

Carotenoid composition of terrestrial Cyanobacteria: response to natural light conditions in open rock habitats in Venezuela

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(Received 1 September 2000; accepted 22 June 2001)

Carotenoid contents of terrestrial cyanobacteria sampled from rock surfaces with different exposure to sunlight were studied in the Guyana-shield region of Venezuela. At the collecting locations of two table mountains Roraima-tepui (2700 m), Auyan-tepui (2000 m) and an inselberg near Puerto Ayacucho (80 m), the most frequent species were *Stigonema ocellatum*, *Stigonema hormoides* and *Scytonema crassum*, respectively. Usually, these species were associated with the unicellular cyanobacterium *Gloeocapsa sanguinea*. Their carotenoid and chlorophyll *a* contents were determined by high-performance liquid chromatography. In general, carotenoids consisted almost equally of myxolglycosides and of β -carotene derivatives, such as β -carotene, echinenone and canthaxanthin. On a chlorophyll *a* basis, myxolglycoside content increased in full exposure to solar irradiation. The relative proportions of the different β -carotene derivatives appeared to be even more strongly influenced by irradiance. Whereas their total amount was increased at full exposure, the content of β -carotene decreased and that of canthaxanthin increased. Maximal ratios of canthaxanthin/ β -carotene of around 5 were observed in the most sun-exposed samples. We conclude that acclimation of the carotenoid content to irradiance, especially the adjustment of the ratio canthaxanthin/ β -carotene, is a response found in many different terrestrial cyanobacterial species. However, when a natural population was artificially shaded for 6 days, short-term acclimation in pigment content was not detected. The results support a photoprotective function of canthaxanthin in terrestrial cyanobacteria under natural environmental conditions.

Key words: β -carotene, canthaxanthin, carotenoid, cyanobacteria, inselberg, light acclimation, myxoxanthophyll, table mountain, Venezuela

Introduction

Cyanobacteria and cyanolichens are often the primary colonizers of exposed rock surfaces in highly insolated environments (Büdel & Wessels, 1991; Büdel *et al.*, 1997). Life at such exposed locations requires special protective mechanisms to avoid photoinhibition of photosynthesis and photo-oxidation. Carotenoids are considered as photoprotective and also antioxidative agents (Krinsky, 1979; Palozza & Krinsky, 1992). A photoprotective function of the ketocarotenoid canthaxanthin has been shown in genetically modified *Synechococcus* (Albrecht *et al.*, 2001). The xanthophyll zeaxanthin plays an important role in green plant protection against photoinhibition (Demmig-Adams, 1990; Niyogi, 1999) and it has been speculated that it could play a similar role in cyanobacteria (Demmig-Adams *et al.*, 1990). Zeaxanthin content can be relatively high in certain aquatic cyanobacteria and be enhanced under high irradiance (Guillard *et al.*, 1985; Bidigare *et al.*, 1989). On the other hand, in

some terrestrial cyanobacterial species, Leisner *et al.* (1993, 1994) could not find a high content of zeaxanthin and it did not increase with light exposure. Instead, these authors observed an apparently irradiance-dependent synthesis of the ketocarotenoid canthaxanthin at the expense of β -carotene. Similar observations were also made by other authors (Bauman *et al.*, 1971; Nonnengiesser *et al.*, 1996; Bilger *et al.*, 1997). In Nostocales-dominated mats from a desert environment, ketocarotenoids were the predominant fraction (Bauman *et al.*, 1971). Vincent *et al.* (1993) investigated benthic microbial mat communities in Antarctica and found enriched contents of canthaxanthin and myxoxanthophyll in the sun-exposed strata of the mats, whereas β -carotene and echinenone were highest in lower strata. Thus, it appears possible that canthaxanthin formation represents a universal response in terrestrial cyanobacteria under light stress.

The tropics belong to the most insolated regions on earth, due to the high zenith angle of the sun. The Guyana shield in Venezuela is characterized by the existence of table mountains (tepui) in the Guyana

highlands and inselbergs (Barthlott *et al.*, 1993) in the Orinoco lowland. The table mountains and inselbergs (isolated outcrops; Porembski & Barthlott, 2000) can be considered as isolated dry habitats in an otherwise rather humid climate (Porembski *et al.*, 1996). Some of the table mountains, such as Auyan-tepui, are accessible only by helicopter and are thus very poorly investigated. Because of their high irradiation and extreme surface temperatures, coupled with low water and nutrient availability, the rock surfaces of these formations represent one of the most difficult environments for photosynthetic organisms. Despite the unfavourable environment, the reddish sandstone and light-coloured granite surfaces of the table mountains and inselbergs are black due to an extensive cover of cyanobacterial crusts and films (Büdel *et al.*, 1994). The mechanisms by which these cyanobacterial populations survive are poorly understood (Büdel, 1999; Büdel *et al.*, 2000). Accordingly, the cyanobacteria from the Venezuelan inselbergs and table mountains are ideal subjects in which to study acclimation responses and carotenoid contents under extremely high natural irradiance.

