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St. Petersburg Culture Collection (CALU): Four decades of storage and research with microscopic algae, cyanobacteria and other microorganisms*

by

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With 2 figures and 1 table

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Abstract: The CALU Culture Collection of microscopic algae and other microorganisms has celebrated its 45th year of continuous operation in 2003. Although it was originally designed to supply live material for aims of biotechnology in the former USSR, it gradually focused itself on isolation, preservation, classification, and polyphasic research with microscopic algae and their microbial partners. Today CALU is among the largest (794 culturable strains) specialized public service collections; it represents all central aspects of biodiversity except for the archaea – viruses, bacteria, algae, protists, and fungi. Some parasites of algae are uniquely deposited in this collection. The basis of CALU is freshwater eukaryotic algae (400 strains from 49 genera) and cyanobacteria (287 strains from 29 genera) – a replica of the Eurasian domain of these microorganisms. The rest is non-photosynthetic bacteria (61 strains from 4 genera), aphelid protists (12 strains from 2 genera), fungi (19 strains from 5 genera), bacteriophages, and viruses of eukaryotes (15 strains from 2 groups). Live material is maintained as axenic, bacteria-free, and unialgal cultures either in liquid media or on agar slants. Except for thermophiles, microbial strains are stored at 12°C, continuously

Dedicated to the memory of Prof. Boris V. Gromov

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or periodically illuminated with ca. 7 μE $m^{-2} s^{-1}$ of cool white light, and reinoculated in 2-3 months. Native research with selected strains of CALU deals with cytology, genetics, molecular biology, biochemistry, physiology, ecology, and taxonomy. All cultures are accessible for free distribution by the official transportation routes.

History and aims of the collection

This contribution gives the reader an opportunity to get familiar with the CALU-culture collection consisting mainly of oxygenic photosynthetic microorganisms. The authors' additional intention is to present a general review on their research on some strains of this collection.

CALU is a large culture collection of free access for home and international scientific users; at the same time, live material is thoroughly characterized and experimentally studied in this laboratory. All cultures can be supplied by the official transportation routes.

The depository in question resides at the Institute of Biology, which is a subdivision of the Faculty of Biology, St. Petersburg State University, Russia. Live material is maintained at the Department of Microbiology, and has retained the original acronym CALU (Collection of Algae of Leningrad University, since in 1924-1991, during the epoch of the USSR, St. Petersburg was called Leningrad).

CALU started its 45th year of operation in the beginning of 2003. It was founded formally in 1959, when B.V. Gromov was offered to organize a small laboratory which later grew to the Department of Microbiology. The bulk progress of this research center called the 'Peterhof School of Microbiology', as well as the creation and propagation of CALU, is a merit of the late Professor Gromov (Fig. 1).

CALU was initially established as supplier of live material for usage in national bioindustrial projects. However, immanent logic of science forced it to reorient itself towards purely academical tasks – the furtherance of isolation, preservation, classification, and polyphasic research with microscopic algae, cyanobacteria, as well as other microorganisms that accompany them in natural habitats.

CALU is indexed as No 461 in the fourth edition of the World Directory of Collections of Cultures of Microorganisms (Sugawara et al. 1993). It is an unaccountable misfortune that CALU lacks reference in the list of culture collections at Cyanosite (http://bilbo.bio.purdue.edu/www-cyanosite/collec.html). It is also unreferred to in a partial list of culture collections of cyanobacteria in the second edition of Bergey's Manual of Systematic Bacteriology (Boone & Castenholz 2001). Both these sources refer to the non-existing 'Bispsu' (sic!) collection of Dr. S.A. Karpov from the Department of Invertebrate Zoology at the same institution. Herewith we try to correct this mistake.

CALU is the property of St. Petersburg State University which partially provides it with financial support. Additional financing comes from the Department of Microbiology (Institute of Biology of St. Petersburg State University), and, occasionally, from national and international grants. CALU is presently directed by

