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RESEARCH PAPERS

Comparative Analysis of Phenolic Compounds in Cyanobacteria and Microalgae from Different Evolutionary Lineages

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Abstract—The study presents the results of an analysis of phenolic compounds in nine strains representing Cyanobacteria, Heterokontophyta, Chlorophyta, and Charophyta, using tandem mass spectrometry. Phenolic compounds were detected in all analyzed strains; however, their qualitative composition varied significantly. In total, 23 phenolic compounds were detected, including 18 phenolic acids, 3 flavonols, 1 hydroxycoumarin, and 1 other polyphenol. Several compounds were confirmed with authentic standards, while the remainder should be considered tentative identifications based on MS/MS spectra and comparison with literature data. For the first time, a compound of the hydroxycumarins class was detected in *Edaphochlorella mirabilis* (Chlorophyta). Among the studied strains, the highest yield of extractable substances and total content of phenolic compounds was observed in *Edaphochlorella mirabilis* (Chlorophyta). The greatest diversity of compounds was recorded in the cyanobacteria *Roholtiella mixta* (9 compounds) and *Anagnostidinema pseudacutissimum* (6 compounds), as well as in the green algae *Edaphochlorella mirabilis* and *Coelastrella terrestris* (6 compounds each). Only a single compound was detected in *Klebsormidium flaccidum* (Charophyta) and *Vischeria magna* (Heterokontophyta, Eustigmatophyceae). The taxonomic positions of all isolated strains were determined using molecular identification methods.

Keywords: cyanobacteria, HPLC-MS/MS, microalgae, phenolic compounds

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INTRODUCTION

Cyanobacteria and microalgae are subjects of increasing interest in various fields, including the food and pharmaceutical industries. The pursuit of improved health and longevity, along with the reduction of land required for crop cultivation, makes them promising sources of biologically active compounds. Cyanobacteria are the most structurally complex photosynthetic prokaryotes, characterized by unique physiology, morphology, and photosynthetic apparatus. Like microalgae, they serve as sources of proteins, fatty acids, polysaccharides, triterpenoids, and other valuable metabolites with applications in both pharmaceuticals and the food industry [1, 2].

Among secondary metabolites, phenolic compounds are of particular importance, comprising over 8000 components across more than ten classes [3]. While the phenolic profiles of macroalgae are relatively well studied, cyanobacteria and microalgae have long remained largely overlooked. It was previously believed that the synthesis of certain compounds, particularly flavonoids, was impossible in these organisms. Low concentrations of these metabolites, lack of genetic evidence, and limitations in analytical methods also contributed to this view. Nevertheless, by the second half of the 20th century, flavonoids were detected in charophyte algae of the genus *Nitella* C. Agardh, which are closely related to higher plants [4]. A major breakthrough occurred in 2014, when key intermediate and final products of flavonoid biosynthesis were identified in algae from different evolutionary lineages (Cyanobacteria, Rhodophyta, Chlorophyta, Haptophyta, Heterokontophyta) [5, 6]. Since then, the number of studies on phenolic compounds in cyanobacteria and microalgae has increased significantly, with potential biotechnological applications being actively explored [7].

The aim of the present study is to expand knowledge on the synthesis, accumulation, and diversity of phenolic compounds in representatives of Cyanobacteria, Heterokontophyta, Chlorophyta, and Charophyta isolated from various ecotopes of the Russian Far East. Despite growing interest, their potential as

Table 1. Primer sequence and supporting information

Region	Primer name	Primer sequence	Average length, bp	References
*rbc*L	DPrbcL1 DPrbcL7	5'-AAGGAGGAADHHATGTCT-3' 5'-AAASHDCCTTGTGTWAGTYTC-3'	1439	[12]
ITS rDNA	Bd18SF1 ITS4R	5'-TTTGTACACACCGCCCGTCGC-3' 5'-TCCTCCGCTTATTGATATGC-3'	835	[13, 14]
16S rRNA	27F 340	5'-AGAGTTTGATCMTGGCTCAG-3' 5'-CTCTGTGTGCCTAGGTATCC-3'	1530	[15, 16]

producers of biologically active compounds remains only partially realized. The results obtained will help refine our understanding of the phenolic composition of these microorganisms.

