



Facile isolation of lipoprotein-bound myxoxanthophyll and oscillaxanthin from *Limnospira (Spirulina) platensis*

R. G. Gevorgiz^{1,2} · A. A. Gontcharov³ · S. R. Abdullin³ · A. Y. Nikulin³ · L. L. Kapranova¹ · S. N. Zheleznova^{1,2} · A. A. Blaginina¹ · T. A. Telegina⁴ · Y. L. Vechtomova⁴ · T. Maoka⁵ · M. V. Nekhoroshev¹

Received: 1 April 2026 / Revised: 5 July 2026 / Accepted: 16 July 2026
© The Author(s), under exclusive licence to Springer Nature B.V. 2026, modified publication 2026

Abstract

A novel approach to the isolation of commercially valuable carotenoids from cyanobacteria is suggested. The protocol avoids the application of costly column chromatography. As an example, the isolation of myxoxanthophyll and oscillaxanthin from *Limnospira (Spirulina) platensis* (Gomont) K.R.S.Santos & Hentschke is described. The method is based on the surface-active properties of a unique carotenoid-lipoprotein complex, which accumulates at the chloroform-water interface and can be easily extracted. The complex is composed of myxol 2'- α -L-quinovoside, oscillol 2,2'-di- α -L-quinovoside, and lipoproteins. The mass fraction of carotenoids in the complex was $23 \pm 7\%$. The ratio of the carotenoids in the complex was $63 \pm 9\%$ myxoxanthophyll to $27 \pm 7\%$ oscillaxanthin. The carotenoids were identified using ultraviolet and visible (UV-Vis) absorption spectroscopy, HPLC analysis, fast atom bombardment mass spectrometry (FAB MS), and proton magnetic resonance spectrometry (^1H NMR). The complex was characterized using vibrational infrared spectroscopy (FTIR). Myxoxanthophyll and oscillaxanthin yield from a dry sample of *L. platensis* was $0.11 \pm 0.03\%$ and $0.05 \pm 0.02\%$, respectively, with 40 mg of myxoxanthophyll and 17 mg of oscillaxanthin being isolated from 36.84 g of *L. platensis* (dry weight). After the carotenoid isolation, further extraction of water-soluble phycobilins from the residual biomass is possible.

Keywords *Limnospira platensis* · Cyanobacterium · Separation · Extraction · Myxoxanthophyll · Oscillaxanthin · Carotenoids

Introduction

Carotenoids are one the most widespread group of pigments synthesized by higher plants, algae, bacteria (including cyanobacteria), some types of archaea and fungi. Carotenoids have extended chain of conjugated double bonds that determine various photophysical and photochemical properties of the molecule. In particular, xanthophylls (carotenoids containing oxygen atoms) show marked structural diversity, with more than 800 kinds reported in nature to date (Maoka 2020). Due to their biological activity, carotenoids are widely used in modern biotechnology to produce various drugs and products for therapeutic and prophylactic purposes (Langi et al. 2018; Ambati et al. 2019; Huang et al. 2025). The carotenoids found in cyanobacteria are particularly intriguing for several reasons. (1) Cyanobacteria produce unique carotenoids that are not found in other organisms (Pagels et al. 2021; Polyakov et al. 2023). (2) Cyanobacterial carotenoids exhibit strong biological activities, including antioxidant (quenching singlet oxygen and

✉ R. G. Gevorgiz
r.gevorgiz@yandex.ru

¹ A.O. Kovalevsky Institute of Biology of the Southern Seas of the Russian Academy of Sciences, 299011, 2 Nakhimov Ave., Sevastopol, Russian Federation

² Novosibirsk State University, 1, Pirogova Str., Novosibirsk 630090, Russian Federation

³ Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences, 690022 Vladivostok, Russian Federation

⁴ Research Center of Biotechnology of the Russian Academy of Sciences, 33 Leninsky Ave., Build. 2, 119991 Moscow, Russian Federation

⁵ Division of Food Function and Chemistry, Research Institute for Production Development, Kyoto 606-0805, Japan

scavenging free radicals) and photoprotective (preventing photooxidative damage under high light) effects. Unlike those of higher plants and algae, cyanobacteria produce glycosylated carotenoids (e.g., myxoxanthophyll, oscillaxanthin) with potent antioxidant and radical scavenging activity. This appears to contribute to *Limnospira*'s anti-cancer activity. (Pagels et al. 2021; Ren et al. 2021; Srivastava et al. 2024; Huang et al. 2025). (3) The rate of cyanobacterial biomass growth significantly exceeds that of plants from which carotenoids are extracted, which makes cyanobacteria a promising source of carotenoids (Gong and Bassi 2016). (4) Modern technologies for intensive cultivation of cyanobacteria on an industrial scale enable the production of these valuable substances at any location and at any time of the year (Novoveská et al. 2019; Chini Zittelli et al. 2023; Huang et al. 2025). (5) Cyanobacteria are the sole source of many carotenoids, as the organic synthesis of complex molecules is often challenging, prohibitively expensive, or even impossible (Ren et al. 2021). In recent years, consumer demand for naturally derived carotenoids used in healthcare and the treatment of various diseases has been steadily increasing, and the scope of carotenoid applications is constantly expanding (Plaza et al. 2010; Vélchez et al. 2011; Nabi et al. 2020; Huang et al. 2025). These compounds are actively used in medicine, food industry, agriculture, cosmetics, etc. (Concepcion et al. 2018). Therefore, various laboratories around the world are in a continuous search for new carotenoid producers, studying biological activity of different carotenoids, and developing innovative technologies for their production (Novoveská et al. 2019; Abidizadegan et al. 2021). Despite the advancements in these fields, the challenge of identifying new producers and developing innovative technologies for valuable carotenoids extraction remains urgent. Additionally, the development of industrial technologies for isolation and purification of these valuable compounds from biomass, often present in small quantities in microorganisms, is a significant task.

The cyanobacterium *Limnospira platensis* (also widely known as *Spirulina platensis*) is a filamentous, spiral-shaped species cultivated on an industrial scale due to its high nutritional value and rich content of bioactive compounds. It contains polar glycosylated cyclic carotenoids myxoxanthophyll and oscillaxanthin, which are found only in cyanobacteria. The sugar moieties of these carotenoids have been reported to consist of quinovose, fucose or rhamnose (Takaichi et al. 2005). These carotenoids have strong photoprotective and antioxidant effects, contribute to the integrity of thylakoids and outer envelope membranes, and are produced in large quantities in response to stress (exposure to bright light and temperature) (Täger et al. 2002; Mohamed et al. 2005; Graham and Bryant 2009; Srivastava et al. 2024). Myxoxanthophyll and oscillaxanthin possess a hydrocarbon skeleton characterized

by conjugated double bonds, along with hydroxyl groups and sugar molecules at their termini. This structural configuration allows them to integrate into the lipid bilayer, with the polar groups positioned on opposite outer sides of the membrane. This arrangement contributes to the stabilization, viscosity, and fluidity of the membrane. Myxoxanthophyll and oscillaxanthin are present in both the outer envelope and thylakoid membranes (Mohamed et al. 2005). The photoprotective ability of these xanthophylls to quench singlet oxygen is explained by the high degree of conjugation of double bonds in their polyene chain and the presence of glycosidic fragments (Steiger et al. 1999; Shindo et al. 2007; Nováková et al. 2020).

Knowledge on the properties of these carotenoids opens up great prospects for their use in the metabolic engineering of cyanobacteria to enhance the stress resistance of useful strains and to improve the industrial synthesis of myxoxanthophyll and oscillaxanthin (Täger et al. 2002; Rodrigues et al. 2014; Nováková et al. 2020; Srivastava et al. 2024). Myxoxanthophyll has been demonstrated to effectively reduce photooxidative damage in *Anabaena* sp. PCC 7120 and *Synechocystis* sp. PCC 6803. Additionally, it exhibits significant antioxidant and antihyperglycemic properties in clinical studies (Steiger et al. 1999; Srivastava et al. 2017, 2024).

The primary method for isolating carotenoids from natural sources involves multi-stage liquid extraction using various solvents (Kultys and Kurek 2022; Wang et al. 2022; López et al. 2023) and the separation of carotenoids through direct- or reversed-phase column chromatography (Kuregyan et al. 2018; Wang et al. 2022; Srivastava et al. 2024). This traditional approach is intricate and requires expensive equipment. In this paper, a novel method is proposed for isolating the minor carotenoids myxoxanthophyll (myxol 2'- α -L-quinovoside) and oscillaxanthin (oscillol 2,2'-di- α -L-quinovoside) from *L. platensis* biomass. This method aims to facilitate the industrial-scale isolation of these valuable carotenoids without relying on liquid chromatography.

Materials and methods

Organism

The cyanobacterium *Limnospira platensis* IBSS-31 strain, from the collection of hydrobionts of A.O. Kovalevsky Institute of Biology of the Southern Seas RAS, was used. The strain was adapted to experimental conditions using a luminostat at 25 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ for 6 days. The culture was grown on Zarrouk medium (Zarrouk 1966) at 33 °C. A pool-type photobioreactor was used to obtain 10 L of suspension.

Microscopic characterization

General morphological observations and measurements were performed using an Olympus BX 53 microscope equipped with Nomarski DIC optics and an Olympus DP27 digital camera. Morphometric data were processed using the software package Statistica 10.0.

