the Polar Regions (Bacillariophyceae), there is only one strain available of the genus *Fistulifera* sp. (0.3% of the total; Fig. 6).

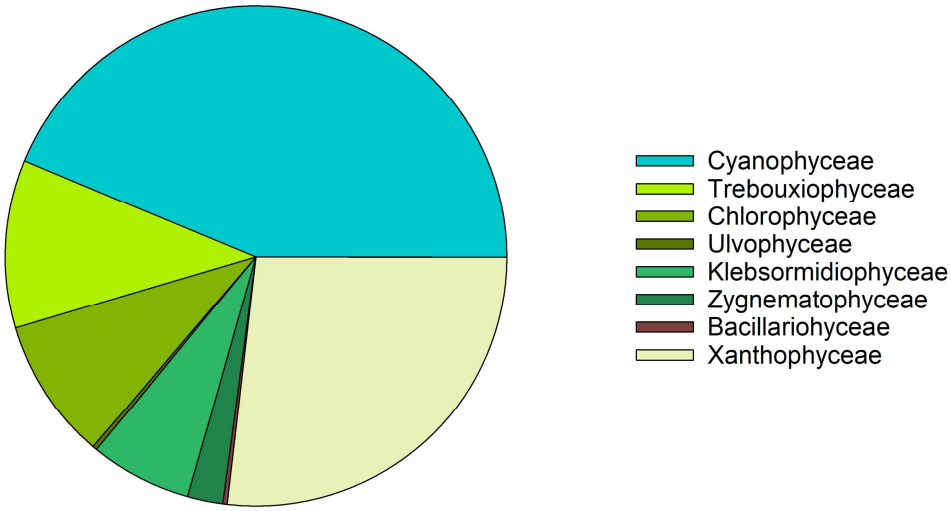

Fig. 6. Proportion of main taxonomic groups (at level of classes) of microalgae in the experimental strain culture collection (n = 350).

Cyanobacteria (153 strains) is most dominant and diverse (22 genera / 38 species) among the strains with nine cyanobacterial orders presented in the culture collection (Fig. 7). The non-heterocytous filamentous cyanobacterial strains are the commonest, 77.8% of all cyanobacterial strains, mainly belonging to the Oscillatoriales (37.9%) and Leptolyngbyales (30.7%) orders with dominant genera *Microcoleus* (28 strains), *Phormidesmis* (27 strains) and *Phormidium* (25 strains; Figs. 7 and 8). The contribution of other non-heterocytous orders is less important (Coleofasciculales – 1.3%, Gomontiellales – 0.7%, Nodosilineales – 0.7%, and Psuanabaenales – 6.8%; Fig. 7). Heterocytous filamentous types are represented by Nostocales (20.9% of all cyanobacterial strains) with the dominant genus *Nostoc* (19 strains; Figs. 7, 8). Unicellular or colonial types (Chroococcales, Synechococcales) are rare (2 strains, 1.3% of all cyanobacterial strains) with only two genera *Chlorogloea* and *Synechococcus* (Figs. 7 and 8).

Chlorophyta (Fig. 9), *i.e.*, classes Trebouxiophyceae, Chlorophyceae and Ulvophyceae are the most diverse group (24 genera / 43 species), although the number of strains (71) is lower than for Cyanophyceae and Xanthophyceae. Of the three chlorophycean classes, Trebouxiophyceae and Chlorophyceae prevail with 38 and 32 strains, respectively (Figs. 9 and 10). Among the Trebouxiophyceae, the order Prasiolales (18 strains) with the dominant genus *Stichococus* (11 strains) makes up almost half of the strains (Figs. 9 and 10).