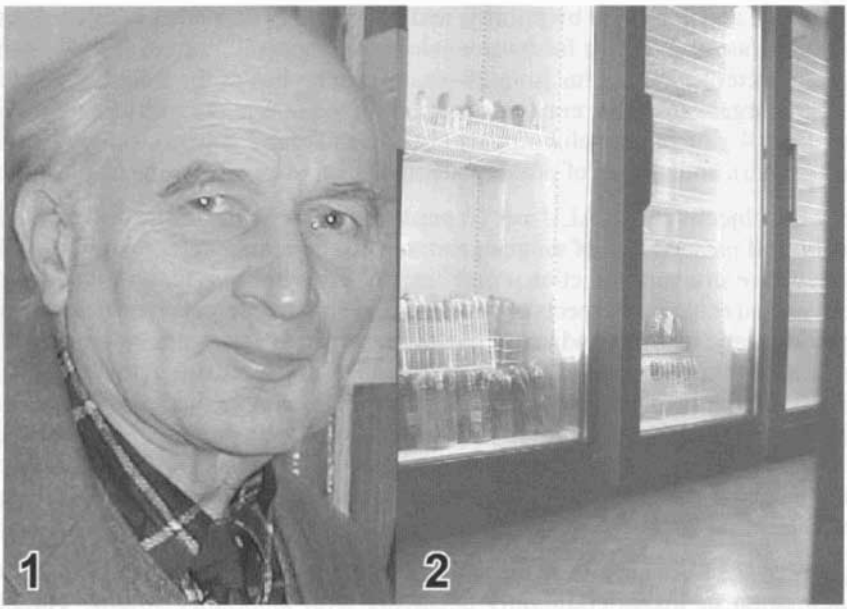

Fig. 1. The initiator of the CALU culture collection, Prof. Boris V. Gromov (18.3.1933- 28.8.2001).
Fig. 2. CALU strains cultivated within a series of thermostated cameras.

Prof. Alexander V. Pinevich, head of the Department of Microbiology (vice-director, Dr. Kira A. Mamkaeva; curator, Dr. Nina N. Titova). Two assistants perform all routine work on local infrastructure, storage, informational background, and strain exchange.

In 2003, CALU numbered 794 original strains isolated personally by the members of the Department of Microbiology from natural samples, or obtained from other collections – principally in Great Britain, Germany, Czech Republic, and the USA (the remaining part was generously supplied by various individual researchers). Geographically, the strains of CALU can be traced back to nearly all parts of the globe.

Original strains issue from the territory of Russia and NIS (New Independent States of former USSR). The list of habitats and niches, which is far from completeness, includes small oligotrophic lakes in Karelia, Baikal Lake, coastal waters of the Baltic Sea, field and forest soils in the Northwest region, tundra and bogs in West Siberia, desert surfaces in Central Asia, mountain slopes in Thien Shan, hydrotherms in Kamchatka Peninsula and Caucasus, rice fields in Azof Sea district, waste treatment installations, outdoor mass cultures, pilot plant bioreactors, etc. A tremendous variety of physical-chemical parameters in these locations and biotopes suggests different adaptational strategies of their inhabitants, as well as extreme diversity of the latter.

CALU deals with all key organisms of the microbial world except for archaea – namely, with viruses, bacteria, algae, protists, and fungi. Certain parasites of algae

were isolated and described by priority, and are uniquely deposited in this collection. The cornerstone of CALU is freshwater eukaryotic algae (400 strains from 49 genera) and cyanobacteria (287 strains from 29 genera) – a replica of the Eurasian domain of these microorganisms. The remaining part comprises non-photosynthetic bacteria (61 strains from 4 genera), aphelid protists (12 strains from 2 genera), fungi (19 strains from 5 genera), and viruses of prokaryotes and eukaryotes (15 strains from 2 groups).

Among the objectives of CALU are: (i) participation in global screening of microbial diversity, and preservation of unique genomes; (ii) obtaining new isolates, especially those with rare structural-function properties; (iii) experimental analysis of cytological, molecular, and ecological aspects of photosynthetic microorganisms and their parasites; (iv) phylogenetic analysis and taxonomy of bacteria and eukaryotic microorganisms; (v) monitoring the outbreaks of water blooms and testing biotoxins in the environment; (vi) study on the involvement of microorganisms in bioremediation; (vii) screening for strains potentially valuable as sources of single cell protein (SCP), pigments, antibiotics, and enzyme inhibitors; (viii) input of primary information to newly created databases; (ix) teaching, foreign consultations, and world-wide dispersal of novel data.

Members of CALU team ensure the safety of live material, and do their best to avoid contamination or losses commonly occurring with laboratory strains. Therefore, prime attention is given to: (a) adequate methods of purification and preservation of cultures; (b) polyphasic comparative analysis with microorganisms of choice; (c) accurate and detailed documentation of strains; (d) effective exchange of live material with allied collections and individual researchers.

