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CAROTENOID PIGMENTS OF *PROTOMYCES INUNDATUS*, DANGEARD

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Abstract—*Protomyces inundatus* contains α, β and γ-carotene, with β-carotene predominating. Total carotenoid pigments have been used as a characteristic in the taxonomy of certain fungi. It is suggested that if such pigments are to be used as a taxonomic tool, absorption spectra of the total extracts give very limited information and that one must know all the carotenoids present using a well-defined medium under strict cultural conditions to be of any use.

INTRODUCTION

DURING an investigation into the life history and taxonomic position of *Protomyces inundatus*,[1] it was observed that the cultures were pigmented, but no detailed observations were made on the pigments involved. Tubaki[2] extracted pigments from various species of *Taphrina* and *Protomyces* and examined the extracts spectrophotometrically and considered the significance of the results in relation to the taxonomy of the species concerned. He found that in contrast to *Rhodotorula*, carotenoid pigments appeared to be absent. In view of the possible taxonomic significance of pigmentation it was decided to re-investigate the pigment composition of *Protomyces inundatus*.

RESULTS

When the pigments in petroleum ether were chromatographed on a MgO–celite column, using increasing concentrations of acetone in *n*-hexane as the developing solvent, four bands appeared. The lowest band was a narrow yellow zone (Fraction 1); above it was the major pigment zone (Fraction 2), dark orange in colour eluted by 2% acetone in *n*-hexane, an orange-pink narrow band (Fraction 3) eluted by 4% acetone in hexane, while near the top of the column were two minor pink bands (Fraction 4) eluted by 5% acetone.

TABLE 1. ABSORPTION SPECTRA OF CAROTENOID FRACTIONS OF *P. inundatus* AND AUTHENTIC CAROTENES (IN VARIOUS SOLVENTS)

Carotenoid fraction	Wavelength of maximal absorption (mμ) in n/hexane	chloroform	carbon disulphide
Total carotenoid fraction	375, 425, 450, 475	—	—
Fraction 1	420, 445, 475	~425, 455, 485	475, 510
Authentic α-carotene	420, 445, 475	454, 485	477, 509
Fraction 2	~425, 450, 480	465, 500	~455, 484, 515
Authentic β-carotene	~425, 451, 482	466, 497	~450, 485, 520
Fraction 3	430, 460, 490	450, 475, 510	465, 495, 530
Authentic γ-carotene	433, 462, 490	447, 475, 508	463, 496, 533

~denotes an inflection.

[1] L. R. G. VALADON, J. G. MANNERS and A. MYERS, *Trans. Brit. mycol. Soc.*, in press.
[2] K. TUBAKI, *Mycologia* **49**, 44 (1957).

Fraction 1 had an absorption spectrum in various solvents (Table 1) which suggested that it was α-carotene. When mixed with a pure sample of α-carotene obtained from *Phycomyces blakesleeanus* the mixture could not be resolved on a MgO–celite column, and it can be concluded therefore that this Fraction is α-carotene.

Fraction 2. The absorption spectra of this fraction in various solvents (Table 1) suggested that it was β-carotene. When the fraction mixed with authentic β-carotene was rechromatographed on a MgO–celite column, no separation was observed. This fraction is therefore β-carotene.

Fraction 3. The absorption spectra of this fraction in *n*-hexane, chloroform and carbon disulphide (Table 1) suggested that it was γ-carotene. Furthermore, there was no separation when this was chromatographed with authentic γ-carotene obtained from *P. blakesleeanus*. This fraction is therefore γ-carotene.

Fraction 4. This pink zone was found in too small a quantity to be examined further, but from its behaviour on the chromatographic column it could conceivably be lycopene, a pigment commonly occurring in fungi.

Quantitative distribution. β-Carotene is the major carotene of *P. inundatus* forming 87 per cent of the total; α- and γ-carotene only comprise 5 and 8 per cent, respectively.

DISCUSSION

Tubaki[2] made a spectrophotometric examination of the total pigments of *Protomyces inouyei, P. lactucae-debilis, P. pachydermus* and compared these with *Taphrina weisneri* and *T. communis* using Ellinghausen and Pelczar's[3] method. He came to the conclusion that "members of the two genera resembled each other closely as to pigmentation". The Ellinghausen and Pelczar[3] method involves washing the cells with a 70% aqueous solution of acetone before extracting the pigments. Most carotenoids are soluble in aqueous acetone and this is suggested as one of the reasons why Tubaki[2] did not obtain any carotenoid pigments.

