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Biochemical characterization of carotenoids in two species of *Trentepohlia* (Trentepohliales, Chlorophyta)

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Abstract Two species of *Trentepohlia*, i.e., *Trentepohlia aurea* and *Trentepohlia cucullata* were collected from walls and tree bark, respectively, at two different seasons in a year. The total carotenoid content in both the species is very high during winter but decreases significantly during summer. By spectroscopic analysis, it was found that. *T. aurea* and *T. cucullata* growing in natural habitats are rich sources of carotenoids. The individual carotenoids were separated, identified, and estimated by HPLC, and identified as β-carotene along with some other carotenoids, i.e., neoxanthin, lutein, β-cryptoxanthin, β,γ-carotene, β, ε-carotene (absent during summer).

Keywords Algae · *Trentepohlia* · Carotenoid · HPLC

Introduction

The Chlorophycean alga *Trentepohlia* Martius is found attached to rocks, moist soil surfaces, wood, moist concrete walls, tree trunks, and leaves, and has the appearance of cushion-like or scattered patches with different shades of colors, usually orange to yellow, facilitates its easy identification in natural habitats. In the present study, the carotenoids of *Trentepohlia aurea* and *T. cucullata* growing in natural habitats have been investigated with a view to assess them as the sources of natural carotenoids for commercial exploitation.

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Materials and methods

Trentepohlia aurea and *T. cucullata* were collected from natural habitats in summer (June and July) and winter (January and February) from walls and tree barks, respectively. *Trentepohlia aurea* was collected from the concrete walls of the botanical garden while *T. cucullata* was collected from a tree bark in the Chemistry Department of Gauhati University, Assam India.

Standard carotenoids were obtained from F. Hoffman-La Roche, Basel, Switzerland. Extraction of carotenoids was carried out under the yellow light of the laboratory. The algal filaments were dried at 4°C. A known amount (1 g) of dry alga was ground with anhydrous Na_2SO_4. The carotenoid pigments, along with the small amount of chlorophylls, were extracted completely in DCM. To remove the chlorophylls, 1 mL of methanolic solution of the extract was saponified with 100 μL of 80% KOH solution in the presence of 50 μL of methanolic BHT (as antioxidant) at 85°C. The carotenoid in the saponified extract was estimated spectrophotometrically following the procedure of Britton (1985).

Each carotenoid was separated, identified, and estimated by HPLC following the procedure of Breithaupt and Bamedi (2001). The HPLC system includes two pumps, LC-8 25×4.6 mm, 5 μm column and a SPD-M10AVP diode array detector set at 450 nm and a gradient solvent system with solvent A (CH_3CN:H_2O=97.50:2.50) and solvent B (CH_3CN:DCM=70:30).The time program was 40 min

(from 0 to 5 min, solvent A concentration 100%, flow rate 0.6 mL min^{-1}; at 25 min, solvent B concentration 100%, flow rate 1.2 mL min^{-1}; at 30 min, solvent B concentration 100%, flow rate 1.2 mL min^{-1}; at 35 min, solvent A concentration 100%, flow rate 0.6 mL min^{-1}; at 40 min, stop time). The estimation of the quantity of the different carotenoids was carried out from the chromatogram peak areas

Results

The two species of algae were identified from their morphological characters. The morphological characters of the alga collected from the wall were similar to that of *T. aurea* (L) Martius as described by Printz (1939, 1964). The alga collected from tree bark was identified to be *T. cucullata* var. *sandvicensis* Willie (Printz 1939).

The separated carotenoids were identified by HPLC to be neoxanthin (retention time 7.511 min and visible spectra peaks at 419, 436, 450 nm), lutein (retention time 8.213 min and visible spectra peaks at 421, 445, 474 nm), β-cryptoxanthin (retention time 13.392 min and visible spectra peaks at 425, 449, 472 nm), β,γ-carotene (retention time 16.890 min), β,ε-carotene (retention time 20.231 min and visible spectra peaks at 435, 449, 472 nm) β,β-carotene (retention time 21.011 min and visible spectra peaks at 425, 453, 479 nm). On injecting the non-hydrolyzed extract, we observed some peaks with a retention time more than that of β-carotene but with negligible area. These peaks were associated with the esters of xanthophylls (identified by their visible spectra). The presence of xanthophyll esters in plants have been also reported by Edelenbos et al. (2001). After saponification, no peaks were found in that zone of the chromatogram (Figs. 1 and 2).