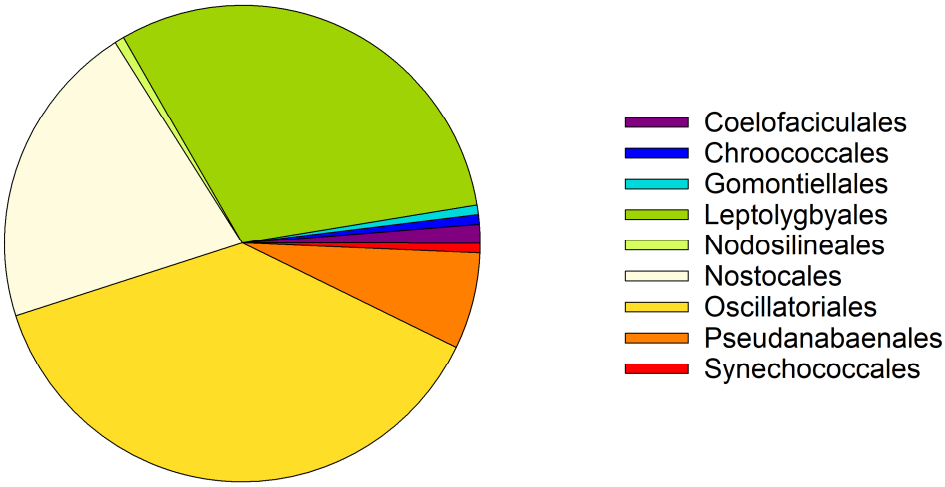

Fig. 7. Proportion of individual orders of class cyanobacteria among the strains in the culture collection (n = 153).

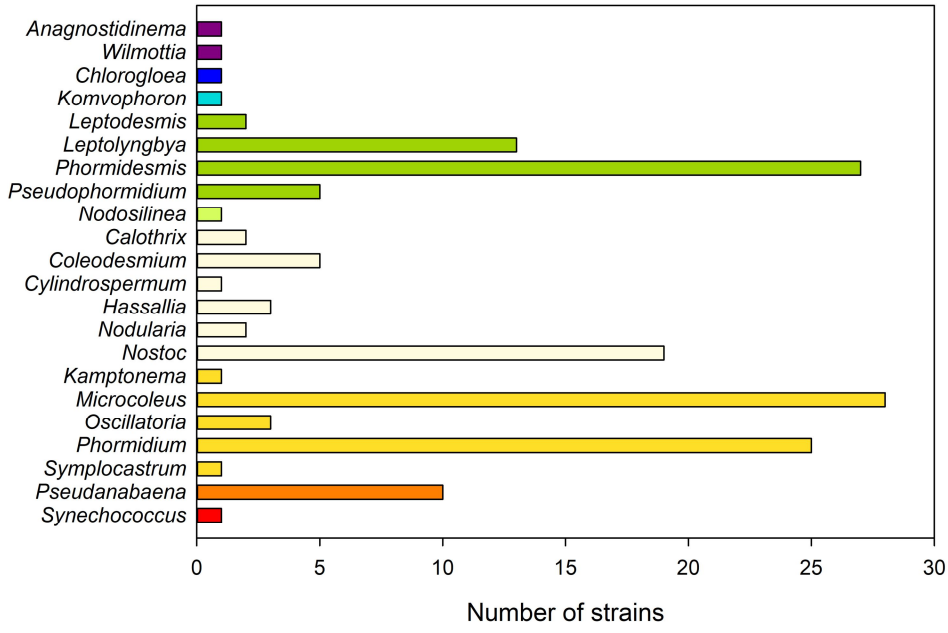

Fig. 8. Number of strains of individual genera of class Cyanophyceae. The bar color corresponds to the pie color of a given order in Fig. 7.

The other half of the strains belong to the orders Chlorellales (10 strains) and Trebouxiophyceae *ordo incertae sedis* (9 strains) with the dominant genera *Chlorella* (6 strains) and *Coccomyxa* (7 strains), respectively (Figs. 9 and 10).

The genus of the snow microalga *Chloromonas* (15 strains) dominates in the Chlorophyceae class with the Volvocales order; other genera contain one to four strains (Figs. 9 and 10). Finally, the Ulvophyceae class is represented by one strain of *Hazenia broadyi* (Ulotrichales; Figs. 9 and 10).