Following Lodder and Kreger-van Rij's[4] taxonomic use of the presence of carotenoid pigments for the subdivisions of the genus *Rhodotorula*, Peterson *et al.*[5] used such a characteristic "to determine the species of yeasts associated with different parts of the cucumber plant". Their "method A" resulted in the extraction of total carotenoid pigments the maximum absorption peaks of which in petroleum ether were in the region 450 and 480 mμ. They found that such total spectra formed the basis of a satisfactory screening procedure for the species they were considering. While methods based on total pigments are satisfactory for screening purposes, the results of Davies[6] who found that the absorption spectra of the total unsaponifiable fraction of *Rhizophlyctis rosea* 440, 465 and 493 mμ, consisting roughly of equal parts of γ-carotene (437, 462, 491·5 mμ) and lycopene (443, 469·5 and 500 mμ) indicate that the exact identification of pigments can be accomplished only after chromatographic separation. Whereas differences in the total spectrum may be useful as a basis of a quick screening method, it is felt that the presence or absence of specific pigments forms a much more certain basis for the elucidation of taxonomic relationships. Experimental conditions, however, have to be clearly defined as certain conditions are essential for pigment production.

[2] H. C. ELLINGHAUSEN, Jr. and M. J. PELCZAR, Jr., *J. Bacteriol.* **70**, 448 (1955).
[4] J. LODDER and N. J. W. KREGER-VAN RIJ, *The yeasts. A taxonomic study.* Interscience, New York (1952).
[5] W. J. PETERSON, T. A. BELL, J. L. ETCHELLS and W. W. G. SMART, Jr., *J. Bacteriol.* **67**, 708 (1954).
[6] B. H. DAVIES, *Phytochemistry* **1**, 25 (1961).

Rhodotorula resembles *P. inundatus* in possessing α- and β-carotene but, in addition, the former contains torularhodin, torulene and other carotenoids.[7] The absence of these pigments from *P. inundatus* tends to suggest a lack of relationship between *Protomyces* and *Rhodotorula* as far as pigmentation is concerned. This had already been suggested by Tubaki[2] on different grounds and a more thorough investigation of the pigments of *Taphrina* and other species of *Protomyces* is essential if one is to compare these two fungi. Valadon *et al.*[1] suggested that as *P. inundatus* is diploid for a large part of its life cycle while *Taphrina* is dicaryotic, the two genera are not closely related. If carotenoid pigments are to be of use in the taxonomy of fungi, one would expect any two genera would have different pigments; a study of the pigments of *Taphrina* and other species of *Protomyces* is therefore being carried out in this department.

EXPERIMENTAL

Diploid cultures of *P. inundatus* were grown either on Lilly and Barnett's[8] semi-synthetic liquid medium or on 2% malt, on a reciprocating shaker at 20°. After 15 days, the cultures were centrifuged, washed with distilled water four times and suspended in methanol. The pigments were extracted several times by "method A" of Peterson *et al.*[5] using methanol instead of acetone. Partition of the pigments between 40–60° light petroleum and aqueous methanol left all the pigments in the epiphase.

Column chromatography was carried out on a MgO-celite (1 : 1) mixture and developed with *n*-hexane containing increasing concentrations of acetone (Goodwin).[9]

The extracts of the pigment were examined in a Unicam SP 500 photoelectric spectrophotometer between 320 and 600 mμ. The $E^{1\%}_{1cm}$ of α-carotene at 445 mμ was taken as 2700, that of β-carotene at 450 mμ as 2560 and that of γ-carotene at 460 as 2720.[9] Pure α, β and γ-carotene were isolated from *Phycomyces blakesleeanus*.[10, 11]

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[7] M. W. MILLER, *The Pfizer handbook of microbial metabolites*, McGraw-Hill, New York (1961).
[8] V. G. LILLY and H. L. BARNETT, *Physiology of the fungi*, McGraw-Hill, New York (1951).
[9] T. W. GOODWIN, *Modern Methods of Plant Analysis*. Edited by K. PAECH and M. V. TRACEY. Vol. 3, Springer-Verlag. Heidelberg (1955).
[10] G. A. GARTON, T. W. GOODWIN and W. LIJINSKY, *Biochem. J.* **48**, 154 (1951).
[11] T. W. GOODWIN, *Biochem. J.* **50**, 550 (1952).