The strategy of CALU is further expansion in the diversity of microscopic algae and cyanobacteria that dwell within cultivated and wild territories of Russia and NIS; purposeful screening for rare and novel forms of free-living photosynthetic microorganisms and their parasites; detailed investigation of microbial genomes and phenotypes based on modern methods in cytology, biochemistry, and molecular biology.

Cultivation methods and strains

Strains from CALU (axenic, bacteria-free, or unialgal) are maintained in a living state either on agar slants or in liquid nutritional media. No cryogenic or other type of conservation has been ever attempted.

In most cases, the background of nutritional media containing essential trace elements is modified mineral 'C' medium designed by Kratz & Myers (1955), or medium 'BG-11' (Blue-Green Medium) introduced by Rippka et al. (see e.g. Rippka & Herdman 1992/93). In some cases 1/5 or more dilutions are practiced. If necessary, organic supplements (glucose, peptone, yeast extract or yeast hydrolysate, casamino acids, etc.) are added to axenic cultures.

The stock of strains is kept in duplicate at 12°C (except for thermophiles), with a continuous irradiation supplied by cool-white luminescent tubes (light intensity of ca. 7 $\mu E\ m^{-2}\ s^{-1}$), and is reinoculated every 2-3 months (Fig. 2).

For the isolation of cyanobacteria possessing rare structural-functional properties, water samples are filtered through a loose layer of cotton or gauze with 0.5 mm pore diameter, supplied with 20% (v/v) of modified BG-11 medium (iron sulfate in place of iron nitrate; 1/5 standard content of sodium nitrate), and kept for two months at 22°C in dim light. An addition of cycloheximide ensures the removal of eukaryotic contaminants, and resultant axenization is achieved by a series of dispersals on fresh agar. Growth of these strains, as well as that of prochlorophytes, is supported by modified BG-11 medium.

Beside the above nutritional receipts, variant cultivation of eukaryotic algae and cyanobacteria is performed in **B**old's **B**asal **M**edium (BBM, see Brown & Bold 1964), as well as in standard organic Becton Dickinson medium (BD BBL™), and ESFl-medium (basal medium with beef extract / '**E**rddekokt + **S**alze + **Fl**eisch') see http://www.epsag.uni-goettingen.de/html/culturemedia.html). The growth of chrysomonads is supported with **M**odified **W**oods Hole **C**ollection medium (MWC) or **S**igma Cereal Leaf-**P**rescott **L**iquid (SPL) media recommended by the **C**ulture **C**ollection of **A**lgae & **P**rotozoa (CCAP; see http://www.ife.ac.uk/ccap/MediaIndex.html).

Wild types and mutants of *Chlamydomonas reinhardtii* Dangeard are maintained on solid **T**ris-**A**cetate-**P**hosphate (TAP) medium of Gorman & Levine (1965) at 18 °C, with reinoculations every three months.

The protistan endotrophic parasites of algae (*Aphelidium* sp. and *Amoeboaphelidium* sp.) are isolated from sensitive cultures of *Chlorococcum minutum* Starr and *Scenedesmus obliquus* (Turpin) Kützing. Microscopic fungi that infect algae and ectotrophically feed on them (*Chytridium lagenula* A. Braun sensu Scherffel, *Entophlyctis* sp., *Mesochytrium* sp., *Rhizophydium* sp., and *Polyphagus* sp.) are obtained from sensitive cultures of *Chlorococcum minutum* and *Tribonema gayanum* Pascher. The above hosts are routinely used for nutritional support and multiplication of their respective parasites.

Quality control includes light microscopy; in the case of initial impurities or indoor contaminations, microorganism-specific methods of axenization are used.

The strains of CALU are grouped in accordance with their taxonomic affiliations, alphabetically listed, and numbered in Table 1. The choice of major assemblages and their consensus names for eukaryotes was made after Margulis et al. (1990), whereas systems by Boone & Castenholz (2001) and Mayo & Pringle (1998) were accepted for bacteria and viruses, respectively.

Past and Current Research by Staff Scientists

The scope of scientific interests of CALU team is demonstrated below by brief summaries of the research on the respective taxonomic groups. The work (local or in the collaboration with native and foreign partners) has been done exclusively or mainly with CALU strains.

CYANOBACTERIA INCLUDING PROCHLOROPHYTES. At the dawn of CALU and henceforth, the preference was on semi-neglected aspects of cyanobacterial structure – budding

Table 1. General inventory of microorganisms cultured in CALU.