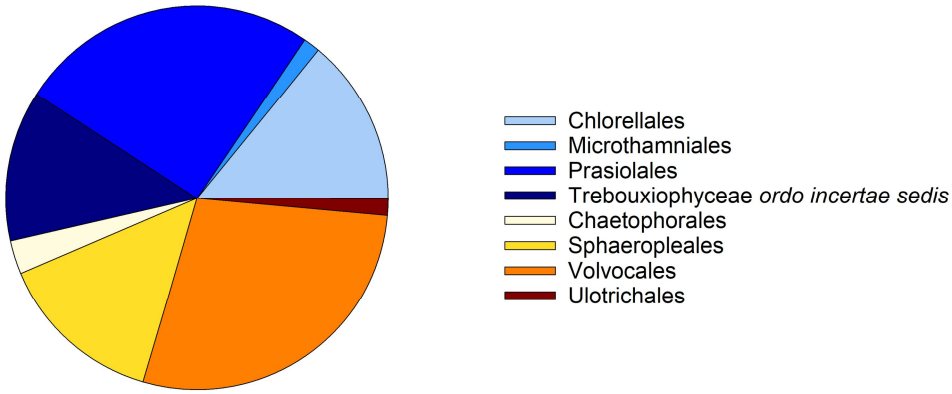

Fig. 9. Proportion of individual orders of Chlorophyta among the strains in the culture collection (n = 71). *Classes*: Blue – Trebouxiophyceae; Yellow and orange – Chlorophyceae; Dark red – Ulvophyceae.

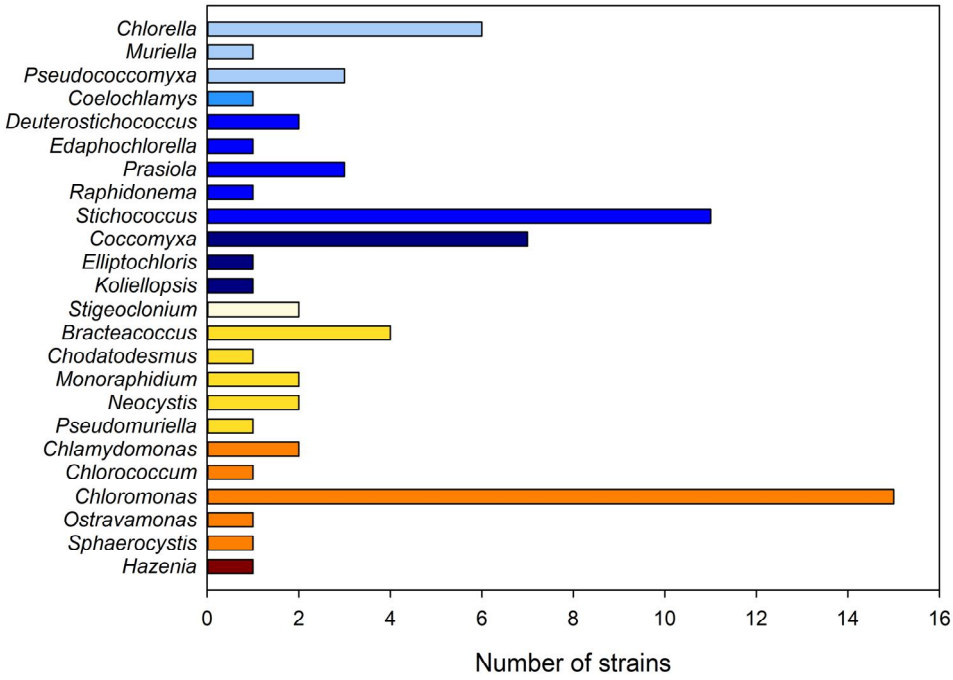

Fig. 10. Number of strains of individual genera of Chlorophyta. The bar color corresponds to the pie color of a given order and class in Fig. 9.

In Streptophyta (Fig. 11), the second evolution line of green algae, almost three quarters of strains belong to a single genus *Klebsormidium* (Klebormidiales, Klebsormidiophyceae, 23 strains; *see* Figs. 11 and 12). The Zygnematophyceae class is only made up of eight strains in four genera of which the genus *Zygnema* is the most common (Figs. 11 and 12).