MATERIALS AND METHODS

Plant material. The study used strains of cyanobacteria and microalgae from various taxonomic groups obtained from the culture collection of Laboratory of Botany of Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences (Table S1).

Microalgae cultivation and sample preparation. Cultures were grown using a batch cultivation method on mineral media prepared with distilled water. In the first stage, algae were adapted to intensive cultivation conditions. Cultures were grown at low light intensity (16 μmol quanta/ m^2 s) in 250 mL glass flasks without stirring under white fluorescent lamps (C∈PIL1LF36W/54-765). As the cultures grew, they were transferred to intensive cultivation: suspension volume was increased to 1 L, light intensity raised to 80 μmol photons/m^2 s, and mixing provided by air bubbling (0.5 L air per 1 L culture per min).

Diatom algae were cultivated at a constant temperature of 20 ± 1°C under continuous illumination in 1 L glass flasks on a medium prepared according to Gerin et al. [8]. Cyanobacteria and green microalgae were cultivated intensively in 1 L glass flasks at 28 ± 1°C under continuous illumination.

For dense cyanobacterial cultures, a BG11-based medium [9] was used with the following composition (g/L): $NaNO_3$—1.5; K_2HPO_4—0.05; $MgSO_4{\cdot}7H_2O$—0.075; $CaCl_2$—0.05; $NaHCO_3$—0.02; Na_2EDTA—10×10^{-3}; $FeC_6H_5O_7$—6×10^{-3}; $CuSO_4{\cdot}5H_2O$—0.08×10^{-3}; $ZnSO_4{\cdot}7H_2O$—0.22×10^{-3}; $CoCl_2{\cdot}6H_2O$—0.05×10^{-3}; $MnCl_2{\cdot}4H_2O$—1.8×10^{-3}; $NaMoO_4{\cdot}H_2O$—0.4×10^{-3}.

For green microalgae, a modified Gromov medium No. 6 [10, 11] was used with the following composition (g/L): KNO_3—1; K_2HPO_4—0.2; $MgSO_4{\cdot}7H_2O$—0.2; $CaCl_2$—0.05; $NaHCO_3$—0.2; $ZnSO_4{\cdot}7H_2O$—0.22×10^{-3}; $MnSO_4$—1.81×10^{-3}; $CuSO_4{\cdot}5H_2O$—0.079×10^{-3}; $NaBO_3{\cdot}4H_2O$—2.63×10^{-3}; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$—$1 \times 10^{-3}$; $FeSO_4{\cdot}7H_2O$—9.3×10^{-3}; $CaCl_2$—1.2×10^{-3}; $Co(NO_3)_2{\cdot}4H_2O$—0.02×10^{-3}; Na_2EDTA—10×10^{-3}.

Once cultures reached the stationary growth phase, cells were separated from the medium by centrifugation at 3000 rpm (1600 *g*), the supernatant was discarded, and the pellet was washed with distilled water. The resulting wet biomass was dried in a warm air stream at 38°C.

Molecular genetic methods. For DNA analysis, cultures in the exponential growth phase were collected and concentrated by centrifugation. Total genomic DNA was extracted according to Abdullin et al. [11]. PCR amplification of the plastid gene *rbc*L (Heterokontophyta, Bacillariophyceae), the internal transcribed spacer (ITS) of rDNA (Heterokontophyta, Eustigmatophyceae; Chlorophyta; Charophyta), and the 16S rRNA gene (Cyanobacteria) was performed using primers listed in Table 1 and the Encyclo Plus PCR Kit (Evrogen, Moscow, Russia).