Major assemblage	Order or family	Genus or group	Amount of strains
BACTERIA			
Cyanobacteria	Chroococcales	*Cyanothece*	5
		Gloeocapsa	1
		Gloeothece	1
		Microcystis	4
		Synechococcus	23
		Synechocystis	19
	Pleurocapsales	*Chroococcidiopsis*	4
		Pleurocapsa	1
		Xenococcus	1
	Oscillatoriales	*Leptolyngbya*	13
		Lyngbya	3
		Microcoleus	1
		Oscillatoria	67
		Prochlorothrix	2
		Pseudanabaena	19
		Spirulina	3
		Symploca	3
	Nostocales	*Anabaena*	34
		Aphanizomenon	1
		Calothrix	13
		Cylindrospermum	2
		Microchaete	2
		Nodularia	1
		Nostoc	38
		Scytonema	5
		Tolypothrix	1
	Stigonematales	*Chlorogloeopsis*	17
		Fischerella	1
		Mastigocladopsis	2
Proteobacteria	Rhodospirillales	*Acetobacter*	15
		Gluconobacter	2
	Pseudomonadales	*Pseudomonas*	32
	'Siderocapsaceae'*	*Siderocapsa*	12
Total			348
ALGAE			
Bacillariophyceae	Bacillariales	*Nitzschia*	1
Chlorophyceae	Chlamydomonadales	*Chlamydomonas*	13
		Haematococcus	3
	'Chlorococcales'	*Ankistrodesmus*	16
		Bracteacoccus	6
		Chodatella	1
		Chlorococcum	30
		Coelastrum	2
		Crucigenia	1
		Dictyococcus	4
		Dictyosphaerium	7
		Ettlia	1
		Kirchneriella	3
		Monoraphidium	1
		Neochloris	8
		Oocystis	4
		Pediastrum	1

Major assemblage	Order or family	Genus or group	Amount of strains
		Planktosphaeria	1
		Pseudodictyococcus	1
		Scenedesmus	85
		Scotiella	1
		Spongiochloris	5
		Tetraedron	3
	Dunaliellales	*Dunaliella*	16
	Volvocales	*Gonium*	1
Chrysophyceae	Chromulinales	*Ochromonas*	12
		Paraphysomonas	1
Conjugatophyceae	Desmidiales	*Cosmarium*	1
		Staurastrum	1
Cryptophyceae	Cryptomonadales	*Campylomonas*	1
Euglenophyceae	Euglenales	*Euglena*	4
Klebsormidiophyceae	Klebsormidiales	*Klebsormidium*	6
Rhodophyceae	Cyanidiales	*Cyanidium*	2
	Porphyridiales	*Porphyridium*	1
Synurophyceae	Synurales	*Synura*	2
Trebouxiophyceae	Trebouxiales	*Chlorella*	119
		Coccomyxa	2
		Koliella	1
		Myrmecia	4
		Stichococcus	19
		Parietochloris	2
Ulvophyceae	Trentepohliales	*Trentepohlia*	1
Xanthophyceae	Botrydiales	*Botrydium*	1
	Vaucheriales	*Vaucheria*	1
	Mischococcales	*Ophiocytium*	1
		Pleurochloris	1
		Pleurogaster	1
	Tribonematales	*Tribonema*	1
Total			400
APHELID PROTISTS			
Aphelidea	Aphelidida	*Amoeboaphelidium*	10
		Aphelidium	2
Total			12
FUNGI			
Chytridiomycetes	Chytridiales	*Chytridium*	1
		Entophlyctis	1
		Mesochytrium	6
		Polyphagus	1
		Rhizophydium	10
Total			19
VIRUSES			
DsDNA	Myoviridae	LPP-*group cyanophage*	3
	Phycodnaviridae	PBCV *phycodnavirus*	12
Total			15
Total amount of strains			794

* Inverted commas are used in the case of provisional taxa.

