Effect of Light on β -Carotene Accumulation in *Blakeslea trispora*

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SUMMARY

Cultures of a plus and a minus strain of *Blakeslea trispora*, grown either separately or together in light, accumulated only 40% of the β -carotene of corresponding cultures grown in darkness. The trisporic acids which accumulated in culture media of plus and minus strains grown together in light differed in their extinction spectra from the trisporic acids in the medium of cultures grown in darkness. Exposure of a trisporic acid extract from dark-grown cultures to light altered its extinction spectrum and decreased its carotenogenic activity. Only the ultraviolet portion of the spectrum of the lamps employed brought about this change in the extinction spectrum of the trisporic acid extract. The visible portion of the spectrum, however, was able to depress β -carotene accumulation in cultures without bringing about a change in the extinction spectrum of the trisporic acids produced. It seems likely that the formation of trisporic acids with altered extinction spectra is not responsible for the decrease in the accumulation of β -carotene.

INTRODUCTION

Blakeslea trispora Thaxter, a zygomycete, order Mucorales, is heterothallic. Plus (+) and minus (–) strains grown separately synthesize small amounts of β -carotene. Plus and minus strains grown together (+/–) reproduce sexually, synthesize more β -carotene than separate + and – strains (Ciegler, 1965; Plempel, 1965; Sutter & Rafelson, 1968) and produce a family of acidic compounds termed β -factor or trisporic acids (Prieto, Spalla, Bianchi & Biffi, 1964; Sebek & Jager, 1964; Caglioti *et al.* 1966). Trisporic acids enhance β -carotene production when added to cultures of the – strain (Prieto *et al.* 1964; Sebek & Jager, 1964; Thomas, Harris, Kirk & Goodwin, 1967; Sutter & Rafelson, 1968; van den Ende, 1968) and are postulated to be sex hormones in *B. trispora* (van den Ende, 1968; Austin, Bu'Lock & Gooday, 1969; Reschke, 1969; van den Ende, Wiechmann, Reyngoud & Hendricks, 1970). This report is on the effect of light on β -carotene accumulation in +, –, and +/– cultures and on the trisporic acids produced by +/– cultures.

METHODS

Strains. Plus and minus strains of *Blakeslea trispora* (USDA, Northern Regional Research Laboratories, no. 9216 and 9159, respectively) were obtained from Dr George Scherr of Colab Laboratories, Chicago Heights, Illinois.

Medium and culture methods. Maintenance of cultures and culture techniques have been described elsewhere (Sutter & Rafelson, 1968). Cultures were grown in 500 ml.

Erlenmeyer flasks containing 150 ml. of medium composed of (% w/v): potato extract, 1.25; glucose, 2.0; thiamine hydrochloride, 0.0002. Cotton plugs were used on culture flasks. Incubation was at 250 rev./min. on an inch stroke Gyrotory-Incubator Shaker Model G-25 (New Brunswick Scientific Co. Inc., New Jersey) at 28°. Cultures grown in darkness were in flasks wrapped with three layers of aluminium foil, which was also placed around the tops of flasks of cultures grown in light.

Light sources and illumination procedures. Cool white, white, red and blue fluorescent (F15T8-CW, F40W, F40R, F40B, respectively) and red incandescent (40 W) lamps were made by Sylvania (Salem, Massachusetts) and black fluorescent (F40T12-BLB) and sun (275 W) lamps by General Electric (Schenectady, New York). Light sources were switched on 5 min. before experiments. The spectral distribution of radiation between 380 and 1100 nm. from light sources was measured with an ISCO (Instrumentation Specialties Company, Lincoln, Nebraska) Spectroradiometer Model SR. The instrument was calibrated with an ISCO Spectroradiometer Calibrator Model SRC with a lamp calibrated against a National Bureau of Standards lamp. The direct readout of the instrument was converted to intensity ($\mu\text{W}/\text{cm}^2$) by the appropriate correction factor. Intensity was plotted against wavelength to give a curve of spectral intensity. The intensity of radiation between given wavelengths was obtained by comparing the area under the corresponding portion of the spectral distribution curve with the total area beneath the curve.