PCR products were purified using ExoSAP-IT PCR Product Cleanup Reagent (Affymetrix, Santa Clara, CA, USA) and sequenced bidirectionally on an ABI 3500 genetic analyzer (Applied Biosystems, Foster City, CA, USA) with the BigDye Terminator v3.1 kit (Life Technologies, Austin, TX, USA) using the same primers as for PCR. Sequences were assembled using the Staden Package v. 1.4 [17] and deposited in GenBank (NCBI, Bethesda, MD, USA) (Table S1).

Following morphological examination, the taxonomic positions of the strains were refined by comparing their sequences with NCBI database entries using the BLAST service (https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed July 10, 2025). For each strain, the closest sequence was determined considering both percent identity and phylogenetic position among related taxa. Comparisons were performed using the "Distance tree of results" option (Fast Minimum Evolution and Neighbor Joining methods) in BLAST (Figs. S2–S10). Identification at the species level was considered reliable if sequences showed 98–100% identity with NCBI entries and a clear placement in the phylogenetic tree.

Phenolic compound extraction. Extraction was performed using 70% methanol (Himmed, Moscow, Russia). Lyophilized biomass was weighed precisely

and mixed with the solvent at a 1 : 10 ratio. Samples were incubated in an ultrasonic bath at 50°C for 1 h (Sapphire, Moscow, Russia). Tubes were then centrifuged for 10 min at 10000 rpm, the supernatant was collected and replaced with fresh solvent. Extraction was repeated three times. Combined extracts were concentrated using a rotary evaporator (Ika RV8, Staufen, Germany) and lyophilized (Martin Christ Alpha 1-2LD, Osterode, Germany). For further analyses, dry extracts were dissolved in 1 mL of 70% methanol.

Yield of extractable substances. Extraction yield (%) was calculated by comparing the dry extract mass with the initial biomass using the formula from Oroian et al. [18]:

$$\text{Yield}\,(\%) = \text{Wex/Wrm} \times 100\,(\%),$$

where Wex is the weight of algae dry extract (g) and Wrm (g) is the weight of the dried sample.

Total phenolic content analysis. Total phenolic content was determined using a modified Singleton and Rossi method [19]. An aliquot of extract (1 mL) was mixed with 1 mL of Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) and 5 mL of distilled water, incubated in the dark at room temperature for 5 min. Then 1 mL of freshly prepared 20% sodium bicarbonate solution (filtered through a 0.45 μm filter) was added, the volume adjusted to 10 mL with purified water, and the mixture incubated in the dark for 60 min at room temperature. Absorbance was measured at 765 nm (UV-2501 PC, Shimadzu, Kyoto, Japan). Calibration was performed using gallic acid (Sigma-Aldrich, St. Louis, MO, USA) standards. Results were expressed as mg gallic acid equivalents per 100 g of dry biomass. For each strain, biomass was obtained from a single cultivation batch due to limited availability of material. To ensure reproducibility, all analyses were performed in three technical replicates, and results are expressed as mean ± SD.

HPLC-MS/MS analysis. UV-Vis spectra and MS_2 fragmentation data were obtained based on the Instrumental Centre of Biotechnology and Gene Engineering of Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS, using an 1260 Infinity analytical HPLC system (Agilent Technologies, Santa Clara, CA, USA), equipped with a G1315D photodiode array detector, G1311C quaternary pump, G1316A column oven, and G1329B auto sampler. The HPLC system was interfaced with a low-resolution ion trap mass spectrometer (Bruker HCT ultra PTM Discovery System, Bruker Daltonic GmbH, Bremen, Germany) equipped with electrospray ionization (ESI) source. The MS analyses were carried out with negative ion detection. The following instrument settings were used: the range of m/z detection was 100–1000, the drying gas (N_2) flow rate was 10.0 L/min, the nebulizer gas (N_2) pressure was 241 kPa, the ion source potential was 4.0 kV, the drying gas temperature was 365°C. Tandem mass spectra were acquired in Auto-MS2 mode (smart fragmentation) using a ramping of the collision energy. The fragmentation amplitude was set to 1 V. MS data were collected using the Bruker Daltonics Compass 1.3 esquire control software (v. 6.2.581.3) and processed the Bruker Daltonics Compass 1.3 Data Analysis software (v.4.0.234.0).