Materials and methods

Collection localities

We collected samples of epilithic cyanobacterial mats from the Auyan-tepui (800–2000 m), the Roraima-tepui (1500–2700 m) and from an inselberg close to Puerto Ayacucho (80 m; Fig. 1). The short-term response of the cyanobacteria to artificial shading was investigated at the Puerto Ayacucho site.

(1) The Roraima-tepui is located in the Guyana uplands on the border with Brazil and Guyana (5°5–15' N; 60°40–50' W). The quartzitic sandstone formation is the highest point of this area, up to 2723 m. Samples were collected from rockpools (Ro1), from rock

surfaces (Ro2) and from the extremely sun-exposed plateau (Ro3), from a moist shaded rock surface at 1700 m in a cloudforest (Ro4) and on a south-east-facing hillside at 1500 m in a dry savannah on sun-exposed rock surfaces (Ro5).

(2) The Auyan-tepui in the western Guyana uplands (5°40'–6°10' N; 62°20'–50' W) is up to 2450 m high. Samples were collected on the plateau from a rock pool (Au1), from rock surfaces (Au2 and Au3), and from the shaded bottom of a rock (AuSh) at 2000 m. Additional populations were sampled near the village of Canaima at 800 m next to the river Caroni (Au4).

(3) In Puerto Ayacucho, which is located in the Orinoco lowlands at the periphery of the Guyana shield, cyanobacterial communities were sampled from rockpools (Pa1) and furrows (Pa2) at 80 m on a granite inselberg (5°35' N; 67°32' W).

Species determination

Determination of the cyanobacterial species mainly followed Geitler (1932), Golubic *et al.* (1981), Anagnostidis & Komárek (1985, 1988, 1990), Komárek & Anagnostidis (1986, 1989), Hoffmann (1991) and Büdel *et al.* (1994) and was performed with biofilms and crusts mounted in water under a light microscope. Species composition was estimated in percentage terms from five samples of each population using five different classes (< 2%, > 2%, > 20%, > 40%, > 70%; Table 1). Photographs were taken with Kodak Ektachrome 64 T films.

Sampling

Samples of the different cyanobacterial populations were collected by scraping the cyanobacterial mats carefully off the rock with a knife. From each population between 2 and 52 different samples were taken (see Table 3), each covering an area of 2 cm². The samples were dried rapidly over silica gel and stored in a dark container over silica gel during transport to the laboratory.

Shading experiment

Irradiance was adjusted to 8%, 15%, 25% and 50% of the incident photosynthetically active radiation (PAR) by



Fig. 1. Location of the study sites within Venezuela.

Table 1. Species composition of populations investigated from different sites and altitudes. The relative abundance is given in categories of at least 70%, 40%, 20%, 2% or less than 2%

Species	Population (Altitude, m)														
	Pa1 (80 m)	Pa2 (80 m)	AuSh (2000 m)	Au1 (2000 m)	Au2 (2000 m)	Au3 (2000 m)	Au4 (800 m)	Ro1 (2700 m)	Ro2 (2700 m)	Ro3 (2700 m)	Ro4 (1700 m)	Ro5 (1500 m)			
<i>Gloeocapsa sanguinea</i>	< 2%	< 2%	> 20%	> 20%	> 2%	< 2%	> 2%	< 2%	> 2%	< 2%	> 2%	> 2%			
<i>Schizothrix</i> sp.	> 70%	> 70%										< 2%			
<i>Scytonema crassum</i>	> 2%	> 20%			> 2%			< 2%				< 2%			
<i>Scytonema hofmannii</i>	> 2%	> 20%			> 2%			< 2%				< 2%			
<i>Scytonema myochrous</i>															
<i>Scytonema ocellatum</i>	> 2%	> 2%			> 2%			< 2%				< 2%			
<i>Stigonema hormoides</i>					> 70%			> 20%				> 20%			
<i>Stigonema manillosum</i>					> 70%			> 20%				> 20%			
<i>Stigonema ocellatum</i>	> 2%				> 70%			> 70%				> 40%			
<i>Stigonema panniforme</i>															
<i>Xenococcus</i> sp.	< 2%	< 2%	< 2%				< 2%	> 70%	> 70%	> 70%	> 70%	< 2%			