Experiments on the effect of light on trisporic acid extracts were performed at 28° in 18 mm. $\times$ 150 mm. test-tubes which were capped with parafilm and contained 5 ml. of extract. Dark controls were wrapped in five layers of aluminium foil. In experiments with filters, a fan circulated air between the light source and filter to prevent destruction of the filter.

Extraction and analytical methods. The extraction and analysis of β -carotene and trisporic acids have been described elsewhere (Sutter & Rafelson, 1968). In preparing tris buffer extracts of trisporic acids, the chloroform extracts of culture filtrates adjusted to pH 2 were flash evaporated at 28°. The residue, which contained trisporic acids, was dissolved in 0.1 M-tris- H_2SO_4 (pH 7.5) and stored at -20°. The relative concentration of trisporic acids in tris buffer extracts was expressed as the total extinction at 328 nm. (i.e. the E_{328} reading times the dilution factor). The relative concentration of trisporic acids in +/— culture filtrates was expressed as the net extinction at 325 nm. (i.e. the E_{325} of the +/— culture filtrates less the E_{325} of the — culture filtrates). Extinction spectra were measured with a Hitachi Double Beam Spectrophotometer Model 124 and Recorder Model 165 (Coleman Instruments, Maywood, Illinois). All operations involving potentially light-labile compounds were performed under red fluorescent lamps. All experiments were performed at least three times.

RESULTS

Effect of light on β -carotene accumulation. The effect of continuous illumination by white fluorescent lamps upon growth, β -carotene accumulation and trisporic acids in *Blakeslea trispora* is shown in Table 1. The dry weights of +, —, and +/— cultures grown in light differed little from comparable cultures grown in darkness. However, +, —, and +/— cultures grown in light accumulated only 40 % of the β -carotene of comparable cultures grown in darkness. This observation suggests a similar mechanism

might be responsible for decreased β -carotene accumulation in both single and combined cultures. *Choanephora cucurbitarum* (Chu & Lilly, 1960) and *Blastocladiella emersoni* (Cantino & Horenstein, 1956) also accumulate less carotene when grown in light than in darkness. In contrast, most carotenoid-producing micro-organisms such as *Fusarium aquaeductuum* (Rau, Feuser & Rau-Hund, 1967), *Mycobacterium* species (Howes & Batra, 1970), *Neurospora crassa* (Harding, Huang & Mitchell, 1969), and *Phycomyces blakesleeanus* (Lilly, Barnett & Krause, 1957) accumulate more carotenoids when grown in light than in darkness.

Table 1. *Effect of light on growth, β -carotene and trisporic acid production by cultures of Blakeslea trispora*

Conditions	Strains	Growth†	β -Carotene‡	Trisporic acids	
				Culture filtrates§	Tris buffer extracts
Darkness	+	753	381	—	—
	—	821	303	—	—
	+/-	795	2550	0.71	2.62
Light*	+	851	161 (42 %)	—	—
	—	916	107 (35 %)	—	—
	+/-	783	1000 (39 %)	0.41	1.14

* Continuous illumination by cool-white fluorescent lamps. Intensity, 413 μ W/cm.²; wavelengths, 380 to 725 nm.

† Dry weight of mycelium (mg.) after 5.2 days growth.

‡ μ g. β -Carotene/g. dried mycelium. Yields in light are also expressed as a percentage of corresponding yields in darkness.

§ Net extinction at 325 nm. of culture filtrate diluted 20-fold with tris buffer.

|| Extinction at 328 nm./extinction at 258 nm.