Molecular identification

For the DNA analysis the culture was harvested in the exponential growth phase and concentrated by centrifugation. Total genomic DNA was extracted according to Echt et al. (1992) with some modifications by Abdullin et al. (2021). Amplification of the 16S rRNA gene was performed using universal cyanobacterial primers 27F (5'-AGAGTTTGATC-MTGGCTCAG-3') (Lane 1991) and 340R (5'-CTCTGT GTGCCTAGGTATCC-3') (Iteman et al. 2000). Polymerase chain reaction (PCR) cycling profile included an initial step of 5 min at 95 °C, followed by 35 cycles of 20 s at 95 °C, 20 s at 51 °C, 2 min at 72 °C, and terminating with a final hold of 5 min at 72 °C using the Encyclo Plus PCR kit (Evrogen, Russia) and a T100 Thermal Cycler (Bio-Rad Laboratories, USA). The PCR products were visualized on 1% agarose gel, then purified by ExoSAP-IT PCR Product Cleanup Reagent (Affymetrix, USA) and sequenced in both directions using an ABI 3500 genetic analyser (Applied Biosystems, USA) with a BigDye terminator v3.1 sequencing kit (Life Technologies Corporation, USA) and the primers 27F, 340R, 1492R (5'-GGTTACCTTGTTACGACTT-3') (Turner et al. 1999). Sequences were assembled with the Staden Package v1.4 (Bonfield et al. 1995). The sequence was deposited in GenBank under accession number MZ408912.

Phylogenetic analysis

The 16S rRNA gene sequence obtained from the strain IBSS-31 was initially compared with sequences available from the National Center for Biotechnology Information database using BLAST (<https://blast.ncbi.nlm.nih.gov>) to assess its phylogenetic affiliation.

A dataset was assembled using 16S rRNA gene sequences retrieved from the NCBI ($\geq 99\%$ identity, 100% coverage, > 1250 bp), supplemented with representative sequences from recent taxonomic revisions of the genus *Arthrospira* and related taxa, including the reclassification proposed by Nowicka-Krawczyk et al. (2019) and Santos et al. (2023). Special attention was given to strains assigned to the genus *Limnospira*, that accommodates commercially cultivated algae previously referred to as *Arthrospira/Spirulina*. The sequences (taxa and accession numbers are given in the tree) were aligned in the SeaView program (Galtier et al. 1996)

with manual corrections. The final alignment consisted of 96 cyanobacterial sequences with 2155 aligned base positions.

The best-fit nucleotide substitution model GTR + I + G was determined using jModelTest 2.1.1 (Darriba et al. 2012). Phylogenetic trees were constructed using maximum likelihood (ML) in RAxML v.7.2.6 (Kozlov et al. 2019) and Bayesian inference (BI) in MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001). For BI, four Markov chains were run executed for 5 million generations, sampling every 100 generations for a total of 50,000 samples. Convergence of the chains was assessed, and stationarity was determined according to the 'sump' plot with the first 12 500 samples (25%) discarded as burn-in; posterior probabilities were calculated from trees sampled during stationary phase. The robustness of the ML trees was estimated by bootstrap percentages (BP) (Stamatakis et al. 2008) and posterior probabilities (PP) in BI. BP < 50% and PP < 0.95 were not considered.

Experimental design and culture conditions

Limnospira platensis was cultivated in an industrial 10-cm-deep pool-type photobioreactor, at 33 ± 2 °C and under 24-h artificial illumination with white mercury lamps (DRL-700 Lisma Russia) which provided $290 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ on the surface of the suspension.

The total volume of the suspension was 730 L. The batch culture was grown on Zarrouk medium (Zarrouk 1966). During cultivation, the culture was stirred with a mechanical stirrer, which created vortex flows inside the suspension (Gevorgiz et al. 2026). To increase the intensification of interfacial mass transfer above the culture, a swirling tornado-type air flow was created, which was generated by the rotation of the paddle wheel above the liquid surface (Naumov et al. 2023).

Dry weight determination

Biomass dry weight was determined by filtering 10 ml of the suspension through dried pre-weighed Whatman GF/C glass microfiber filters with a pore size of 1.2 μm and drying at 105 °C for 24 h (Michael et al. 2019). In addition, biomass dry weight concentration was measured photometrically (Gevorgiz et al. 2005). The optical density of the cyanobacteria suspension at 750 nm (OD_{750}) was measured on a photometer Unico 2100 (United Products & Instruments, USA) in 5-mm-pathlength cuvettes. The absolute measurement error did not exceed 1%.

The optical density unit (OD_{750}) conversion to biomass dry weight (DW) values was as follows:

$$DW = k \times \text{OD}_{750}, \quad (1)$$

where DW is biomass dry weight, OD_{750} is culture optical density, and $k=0.85$ ($R^2=0.98$) is conversion factor. The conversion factor was determined empirically for *L. platensis* under our cultivation conditions (Gevorgiz et al. 2005). Linear regression of OD_{750} on gravimetrically measured dry weight. For dry weight determination, cells were washed with isotonic $(NH_4)_2CO_3$ solution to remove salts, following the recommendations on processing marine microalgal biomass (Zhu and Lee 1997).

Biomass harvesting

After reaching the stationary growth phase, the cyanobacterial suspension was filtered through a cloth with a mesh size of 45 μm . The crude mass of *L. platensis* obtained during filtration was washed three to four times with a large volume of distilled water to remove any residual culture medium. The biomass was dried in a stream of warm air at a temperature of 37–40 °C to a residual moisture content of 10–12%.

Lipid extraction

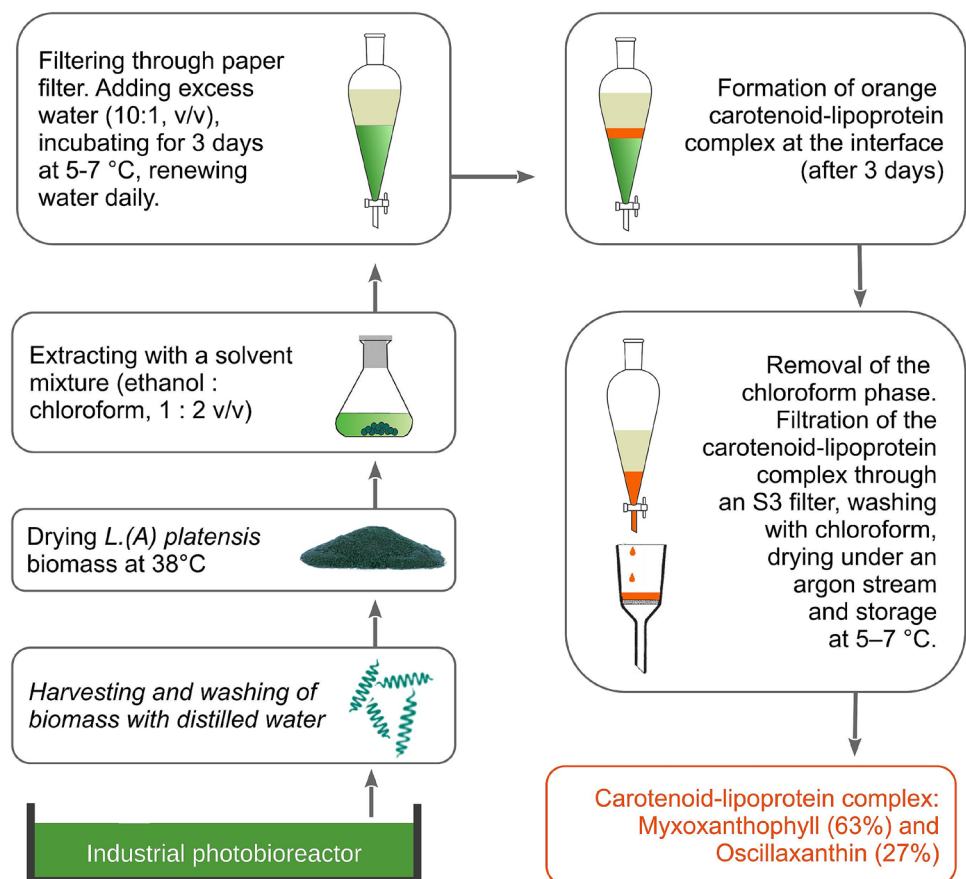
To 36.84 g crushed dry weight of *L. platensis*, 200 mL of Folch-type solution (ethanol:chloroform, 1:2) was added

(Takemura et al. 2026). The extraction was conducted in the dark at 5–7 °C over a period of three days with continuous stirring. The residual biomass was then removed by filtration and washed twice with ethanol:chloroform, 1:2 (100 mL). Analytical grade solvents were used in the extraction.

Isolating a carotenoid complex

600 mL of distilled water was added to the lipid extract and stored over the period of three days in a refrigerator at 5–7 °C. The aqueous fraction was changed daily, with the equal volume of distilled water being added for reconstitution. As the aqueous phase changed, a red–orange complex formed at the interface. The carotenoid complex was separated and washed with chloroform until the washing fraction was completely decolorized. As a result, a red–orange carotenoid complex insoluble in chloroform was obtained, which was dried in argon flow to remove traces of chloroform. For further analysis, the obtained precipitate was dissolved in 10 mL of ethanol. An outline of the *L. platensis* pigment complex isolation procedure is presented in Fig. 1.