Pa, Puerto Ayacucho; Au, Auyan-tepui; Ro, Roraima-tepui.

various combinations of filter foils (Nos. 201, 209, 211, 226 and 253; LEE Filters, Hampshire, UK) attached to aluminium frames (40 × 40 cm). The frames were placed 12 cm above the crusts and films on the inselberg at Puerto Ayacucho. Air circulation and heat exchange below the filters were sufficient to keep the temperature of the shaded cyanobacteria at less than 2 °C above that of unshaded controls. Filters were removed during the night. The crusts were frequently wetted by rainfall, dew and runoff water. Three samples of each treatment were taken every day for 6 days.

Pigment analysis

Before extraction, sand and soil particles were removed as completely as possible from the cyanobacterial mats. Since even a small amount of remaining contamination would strongly interfere with the determination of dry weight, the carbon (C) content of a fraction of each sample (15–30 mg) was determined by elemental analysis (CHN-O-Rapid, Heraeus, Hanau, Germany). Contamination-free cyanobacterial populations had a carbon content of 40.6% (SD = 2.3%, $n = 14$). The dry weight of all samples was calculated from their C content, assuming 40% C content.

The cleaned samples were moistened with distilled water (10 times the sample dry weight), frozen in liquid nitrogen and ground in a mortar with quartz sand, about 1.5 ml acetone and a small amount of MgCO₃ as a buffer. Prior to grinding, a defined volume of a methanolic solution of chlorophyll (Chl) *b* (Sigma, Deisenhofen, Germany) was added as internal standard. Recovery of the standard was regularly about 90%. The homogenate was sonified (Sonic Power Sonifier, Branson Instruments, Danbury, USA) for 5 min and incubated for 2 h at 4 °C under N₂ in darkness. Subsequently, the extract was centrifuged at 14000 × *g* and the pellet resuspended two or three times in acetone (100%) allowing an extraction period of 0.5 h each time. The acetone concentration of the unified supernatants was adjusted to 80% by adding an appropriate amount of water. The extract was filtered through a PTFE membrane (0.2 μm pore size) and stored under N₂ at 4 °C in darkness until the HPLC analysis within 1 h of extraction.

HPLC analysis followed the procedure described in Ehling-Schulz *et al.* (1997). Standards for identification and quantification of pigments were either purchased (Chl *a* from Sigma, β-carotene from Merck, canthaxanthin and zeaxanthin from Roth, Karlsruhe, Germany) or prepared by TLC (myxoglycosides and scytonemin) according to the method described by Garcia-Pichel & Castenholz (1991). Echinenone was a gift from Prof. F.-C. Czygan, Würzburg. Extinction coefficients used in calibration were from Davies (1976) and Porra *et al.* (1989). Absorbance of the standards was determined using a UVIKON 930 spectrophotometer (Kontron, Eching, Germany).

Radiation measurements

PAR was measured with recently calibrated 2π quantum-sensors (LI-190SA, Li-Cor, Lincoln, NE, USA) and a datalogger (LI-1000, Li-Cor). Values were measured every 10 s and minimum, maximum and average values were stored every 10 min.

Statistical analyses

The data were analysed in Shapiro-Wilks and ANOVA tests with sampling locations grouped in three classes as fixed factors and checked assuming equal variances by Scheffé's post-hoc test. The non-parametric data of the canthaxanthin/ β -carotene ratio were tested by Kruskal-Wallis ANOVA.

Results

The cyanobacterial mats collected were composed of different species (Table 1). However, each population was dominated by a single species with at least 70% relative abundance: *Stigonema ocellatum* on the Roraima-tepui, *Stigonema hormoides* on the Auyan-tepui and *Scytonema crassum* on the inselberg at Puerto Ayacucho. *Gloeocapsa sanguinea* was present in almost all populations, but its abundance varied.

Daily light exposure measured at the surface of the cyanobacterial films during the experimental period varied depending on weather conditions from 10 to 70 mol m⁻² day⁻¹ (Pto. Ayacucho: mean = 34.8; SD = 22.15; n = 6; Auyan-tepui: mean = 50.65; SD = 23.66; n = 4). Peak irradiances of 2500–3400 μ mol m⁻² s⁻¹ were common.