Effect of light on trisporic acids accumulation. Plus/minus cultures grown in darkness appeared to accumulate twice as much trisporic acid as cultures grown under fluorescent lamps, as judged by the extinction at 325 nm. of their culture filtrates. However, when the spectra of tris buffer extracts of trisporic acids were examined, it was found that the trisporic acids from cultures grown under white fluorescent lamps had a spectrum different from that of the trisporic acids from cultures grown in darkness (Fig. 1). The ratio of extinction at 328 nm. to extinction at 258 nm. (E_{328}/E_{258}) of trisporic acid extracts from cultures grown in light was 1.14 instead of 2.62, the value with extracts from dark-grown cultures. The extinction at 328 nm. was decreased, the 328 nm. peak was shifted to 303 nm., and the extinction at 258 and 234 nm. was increased.

Ultraviolet irradiation of extracted trisporic acids. Light has been shown to alter the extinction spectrum of extracts of trisporic acids (Caglioti *et al.* 1966; van den Ende, 1967). Van den Ende (1967) observed that the alteration was accompanied by decreased sex hormone activity. To test if light also decreased carotenogenic activity, 8 ml. samples of filter-sterilized tris buffer extracts of trisporic acid ($E_{328} = 438$) were placed 36 in. from a sun lamp (which emits mainly u.v. radiation) for various times (0 to 300 min.) to obtain extracts with a series of different E_{328}/E_{258} ratios from 2.74 to 1.30. Three ml. of each extract were added to each of two 26 h. —cultures in 70 ml. medium in 250 ml. flasks. Cultures were incubated for 4 days and the amounts of β -carotene determined. The production of β -carotene decreased linearly with the E_{328}/E_{258} ratio

of added trisporic acid (Fig. 2). In another experiment with trisporic acid extracts with E_{328}/E_{285} ratios ranging from 2.5 to 0.8, β -carotene production also decreased linearly. Thus loss of carotenogenic activity accompanied the changes in the spectrum of irradiated trisporic acids. It was also shown that the rate at which the extinction spectrum was altered was dependent upon the concentration of trisporic acids, light intensity and the type of light-source employed.

Fig. 1

Fig. 2

Fig. 1. Extinction spectra of tris buffer extracts of culture filtrates from 5.2 day +/– cultures grown in the presence (dotted) and absence (solid line) of cool-white fluorescent light (intensity, $413 \mu\text{W}/\text{cm}^2$; wavelength, 380 to 725 nm.). Portions of tris buffer extract (7 ml.) were diluted 200-fold for measurements.

Fig. 2. Effect of near u.v. radiation upon carotenogenic properties of trisporic acids. Trisporic acid extracts ($E_{328} = 438$) were exposed to a sun lamp for 0 to 300 min. to obtain acids with different E_{328}/E_{258} ratios prior to their addition to – cultures.

Effect of visible radiation on trisporic acids and carotene. Since trisporic acids absorb only in the u.v. region of the spectrum, lamps not emitting u.v. radiation should not affect the extinction spectrum of trisporic acids. This was confirmed as no detectable changes occurred in the spectrum of extracts ($E_{328} = 1.2$) exposed to red fluorescent ($107 \mu\text{W}/\text{cm}^2$; 575 to 850 nm.) or red incandescent ($533 \mu\text{W}/\text{cm}^2$; 575 to 1100 nm.) lamps for 48 h. although extracts exposed to black fluorescent or sun lamps (both of which emit large amounts of u.v. radiation) exhibited rapid changes in extinction spectra. To test whether u.v. radiation alone was responsible for altering the spectrum of extracts, samples were exposed for 4 h. to light from one blue and two white fluorescent lamps which had been passed through filters to eliminate u.v. radiation. No detectable changes occurred in the spectrum of extracts when filters excluded radiation below either 475 or 400 nm. although extensive alteration occurred in controls not protected by filters (Table 2). Separate experiments showed that slight changes occurred in the spectrum of extracts exposed for 24 h. to light from which u.v. radiation

was excluded by a filter of Mylar W (DuPont, Delaware). This can be accounted for as Mylar W transmits and trisporic acids absorb slight amounts of radiation at 400 nm.