Although the number of strains, 94, in the Xanthophyceae class (Fig. 13) is the second highest number within the main groups in the culture collection, the diversity of this group is low (5 genera/10 species). The majority of strains belong to the order Tribonematales with the dominant genera *Tribonema* (41 strains) and *Heterococcus* (34 strains; Figs. 13 and 14). Moreover, *Tribonema* is the most common genus among all the strains in the culture collection.

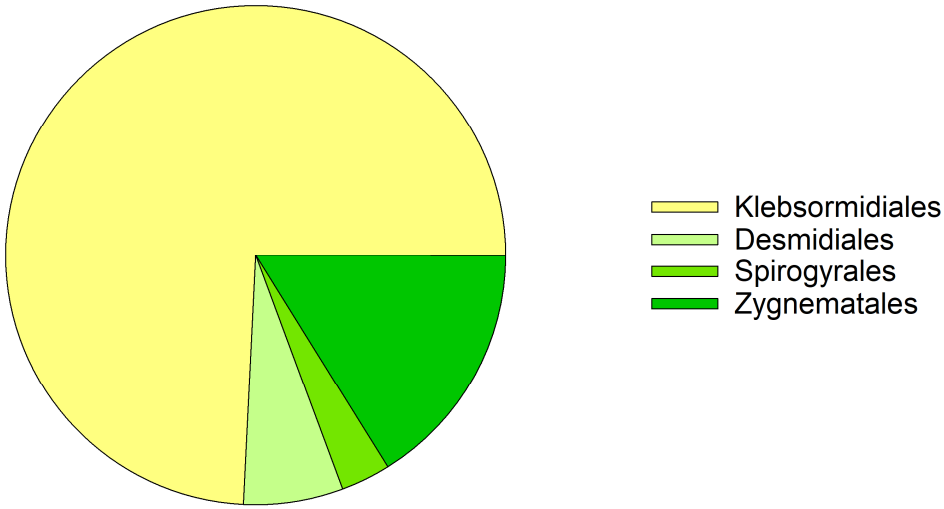

Fig. 11. Proportion of individual orders of Streptophyta among the strains in the culture collection (n = 31). *Classes*: yellow – Klebsormidiophyceae, green – Zygnematophyceae.

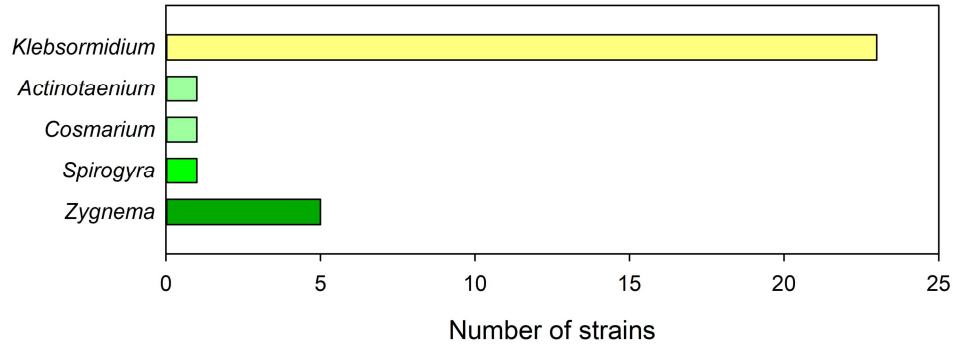

Fig. 12. Number of strains of individual genera of Streptophyta. The bar color corresponds to the pie color of a given order and class in Fig. 11.