Fig. 1 Procedure of isolating the carotenoid-lipoprotein complex from dry biomass of *Limnospira platensis*



Thin layer chromatography of the carotenoid complex and quantification of pigments

The ethanolic solution of the carotenoid complex was concentrated under argon flow, and (0.05 mL) was applied to glass-substrate thin-layer chromatography (TLC) plates coated with a 0.5 mm layer of silica gel. The plates were home-made using silica gel 60 G (Kieselgel 60 G, Merck No. 7731) with gypsum as a binder. The silica gel particle size is in the range of 10–40 μm , which is standard for analytical TLC (Stahl 1969). "Home-made" TLC plates were used, prepared on a TLC plate-covering applicator (Sandoz, Switzerland). The pigments were eluted in hexane:acetone (1:1).

The extinction coefficient ($E_{1\text{ cm}}^{1\%}$) value of 2500 was applied by Aakermann et al. (1992) to quantify the carotenoid pool from cyanobacterial strains. This value has also been adopted in the present study. For myxoxanthophyll, the wavelength corresponding to the highest peak is 473 nm, and for oscillaxanthin, it is 492 nm. The concentration of carotenoids (C , %) in the complex was calculated using the formula:

$$C_{\text{car}} = \frac{10}{E_{1\text{ cm}}^{1\%}} \frac{OD \cdot V}{L \cdot m}, \quad (2)$$

where OD is the optical density (measured at the appropriate wavelength); V is the carotenoid solution volume, mL; $E_{1\text{ cm}}^{1\%} = 2500$ is the carotenoid extinction coefficient in ethanol; L is the cuvette pathlength, cm; and m is dry complex mass, mg.

Identification of carotenoids

The UV–Vis spectra were recorded on a Hitachi U-2001 spectrophotometer in diethyl ether. Commercial diethyl ether (Sigma-Aldrich) containing BHT (2,6-di-*tert*-butyl-4-methylphenol) as a stabilizer was used for all procedures. The positive ion FAB MS spectra were recorded using a JEOL JMS-HX 110 A mass spectrometer with *m*-nitrobenzyl alcohol as matrix. The ^1H NMR (500 MHz) spectra were measured on a Varian Unity INOVA 500 spectrometer in CDCl_3 with TMS as an internal standard. Myxoxanthophyll (myxol 2'- α -L-quinovoside) and oscillaxanthin (oscillol 2,2'-di- α -L-quinovoside) were identified as corresponding acetate derivatives (tetraacetate and hexaacetate) from the UV–Vis, FAB MS and ^1H NMR spectral data. Acetylation of myxoxanthophyll and oscillaxanthin with acetic anhydride in dry pyridine at room temperature for 10 h yielded myxoxanthophyll tetraacetate and oscillaxanthin hexaacetate, respectively (Aakermann et al. 1992). The HPLC separation of the acetylated carotenoids was performed using a Shimadzu LC-6AD

instrument (L-6200 Intelligent pump) with a L-4250 UV–Vis detector (Hitachi, Japan), with acetone: hexane (4:6, v/v) at a flow rate of 1.0 mL min^{-1} (Maoka et al. 2011, 2014). The column used was 10 μm Cosmosil 5C18-II, $250 \times 10\text{ mm i.d.}$ (Nacalai Tesque, Japan).

FTIR analysis of the carotenoid complex

The measurements were performed using a Fourier transform infrared (FTIR) spectrometer FT/IR-6700 (Jasco, Japan). The samples were studied in the attenuated total reflection (ATR) mode. For the IR measurements, the dried carotenoid complex was dissolved in ethanol. First, the spectrum of a pure prism (ATR crystal – single-crystal diamond) was recorded in the absence of a sample (air served as the reference sample). Then 10 μL of the sample in ethanol was applied, dried in a stream of air and pressed tightly onto the crystal using a micrometer screw. The measurements were carried out with one hundred scans in the range from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} . The spectra were obtained by subtracting the spectrum of the reference sample.

CHN analysis of the carotenoid complex

CHN analysis of the carotenoid complex was performed on a Flash EA 1112 analyzer (Thermo Finnigan, Italy). Acetanilide was used as a standard.

Phosphorus analysis of the carotenoid complex

Presence of phosphorus in the phospholipids of the carotenoid complex was determined by the classical colorimetric method using molybdenum blue (Worsfold et al. 2005). To achieve this, phospholipids were first mineralized with concentrated nitric acid to convert phosphorus to H_3PO_4 . Then, phosphate ions in an acidic medium in the presence of excess molybdenum reagent (ammonium molybdate in 5 M sulfuric acid) formed phosphomolybdic heteropoly acid $\text{H}_3[\text{P}(\text{Mo}_3\text{O}_{10})_4] \cdot n\text{H}_2\text{O}$, which absorbs radiation in the UV spectral range. By reducing phosphomolybdic acid under mild conditions with Mohr's salt ($(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$), an intensely colored phosphomolybdic blue (with an absorption maximum of 710 nm) was obtained after 20 min. Determination of phosphorus was carried out using a calibration graph previously constructed using phosphate ion.

Calculation of means and experimental uncertainty

Triplicate biological and analytical samplings were used in the experiments. Statistical analysis was performed using Microsoft Excel built-in statistical tools. The experimental

results were expressed as mean value $\pm$ standard deviation (SD) (McDonald 2009).

Results and discussion

Phylogenetic position and taxonomic interpretation

Results of the BLAST searches showed that the sequence of the 16S rRNA gene (1281 bp) obtained for the strain IBSS-31 was highly similar (> 99.5%) to sequences annotated as *Arthrospira platensis* and *A. maxima* but recently assigned to the genus *Limnospira* (Nowicka-Krawczyk et al. 2019; Santos et al. 2023). Phylogenetic reconstructions based on the expanded dataset (Fig. 2) separated traditional *Arthrospira*-like cyanobacteria into two distinct lineages: *Arthrospira* sensu stricto (represented by the type species *Arthrospira jenneri*) and *Limnospira*. This topology is fully congruent with previous studies. Strain IBSS-31 was a part of the strongly supported *Limnospira* clade (BP 100/PP 1.00). It was clustered in the subclade that includes the majority of strains previously identified as "*A. platensis*", "*A. maxima*", "*A. fusiformis*", and "*Arthrospira*" sp. – a homogeneous group corresponding to *Limnospira platensis*. Our strain showed the highest similarity (100% identity) to the type strain originally annotated as *A. platensis* (PCC 7345, JN831264) and to the reference strain of *L. platensis* (CCIBt3254, OR142672) as revised by Santos et al. (2023). No phylogeographic structure was evident in this clade, as IBSS-31 grouped with isolates from Mongolia, Mexico, China, Africa, and South America, confirming high genetic homogeneity of globally distributed *L. platensis*. Therefore, based on the BLAST results, phylogenetic placement, and current taxonomic framework, the strain IBSS-31 was identified as *Limnospira platensis*.

Microscopic characterization

Trichomes are solitary, cylindrical, green–blue in color, usually laterally compressed, surrounded by sheath. Only straight trichomes were observed. The apical cells are either rounded or bearing a head (capitate), often surrounded by a calyptra (more frequently at one end and always in the capitate end cells) (Fig. 3). The cells were 4.10–8.72 μm wide, 4.36–8.46 μm long; end cells were 3.59–9.23 μm wide, 4.10–8.21 μm long. The trichomes did not settle and remained suspended even without mixing.

Although helical trichomes are typical of *L. platensis* cultures, linear or nearly linear filaments are also common, particularly under artificial laboratory and mass cultivation conditions (Wang and Zhao 2005; Papapanagiotou et al. 2019; Yadav et al. 2020). The plasticity of morphological and morphometric features, such as filament length,

spiral width, helix pitch, tightness of coils, abundance of aerotopes and helix orientation in *L. platensis* under different growth conditions (e.g., temperature, light source and intensity, salinity, alkalinity, turbulence, and nutrient availability) is well known (Mühling et al. 2003; Ma and Gao 2009; Kaggwa et al. 2013).

Carotenoid complex isolation

Formation of the carotenoid complex was observed at the chloroform–water interface. The obtained solid complex was red–orange in color. The complex was readily soluble in ethanol and virtually insoluble in chloroform and hexane. When acetone was added, the complex was disrupted, leaving the carotenoids dissolved in the acetone fraction and causing a white substance, likely consisting of proteins and phospholipids (Lu et al. 2012), to precipitate from the solution.