To determine if the decreased accumulation of β -carotene in $+/-$ cultures grown under white fluorescent lamps resulted from alteration of trisporic acids or whether the two events occurred independently, $+$, $-$, and $+/-$ cultures were grown in darkness and in light from which u.v. radiation was excluded by Mylar W. All the illuminated cultures accumulated less than half the β -carotene of comparable cultures in darkness (Table 3), a result similar to that obtained in experiments in which cultures were grown in light from which u.v. radiation was not excluded (Table 1). The trisporic acid extracts from both light-grown and dark-grown $+/-$ cultures, however, exhibited

Table 2. *Effect upon trisporic acids of light of various wavelengths and intensities*

Filters	Illumination†		Trisporic acids§ E_{328}/E_{258}	
	Intensity‡	Wavelength	Light	Darkness
None	150	380 to 725 nm.	1.25	2.61
Kapton 100H*	75	475 to 725 nm.	2.61	2.61
Mylar W*	148	400 to 725 nm.	2.61	2.60

* Names are registered trademarks of DuPont. Two sheets of filter were placed between two sheets of window glass over a black box containing the samples of trisporic acid.

† Two white and one blue fluorescent lights. Exposure time 4 h.

‡ Intensity in $\mu\text{W}/\text{cm}^2$.

§ $E_{328} = 1.2$.

Table 3. *Effects upon Blakeslea trispora of light from which u.v. radiation was excluded by Mylar W*

Conditions	Strains	Growth†	β -Carotene‡	Trisporic acids	
				Culture filtrates§	Tris buffer extracts
Darkness	+	823	329	—	—
	—	860	321	—	—
	$+/-$	774	3095	0.63	2.65
Light*	+	843	237	—	—
	—	975	114	—	—
	$+/-$	816	1350	0.61	2.35

* Continuous illumination by cool-white fluorescent lamps. Intensity, $290 \mu\text{W}/\text{cm}^2$; wavelengths, 400 to 725 nm.

† Dry weight of mycelium (mg.) after 5.3 days growth.

‡ $\mu\text{g. } \beta$ -Carotene/g. dried mycelium.

§ Net extinction at 325 nm. of culture filtrate diluted 20-fold with tris buffer.

|| Extinction at 328 nm./extinction at 258 nm.

similar extinction spectra. Thus less β -carotene accumulated in $+/-$ cultures grown in light from which u.v. radiation was excluded even though the nature and amounts of trisporic acids was unchanged by illumination. Therefore, the accumulation of less β -carotene and the alteration of the extinction spectrum of trisporic acids in $+/-$ cultures grown under white fluorescent lamps are independent events even though the change in extinction spectrum of extracted trisporic acids is accompanied by a reduced carotenogenic activity.

Possible relationship between trisporic acids and β -carotene accumulation. Trisporic acids are synthesized by – cultures (Sutter, 1970; van den Ende *et al.* 1970) and can stimulate carotenogenesis in – cultures. Since the latter reaction is inhibited by actidione (Thomas *et al.* 1967) and since labelled trisporic acids are not incorporated into β -carotene (Sebek & Jager, 1964), trisporic acids presumably act by inducing *de novo* synthesis of rate-limiting enzymes in the carotenoid pathway. It seems likely that in +/– cultures grown in light the trisporic acids synthesized by minus cultures induce carotenogenesis before being released into the medium where their extinction spectra are altered with a concomitant loss of biological activity. A remote possibility does exist that trisporic acids in the mycelium might be acted upon by visible light via an endogenous photosensitizer such as riboflavin prior to the induction step, thereby reducing carotene synthesis in +/– cultures. However, the percentage decrease in accumulation of β -carotene in all light-grown as compared with dark-grown cultures (+, –, +/–) is very similar. This strongly suggests that the same mechanism, such as increased destruction of β -carotene, is operative in all light-grown cultures.

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