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SOME OBSERVATIONS ON THE CAROTENOGENESIS IN THE YEAST *RHODOTORULA MUCILAGINOSA*

P. MARGALITH and S. MEYDAV

Laboratory of Microbiology, Department of Food and Biotechnology,
Technion, Israel Institute of Technology, Haifa, Israel

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Abstract—The effect of some compounds on the carotenogenesis of *Rhodotorula mucilaginosa* was examined at growth subinhibitory levels. Ethanol, diphenylamine and isopropanol all reduced the torularhodin: torulene ratio, but only ethanol had a stimulatory effect on total carotenogenesis. Chloramphenicol was without effect on growth or carotenoid biosynthesis. These results are discussed in light of the current concepts on yeast carotenogenesis.

INTRODUCTION

THE biosynthesis of carotenoids in the carotenogenic yeasts of the genus *Rhodotorula* (Family *Cryptococcaceae*) has been the subject of numerous investigations. Essentially two pathways have been suggested. According to Bonner *et al.*[1] the principal yeast carotenoids, torulene, β-carotene and γ-carotene, are produced separately from one C_{40} precursor. However, Simpson *et al.*,[2] in a more recent paper, put forward evidence in favour of γ-carotene being the precursor of both β-carotene and torulene (Fig. 1). The carboxylic acid torularhodin, another major carotenoid of the yeast, was suggested to arise by oxidation of torulene, a C_{40}-aldehyde being suggested as intermediate. Simpson *et al.*[3] demonstrated the incorporation of one atmospheric oxygen atom into torularhodin by growing *R. rubra* in an enriched atmosphere of $^{18}O_2$.

The production of carotenoids by yeasts has assumed new interest since Schwartz and Margalith[4] have shown that supplementation of poultry feed with a preparation of *R. mucilaginosa*, instead of conventional food yeasts, promotes pigmentation of egg yolk. However, field experiments resulted in slight, undesirable violet hues in the yolks of eggs laid by chickens on a diet containing such pigmented yeast.

Since most of the colouring material derived from *Rhodotorula* is due to torulene and torularhodin, it was of interest to investigate the possibility of changing the ratio of these pigments by suitable techniques. We have reason to believe that a substantial reduction in the torularhodin content may solve the problem of the undesirable violet hue in yolks.

It is known that both the total carotenoid level as well as the quantitative relationship between the different pigments varies according to strain, media and culture conditions.

[1] J. BONNER, A. SANDOVAL, Y. W. TANG and L. ZECHMEISTER, *Arch. Biochem.* **10**, 113 (1946).
[2] K. L. SIMPSON, T. O. M. NAKAYAMA and C. O. CHICHESTER, *J. Bacteriol.* **88**, 1688 (1964).
[3] K. L. SIMPSON, T. O. M. NAKAYAMA and C. O. CHICHESTER, *Biochem. J.* **92**, 508 (1964).
[4] Y. SCHWARZ and P. MARGALITH, *J. Appl. Microbiol.* **13**, 876 (1965).

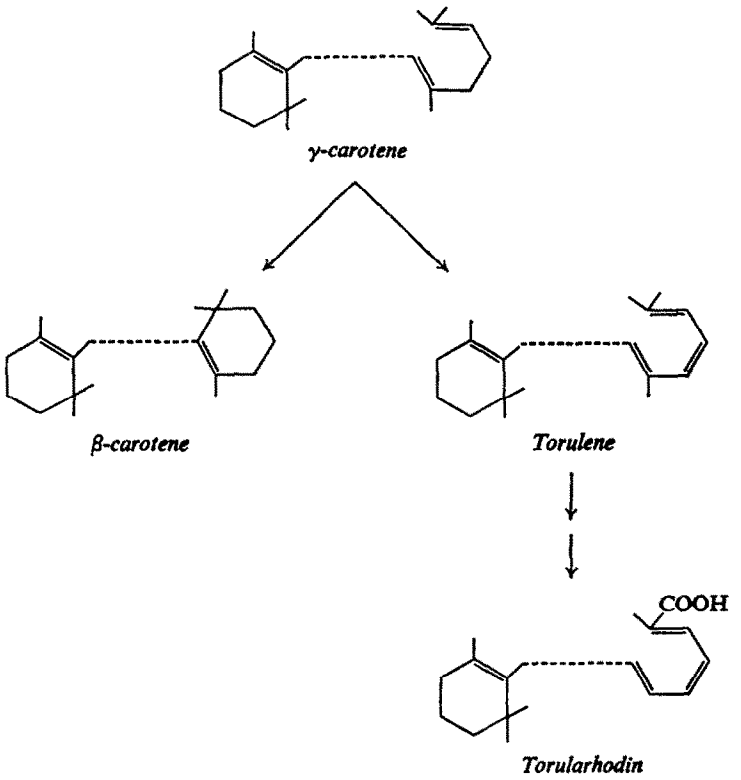

FIG. 1. BIOGENESIS OF YEAST CAROTENOIDS, AFTER SIMPSON *et al.*[2]