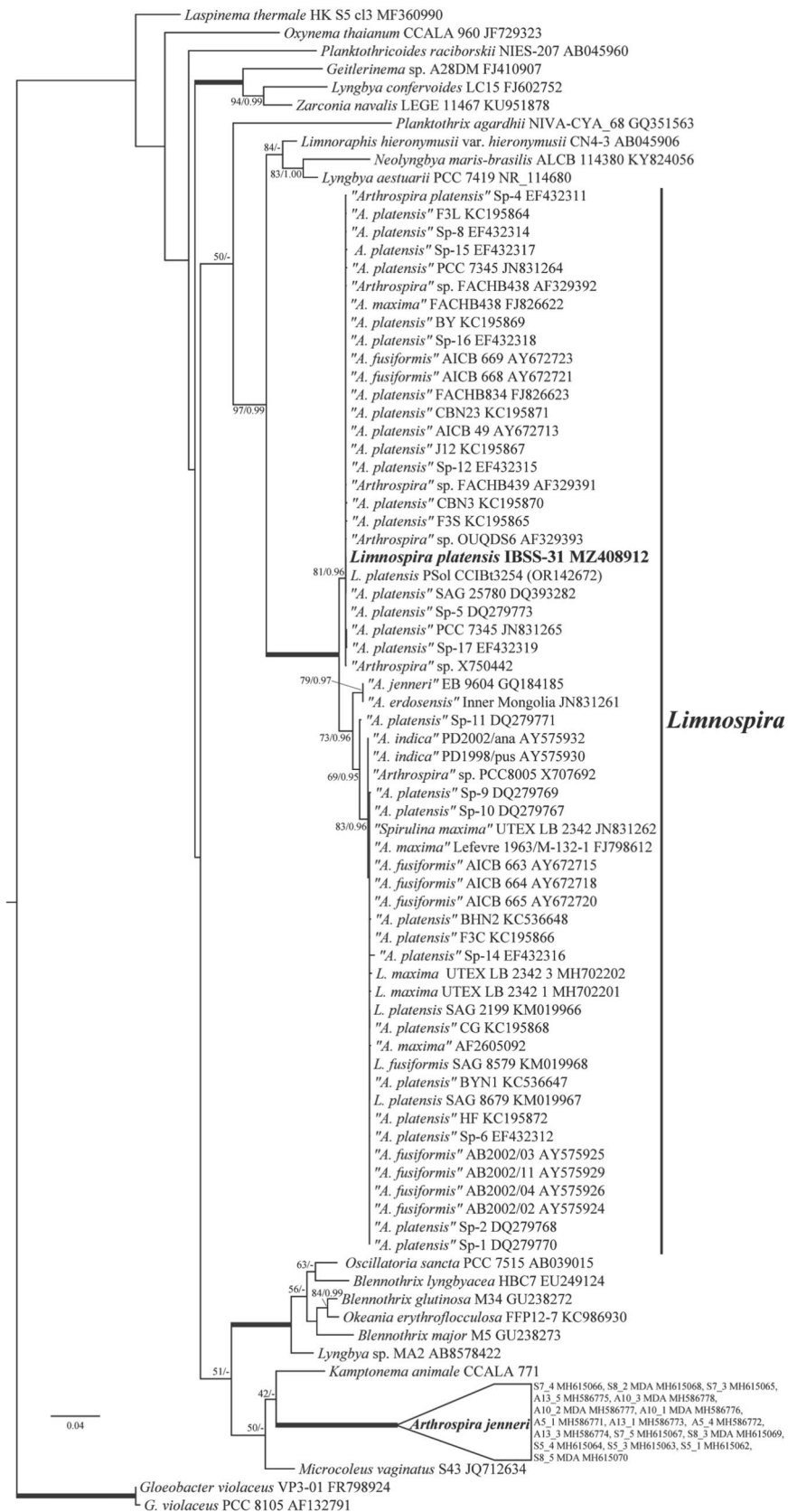
The capability of the carotenoid complex to concentrate at the chloroform–water interface was used as a basis for the proposed simple method of myxoxanthophyll and oscillaxanthin extraction from the pigment mixture. The complex contains carotenoids with polar terminal groups, including carbohydrates, attached to a non-polar polyene chain, and a lipoprotein part, which also contains polar (protein) and non-polar (lipid) components, providing the complex with the surface-active properties and making it accumulate at the chloroform–water interface.

Identification of carotenoids in the complex

The spectrum of the carotenoid complex in ethanol and TLC of the complex are given in Fig. 4. Absorption spectrum of the complex (Fig. 4a) shows presence of two absorption peaks (480 and 515 nm) and a shoulder at 450 nm corresponding to the sum of myxoxanthophyll (448, 473, 509 nm) and oscillaxanthin (465, 492, 525 nm). A weak characteristic band of chlorophyll *a* (665 nm) is also present, as confirmed by TLC (Fig. 4b). Chlorophyll and some other compounds could be retained in trace amounts in the complex during precipitation. It should be noted that the objective of this work was to examine the pigment–protein complex itself. Instead, the complex was used as the means only to isolate carotenoids from the cyanobacterial biomass for further characterization. Although the data presented in Fig. 4 cannot be used as a basis for an analytical study, they indicate two major pigments that comprise the complex. More studies are necessary to understand the biological role of the carotenoid–lipoprotein complex and to optimize protocols for the extraction of valuable carotenoids from *L. platensis*.

The UV–Vis, FAB MS and ^1H NMR spectral data of myxol 2'- α -L-quinovoside tetraacetate and oscillol 2,2'-di- α -L-quinovoside hexaacetate obtained from *L. platensis* are summarized in Table 1. Since signals of the sugar moiety in

Fig. 2 Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences showing position of *Limnospira platensis* IBSS-31 (in bold). Bootstrap support values (ML, $\geq 50\%$) and Bayesian posterior probabilities (BI, ≥ 0.95) are shown at the nodes. Nodes with maximal support (100% BP/1.00 PP) are shown in bold. The tree includes representatives of *Arthrospira* sensu stricto, *Limnospira*, and related oscillatorialean cyanobacteria. Scale bar represents substitutions per site



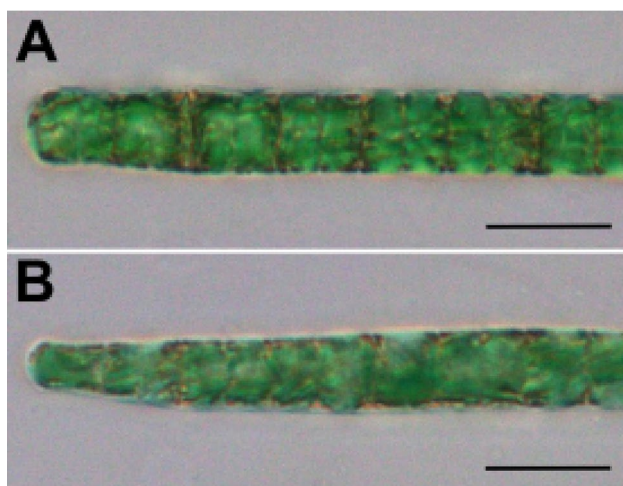


Fig. 3 General morphology of *Limnospira* (Spirulina) *platensis* using light microscopy. **(A)** Trichome with rounded apical cell. **(B)** Trichome with capitate apical cell. Scale bar = 10 μm

myxoxanthophyll and oscillaxanthin overlapped with peaks of other protons, to identify the sugar moieties in myxoxanthophyll and oscillaxanthin, they were converted to tetraacetate and hexaacetate, respectively. ^1H NMR signals of the sugar moieties of myxoxanthophyll tetraacetate and oscillaxanthin hexaacetate were identical with those of α -quinovose acetate reported by Aakermann and Takaichi and co-authors (Aakermann et al. 1992; Takaichi et al. 2005).

In total, 281 ± 66 mg of the carotenoid complex ($0.76 \pm 0.18\%$) was obtained from dry biomass weighing 36.84 g. The mass of carotenoids in the complex was 63.7 ± 19 mg, which is $0.17 \pm 0.05\%$ on *L. platensis* dry weight basis. The proportion of carotenoids in the complex

was $23 \pm 7\%$ and the percentage of the lipoprotein part was $77 \pm 7\%$. According to the HPLC analysis after the acetylation with acetic anhydride in pyridine, the carotenoids ratio (myxoxanthophyll: oscillaxanthin) in the complex was $63 \pm 11\%$ myxoxanthophyll to $27 \pm 10.7\%$ oscillaxanthin (10% unidentified pigments). Minor peaks were not identified due to low abundance (Fig. 5). Thus, 40 ± 11 mg of myxoxanthophyll and 17 ± 7 mg of oscillaxanthin were obtained. On *L. platensis* dry weight basis, the yields of the carotenoids were $0.11 \pm 0.03\%$ myxoxanthophyll and $0.05 \pm 0.02\%$ oscillaxanthin.

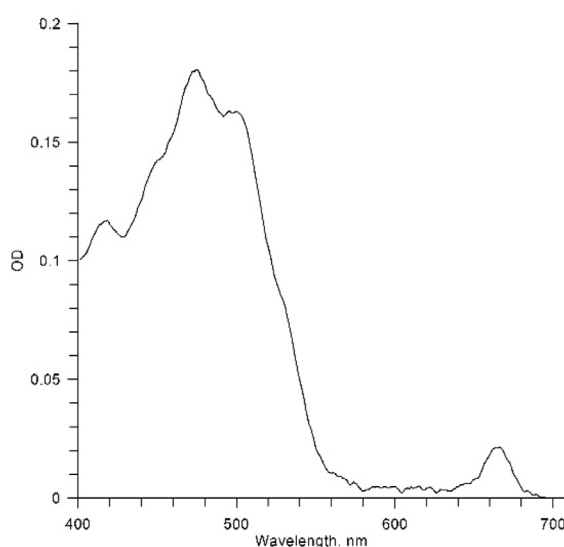
Fourier-transformed infrared (FTIR) spectrometry of the complex

Isolated complex was examined by ATR-FTIR spectrometry to identify components such as carotenoids, proteins and lipids, including phospholipids (Fig. 6).