All solvents were of HPLC grade. An analytical reverse phase column (Zorbax C18, 150 mm, 2.1 mm i.d., 3.5 μm part size, Agilent Technologies, Santa Clara, CA, United States) for separation was applied. Separation was carried out at following conditions: the column temperature was 40°C, the mobile phase consisted of 0.1% aqueous formic acid (A) and acetonitrile (B). The following elution gradient with a flow rate of 0.2 mL/min was used: 0 min 0% B; 35 min 40% B; 40 min 50% B; 50 min 100% B; and then eluent B for 60 min.

Statistical analysis. Data were processed using STATISTICA 6.0 (StatSoft Inc., Tulsa, OK, USA). Results are presented as mean ± standard deviation ($n = 3$). Comparisons were performed by ANOVA, with differences considered significant at $P \leq 0.05$.

RESULTS

Molecular Identification

The taxonomic position of all isolated strains was determined using molecular identification methods. Sequences of *rbc*L (Heterokontophyta, Bacillariophyceae), ITS rDNA (Heterokontophyta, Eustigmatophyceae, Chlorophyta, Charophyta), and 16S rRNA (Cyanobacteria) enabled unambiguous identification of the isolates, which showed 98–100% similarity to sequences in the NCBI database (Table 2 and Table S1).

Extraction Yield and Total Phenolic Content Analysis

The above-mentioned microorganism's strains were analyzed for extraction yield and total phenolic content. The obtained values correspond to the literature data and are close to their average levels (Table 2).

HPLC–MS Analysis of Phenolic Compounds

Identification of phenolic compounds was based on retention times, UV–Vis spectra and MS/MS fragmentation patterns using available databases and literature sources. In total, 23 phenolic compounds were observed in the studied strains, comprising 18 phenolic acids, 3 flavonols, 1 hydroxycoumarin, and 1 other polyphenol. Four metabolites (p-coumaric acid, rutin, gallic acid monohydrate, and caffeic acid) were confirmed with authentic standards (MSI Level 1). Several others, including caffeic acid hexoside, phenylacetic acid conjugates, and galloyl derivatives, were assigned with high probability based on MS/MS fragmentation and literature references (MSI Level 2).

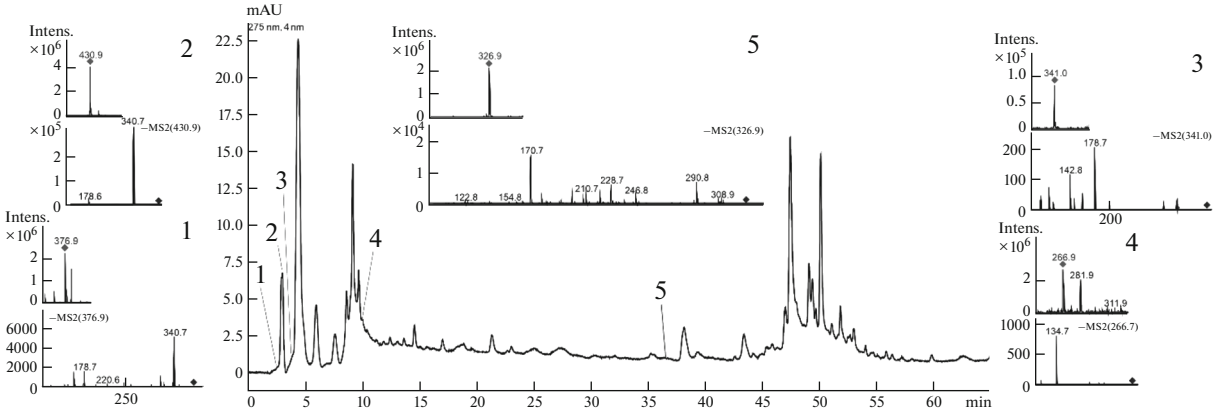

Fig. 1. Representative HPLC–MS/MS chromatogram of the *C. terrestris* strain. (1) Caffeic acid hexoside; (2) Caffeic acid derivative; (3) Caffeoyl glucose; (4) Phenylacetic acid pentoside; (5) Kaempferol-3,4',7-trimethyl ether, or 3-hydroxy-4',5,7-trimethoxyflavone.