A typical chromatogram from an HPLC analysis of an extract of *Scytonema crassum* from Puerto Ayacucho is shown in Fig. 2. Retention times and the maxima of online absorbance spectra are given in Table 2. Peaks 1 and 5–10 were identified by their absorbance maxima and by comparison with standards which were purchased or prepared by TLC. The carotenoids eluting as peaks 2 and 4 had an apparently identical chromophore. On the basis of their relatively short retention time and their R_f-value in a TLC system (Garcia-Pichel & Castenholz, 1991), they were tentatively identified as myxolglycosides. Carotenoid 3 showed a bathochromic shift of the absorbance maximum and a pronounced

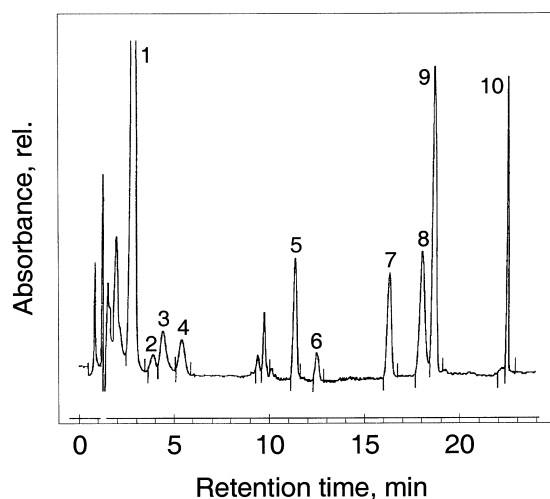


Fig. 2. HPLC chromatogram of an extract of a *Scytonema crassum* dominated mat from Puerto Ayacucho. The numbers refer to the pigments indicated in Table 2.

Table 2. Pigment number, retention time and online absorbance maxima for the pigments separated by HPLC. Wavelengths for shoulders in the absorbance spectra are indicated in parentheses

No.	Pigment	Retention time (min)	Absorbance maxima (nm)
1	Scytonemin	2.9	391
2	Myxolglycoside 1	4.0	452, 479, 509
3	Ketomyxolglycoside-like	4.5	(466), 488, 508
4	Myxolglycoside 2	5.5	452, 480, 508
5	<i>Trans</i> -canthaxanthin	11.40	476
6	<i>Cis</i> -canthaxanthin	12.4	372, 468
7	Chlorophyll <i>b</i> (internal standard)	16.3	460
8	Echinenone	18.1	462
9	Chlorophyll <i>a</i>	18.7	431
10	β -Carotene	22.5	(435), 452, 480

loss of spectral fine structure relative to 2 and 4. These characteristics are indicative of a ketocarotenoid (Britton, 1991) and we assume that this carotenoid is a ketomyxolglycoside (Francis *et al.*, 1970). Peak 6 was also present in solutions of commercially available canthaxanthin and was enhanced upon light exposure of these solutions. From this and its spectral characteristics, it was identified as a *cis*-isomer of *trans*-canthaxanthin and it was added to the content of *trans*-canthaxanthin. Note that Chl *b* was added as an internal standard. Extracts without the addition of Chl *b* verified the absence of Chl *b* in the cyanobacterial film, and so excluded contamination by green algae. Peak 1 was attributed to scytonemin, an ultraviolet-protective cyanobacterial pigment (Garcia-Pichel *et al.*, 1992). A zeaxanthin standard eluted with a retention time of 9.5 min, and a small peak appeared in the chromatograms of some samples at this time (cf. Fig. 2). This peak was too small to be identified as zeaxanthin by its absorbance spectrum and its area was always less than 1% of the summed area of the remaining carotenoid peaks. Based on its absorbance spectrum, the peak at 9.8 min was not a carotenoid but probably a degradation product of scytonemin. Despite the variability in species composition (Table 1), the qualitative composition of the pigment contents from the different locations was remarkably similar. All carotenoids were found in all samples with the exception of myxolglycoside 1 (peak 2) which was missing in three samples from Roraima-tepui and Auyan-tepui (Table 3).