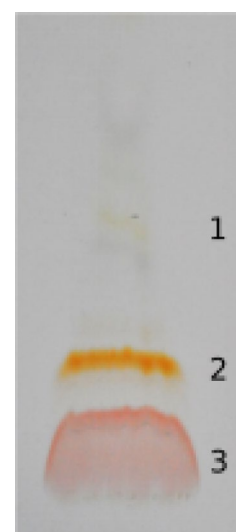
FTIR spectral data shown in Fig. 6 suggest as follows.

- (1) Characteristic bands of carotenoids in the IR region are the bands at $1550\text{--}1520\text{ cm}^{-1}$ (C=C stretching in polyene chain) and $1170\text{--}1150\text{ cm}^{-1}$ (C-C stretching in polyene chain). Obviously, the stretching vibrations of the C-C bonds are present in a strong band with the maximum at 1165 cm^{-1} (Lóránd et al. 2002; Quijano-Ortega et al. 2020). The C=C bond stretching vibrations are not clearly manifested, since they apparently participate in the formation of complexes with proteins, and this leads to a bathochromic shift of the absorption band to the region of 1542 cm^{-1} which overlaps with the Amide II protein band. Aliphatic C-H stretches with peaks at 2920 cm^{-1} (asymmetric) and 2851 cm^{-1}

Fig. 4 **(a)** UV-Vis spectrum of the carotenoid complex in ethanol and **(b)** TLC of the carotenoid complex solution in ethanol. Bands: (1) chlorophyll *a* (traces), (2) myxoxanthophyll (myxol 2'- α -L-quinovoside), (3) oscillaxanthin (oscillol 2,2'-di- α -L-quinovoside)



(a)



(b)

Table 1 UV–VIS, FAB MS and ^1H -NMR spectral data of myxol 2'- α -L-quinovoside tetraacetate and oscillol 2,2'-di- α -L-quinovoside hexaacetate obtained from the *Limnospira* (*Spirulina*) *platensis* carotenoid complex

Myxoxanthophyll tetraacetate			Oscillaxanthin hexaacetate		
FAB MS m/z	957 [M + H ⁺]		1145 [M + H ⁺]		
VIS nm	448, 473. 509		465, 492, 525		
¹ H-NMR carot-enoid moiety					
Position	δ	mult. (Hz)	Position	δ	mult. (Hz)
2	α 1.78	ddd (12, 4, 1)	2	3.75	d (9)
2	β 1.58	dd (12, 12)			
3	5.06	m	3	5.65	dd (15,0.5, 0.9)
4	α 2.45	ddd (16, 6, 1.5)	4	6.40	d (15.5)
4	β 2.11	dd (16, 9)	6	6.20	d (11)
7	6.10	d (16)	7	6.59	dd (15.5, 11)
8	6.13	d (16)	8	6.40	d (15.5)
10	6.16	d (11.5)	10	6.20	d (11)
11	6.65	d (15.5, 11.5)	11	~6.65	overlapped
12	6.36	d (11.5)	12	6.34	d (15)
14	6.26	d (11)	14	6.25	m
15	6.64	m	15	~6.65	overlapped
16	1.11	s	16	1.17	s
17	1.08	s	17	1.21	s
18	1.74	s	18	1.94	s
19	1.97	s	19	1.98	s
20	1.97	s	20	2.00	s
2'	3.75	d (9)	2'	3.75	d (9)
3'	5.65	dd (15,0.5, 0.9)	3'	5.65	dd (15,0.5, 0.9)
4'	6.40	d (15.5)	4'	6.40	d (15.5)
6'	6.20	d (11)	6'	6.20	d (11)
7'	6.59	dd (15.5, 11)	7'	6.59	dd (15.5, 11)
8'	6.40	d (15.5)	8'	6.40	d (15.5)
10'	6.20	d (11)	10'	6.20	d (11)
11'	~6.65	overlapped	11'	~6.65	overlapped
12'	6.34	d (15)	12'	6.34	d (15)
14'	6.25	m	14'	6.25	m
15'	~6.65	overlapped	15'	~6.65	overlapped
16'	1.17	s	16'	1.17	s
17'	1.21	s	17'	1.21	s
18'	1.94	s	18'	1.94	s
19'	1.98	s	19'	1.98	s
20'	2.00	s	20'	2.00	s
sugar moiety					
1''	5.11	d (3.5)	1''	5.11	d (3.5)
2''	4.92	dd (3.5, 2)	2''	4.92	dd (3.5, 2)
3''	5.43	dd (10, 3)	3''	5.43	dd (10, 3)
4''	4.78	dd (10, 3)	4''	4.78	dd (10, 3)
5''	3.96	m	5''	3.96	m
6''	1.04	d (6)	6''	1.04	d (6)
acetyl moiety					
	2.02	s		2.02	s
	2.05	s		2.05	s
	2.07	s		2.07	s

Fig. 5 Absorption spectra of myxoxanthophyll tetraacetate (**A**) and oscillaxanthin hexaacetate (**B**) after HPLC separation. (**C**) HPLC of the obtained carotenoids myxoxanthophyll tetraacetate and oscillaxanthin hexaacetate, mobile phase acetone: hexane (4: 6)

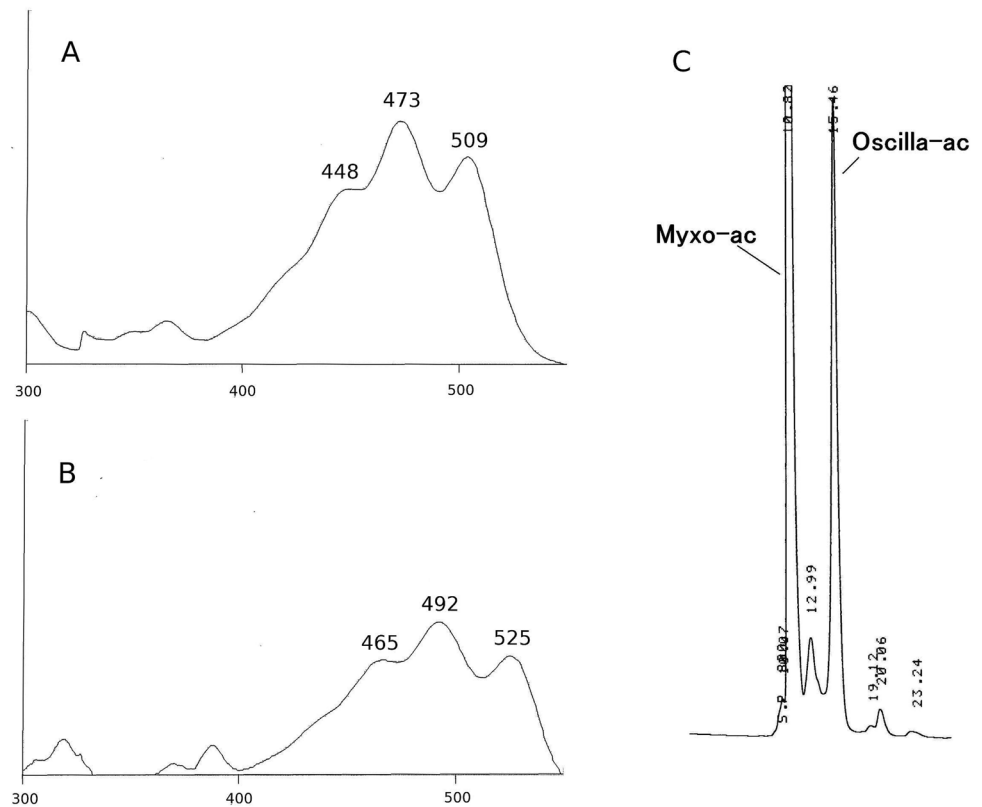
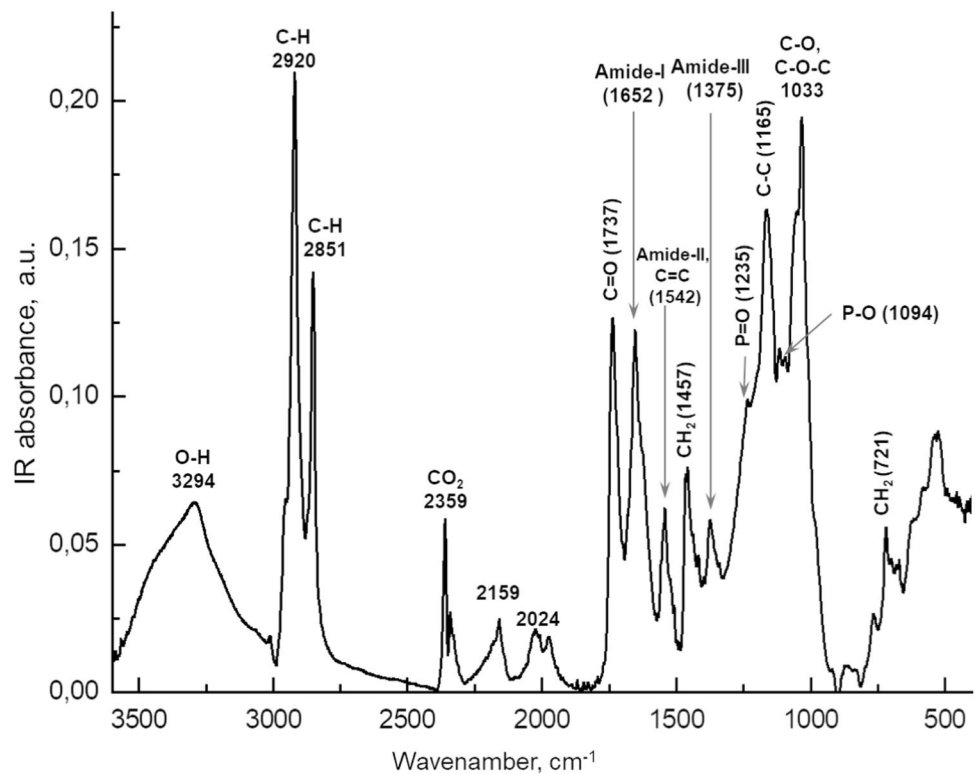


Fig. 6 The averaged FTIR spectrum of the dry carotenoid complex at room temperature



(symmetric) are associated with the alkyl groups in the carotenoid backbone, methyl groups and glycosides (Várkonyi et al. 2002).