The remaining compounds, such as benzoic acid derivatives, sinapic acid-like metabolites, methoxylated flavonoids, and dicoumarol-like structures, were classified as tentative identifications (MSI Level 3) (Table 3, Fig. S1). Representative chromatograms obtained by HPLC–MS/MS are shown in Fig. 1. The selected example corresponds to the *C. terrestris* strain with most abounded identified compounds. The chromatogram is accompanied by MS/MS spectra. These spectra illustrate the diagnostic fragmentation patterns used for metabolite identification.

DISCUSSION

Microalgae and cyanobacteria represent a large and diverse group of microorganisms with pronounced taxonomic and metabolic heterogeneity, encompassing both obligate phototrophs and organisms with a mixotrophic type of nutrition. Mixotrophy has been demonstrated, for example, in certain representatives of Chlorophyta (*Coelastrella*) as well as in the cyanobacterium *N. punctiforme* [32, 33]. Despite their considerable phylogenetic distance from higher plants, these organisms synthesize a broad spectrum of phenolic compounds, which play key roles in protection against oxidative stress, UV radiation, and adverse environmental conditions.

Published data indicate that the phenolic content of cyanobacterial and microalgal strains, as determined by the Folin–Ciocalteu method, varies over a very wide range—from near-zero values to relatively high concen-

Table 2. Total phenolic content and yield of extraction of different cyanobacteria/microalgae strains

Cyanobacterial/Microalgal Taxon	Phylum	Strain	Yield of extraction, %	Total phenolic content (mg/100 g GAE)
Anagnostidinema pseudacutissimum (Geitl.) Strunecký, Bohunická, J.R. Johansen & J. Komárek	Cyanobacteria	VCA-210	11.59	483.70 ± 26.12
Nostoc punctiforme Hariot	Cyanobacteria	VCA-247	13.46	224.98 ± 13.38
Roholtiella mixta Abdullin & Gontcharov	Cyanobacteria	VCA-31	11.88	392.31 ± 10.2
Vischeria magna (J.B.Petersen) Kryvenda, Rybalka, Wolf & Friedl	Heterokontophyta Eustigmatophyceae	VCA-248	8.13	183.94 ± 3.67
Sellaphora atomoides (Grun.) Wetzel & Van de Vijver	Heterokontophyta Bacillariophyceae	VCA-249	17.83	523.48 ± 21.98
Hantzschia calcifuga E.Reichardt & Lange-Bertalot	Heterokontophyta Bacillariophyceae	VCA-250	18.25	426.41 ± 17.06
Coelastrella terrestris (Reisigl) Hegewald & N. Hanagata	Chlorophyta	VCA-251	10.59	217.87 ± 12.96
Edaphochlorella mirabilis (V.M. Andreeva) Darienko & Pröschold	Chlorophyta	VCA-215	22.93	590.30 ± 39.81
Klebsormidium flaccidum (Kütz.) P.C. Silva, Mattox & W.H. Blackwell	Charophyta	VCA-253	9.08	560.18 ± 48.15

Table 3. Characterization of phenolic compounds in cyanobacteria and microalgae by using HPLC-MS/MS