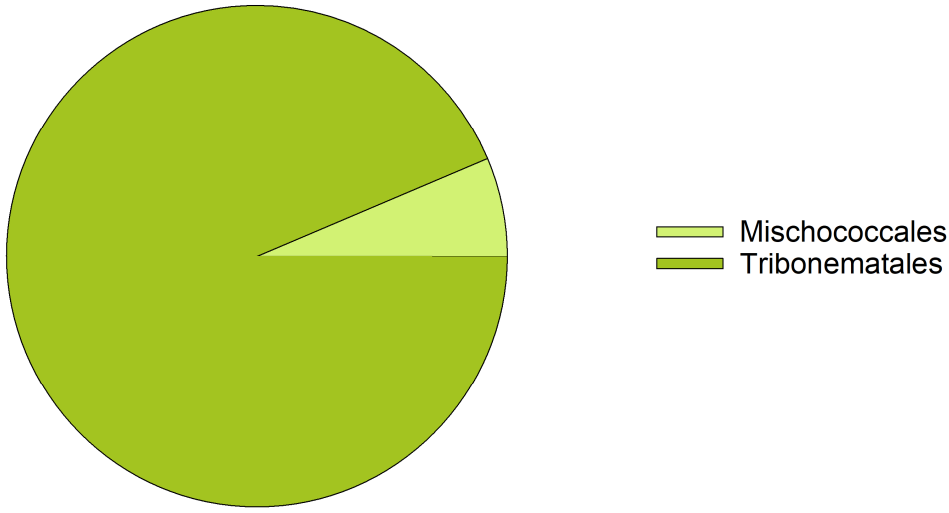

Fig. 13. Proportion of individual orders of class Xanthophyceae among the strains in the culture collection (n = 94).

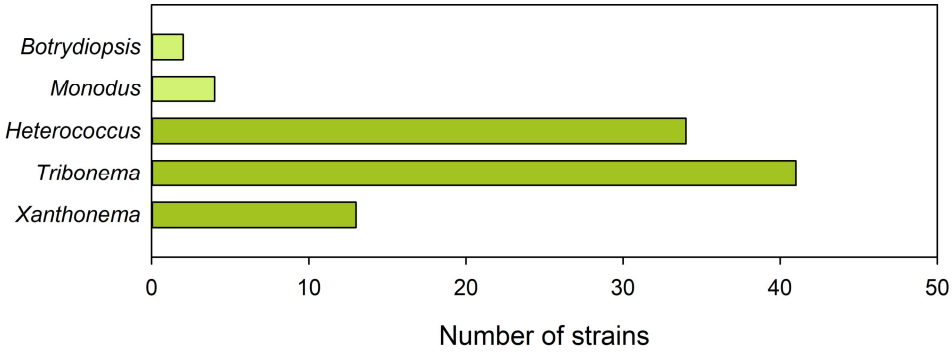

Fig. 14. Number of strains of individual genera of class Xanthophyceae. The bar color corresponds to the pie color of a given order and class in Fig. 14.

6. Exploitation of isolated strains

6.1. Basic research

Molecular taxonomic data from the isolated strains provides valuable information for biological research. The deposited sequences of these strains are used in metagenomic profiling and DNA metabarcoding, significantly accelerating the study of the organismal diversity of a particular location (Pushkareva et al. 2018, Mikhailyuk et al. 2025). This approach enables a rapid and efficient assessment of the community composition and abundance, which is essential in ecosystem monitoring and environmental protection (Kacheva et al. 2022).

Since the collection includes numerous strains belonging to the same taxa (genera and species) but isolated from different locations, these strains may serve as a valuable resource for large-scale studies on phylogeny, evolution, and species distribution (*e.g.* Agostini et al. 2024, Strunecký et al. 2010, 2012a, 2012b).

Isolated strains enable a variety of laboratory experiments aimed at studying the mechanisms of cyanobacterial and microalgal adaptation to environmental conditions (Agostini et al. 2024). Using transcriptomic methods, analyses of metabolic pathway regulation, and photosynthetic activity measurements, it becomes possible to investigate survival processes and functional cellular responses to stress factors (Mock et al. 2008, Hopes et al. 2017, Kolomiiets et al. 2025). Temperate strains from CCALA or other culture collections, or isolated strains such as the one from Belgium, serve as reference strains for comparisons or inner laboratory standards. Such data helps an understanding of the limits of organismal resilience and the ecophysiological mechanisms of survival (Orekhova et al. 2018). The application of these data is valuable for biotechnological development (Teneva et al. 2025) as well as for predictions of ecosystem responses to climate change (Holzinger et al. 2018, Permann et al. 2022).