- (2) Lipids in the complex can be identified by peaks related to C-H stretching vibrations in CH_3 and CH_2 at 2851 and 2920 cm^{-1} , C=O stretching in esters at 1737 cm^{-1} , CH_2 -bending at 1457 cm^{-1} , C-O-C stretching in esters ($1250\text{--}1070\text{ cm}^{-1}$) and CH_2 rocking at 721 cm^{-1} (Forfang et al. 2017; Olsztyńska-Janus et al. 2018). The presence of phospholipid functional groups in the complex can be established based on peaks related to P=O and P-O stretching vibrations at 1235 cm^{-1} and 1094 cm^{-1} (Forfang et al. 2017). Phospholipids are present in membranes mainly in the composition of lipoproteins. The presence of phosphorus in the carotenoid complex was confirmed by the analysis using the molybdenum blue method. The complex was shown to contain $0.70 \pm 0.04\%$ phosphorus, and this can serve as an additional indication of the presence of phospholipids in the complex.
- (3) The presence of protein in the complex was detected by analyzing the recorded spectra in the region of $1700\text{--}1300\text{ cm}^{-1}$ (Fig. 6). For various strains of *L. platensis*, it was found that: the Amide I band, which results from the C=O stretching vibrations of peptide bonds, in the region 1650 cm^{-1} . The Amide II band, which results from N-H bending and C-N stretching vibrations, is observed in the region 1550 cm^{-1} ; and at $1426\text{--}1377\text{ cm}^{-1}$ and $1329\text{--}1290\text{ cm}^{-1}$, the protein COO-side chain and Amide III ($\approx 1350\text{--}1200\text{ cm}^{-1}$) bands are present (Singh et al. 1993; Bataller and Capareda 2018; Tatulian 2019). When proteins are complexed by donor-acceptor interactions with carotenoids having C-C stretching vibrations at 1525 cm^{-1} , the absorption band of Amide II (in the region of 1550 cm^{-1}) can shift hypsochromically to the region of 1542 cm^{-1} . Indeed, the bands of Amide I (1652 cm^{-1}), Amide II (1542 cm^{-1}) and Amide III (1375 cm^{-1}) were identified in the isolated complex, indicating the presence of protein in the complex.
- (4) Carbohydrates, including the characteristic bands of glycosides that have the C-O-C, C-OH and C-C stretching, were observed at $1170\text{--}955\text{ cm}^{-1}$. Broad band in the region of 3300 cm^{-1} indicates strong stretching vibrations of the O-H bond (hydroxyl groups mainly in sugars). In general, the $1150\text{--}1000\text{ cm}^{-1}$ region is considered to be the "fingerprint" of the carbohydrate ring, which is dominated by strong C-O and C-C stretching vibrations and the glycosidic C-O-C linkage to the myxol backbone (Nikonenko et al. 2000; Wiercigroch et al. 2017; Bataller and Capareda 2018; Olsztyńska-Janus et al. 2018; Figueroa et al. 2022; Moge et al. 2023). At the same time, there is an overlap

of C-C stretching vibrations of the carotenoid polyene chains in the region of 1150 cm^{-1} .

Analysis of the non-carotenoid part of the complex

Nitrogen content was analyzed in the white substance obtained after the extraction of carotenoids from the complex with acetone. Results of the CHN-analysis show that the white substance contains $6.7 \pm 0.2\%$ nitrogen and $47.7 \pm 1.1\%$ carbon. High nitrogen content indicates that the non-carotenoid portion of the complex includes proteins, which is consistent with FTIR spectroscopy data.

Challenges and prospects

Myxoxanthophyll and oscillaxanthin are promising molecules for medicine and biotechnology (Tsushima et al. 1995; Nováková et al. 2020; Srivastava et al. 2024). However, despite their significance and potential, there is limited information available regarding the biomedical applications of these carotenoids. The challenges associated with the extraction of myxoxanthophyll and oscillaxanthin, which involve complex multistage procedures, limit access to these substances (Aakermann et al. 1992; Nováková et al. 2020).

Success of new biotechnologies in the production of biologically active materials depends on three key factors: (1) access to inexpensive raw materials with a high concentration of the target substance, (2) effective methods for extracting target molecules and purifying them from impurities, and (3) adaptation and cost reduction of technology for industrial-scale applications. To date, many efforts have been focused on search, structure analysis and identification of glycosylated carotenoids from different cyanobacterial species. New perspective strains were identified and influence of different environmental and nutritional factors on carotenoid production in cyanobacteria was examined (Pfander 1976; Pagels et al. 2021; Srivastava et al. 2024). Significantly less progress was achieved in development of new methods for the extraction of myxoxanthophyll and oscillaxanthin, as well as in comparing their effectiveness (Aakermann et al. 1992; Nováková et al. 2020). It is important to note that there is no information available on any industrial methods for the production of these substances.

The methods for isolating glycosylated carotenoids from various cyanobacterial species that have been known to date primarily involved chromatography techniques, which necessitate costly equipment for the pigment separation. A fundamentally new approach is proposed for this purpose. The pigment separation is achieved by carotenoid-lipoprotein complex formation that concentrates at the chloroform-water interface and can be easily separated afterwards, after which the pigments are easily isolated.

Thus, the isolation of this complex eliminates the need for chromatographic separation and can be easily scaled up.

The advantages of the proposed approach are as follows: (1) the carotenoid extraction process consists of only a few steps; (2) each step is facile; and (3) chromatography is not involved. It can be hypothesized that additional carotenoid-lipoprotein complexes may be discovered in the future, enabling the simple and cost-effective isolation of various pigments from biomass.

Conclusions

A new approach to the isolation of commercially valuable carotenoids from cyanobacteria has been proposed. A facile method for isolating the carotenoids myxoxanthophyll and oscillaxanthin from *L. platensis* has been developed, paving the way for the green and cost-effective industrial production of these carotenoids. The method described is based on the ability of the unique amphiphilic pigment-lipoprotein complex to concentrate at the chloroform-water interface. It does not require any chromatographic technique for the isolation and purification of the carotenoids. The chromatographic separation (HPLC) was used in this study only for the purpose of carotenoid identification and quantification.

Carotenoids comprised 22.7% of the complex, while the remainder was composed of lipoproteins. The carotenoid ratio (myxoxanthophyll: oscillaxanthin) was 63%: 27%, with 40 mg of myxoxanthophyll (0.1% of *Limnospira platensis* dry weight) and 17 mg of oscillaxanthin (0.05% of *L. platensis* dry weight) isolated from 36.84 g of *L. platensis* (dry weight).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10811-026-03976-9>.

Author's contribution R.G.G., M.V.N. and A.A.G.: conceptualization. R.G.G., M.V.N.: designed the study. R.G.G., A.A.G., S.R.A., A.Y.N., T.A.T., Y.L.V.: analyzed the data and wrote the original draft. S.R.A., A.Y.N., T.A.T., Y.L.V.: software. A.A.G., T.M.: validation. S.N.Z., A.A.B. and L.L.K.: performed the laboratory work. M.V.N., A.Y.N., T.A.T., Y.L.V., T.M.: performed the analyses. R.G.G. and A.A.G.: edited the final version of the manuscript. R.G.G. and A.A.G.: received the funding. R.G.G.: supervised the project. All authors have read and agreed to the published version of the manuscript.

Funding The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation (themes No 124022400152–1; No 124012400285–7; No 126031818944–7). Experiments on *L. platensis* cultivation in the industrial vortex-type photobioreactor were carried out in the frame of research project supported by the Russian Science Foundation, RSF No 25–79–30002.

Data availability Data can be made available upon reasonable request.

Declarations

Competing interest The authors declare no competing interests.