Proposed compounds	Observed, *m/z*	MS/MS product ions	The presence in the studied Cyanobacterial/ Microalgae strain	References	Confidence level (MSI)*
Phenolic acids					
Benzoic acid derivative	187	125	*A. pseudacutissimum* *N. punctiforme* *R. mixta* *H. calcifuga* *E. mirabilis*	[20]	3
Hydroxybenzoic acid, such as Salicylic Acid	137	119	*K. flaccidum*	[20]	3
Syringic acid-O-glucuronide	373	197, 175	*S. atomoides*	[21]	3
Galloyl deoxyhexoside	315	297, 256, 229, 169	*R. mixta* *S. atomoides*	[22]	2
Gallic acid monohydrate	187	169, 125	*H. calcifuga*	[23]	1
Gallic acid O-(6-galloylglucoside)/Di-galloyl-hexoside	483	331, 313, 271, 169, 150, 125	*N. punctiforme*	[24]	2
Phenylacetic acid	135	117	*N. punctiforme*	Authors' elaboration	2
Phenylacetic acid pentoside	267	135	*A. pseudacutissimum* *R. mixta* *C. terrestris*	Authors' elaboration	2
Phenylacetic acid hexoside	297	135	*R. mixta*	Authors' elaboration	2
Sinapic acid-like (plus water)	241	223, 197, 179, 161	*H. calcifuga*	[20]	3
Sinapic acid-like (plus two water)	259	241, 223, 197, 128	*A. pseudacutissimum* *R. mixta* *S. atomoides* *C. terrestris*	Authors' elaboration	3
Caffeoyl glucose	341	179, 161	*N. punctiforme* *C. terrestris* *E. mirabilis*	[25]	2
Caffeic acid	179	135, 121	*R. mixta*	[26]	1
Caffeic acid derivative	431	341, 179, 135	*C. terrestris* *E. mirabilis*	[27]	2
Caffeic acid hexoside	377	341, 179	*R. mixta* *C. terrestris*	Authors' elaboration	2
Coumaric acid	163	119	*V. magna*	[5]	1
5-O-p-coumaroylshikimic acid (5-p-CoSA)	319	301, 275, 257, 243, 231, 217, 203, 171, 163, 155, 145, 137, 119, 93	*A. pseudacutissimum* *R. mixta*	[28]	3
Phloretic acid (Hydroxyphenyl)propionic acid	165	121	*R. mixta*	[29]	3
Flavonols					
Pentahydroxyflavone	301	265, 229, 211, 187 171	*H. calcifuga*	[20]	3
Compound similar to: Kaempferol-3,4',7-trimethyl ether, or 3-hydroxy-4',5,7-trimethoxyflavone	327	309, 291, 229, 211, 209, 185, 171	*A. pseudacutissimum* *C. terrestris* *E. mirabilis*	[20]	3
Rutin	609	(301)	*S. atomoides*	[30]	1

Table 3. (Contd.)

Proposed compounds	Observed, *m/z*	MS/MS product ions	The presence in the studied Cyanobacterial/ Microalgae strain	References	Confidence level (MSI)*
Hydroxycoumarins					
3,3'-Methylenebis[4-hydroxycoumarin] (a.k.a. Dicoumarin) or Coumarin-6-(7-hydroxycoumarin-8-yl)-7-methoxy	335	317, 291, 273, 173	*E. mirabilis*	[20]	2–3
Other polyphenols					
Caffeoyl spermine conjugate	611	593, 482, 306, 272, 254, 179	*A. pseudacutissimum* *E. mirabilis*	[31]	2

* MSI Level 1, confirmed with authentic standard. MSI Level 2, probable structure based on MS/MS and literature/database match, but without standard. MSI Level 3, tentative assignment; general class defined, but exact structure uncertain.

trations [34, 35]. These differences occur both at the interspecific and intraspecific levels, which highlights the influence of cultivation conditions, extraction parameters, and a range of epigenetic factors. Interpretation of such data is further complicated by the presence of co-extracted compounds (sugars, ascorbic acid, amino acids, proteins), which can yield false-positive signals when reacting with the Folin–Ciocalteu reagent.