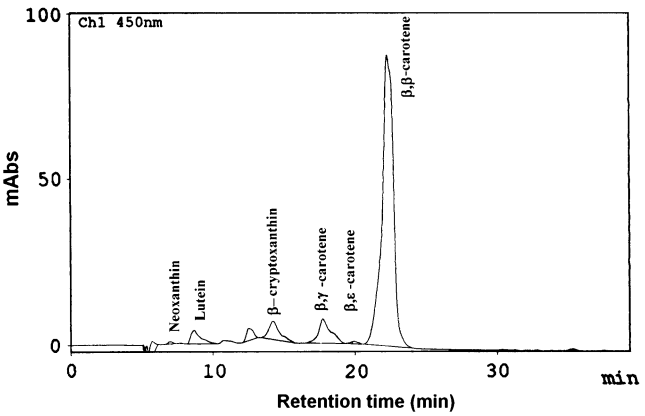

Fig. 1 HPLC chromatogram for *T. aurea*. Column LC-8, 25×4.6 mm, 5 μm column, mobile phase gradient of acetonitrile–water and acetonitrile–dichloromethane, detection wave length 450 nm

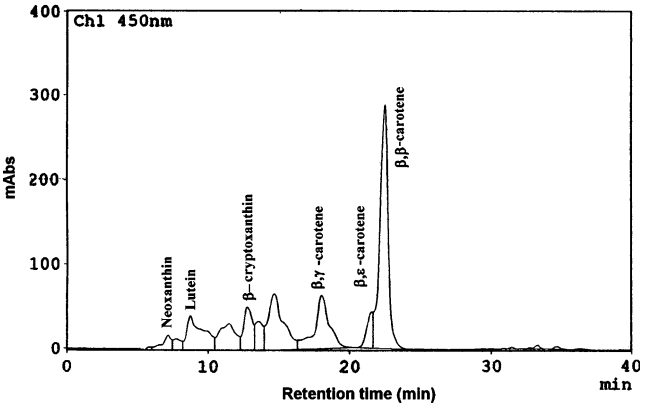

Fig. 2 HPLC chromatogram for *T. cucullata*. Column LC-8, 25×4.6 mm, 5 μm column, mobile phase gradient of acetonitrile–water and acetonitrile–dichloromethane, detection wave length 450 nm

The fraction of carotenoid in the HPLC chromatogram which was identified as neoxanthin was isolated by column chromatography and dissolved in methanol. On addition of 0.1 M methanolic HCl solution, the carotenoid showed a hypsochromic shift of almost 20 nm (436 to 421 nm) confirming the presence of a single 5,6-epoxy group which, on acid treatment, was converted to furanoid (Isler 1971).

The total amount as well as different carotenoids in *T. aurea* and *T. cucullata* during summer (June–July) and winter (January–February) are shown in Table 1. In winter, the total amount of carotenoids of *T. aurea* was 6.31± 1.24 mg g^{-1} dry weight while that of *T. cucullata* was 3.88± 0.409 mg g^{-1} dry weight. The total amount of carotenoids of *T. aurea* collected in July was 838.56±0.23 μg g^{-1} dry weight and that of *T. cucullata* was 341.05±0.34 μg g^{-1} dry weight.

Table 1 Total and individual carotenoid contents of two species of *Trentepohlia* in summer and winter (mean±S.E.)

Carotenoids	*Trentepohlia aurea*		*Trentepohlia cucullata*	
	Summer (μg g^{-1})	Winter (mg g^{-1})	Summer (μg g^{-1})	Winter (mg g^{-1})
Total	838.56 ± 0.23	6.31±1.24	341.05± 0.34	3.88±0.41
Neoxanthin	8.33±1.83	0.017±0.005	11.52± 0.02	0.131±0.020
Lutein	34.52± 2.99	0.204±0.055	13.32± 0.96	0.306±0.046
β-cryptoxanthin	50.03± 0.60	0.412±0.281	19.93± 0.78	0.516±0.080
β,γ-carotene	77.81± 2.75	0.371±0.140	27.25± 0.32	0.474±0.027
β,ε-carotene	–	0.0621±0.066	–	0.101±0.092
β.β-carotene	528.05± 2.75	4.886±0.839	236.33± 0.98	1.156±0.350

Discussion

Different species of *Trentepohlia* have been studied; for example, *T. aurea* has been used to remove nitrate and phosphate from a water body (Abe et al. 2008).

In 1892, carotenoids in *Trentepohlia iolithus* were first studied by Zopf (1892) and a detailed study was carried out by Kjosen et al. (1972). Abe et al. (1998) successfully increased the amount of carotenoid (β-carotene) several fold in *Trentepohlia* by using a two-stage culture. They reported that β-carotene productivity increased significantly with an increase of light intensity from 3,000 to 10,000 lx. They also mentioned that a dense algal cell culture is required to increase β-carotene productivity but growth of *Trentepohlia* is inhibited significantly beyond 30°C. Tan et al. (1993) also reported that carotenoid content increased in *T. odorata* with high light intensity.

We found that carotenoid content increased several fold in winter. In summer, the weather is very hot (30–40°C) which is very unfavorable for the growth of *Trentepohlia*. In this season, the sky of Assam remains covered with cloud. Thus, the light intensity is reduced. On the other hand, the lower temperatures (~25°C) and cloudless clear sky in winter provide perfect conditions favoring the growth and carotenogenesis of the two species of *Trentepohlia*.

This report provides new information on the nature of different carotenoids biosynthesized by *T. aurea* and *T. cucullata* collected from natural sources without culturing the algae in any artificial medium.

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