in Chroococcales, baeocytes in Pleurocapsales, capsules and sheaths in Oscillatoriales, heterocysts and akinetes in Nostocales, true branching in Stigonematales, etc. This interest of cyanobacterial ultrastructure was not limited to the scrutiny of TEM or freeze-fracture images; pioneering results on cellular alterations under environmental stress have been obtained as well (Voloshko et al. 2001). With respect to the diversity of cyanobacteria, special attention is paid to those objects which demonstrate rare structure-function features. These include *Pseudanabaena* sp. with oligomeric trichomes, *Synechococcus* sp. containing 'purple' phycobilisomes permanently adapted to phycoerythrin-rich state, and *Pleurocapsa* sp. characterized by the release of water-soluble UV-absorbing pigment (Pinevich et al. 1997a). Prochlorophytes, which contrast with closely related cyanobacteria by the presence of accessory chlorophyll *b*, represent an arcane aspect of the diversity of oxygenic photosynthetic bacteria. Their cell structure, molecular details, and metabolism, as well as their natural dispersal and population density, remain obscure. Taking into account that general attention is now being paid to *Prochlorococcus marinus* Chisholm et al. which is among the leading contributors to primary productivity in oceans, similar research with freshwater strains of *Prochlorothrix* spp. is underway (Pinevich et al. 1999). At the peak of general enthusiasm with utilitarian usage of SCP between 1960-1990 and thereafter, growth conditions and optimum parameters of productivity in microscopic algae and cyanobacteria were questioned. Besides, successful screening of strains perspective as over-producers of pigments, e.g. phycoerythrin, was performed. During the search of secondary metabolites of cyanobacterial origin, novel antibiotics from the class of PSII-affecting cyanobacterins, as well as effective inhibitors of human proteases and hepatotoxic microcystin-type polypeptides, were discovered (Gromov et al. 1991; Sivonen et al. 1992).

Chlorophyceae. Members of the CALU team are at the top of the list of researchers who in the early 1970s analysed cytology of eukaryotic photosynthetic microorganisms. Of significance is a series of studies on the architecture and morphometry in vegetative cells, as well as on general anatomy and flagellar structure in zoospores of Chlorophyceae and Trebouxiophyceae. In this connection, among the priorities is the choosing of morphological criteria to delimit individual taxa. For example, the fine organization of the flagellar apparatus helps to identify the strains with undetermined phylogenetic status, and elucidate their positions within the taxonomic groups (Gavrilova & Gromov 1991).

Behavioural responses in *Chlamydomonas*. The chlorophycean alga *Chlamydomonas reinhardtii* is increasingly employed in genetic studies on regulatory responses of microorganisms; besides the light-dependent changes in cell motility, flagellated *C. reinhardtii* demonstrate specific attraction towards sugars or ammonium ions. In search for novel facets of this chemosensory system, mutants were selected by means of UV-, NG-, and insertion mutagenesis (Ermilova et al. 1996). Basing on experimental results, a new mechanism was suggested shedding light on the interrelationship of chemo- and photosensory pathways within the unicellular organism. Chemotaxis-based behaviour was also traced in analysis of algal sexuality – unlike vegetative cells, mature gametes lack attraction to ammonium; according to the present data, the regulatory pathway of chemotaxis and those that govern mating competence have elements in common.

Chrysophytes (Chrysophyceae and Synurophyceae). These algae are promising objects for the investigation of evolutionary events in heterokonts, whereas scaled chrysophytes are of importance in the light of global silica biomineralization. Unfortunately, only few strains are maintained in collections, and the description of species is incomplete. Metastable classification of chrysophytes is based on the accentuated cytological analysis of the genera *Ochromonas* and *Synura*. Since corresponding strains are available at CALU, they were specially investigated with respect to stomatocyst formation, cytoskeleton organization, and polymorphism of scale structure under a broad range of environmental conditions (Voloshko & Gavrilova 2001).

Xanthophyceae. The siphonous alga *Vaucheria* is a privileged object when the involvement of the cytoskeleton in polar growth is analysed; an intriguing aspect is cell morphogenesis, ultrastructure, and inhibitor responses. Under separate investigation is internal orientation of organelles, primarily the nucleus, mediated by cytoskeleton and influenced by the direction of the gravity vector (Gavrilova et al. 1997).