In contrast to the qualitative uniformity of the pigment composition, the quantitative content varied substantially among different sites and samples. Carotenoid contents ranged from *c.* 100 to 1400 nmol g⁻¹ dry weight (DW), and Chl *a* contents from 72 to 830 nmol g⁻¹ DW (Table 3). The highest

Table 3. Average contents (\pm standard deviation) of carotenoids and chlorophyll *a* per unit dry weight (nmol g⁻¹ DW), mean content (\pm standard deviation) of carotenoids for each group of sites (nmol g⁻¹ DW and nmol g⁻¹ Chl), and ratio of canthaxanthin: β -carotene (mol mol⁻¹)

Population	(n)	Myxolglycoside 1	Ketomyxolglycoside-like	Myxolglycoside 2	Canthaxanthin	Echinenone	β -Carotene	Chlorophyll <i>a</i>	Canthaxanthin/ β -carotene
<i>'Shade'</i>									
AuSh	7	5 \pm 1	10	20 \pm 19	15 \pm 7	23 \pm 7	20 \pm 7	72 \pm 22	1.2 \pm 0.1
Ro4	2	30	179	36	62	117	59	280	1.1
Mean (g ⁻¹ DW)	9	18 \pm 14	123 \pm 107	24 \pm 20	25 \pm 22	44 \pm 42	28 \pm 19	118 \pm 94	
Mean (g ⁻¹ Chl)	9	0.09 \pm 0.0	0.47 \pm 0.3	0.23 \pm 0.2	0.21 \pm 0.1	0.34 \pm 0.1	0.26 \pm 0.0		0.8 \pm 0.3
<i>'Partial sun'</i>									
Pa1	52	80 \pm 41	219 \pm 64	163 \pm 44	197 \pm 68	270 \pm 59	171 \pm 41	646 \pm 140	1.2 \pm 0.1
Pa2	28	161 \pm 46	202 \pm 63	161 \pm 60	263 \pm 51	336 \pm 66	225 \pm 48	829 \pm 185	1.2 \pm 0.1
Au1	2	26	42	25	49	47	30	94	1.6
Au2	8		125 \pm 36	38 \pm 17	86 \pm 33	79 \pm 15	53 \pm 11	169 \pm 38	1.6 \pm 0.1
Au4	7	35 \pm 15	40 \pm 12	25 \pm 16	59 \pm 12	65 \pm 12	27 \pm 7	184 \pm 29	2.2 \pm 0.1
Mean (g ⁻¹ DW)	97	101 \pm 59	191 \pm 80	141 \pm 67	195 \pm 85	256 \pm 104	165 \pm 73	620 \pm 259	
Mean (g ⁻¹ Chl)	97	0.15 \pm 0.1	0.34 \pm 0.2	0.23 \pm 0.1	0.32 \pm 0.1	0.42 \pm 0.0	0.26 \pm 0.1		1.3 \pm 0.5
<i>'Full sun'</i>									
Ro1	2		28	28	66	43	19	90	3.3
Ro2	4	7	55 \pm 38	75 \pm 26	30 \pm 3	30 \pm 5	20 \pm 3	73 \pm 11	1.6 \pm 0.1
Au3	7	26 \pm 16	18 \pm 11	30 \pm 15	47 \pm 8	22 \pm 4	9 \pm 2	72 \pm 12	5.3 \pm 0.1
Ro3	3		250 \pm 97	45 \pm 24	118 \pm 21	86 \pm 17	28 \pm 4	167 \pm 31	4.2 \pm 0.0
Ro5	2	6	100	202	208	75	31	251	6.8
Mean (g ⁻¹ DW)	18	21 \pm 16	77 \pm 98	51 \pm 47	75 \pm 60	43 \pm 28	18 \pm 9	110 \pm 64	
Mean (g ⁻¹ Chl)	18	0.27 \pm 0.2	0.61 \pm 0.5	0.51 \pm 0.3	0.64 \pm 0.2	0.38 \pm 0.1	0.18 \pm 0.1		4.3 \pm 2.1

Pa, Puerto Ayacucho; Au, Auyan-tepui; Ro, Roraima-tepui.