6.2. Applied research

The biotechnological exploitation of cyanobacteria and microalgae covers a broad spectrum of applications, from waste-water treatment to the production of high-value compounds used in pharmacology (Arora et al. 2021, Ahmad et al. 2022, Dawiec-Liśniewska et al. 2022). The cyanobacteria and microalgae from cold environments have developed a complex system of adaptation-acclimation mechanisms including the production of biotechnologically valuable compounds, for instance, polysaturated fatty acids (Řezanka et al. 2025) or astaxanthin (Leya 2022). Since warming of the Arctic opens up new opportunities for the exploitation of natural resources, and hence an increase in the human population in the area, the biotechnological application based on local cyanobacterial or microalgal strains could mitigate the impact of human activities (*e.g.* bioremediation or waste-water treatment) and open up new business opportunities for the local people (*e.g.* biotechnological companies).

The ideal strain for putative biotechnological applications in low-temperature conditions should have the following characteristics (Kvíderová et al. 2017, extended):

- good growth/high biomass productivity at low temperatures (0-10°C);
- tolerance to higher temperatures (above 15°C);
- adapted to lower irradiances, but able to photoacclimate to higher irradiance;
- low nutrient demand;
- easy biomass harvest and processing;
- production of high-value compounds;
- local origin.

Bioprospection starts with small-volume (200 µL to 100 mL) screening and testing of several strains (*e.g.* Vasileva et al. 2025). Up-scaling of the cultivars of perspective strains are performed through medium-scale (1 to 20 L) and large-volume (150/250 L) pilot plant cultivation (*see* Fig. 15, Table 5) during which the environmental and biological parameters are continuously monitored to establish the optimum biomass/specific compound production.

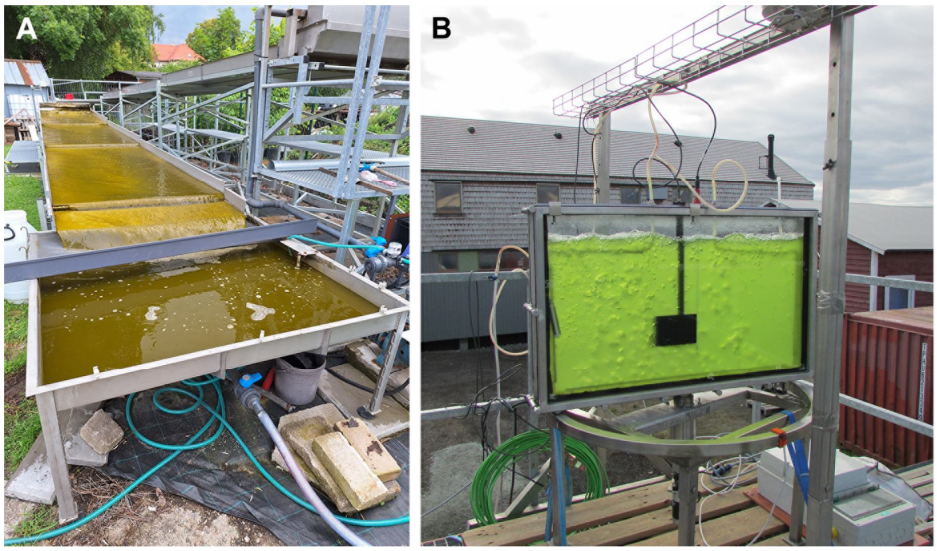

Fig. 15. (**A**) Outdoor large-volume (250 L) thin-layer photobioreactor pilot plant with Antarctic *Tetradesmus obliquus* culture in late stationary phase at Institue of Botany CAS, Třeboň, Czech Republic, June 2025, and (**B**) middle-volume (20 L) rotary panel photobioreactor containing *Neocystis mucosa* CCALA 1141 culture in early exponential phase at Julius Payer House, Czech Arctic Research Infrastructure, University of South Bohemia in České Budějovice, Longyearbyen, Svalbard, June-July 2022.