Pathology of algae. In the early 1960s Prof. B.V. Gromov came across the dramatic phenomenon of viruses-, bacteria-, protists-, and fungi-mediated lysis of microscopic algae and cyanobacteria (which at that time were also considered as algae) cultivated in pilot plant bioreactors. This made him understand the role of parasites of algae, and for a long period of time the CALU team concentrated on the problems of microbial interrelationships. Sporadic developments of parasites of algae cause extensive death to their respective hosts, this is crucial for the circulation of organic matter in aquatic ecosystems. From fundamental point of view, the study of these microorganisms sheds light on their structure, physiology, behaviour, and taxonomy. The CALU research group not only merits the credit as pioneers in algal pathology, but it also retains its productivity in this peculiar field of research (cross-road of phycology, bacteriology, protistology, mycology, and virology). Strategic screening of biodiversity, structure, distribution, and ecology of algal parasites yielded plenty of original isolates, and new taxa were legitimately described, in particular, the protistan class Aphelidea and the bacterial genus *Vampirovibrio* (Gromov & Mamkaeva 1980; Gromov 2002) – to say nothing about the previously unknown cyanophages and viruses of green algae. The unique subcollection of endotrophic parasites of algae embraces 12 strains of protists attributed to the genera *Aphelidium* and *Amoeboaphelidium* from the class Aphelidea. Correspondingly, algal ectoparasites are represented by 19 strains of chytrid fungi from the genera *Chytridium*, *Entophlyctis*, *Mesochytrium*, *Polyphagus*, and *Rhizophydium*. The strains of *Mesochytrium* have a narrow host range and demonstrate a unique mode of development of sporangia, whereas those of *Rhizophydium* are characterized by a broad spectrum of hosts, the ability of saprophytic growth, and the possession of polymorphous sporangia (Gromov et al. 2000). Close to the end of the 1990s, the traditionally used morphological-physiological approach in phycopathology was firstly complemented by methods of molecular biology. In particular, the polymorphism of karyotypes in *Amoeboaphelidium* sp. was characterized by PFGE (Pinevich et al. 1997b).

ALGAL SYMBIOSES. In the 1970s-1980s, the CALU team came across the symbiotic systems with participation of microorganisms (among which parasitism is actually the extreme). In the initial phase of this research, preference was given to stable symbiotic associations – like intranuclear symbiosis of *Paramecium* with heterotrophic bacteria from the genus *Holospora* or intracytoplasmic symbiosis events with other non-photosynthetic protists as hosts. Viruses of eukaryotic algae were initially represented in CALU by a single strain that infected zoospores in *Chlorococcum minutum* (Gromov & Mamkaeva 1981); however, it has been subsequently lost. Another virus participates in the sophisticated triple symbiotic system *Paramecium bursaria-Chlorella* sp.-virus PBCV (Kvitko & Gromov 1984). The findings of this globally distributed agent are documented in Karelia, Armenia, Far East, and North America. Physiological, biochemical, and molecular analyses of PBCV, as well as these of its host ('zoochlorella'), gives further insight into biodiversity and distribution of *Chlorella*, transducing ability of the virus, and factors regulating the establishment and propagation of the entire symbiotic association. Although viruses of cyanobacteria (cyanophages) have been once overestimated as possible remedy against hazardous water blooms, the significance of their basic investigation is beyond doubt. DNA-containing cyanophages can infect both unicellular and filamentous cyanobacteria, and initial information on CALU isolates can be gained from a review by Gromov (1983). At present, cyanophages belonging to LPP-group (from ***Lyngbya-Plectonema-Phormidium***) or L series (the latter is an abbreviation for 'Leningrad') became an effective tool of genetic and molecular biology research with cyanobacteria and are, in particular, employed in the study of heterocyst differentiation and pattern formation (Khudyakov & Wolk 1996).

WATER BLOOMS. A subcollection of cyanobacteria (48 strains) has been obtained from continuous or sporadic blooms of water bodies in Northwest Russia (Karelian lakes, Ladoga Lake, Finland Bay, coastal waters of Baltic Sea, etc.). The bioactivity of these isolates was tested by means of viable and chemical systems; the degree of toxicity proved variant depending on strain and its growth conditions (Gromov et al. 1996).

BIOREMEDIATION. The general problem of environmental pollution can be solved in part by the application of biofilters with immobilized cells of *Stichococcus*. This microscopic trebouxiophycean alga not only withstands high inputs of simple and condensed aromatic compounds, but also effectively decomposes them into water-soluble nontoxic products, which enables the removal of spilled motor oil and other xenobiotics from the environment (Safonova et al. 1998). CALU includes 19 strains of *Stichococcus* sp. isolated from oil-polluted soil and water bodies in the environs of St. Petersburg, or obtained from Prof. W. Reisser (Leipzig University). A subcollection of so-called 'ecological strains' including cyanobacteria, microscopic algae, and microscopic fungi is also incorporated in CALU; all of them are tolerant to phenolic compounds and can be used as components of oil-absorbing biofilters.

To summarize, CALU is an unlimited field of research with photosynthetic microorganisms and their symbionts. Both the genome-based and phenotype-based approaches to this part of global biodiversity are used; polyphasic analysis is preferred, that combines morphology, physiology-biochemistry, genetics, ecology, and taxonomy.

CALU is open to home and international collaboration. Its management is planning to broaden the list of strains, and to improve the maintenance and informational background of the latter.

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