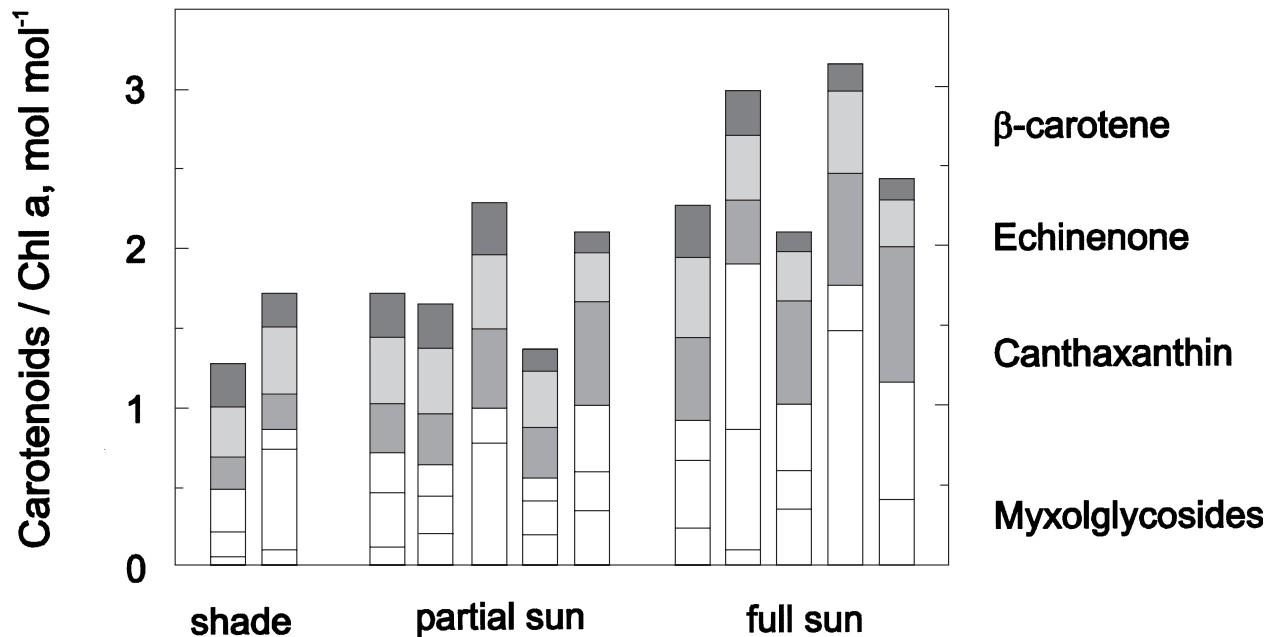


Fig. 3. Carotenoid/chlorophyll *a* ratio in sites with different light exposure. Each bar represents a different collecting site. Myxolglycosides include pigments 2, 3 and 4 (Fig. 2) and the bars are subdivided in this sequence, beginning at the bottom. In bars with only two divisions, pigment 2 (myxolglycoside 1) is missing.

contents were found in the *Scytonema crassum*-dominated mats from Puerto Ayacucho. Calculated on a surface area basis, the latter crusts possessed 133 mg Chl *a* m⁻² (SD = 40; *n* = 5), the Auyan-tepui crusts (Au2–Au4) 211 mg Chl *a* m⁻² (SD = 50; *n* = 10) and crusts in which *Gloeocapsa sanguinea* represented more than 20% (AuSh and Au1) contained 122 mg Chl *a* m⁻² (SD = 15; *n* = 11; see also Büdel, 1999).

The individual sampling locations at each site were grouped into three categories with different light exposure: 'shade', 'partial sun', and 'full sun' (Table 3). 'Shade' comprised two samples: one from a 1.5 m² large underside of a rock at the Auyan-tepui (AuSh) and the other from a moist, steeply inclined overhang within a cloudforest at the Roraima-tepui (Ro4). The group is characterized by permanent shading through forest vegetation and obstructing rock surfaces. The group 'full sun' included populations that were never shaded during the day: sun-exposed rock surfaces in a dry savannah (Ro5) and on the elevated plateau of Roraima-tepui (Ro1, Ro2 and Ro3) and exposed rock surfaces at the Auyan-tepui. The remaining populations were assigned to the group 'partial sun' because they were exposed but surrounded by shrubby vegetation and rock formations within 10 m. Thus these sites were temporarily shaded, particularly when the sun was low.

The pigment contents related to dry-weight (Table 3) showed no significant differences among the groups of sites with different degrees of shading. However, when carotenoid contents were expressed on a Chl *a* basis or related to each other, some trends became apparent (Fig. 3). Canthaxanthin/

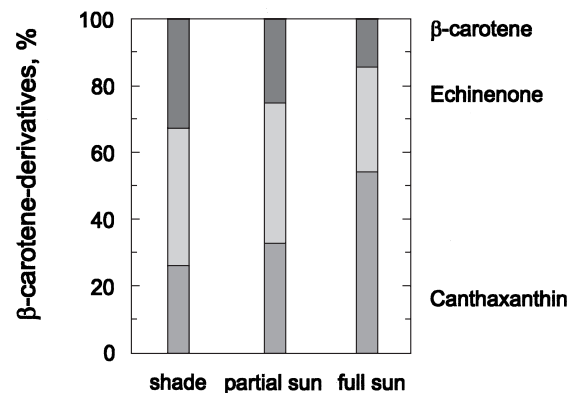


Fig. 4. Mean of the composition of β -carotene derivatives as percentage of their total content in sites with different light exposure.