References

- Aakermann T, Skulberg OM, Liaaen-Jensen S (1992) A comparison of the carotenoids of strains of *Oscillatoria* and *Spirulina* (Cyanobacteria). *Biochem Syst Ecol* 20:761–769
- Abdullin SR, Nikulin AY, Bagmet VB, Nikulin VY, Gontcharov AA (2021) New cyanobacterium *Aliterella vladivostokensis* sp. nov. (Aliterellaceae, Chroococcidiopsidales), isolated from temperate monsoon climate zone (Vladivostok, Russia). *Phytotaxa* 527:221–233
- Abidizadegan M, Peltomaa E, Blomster J (2021) The potential of cryptophyte algae in biomedical and pharmaceutical applications. *Front Pharmacol* 11:618836
- Ambati RR, Gogisetty D, Aswathanarayana RG, Ravi S, Bikkina PN, Bo L, Yuepeng S (2019) Industrial potential of carotenoid pigments from microalgae: current trends and future prospects. *Crit Rev Food Sci Nutr* 59:1880–1902
- Bataller BG, Capareda SC (2018) A rapid and non-destructive method for quantifying biomolecules in *Spirulina platensis* via Fourier transform infrared — attenuated total reflectance spectroscopy. *Algal Res* 32:341–352
- Bonfield JK, Smith KF, Staden R (1995) A new DNA sequence assembly program. *Nucleic Acids Res* 23:4992–4999
- Chini Zittelli G, Lauceri R, Faraloni C, Silva Benavides AM, Torzillo G (2023) Valuable pigments from microalgae: phycobiliproteins, primary carotenoids, and fucoxanthin. *Photochem Photobiol Sci* 22:1733–1789
- Concepcion MR, Avalos J, Bonet ML, Boronat A, Gomez-Gomez L, Hornero-Mendez D, Limon MC, Meléndez-Martínez AJ, Olmedilla-Alonso B, Palou A, Ribot J, Rodrigo MJ, Zacarias L, Zhu C (2018) A global perspective on carotenoids: metabolism, biotechnology, and benefits for nutrition and health. *Prog Lipid Res* 70:62–93
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9:772
- Echt CS, Erdahl LA, McCoy TJ (1992) Genetic segregation of random amplified polymorphic DNA in diploid cultivated alfalfa. *Genome* 35:84–87
- Figuerola FA, Abdala-Díaz RT, Pérez C, Casas-Arrojo V, Nesic A, Tapia C, Durán C, Valdes O, Parra C, Bravo-Arrepol G, Soto L, Becerra J, Cabrera-Barjas G (2022) Sulfated polysaccharide extracted from the green algae *Codium bernabei*: physicochemical characterization and antioxidant, anticoagulant and antitumor activity. *Mar Drugs* 20:458
- Forfang K, Zimmermann B, Kosa G, Kohler A, Shapaval V (2017) FTIR spectroscopy for evaluation and monitoring of lipid extraction efficiency for oleaginous fungi. *PLoS ONE* 12:e0170611
- Galtier N, Gouy M, Gautier C (1996) Seaview and phylo_win: two graphic tools for sequence alignment and molecular phylogeny. *Comput Appl Biosci* 12:543–548
- Gevorgiz RG, Alisieva AV, Shmatok MG (2005) Estimation of biomass *Spirulina platensis* (Nordst.) Geitl with use of optical density of culture. *Ecologiya Morya* 70:96–106 (in Russian)
- Gevorgiz RG, Naumov IV, Sharifullin BR, Skripkin SG, Zheleznova SN, Klochkova VS, Kapranov SV (2026) A new approach to microalga *Porphyridium purpureum* (Bory) Ross cultivation based on a pilot-scale vortex photobioreactor. *Bioresour Technol Rep* 34:102773

- Gong M, Bassi A (2016) Carotenoids from microalgae: a review of recent developments. *Biotechnol Adv* 34:1396–1412
- Graham JE, Bryant DA (2009) The biosynthetic pathway for myxol-2' fucoside (myxoxanthophyll) in the cyanobacterium *Synechococcus* sp. strain PCC 7002. *J Bacteriol* 191:3292–3300
- Huang JJ, Xu W, Lin S, Cheung PCK (2025) The bioactivities and biotechnological production approaches of carotenoids derived from microalgae and cyanobacteria. *Crit Rev Biotechnol* 45:276–304
- Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755
- Iteman I, Rippka R, de Marsac NT, Herdman M (2000) Comparison of conserved structural and regulatory domains within divergent 16S rRNA–23S rRNA spacer sequences of cyanobacteria. *Microbiol* 146:1275–1286
- Kaggwa MN, Burian A, Oduor SO, Schagerl M (2013) Ecomorphological variability of *Arthrospira fusiformis* (Cyanoprokaryota) in African soda lakes. *MicrobiolOpen* 2:881–891
- Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A (2019) RAXML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* 35:4453–4455
- Kultys E, Kurek MA (2022) Green extraction of carotenoids from fruit and vegetable byproducts: a review. *Molecules* 27:518
- Kuregyan AG, Pechinskij SV, Stepanova EF (2018) Method for obtaining individual carotenoids. Russian Patent RU 2648452C1
- Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) *Nucleic acid techniques in bacterial systematics*. John Wiley and Sons, New York, pp 115–175
- Langi P, Kiokias S, Varzakas T, Proestos C (2018) Carotenoids: from plants to food and feed industries. *Meth Mol Biol* 1852:57–71
- López G-D, Álvarez-Rivera G, Carazzone C, Ibáñez E, Leidy C, Cifuentes A (2023) Bacterial carotenoids: extraction, characterization, and applications. *Crit Rev Anal Chem* 53:1239–1262
- Lóránd T, Deli J, Molnár P, Tóth G (2002) FT-IR study of some carotenoids. *Helv Chim Acta* 85:1691–1697
- Lu FS, Nielsen NS, Baron CP, Diehl BW, Jacobsen C (2012) Oxidative stability of dispersions prepared from purified marine phospholipid and the role of α -tocopherol. *J Agric Food Chem* 60:12388–12396
- Ma Z, Gao K (2009) Photoregulation of morphological structure and its physiological relevance in the cyanobacterium *Arthrospira* (*Spirulina*) *platensis*. *Planta* 230:329–337
- Maoka T (2020) Carotenoids as natural functional pigments. *J Nat Med* 74:1–16
- Maoka T, Akimoto N, Tushima M, Komemushi S, Mezaki T, Iwase F, Takahashi Y, Sameshima N, Mori M, Sakagami Y (2011) Carotenoids in marine invertebrates living along the Kuroshio Current coast. *Mar Drugs* 9:1419–1427
- Maoka T, Kuwahara T, Narita M (2014) Carotenoids of sea angels *Clione limacina* and *Paedoclione doliiformis* from the perspective of the food chain. *Mar Drugs* 12:1460–1470
- McDonald JH (2009) *Handbook of Biological Statistics*, 2nd ed. Sparky House Publishing, Baltimore, MD
- Michael A, Kyewalyanga MS, Lugomela CV (2019) Biomass and nutritive value of *Spirulina* (*Arthrospira fusiformis*) cultivated in a cost-effective medium. *Ann Microbiol* 69:1387–1395
- Moge B, Schindler B, Yeni O, Compagnon I (2023) Fucose migration pathways identified using infrared spectroscopy. *Angew Chem Int Ed Engl* 62:e202300538
- Mohamed HE, van de Meene AM, Roberson RW, Vermaas WF (2005) Myxoxanthophyll is required for normal cell wall structure and thylakoid organization in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *J Bacteriol* 187:6883–6892
- Mühling M, Harris N, Belay A, Whitton BA (2003) Reversal of helix orientation in the cyanobacterium *Arthrospira*. *J Phycol* 39:360–367
- Nabi F, Arain MA, Rajput N, Alagawany M, Soomro J, Umer M, Soomro F, Wang Z, Ye R, Liu J (2020) Health benefits of carotenoids and potential application in poultry industry: a review. *J Anim Physiol Anim Nutr* 104:1809–1818
- Naumov IV, Gevorgiz RG, Skripkin SG, Tintulova MV, Tsoi MA, Sharifullin BR (2023) Experimental study of the topological flow transformations in an aerial vortex bioreactor with a floating washer. *Biotechnol J* 18:e2200644
- Nikonenko NA, Buslov DK, Sushko NI, Zhabankov RG (2000) Investigation of stretching vibrations of glycosidic linkages in disaccharides and polysaccharides with use of IR spectra deconvolution. *Biopolymers* 57:257–262
- Nováková M, Fábryová T, Vokurková D, Dolečková I, Kopecký J, Hrouzek P, Tůmová L, Cheel J (2020) Separation of the glycosylated carotenoid myxoxanthophyll from *Synechocystis salina* by HPLC and evaluation of its antioxidant, tyrosinase inhibitory and immune-stimulating properties. *Separations* 7:73–88
- Novoveská L, Ross ME, Stanley MS, Pradelles R, Wasiolek V, Sassi J-F (2019) Microalgal carotenoids: a review of production, current markets, regulations, and future direction. *Mar Drugs* 17:640
- Nowicka-Krawczyk P, Mühlsteinová R, Hauer T (2019) Detailed characterization of the *Arthrospira* type species separating commercially grown taxa into the new genus *Limnospira* (Cyanobacteria). *Sci Rep* 9:694
- Olsztyńska-Janus S, Kielbowicz Z, Czarnecki MA (2018) ATR-IR study of skin components: lipids, proteins and water. Part II: near infrared radiation effect. *Spectrochim Acta A Mol Biomol Spectrosc* 202:93–101
- Pagels F, Vasconcelos V, Guedes AC (2021) Carotenoids from cyanobacteria: biotechnological potential and optimization strategies. *Biomolecules* 11:735
- Pfander H (1976) Carotenoid glycosides. *Pure Appl Chem* 47:121–128
- Plaza M, Santoyo S, Jaime L, García-Blairsy Reina G, Herrero M, Señoráns FJ, Ibáñez E (2010) Screening for bioactive compounds from algae. *J Pharm Biomed Anal* 51:450–455
- Polyakov NE, Focsan AL, Gao Y, Kispert LD (2023) The endless world of carotenoids—structural, chemical and biological aspects of some rare carotenoids. *Int J Mol Sci* 24:9885
- Quijano-Ortega N, Fuenmayor CA, Zuluaga-Dominguez C, Diaz-Moreno C, Ortiz-Grisales S, García-Mahecha M, Grassi S (2020) FTIR-ATR spectroscopy combined with multivariate regression modeling as a preliminary approach for carotenoids determination in *Cucurbita* spp. *Appl Sci* 10:3722
- Ren Y, Sun H, Deng J, Huang J, Chen F (2021) Carotenoid production from microalgae: biosynthesis, salinity responses and novel biotechnologies. *Mar Drugs* 19:713
- Rodrigues DB, Flores ÉMM, Barin JS, Mercadante AZ, Jacob-Lopes E, Zepka LQ (2014) Production of carotenoids from microalgae cultivated using agroindustrial wastes. *Food Res Int* 65B:144–148
- Santos KRS, Hentschke GS, Ferrari G, Andreote APD, Fiore MF, Vasconcelos V, Sant'Anna CL (2023) Molecular, morphological and ecological studies of *Limnospira platensis* (Cyanobacteria), from saline and alkaline lakes, Pantanal Biome, Brazil. *Front Environ Sci* 11:1204787
- Singh BR, DeOliveira DB, Fu F-N, Fuller MP (1993) Fourier transform infrared analysis of amide III bands of proteins for the secondary structure estimation", *Proc. SPIE* 1890, *Biomolecular Spectroscopy III*, <https://doi.org/10.1117/12.145242>
- Shindo K, Kikuta K, Suzuki A, Katsuta A, Kasai H, Yasumoto-Hirose M, Matsuo Y, Misawa N, Takaichi S (2007) Rare carotenoids, (3R)-saproxanthin and (3R,2'S)-myxol, isolated from novel marine bacteria (*Flavobacteriaceae*) and their antioxidative activities. *Appl Microbiol Biotechnol* 74:1350–1357
- Srivastava A, Brilisaue K, Rai AK, Ballal A, Forchhammer K, Tripathi AK (2017) Down-regulation of the alternative sigma factor