7. Future development of culture collection

The collection of polar and alpine phototrophic microorganisms is expected to be expanded through the isolation of new strains from a variety of cold habitats. Special attention will be given to poorly studied regions that may contain unique or endemic microalgal species. The exchange of strains with other microalgal collections is also planned, with the aim of the mutual enrichment of strain repositories and the enhancement of the long-term preservation of unique isolates.

Being competitive, our running project (Biomass production from polar microalgae in Central European winter conditions, TAČR) aligns with the EU Bioeconomy Strategy, creating an impact on the sustainable European bioeconomy. In addition, designing and implementing innovative bioprocesses are opening the way to new circular value chains and biomanufacturing.

The isolation of polar and alpine microalgal strains which: (i) can produce valuable metabolites, leading to an in-depth understanding of the mutual interactions between environmental conditions, their biological functions and biotechnological potential, and the cryopreservation of threatened extremophilic genetic diversity; (ii) enhance green bioprospecting for aquatic bioactive substances, focusing on sustainable biotechnological processes and applications for bio-based product development, and testing a new branch of biomanufacturing: low-temperature green biotechnology performed in the winter conditions of Central Europe.

	Stock solution		**Quantity used per L of media**	**Final concentration in medium**
Stock solution 1			10 mL	
MgSO4.7H2O	98.8 g L^{-1}			0.988 g L^{-1}
Trace metals elements	100 mL			*see* below
Stock solution 2			10 mL	
KNO_3	202 g L^{-1}			2.02 g L^{-1}
$CaCl_2.6H_2O$	1.1 g L^{-1}			11 mg L^{-1}
Fe-EDTA	1.8 g L^{-1}			18 mg L^{-1}
Stock solution 3			10 mL	
KH_2PO_4	34 g L^{-1}			0.34 g L^{-1}
Trace metals solution		**Concentration in Stock sol. 1**		
H_3BO_3	3.086 g L^{-1}	0.3086 g L^{-1}		3.086 mg L^{-1}
$MnSO_4.4H_2O$	1.18 g L^{-1}	0.118 g L^{-1}		1.18 mg L^{-1}
$CoSO_4.7H_2O$	1.404 g L^{-1}	0.1404 g L^{-1}		1.404 mg L^{-1}
$CuSO_4.5H_2O$	1.244 g L^{-1}	0.1244 g L^{-1}		1.244 mg L^{-1}
ZnSO4.7H2O	1.43 g L^{-1}	0.143 g L^{-1}		1.43 mg L^{-1}
$(NH_4)_6Mo_7O_{24}.4H_2O$	1.84 g L^{-1}	0.184 g L^{-1}		1.84 mg L^{-1}
NaOH	10 g 250 mL^{-1}	= 40 gL^{-1}	1.5 mL	60 mg L^{-1}

Table 4. The composition of ½ SŠ medium (Zachleder and Šetlík 1982).

Microorganism	**Strain**	**Origin**	**Reference**
Ankistrodesmus antarcticus	CCAP 202/25	Antarctica	Nedbalová et al. (2024)
Bracteacoccus bullatus[*]	CCALA 1120	Sierra Nevada, Spain	Lukavský et al. (2023)
Chodatodesmus australis	CCALA 1131	Antarctica	Řezanka et al. (2021)
Monoraphidium sp.	CCALA 1094	Antarctica	Řezanka et al. (2017)
Neocystis mucosa	CCALA 1141	Svalbard	Řezanka et al. (2025)
Tetradesmus obliquus[*]	Kaštánek/Lukavský	Antarctica	-
Tribonema cf. *minus*[*]	CCALA 1160	Svalbard	Lakatos et al. (2025)

Table 5. Strains from the cold environments used for bioprospection and large-scale pilot plant cultivation. *Note*: [*]Not included in culture collection of experimental strains from cold environments.

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