Chl *a* rose significantly from 'shade' to 'partial sun' to 'full sun' (Scheffé's post-hoc test: $p \leq 0.005$) and all three myxoxanthophylls and canthaxanthin were significantly higher in 'full sun' than in the two other groups ($p \leq 0.0001$). However, with the exception of canthaxanthin, there was no difference between 'shade' and 'partial sun'. β -Carotene was significantly lower in 'full sun' than in 'shade' or 'partial sun' (Table 3) and the relative proportion of β -carotene to other β -carotene derivatives declined in full sun exposure ($F = 23.67$; d.f. = 2, 122; $p \leq 0.0001$), although such a trend was not obvious for this pigment when its contents were related to Chl *a* (Fig. 3). A rising tendency in favour of canthaxanthin could be observed with increasing exposure ($F = 108.75$; d.f. = 2, 122; $p \leq 0.0001$; Fig. 4). The average ratio of canthaxanthin to β -carotene rose from 0.8 and 1.3 in the two groups 'shade' and 'partial sun' respectively to 4.3 in

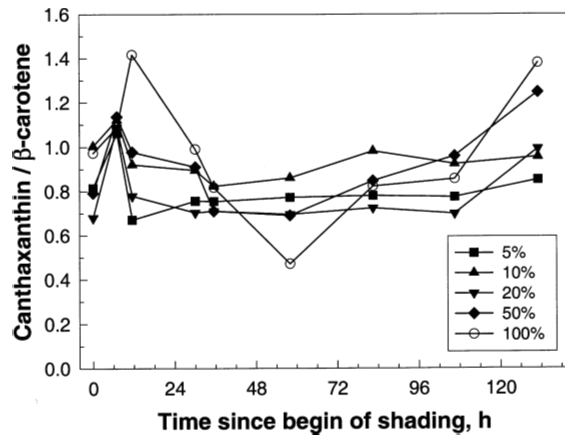


Fig. 5. Ratio of canthaxanthin/ β -carotene (mol mol⁻¹) in different shading treatments (light exposure indicated as a percentage of incident irradiance). The experiment started at 0600 hours, and samples were taken from site Pa2 around midday and at sunset on days 1+2 and at sunset on days 3–6.

the group 'full sun' ($F = 45.85$; d.f. = 2, 122; $p \leq 0.0001$; Table 3).

In order to test the short-term influence of irradiance on pigment contents, six subcommunities at Puerto Ayacucho were shaded to different degrees and sampled over 6 days. The time course of the ratio between canthaxanthin and β -carotene is shown in Fig. 5. Apart from some oscillations in the control under a fully transparent filter and the 50% shaded samples, the ratio remained constant over six light periods. Also, for all other pigment contents, no significant changes were found.

Discussion

In this study we searched for light-exposure-dependent differences in carotenoids of cyanobacteria at extreme locations in Venezuela. When the carotenoids were related to Chl *a*, we found a significant increase in all pigments at the highest solar exposure, whereas most pigments showed no difference between the groups 'shade' and 'partial sun'. While the locations defined as 'shade' and 'full sun' were clearly separated, since they either received no direct solar radiation or were continually exposed to the sun, the exposure of the group 'partial sun' was less well defined. These sites were shaded during parts of the day. Since cyanobacteria are poikilohydric, they can be in a dry and, hence, metabolically inactive, state during extended periods of time. Only during the active periods is acclimation possible. Throughout most of their phases of activity, cyanobacteria in 'partial sun' will be in shaded conditions, but they dry out rapidly and soon become inactive during the occasional sunny periods (cf. Lange et al. 1999). Their adaptation, therefore will be to shade and

not to sunny conditions. This could account for the insignificant differences between 'shade' and 'partial sun'.