- SigJ confers a photoprotective phenotype to *Anabaena* PCC 7120. *Plant Cell Physiol* 58:287–297
- Srivastava A, Thapa S, Chakdar H, Babele PK, Shukla P (2024) Cyanobacterial myxoxanthophylls: biotechnological interventions and biological implications. *Crit Rev Biotechnol* 44:63–77
- Stahl E (ed) (1969) *Thin-layer Chromatography: A Laboratory Handbook*, 2nd ed. Springer, Berlin
- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. *Syst Biol* 57:758–771
- Steiger S, Schäfer L, Sandmann G (1999) High-light-dependent upregulation of carotenoids and their antioxidative properties in the cyanobacterium *Synechocystis* PCC 6803. *J Photochem Photobiol B* 52:14–18
- Täger C, Sättler A, Schröder KR, Rögner M (2002) Use of myxoxanthophyll and/or echinenone for the prophylactic and/or therapeutic treatment of undesirable physical conditions conditioned or favoured by oxidative processes. WO Patent 2002024183A1
- Takaichi S, Mochimura M, Maoka T, Katho HI (2005) Myxol and 4-ketomyxol 2'-fucosides, not rhamnosides, from *Anabaena* sp. PCC 7120 and *Nostoc punctiforme* PCC 73102, and proposal for the biosynthetic pathway of carotenoids. *Plant Cell Physiol* 46:497–504
- Takemura M, Maoka T, Misawa N (2026) Heterologous production of torularhodin, the monocyclic carotenoid with a terminal carboxyl group, in *Escherichia coli*. *BioTech* 15:3
- Tatulian SA (2019) FTIR analysis of proteins and protein-membrane interactions. *Methods Mol Biol* 2003:281–325
- Tsushima M, Maoka T, Katsuyama M, Kozuka M, Matsuno T, Tokuda H, Nishino H, Iwashima A (1995) Inhibitory effect of natural carotenoids on Epstein-Barr virus activation activity of a tumor promoter in Raji cells. A screening study for anti-tumor promoters. *Biol Pharmaceut Bull* 18:227–233
- Turner S, Pryer KM, Miao VP, Palmer JD (1999) Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *J Eukaryot Microbiol* 46:327–338
- Várkonyi Z, Masamoto K, Debreczeny M, Zsiros O, Ughy B, Gombos Z, Domonkos I, Farkas T, Wada H, Szalontai B (2002) Low-temperature-induced accumulation of xanthophylls and its structural consequences in the photosynthetic membranes of the cyanobacterium *Cylindrospermopsis raciborskii*: an FTIR spectroscopic study. *Proc Natl Acad Sci U S A* 99:2410–2415
- Vílchez C, Forján E, Cuaresma M, Bédmar F, Garbayo I, Vega JM (2011) Carotenoids: biological functions and commercial applications. *Mar Drugs* 9:319–333
- Wang J, Hu X, Chen J, Wang T, Huang X, Chen G (2022) The extraction of β -carotene from microalgae for testing their health benefits. *Foods* 11:502
- Wang ZP, Zhao Y (2005) Morphological reversion of *Spirulina* (*Arthrospira*) *platensis* (Cyanophyta): from linear to helical. *J Phycol* 41:622–628
- Wiercigroch E, Szafraniec E, Czamara K, Pacia MZ, Majzner K, Kochan K, Kaczor A, Baranska M, Malek K (2017) Raman and infrared spectroscopy of carbohydrates: a review. *Spectrochim Acta A* 185:317–335
- Worsfold PJ, Gimbert LJ, Mankasingh U, Omaka ON, Hanrahan G, Gardolinski PC, Haygarth PM, Turner BL, Keith-Roach MJ, McKelvie ID (2005) Sampling, sample treatment and quality assurance issues for the determination of phosphorus species in natural waters and soils. *Talanta* 66:273–293
- Yadav A, Monsieurs P, Misztak A, Waleron K, Leys N, Cuypers A, Janssen PJ (2020) Helical and linear morphotypes of *Arthrospira* sp. PCC 8005 display genomic differences and respond differently to Co-60 gamma irradiation. *Eur J Phycol* 55:129–146
- Zarrouk C (1966) Contribution à l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima* (Setch et Gardner) Geitler. PhD thesis. University of Paris, Paris
- Zhu CJ, Lee YK (1997) Determination of biomass dry weight of marine microalgae. *J Appl Phycol* 9:189–194

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

Terms and Conditions

Springer Nature journal content, brought to you courtesy of Springer Nature Customer Service Center GmbH ("Springer Nature").

Springer Nature supports a reasonable amount of sharing of research papers by authors, subscribers and authorised users ("Users"), for small-scale personal, non-commercial use provided that all copyright, trade and service marks and other proprietary notices are maintained. By accessing, sharing, receiving or otherwise using the Springer Nature journal content you agree to these terms of use ("Terms"). For these purposes, Springer Nature considers academic use (by researchers and students) to be non-commercial.

These Terms are supplementary and will apply in addition to any applicable website terms and conditions, a relevant site licence or a personal subscription. These Terms will prevail over any conflict or ambiguity with regards to the relevant terms, a site licence or a personal subscription (to the extent of the conflict or ambiguity only). For Creative Commons-licensed articles, the terms of the Creative Commons license used will apply.

We collect and use personal data to provide access to the Springer Nature journal content. We may also use these personal data internally within ResearchGate and Springer Nature and as agreed share it, in an anonymised way, for purposes of tracking, analysis and reporting. We will not otherwise disclose your personal data outside the ResearchGate or the Springer Nature group of companies unless we have your permission as detailed in the Privacy Policy.

While Users may use the Springer Nature journal content for small scale, personal non-commercial use, it is important to note that Users may not:

1. use such content for the purpose of providing other users with access on a regular or large scale basis or as a means to circumvent access control;
2. use such content where to do so would be considered a criminal or statutory offence in any jurisdiction, or gives rise to civil liability, or is otherwise unlawful;
3. falsely or misleadingly imply or suggest endorsement, approval, sponsorship, or association unless explicitly agreed to by Springer Nature in writing;
4. use bots or other automated methods to access the content or redirect messages
5. override any security feature or exclusionary protocol; or
6. share the content in order to create substitute for Springer Nature products or services or a systematic database of Springer Nature journal content.

In line with the restriction against commercial use, Springer Nature does not permit the creation of a product or service that creates revenue, royalties, rent or income from our content or its inclusion as part of a paid for service or for other commercial gain. Springer Nature journal content cannot be used for inter-library loans and librarians may not upload Springer Nature journal content on a large scale into their, or any other, institutional repository.

These terms of use are reviewed regularly and may be amended at any time. Springer Nature is not obligated to publish any information or content on this website and may remove it or features or functionality at our sole discretion, at any time with or without notice. Springer Nature may revoke this licence to you at any time and remove access to any copies of the Springer Nature journal content which have been saved.

To the fullest extent permitted by law, Springer Nature makes no warranties, representations or guarantees to Users, either express or implied with respect to the Springer nature journal content and all parties disclaim and waive any implied warranties or warranties imposed by law, including merchantability or fitness for any particular purpose.

Please note that these rights do not automatically extend to content, data or other material published by Springer Nature that may be licensed from third parties.

If you would like to use or distribute our Springer Nature journal content to a wider audience or on a regular basis or in any other manner not expressly permitted by these Terms, please contact Springer Nature at

onlineservice@springernature.com