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Lycopene, the Major Pigment of *Podosordaria leporina*

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The lipid-soluble pigments were extracted from the mycelium of *Podosordaria leporina* and analyzed by thin-layer chromatography and absorption spectroscopy. Lycopene was found to be the major pigment produced when mycelium is exposed to blue light.

INDEX DESCRIPTORS: Lycopene, *Podosordaria leporina,* Podosordaria Lycopene.

Pigments of a carotenoid nature have been known in the ascomycetes for a long time. Zopf reported such pigments in his 1890 treatise. Over the years pigment production and ascocarp formation by fungi have been observed simultaneously when the fungus was exposed to light. Hence, the two events were frequently linked and thought to be photochemically induced. A copious amount of literature exists concerning pigments of fungi but only those which involve carotenoids or the perfect or ascogenous stage are included here. Review articles by Goodwin (1952, 1965a, 1965b), Burnett (1965), Carlile (1965), and Page (1965) have been relied on heavily for much of the literature cited here.

Ascomycetes were first cited as producing carotene pigments by Zopf (1890) and by Kohl (1902), whose list included 10 ascomycetous species. Zopf first extracted carotenoid pigments from the ascomycete *Polystigma rubrum* (Persoon) DC. Later, Lederer (1938) confirmed the findings of Zopf. They found two pigments, one of which they identified as being lycoxanthin; the other remained unidentified (Goodwin, 1952). Much later Heim (1946) reported carotenoids in at least five discomycete species.

Much of the work on carotenoids in ascomycetes has been done on *Neurospora crassa* Shear and Dodge. Haxo (1949) utilized chromatographic and spectrophotometric procedures to determine that lycopene was the main orange pigment.

Zalokar (1954) found that when the mycelium of *Neurospora crassa* is exposed to light, the production of carotenoids is stimulated. He found that a 1 min. exposure was enough to bring about this effect. Later, Zalokar (1955) reported that the amount of carotenoid produced is proportional to the light dosage. He also reported that pigment production is temperature dependent and is induced by light of 400 to 500 nm wavelengths. About four molecules of carotenoids are produced for each quantum of light absorbed.

Light was shown to affect pigmentation in several species including *Pyronema confluens* Tul., *Fusarium aquaeductum* Lagh., and *Cephalosporium diospyres* Crandall (Carlile, 1965). In *Neurospora crassa* dark-grown cultures contained carotenoids only in the conidia; however, light was necessary for mycelial carotenoid synthesis. In darkness, colorless polyenes were present but their subsequent conversion to related unsaturated carotenoids required light and oxygen.

Garton, Goodwin, and Lijinsky (1951) found in *N. crassa* that cultures exposed to light produced normal amounts of beta carotene irrespective of the wavelength of light. When cultures were kept in daylight for three days and then placed in total darkness, they continued to produce carotene at the daylight rate. Cultures grown in complete darkness produced only half the amount of carotene of those grown in light (Garton, et al., 1951).

Formation of perithecia and mature ascospores of *Hypomyces solani* depends upon light (Snyder and Hansen, 1941). Perithecial primordia appeared abundantly on cultures exposed to light, whereas no primordia were formed on dark-grown cultures. Although they did not link the two events, they did mention that flesh-ochre and cinnamon-pink pigments developed in the presence of light.

Robinson (1926) showed that blue light is responsible for mycelial changes leading to development of a pink pigment and inception of reproductive bodies in *Pyronema confluens.* Twenty-four hours of 40 candlepower illumination at a distance of 50 cm will induce formation of normal apothecia, asci, and viable ascospores. This effect of light was not transmitted from illuminated portions of a mycelium to a shaded part. He concluded that the same photochemical events induce both pigmentation and reproductive bodies. He could find no difference in respiration rates of illuminated and shaded cultures and therefore did not believe that the pigment had a respiratory function. A culture of a white form of *P. confluens* established in the laboratory of Bean and Brooks (1932) required light for apothecial formation. These investigators therefore questioned the cause and effect relationship of pigmentation and reproductive abilities. More recently, Carlile and Friend (1956) have shown that apothecial production occurs when this species is grown in light of 300 to 400 nm. When grown in total darkness, the cultures remain vegetative. Since albino mutants are able to produce the sexual phase, they concluded that two distinct photochemical events occur, one leading to carotenoid synthesis, and the other to apothecial production. Although Carlile and Friend did not associate pigment production with the sexual phase, they have found that four carotenoids are produced by the mycelium, namely beta carotene, torulene, gamma carotene, and neotorulene.