In recent years, this method has been increasingly supplemented or replaced by the Fast Blue BB assay, which is less sensitive to interference from non-phenolic compounds [36]. Nevertheless, this approach also has limitations and does not allow direct comparison with previously accumulated results. Therefore, it is advisable to employ multiple methods in parallel and to minimize co-extraction of ballast compounds. Importantly, the Folin–Ciocalteu assay retains its relevance, since in most cases phenolic compounds constitute the major antioxidants, and the values obtained provide a satisfactory estimate of their total content [37, 38].

In the present study, 23 phenolic compounds were detected, including shikimate pathway derivatives, such as shikimic acid. Only a subset of them was reliably confirmed with authentic standards, while the others were tentatively identified by comparison of MS/MS fragmentation patterns with literature and databases. Therefore, these latter compounds should be considered as putative candidates rather than unambiguously identified metabolites. The detection of C6–C1 phenolics (e.g., gallic acid) does not necessarily imply the operation of the full pathway, as these compounds may also be formed directly from shikimic or 3-dehydroshikimic acid. These findings are consistent with the results of Del Mondo et al. [39], where *in silico* analysis confirmed the presence of key enzymes of phenolic metabolism across all major taxonomic groups of microalgae. An exception is PAL (phenylalanine ammonia-lyase), which is absent in several taxa, suggesting the existence of alternative biosynthetic routes.

Most of the compounds identified belong to phenolic acids, which serve as precursors of more complex phenolic metabolites, the diversity of which was found to be limited. Their relatively narrow spectrum is likely related to the absence of conditions that trigger further synthesis. Several studies indicate that stress factors may act as the key drivers of such induction [5–7]. Even within a single taxon, however, strains may respond differently to stress, reflecting their specific adaptive mechanisms.

The greatest diversity of phenolic compounds was found in representatives of Cyanobacteria (*R. mixta*) and Chlorophyta (*E. mirabilis, C. terrestris*). The least diverse composition was detected in *K. flaccidum* (Charophyta) and *V. magna* (Heterokontophyta), in which only a single compound was identified (salicylic and coumaric acids, respectively). Notably, *K. flaccidum* exhibited a high total phenolic content (Table 2), consistent with reports on the richness of polyphenols in *K. flaccidum* var. zivo [40]. However, no data on the structural diversity of these compounds are available for this species, making it impossible to assess their chemical variability.

Flavones were detected only in *A. pseudacutissimum* (Cyanobacteria), *E. mirabilis* and *C. terrestris* (Chlorophyta), as well as *H. calcifuga* and *S. atomoides* (Heterokontophyta). In three strains (*A. pseudacutissimum, E. mirabilis*, and *C. terrestris*), a compound with a structure close to kaempferol-3,4',7-trimethyl ether or 3-hydroxy-4',5,7-trimethoxyflavone was identified. Rutin was detected in only one strain, *S. atomoides*.

Proanthocyanidins and anthocyanidins, which are the end products of flavonoid biosynthesis in higher plants, were not found in the samples. This may indicate that flavonoid biosynthetic pathways in cyanobacteria and microalgae do not reach the level of complexity typical of higher plants. However, their possible for-

mation cannot be excluded given the insufficient knowledge of phenolic metabolism in these groups.

A hydroxycoumarin compound (3,3'-methylene-bis[4-hydroxycoumarin]), also known as dicoumarol, was identified for the first time in *E. mirabilis*.

Along with various forms of cinnamic acids, higher plants are characterized by the accumulation of their conjugates with spermidine. Spermidine itself is a polyamine present in all photosynthetic cells, participating in pigment formation and enhancing stress resistance [11–13]. In our study, a similar compound was detected in *E. mirabilis* and *A. pseudacutissimum* and was tentatively identified as a caffeoyl spermidine conjugate.

Despite the limited occurrence of flavonoids in the studied strains, the presence of their precursors and immediate derivatives is noteworthy. As shown in Table 3, phenolic acids were the most widely represented class of compounds. These relatively simple metabolites possess biological activity of their own and serve as precursors in the synthesis of more complex phenolic structures.