Canthaxanthin was the only carotenoid that already showed an increase in 'partial sun'. The data presented here provide further evidence that formation of canthaxanthin is stimulated by high irradiance and that this response is present in a number of different cyanobacterial genera (Leisner et al., 1994; Lange et al., 1999). Originating from one of the most sun-exposed locations on earth, the investigated cyanobacterial films showed canthaxanthin/ β -carotene ratios of 4 to 6, which are among the highest recorded. At locations in Germany, with a more moderate irradiance level, Lange et al. (1999) observed ratios between 0.3 and 2.0 during the course of a year for a *Nostoc*-containing lichen, whereas Bilger et al. (1997) found values between 0.2 and 1.0 for field-collected *Nostoc commune*. For five different cyanobacterial lichens from Colorado, USA, Adams et al. (1993) reported ratios between 0.1 and 3.0 in thalli from the most shaded and the most exposed location, respectively. On the other hand, the data from Venezuela compare well with ratios for cyanolichens from the Negev desert, another extremely exposed location, where values of about 5 were observed (Leisner et al., 1994). Furthermore, within the present dataset, an increase in canthaxanthin/ β -carotene ratios with increased exposure was apparent. The ratios ranged from 1.1 in the most shaded samples to a maximum of 6.8 in an extremely irradiated sample. Of course, with the exception of the above-mentioned *Nostoc* data collected in Germany (Lange et al., 1999, Bilger et al., 1997), different genera are being compared, and some of the differences in canthaxanthin/ β -carotene ratios might be caused by species-specific differences in pigment biosynthesis. In fact, it is not possible to determine whether the observed trends were due to genetic adaptation or phenotypic modification.

The short term time course in the ratio between β -carotene and canthaxanthin following shading (Fig. 5) gives no indication of short-term acclimation of carotenoid contents in the mats dominated by *Scytonema crassum*, in contrast to observations with the cyanobacterial lichens *Peltigera rufescens* and *P. praetextata* (Leisner et al., 1994). Hence, canthaxanthin formation in the slowly growing cyanobacterial population in this study is either genetically fixed or acclimation operates over a longer time period than 6 days. Under natural conditions it was not possible to increase irradiance even further and one should consider that our observations pertain only to acclimation to a reduction in irradiance. A sudden increase in irradiance could have caused much more rapid pigment changes.

We tentatively identified three different myxolglycosides, of which one may have been myxol- α -L-chinovoside, which is identical with myxoxanthophyll (Foss *et al.*, 1986). In addition to myxoxanthophyll, several other myxolglycosides have been found in cyanobacteria (Hertzberg *et al.*, 1971; Foss *et al.*, 1986; Aakermann *et al.*, 1992; Karsten & Garcia-Pichel, 1996). The myxolglycosides also showed irradiance-dependent acclimation, although the pattern was less pronounced. There are numerous reports of high-light-induced increases in myxoxanthophyll contents in various cyanobacterial genera (Millie *et al.*, 1990; Nonnengieser *et al.*, 1996; Bilger *et al.*, 1997; Steiger *et al.*, 1999). One report indicates that low temperature inhibits accumulation of myxoxanthophyll in thylakoid membranes (Ivanov *et al.*, 2000). The presence of ketomyxolglycoside in all samples may indicate that the ketolase, presumably involved in echinenone formation (Lagarde *et al.*, 2000), can use myxol as substrate as well. High-light-induced ketomyxolglycoside formation was also observed in cultures of *Nostoc* sp., isolated from *Peltigera rufescens* (M. Woitke and W. Bilger, unpublished).

The present data point to a photoprotective function of canthaxanthin. Very recently, Albrecht *et al.* (2001) showed that *Synechococcus* PCC7942 transformed to synthesize canthaxanthin was significantly better protected against high-light-induced damage of the photosynthetic apparatus than the wild-type, which cannot form canthaxanthin. Our data suggest that the formation of canthaxanthin may be a photoprotective strategy widely distributed in terrestrial cyanobacterial genera.

Acknowledgements

This research was supported by the Deutsche Forschungsgemeinschaft (BU666/5-1), the Sonderforschungsbereich 251 of the University of Würzburg, the Schimper Stiftung and the Hermann-Willkomm Stiftung. The authors thank Dr Otto Huber (Universidad Simón Bolívar, Caracas, Venezuela) and Dr Ernesto Medina (Instituto Venezolano de Investigaciones Científicas) for their cooperation and logistical help, Markus Woitke and Dr Rainer Wirth for discussion and help on many occasions, Matthias Bohuschke for help with the HPLC, Elfriede Reisberg for CHN analyses, and Dr Jon Swenson for linguistic corrections.

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