Phototropic responses and yellow pigment production in the presence of light are reported for *Sordaria* sp. by Ingold and Hadland (1959). However, the pigment in this case was not identified and the quality of light was not limited to a specific spectral region.

Inhibitory effects are also known. Chona (1932) reports that light inhibits ascocarp production but enhances conidial production in *Aspergillus glaucus.* Tatarenko (1954) found that blue light depresses mycelial growth and the develop-

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ment of asci and sclerotia. Blue light does not allow the formation of red, orange, and yellow pigments in *Penicillium rubrum, A. amylovorus,* and *P. purpurogenum,* but stimulates conidial formation. Red light, in these cases, does not depress growth but fails to ensure pigmentation.

Valadin and Mummery (1968) in their carotenoid studies have extracted at least seven carotenoids from *Peziza aurantia.* Although they find variation in pigments under varying habitats, they do report alpha, beta, and gamma carotenes plus neurosporene, rubixanthin, aleuriaxanthin, and lycopene present in this discomycete.

Bindl et al. (1970) have identified sigma and gamma carotene, torulene, neurosporaxanthin, and lycopene in *Fusarium aquaeductum.* They have shown that production of carotenogenic enzymes is photo-induced in *F. aquaeductum.*

In this paper we report the results of chromatographic and spectrophotometric examination of the pigments of *Podosordaria leporina;* studies resulting from the discovery that the mycelium of this species produces a pinkish-orange pigment (Koehn, 1971).

Materials and Methods

All *P. leporina* cultures used in pigment analyses were derived from a single ascospore produced from a culture isolated from rabbit dung collected in Metropolitan Park, Austin, Texas, on April 25, 1971. A synthetic medium (Koehn, 1971) developed for this species was used throughout this study.

Using this medium the mycelial cultures were grown under controlled light and temperature conditions. The incubator-controlled light period was a 12-hour light cycle. Cool white, 20-watt, G.E. fluorescent bulbs located 25 cm above the plates provided 140 footcandles of light. An optimum temperature of 25°C was maintained throughout the growing period.

P. leporina cultures for pigment analysis were grown on dialyzing membranes placed over synthetic medium in 60 x 15 mm petri plates. After 10 or 11 days the membranes were lifted from the medium and the mycelium removed to a Ten-Broeck tissue homogenizer containing acetone. The acetone-mycelial mixture was homogenized at 0-5°C and the resulting suspension centrifuged to remove the hyphal debris. The supernatant was decanted and concentrated by vacuum evaporation. All of these procedures were performed in a temperature range of 0-5°C. The concentrated extract was then spotted on Eastman Kodak (#6061) Silica Gel G thin layer sheets with a developing solvent of benzene: petroleum ether (b.p. 38-47 C): methanol (1:1:0.01 v/v/v). This particular solvent system provided maximum separation of the pigments. Other solvent systems used to determine R_f values were benzene: petroleum ether (2:98 v/v) and ethyl acetate: methylene chloride (2:8 v/v). The sample was applied as a band extending across the entire thin layer sheets and allowed to develop in a saturated chamber at 28°C. The chromatograms were immediately removed to a cold chamber where the color bands were measured and their R_f values calculated.

Separated pigments were scraped from the plates and the samples extracted from the silica gel by various solvents selected for spectrophotometric analysis: absolute ethanol, benzene, and chloroform. A beta carotene sample purchased from Sigma Chemical Co., St. Louis, Mo., was used as a control. The first spectrophotometric studies were carried out on a Beckman DU spectrophotometer. Later studies were recorded on a Cary 14 spectrophotometer at Southwest Research Institute, San Antonio, Texas.