Benzoic acid is a widespread metabolite serving as a precursor to numerous synthetic pathways leading to the formation of more complex compounds in both plants and algae. Benzoic acid derivatives, detected in five microbial strains, can be converted into salicylic acid (identified in *K. flaccidum*) via β-oxidation [14].

Caffeic acid, a precursor of the flavanone eriodictyol, was found in free form in *R. mixta*, as a hexoside in *C. terrestris* and *R. mixta*, and as a derivative in *E. mirabilis* and *C. terrestris*. Coumaric acid, detected only in *V. magna*, may participate in the biosynthesis of flavonoids and coumarins.

Syringic acid, synthesized through the shikimate pathway, was tentatively identified as a glucuronide derivative in *S. atomoides*. It serves as a precursor of several compounds, including sinapic acid (a phenylpropanoid), which was detected as two derivatives in *R. mixta, A. pseudacutissimum, S. atomoides, C. terrestris*, and *H. calcifuga*. Sinapic acid and catechin are known to play an important role in iron oxidation processes and in enhancing plant resistance to pathogens [15, 16].

Gallic acid is a precursor of a wide range of secondary metabolites. Its derivatives (galloyl-deoxy-hexoside, gallic acid monohydrate, gallic acid O-(6-galloylglucoside)/di-galloyl-hexoside) were identified in representatives of Cyanobacteria and Heterokontophyta—*R. mixta, S. atomoides, H. calcifuga*, and *N. punctiforme*. Phloretic acid, which has attracted attention for the development of environmentally friendly materials due to its unique structure [17], was found only in *R. mixta*.

Among phenolic compounds newly identified in microalgae, phenylacetic acid deserves attention. It is known as a plant auxin with antimicrobial activity. Oroian et al. [18] questioned the possibility of its synthesis by microalgae in axenic cultures, suggesting a bacterial origin of the metabolite. In our study, phenylacetic acid was tentatively identified in free form in *N. punctiforme*, as a pentoside in *R. mixta, A. pseudacutissimum, C. terrestris*, and as a hexoside in *R. mixta*.

Comparison of the results with the phylogenetic position of the strains revealed no strict correlation between taxonomy and either the qualitative or quantitative profiles of phenolic compounds. For example, the highest values were recorded in representatives of distinct evolutionary lineages—*E. mirabilis* (Chlorophyta) and *K. flaccidum* (Charophyta), whereas the lowest levels were observed in *V. magna* (Heterokontophyta) and *N. punctiforme* (Cyanobacteria). These findings confirm that phenolic profiles are primarily determined by species-specific traits and environmental factors, rather than phylogenetic relatedness. Comparable conclusions have been drawn in earlier studies on other taxa [5].

Thus, using HPLC–MS/MS, phenolic compounds were detected in all studied strains of microalgae and cyanobacteria. A set of 23 metabolites was observed, including several compounds reliably confirmed with authentic standards and others tentatively identified based on MS/MS spectra and literature data. The greatest diversity and overall abundance were observed in extracts of *R. mixta*, *E. mirabilis*, and *C. terrestris*, highlighting these strains as promising producers of bioactive compounds. The results expand current knowledge on the ability of lower phototrophs to synthesize phenolics and emphasize their potential for biotechnological applications. The absence of a clear link between taxonomic position and phenolic profile underscores the significant role of strain-specific features and environmental conditions in regulating metabolism.

SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTION

Conceptualization, supervision, and project administration, A.Y.M. and S.R.A.; culture collection, strain setup and culturing, experiment, and microscopic analysis, V.B.B., R.G.G., S.N.Zh.; data analyses, writing—original draft preparation, A.Y.M., S.R.A., A.Y.N., V.Y.N., Ch.M., G.L.; writing—review and editing, A.A.G.; molecular and phylogenetic analyses, A.Y.N. and V.Y.N. All authors have read and agreed to the published version of the manuscript.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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