A second study involved a color filter experiment to determine what quality of light was needed to induce pigmentation. Corning color filters with light transmittance in the ranges of 360-450 nm (blue CS #5-58), 480-545 nm (green CS #4-64), and 600-650 nm (red CS #2-60) were placed over metal pans measuring 22 cm x 25 cm x 6 cm. A clear plate was used as a control. All other possible light was blocked by black tape and paint. Four cultures were placed under each light filter in the incubator, 100 cm from the 110-115 footcandle light source. The cultures were allowed to develop at their normal growth rates for 28 days. The cultures were then removed from the chambers in a dark room, observed under red light for various stages in the life cycle, and immediately extracted, using the same procedure described above. These extracts were later chromatographed and examined by spectral analysis.

Results

Chromatographic studies first revealed one major pigment band extracted from three mycelial colonies. Later, when the amount of mycelium was tripled and concentrated, three other weak bands appeared on the chromatograms. The major pigment band was designated as R_2; the others were designated as R_1, R_3, and R_4 according to their respective migration patterns.

An average of 65 chromatograms gave R_f values of 0.33, 0.73, 0.85, and 0.96, respectively, for R_1 through R_4. In this solvent system (benzene: petroleum ether: methanol), Davies (1965) listed the R_f value for lycopene as 0.74 and that of beta carotene as 0.82, indicating a possibility that these two pigments were present in the mycelial extract obtained from *P. leporina.* A second solvent system of benzene and petroleum ether gave R_f values of 0.16 for the major band and 0.80 for the third band, which compared with Davies' (1965) R_f values, lycopene 0.19 and beta carotene 0.82. A solvent system of ethyl acetate and methylene chloride gave R_f values of 1.0 for all pigment bands. Davies (1965) listed lycopene and beta carotene as having R_f values of 1.0. The results are summarized in Table 1.

TABLE 1. R_f VALUES FOR CAROTENE PIGMENTS FOUND IN *P. leporina*

Pigment	*Solvent* *ethyl acetate: methylene chloride (2:8 v/v)*	*benzene: petroleum ether (2:98 v/v)*	*benzene: petroleum ether: methanol (1:1:0.01 v/v/v)*
R_1	1.0	. .	0.33
R_2	1.0	0.16	0.725
R_3	1.0	0.80	0.85
R_4	1.0	. .	0.957
Lycopene*	1.0	0.19	0.74
Beta Carotene*	1.0	0.82	0.82

* According to Davies (1965).

The absorption peaks for the major pigment and those given by Davies for lycopene are compared in Table 2.

The light quality experiment indicated that light in the blue region of the visible spectrum was necessary to produce any pigment in the mycelium. Cultures grown under the blue wavelengths consistently produced pigment which, upon analysis, proved to be the same as those previously extracted. Cultures grown under the red and green portions of the spectrum did not produce pigment.

TABLE 2. PEAK ABSORPTION IN NANOMETERS OF THE MAJOR (R_2) PIGMENT COMPARED TO KNOWN VALUES FOR LYCOPENE

Solvent	*R_2, DU*	*R_2, CARY*	*Lycopene**
Ethanol	443		443
	472	472	472
	502	502	502
Chloroform	455		456
	485		485
	500		520
Benzene	487	490	487
		522	

* Values listed by Davies (1965).

DISCUSSION

Spectral data and R_f values indicate that lycopene is the major lipid-soluble pigment which accumulates in the mycelium of *P. leporina*. Light experiments support the previous contention that lycopene is produced only under the influence of blue light. At present it is still not known if the biosynthetic pathways leading to stromatal production and carotenoid production are interrelated. Recent work by Bindl, Lang, and Rau (1970) indicates that in *Fusarium aquaeductum*, the carotenogenic enzymes are photo-induced. However, their work does not indicate if reproductive processes are subsequently or simultaneously inducted. The function of lycopene and related carotenoids in *P. leporina* still eludes us.

R_f values for the minor pigment band, R_3, indicated that it might be beta carotene. However, absorption spectroscopy did not substantiate that conclusion. The other two minor bands could not be determined, but are thought to be intermediates or degradation products resulting from extraction procedures.

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