Peterson *et al.*[5] claimed that a high yield of total carotenoids (358 μg/g dry weight) with a comparatively low torularhodin/torulene ratio (TR/T) of 0·48 could be produced in *R. mucilaginosa* cultivated on a glucose–asparagine medium, while Villoutreix[6] obtained yields of 122 μg/g dry weight with a TR/T ratio as high as 6·12 from this yeast grown on a sucrose–ammonium sulphate medium. In our laboratory[7] yields of total carotenoids of 100–200 μg/g dry weight with a TR/T ratio of 0·8–1·0 were obtained employing a glucose–ammonium lactate medium. It seems likely that the nitrogen source has a considerable effect on the production of these pigments. Several specific compounds are also known to affect carotenoid biosynthesis both quantitatively and qualitatively. Diphenylamine has been shown to suppress carotenogenesis in a large number of mycelial fungi,[8] pigmented yeast[6] and a number of bacteria.[9] The inhibition of carotenogenesis by diphenylamine has become a standard procedure in the characterization of the biosynthesis of these compounds in various micro-organisms (e.g. *Mycobacterium marinum*,[10] *Blakeslea trispora*[11]). Some

[5] W. J. PETERSON, W. R. EVANS, E. LECCE, T. A. BELL and J. L. ETCHELLS, *J. Bacteriol.* **75**, 586 (1958).
[6] J. VILLOUTREIX, *Biochem. Biophys. Acta* **40**, 434 (1960).
[7] P. MARGALITH and M. A. FUCHS. Unpublished observations.
[8] T. W. GOODWIN, *Biochem. J.* **50**, 550 (1952).
[9] G. TURIAN and F. T. HAXO, *J. Bacteriol.* **63**, 690 (1952).
[10] M. M. MATHEWS, *Photochem. Photobiol.* **2**, 1 (1963).
[11] D. M. THOMAS and T. W. GOODWIN, *Phytochem.* **6**, 355 (1967).

observations on the effect of certain other compounds on the relative proportions of torularhodin and torulene in *R. mucilaginosa* are described below.

RESULTS

The effect of diphenylamine on the carotenogenesis in *Rhodotorula mucilaginosa* is shown in Table 1. Similar experiments were carried out with ethanol and isopropanol and, following the observations of Goodwin[12] and Thomas *et al.*,[13] it was interesting also to examine the effect of chloramphenicol. These results are also summarized in Table 1. In all cases inhibitors were used at both growth subinhibitory levels as well as concentrations that were previously found to be not, or only slightly, inhibitory to pigment formation.

TABLE 1. THE EFFECT OF VARIOUS COMPOUNDS ON CAROTENOGENESIS IN *R. mucilaginosa*
CAROTENOIDS μg/g DRY WEIGHT

Treatment	Dry weight (%)	Total	β-carotene	γ-carotene	Torularhodin	Torulene	Torularhodin / Torulene
Control	1·16	186	9	10	85	82	1·04
Diphenylamine (5 ppm)	1·13	102	21	24	14	43	0·33
Control	1·16	206	13	6	93	94	0·99
Ethanol (2% v/v)	1·11	244	20	1	55	168	0·33
Control	1·24	182	12	8	82	81	1·01
Isopropanol (1% v/v)	1·19	195	15	8	61	111	0·54
Control	1·18	186	10	7	85	84	1·01
Chloramphenicol (1000 ppm)	1·15	208	12	6	95	95	1·00

DISCUSSION

Very low concentrations of diphenylamine had no observable effect on the dry weight of the yeast, but carotenogenesis was considerably inhibited, mainly due to the reduction of torularhodin, resulting in a low TR/T ratio. Ethanol also reduced the TR/T ratio but the total carotenoid content was increased by about 20 per cent. The effect of isopropanol was somewhat intermediate, with only a slight increase in carotenoid production. Chloramphenicol was not inhibitory to growth or carotenoid production and did not affect the TR/T ratio.

In accordance with the biosynthetic scheme postulated by Simpson *et al.*,[2] inhibition of torulene oxidation by ethanol or diphenylamine was accompanied by a slight, but significant increase in β-carotene formation, thus displaying a shift in the metabolic pathway favouring ring closure (see Fig. 1). While ethanol had, in addition, a stimulatory effect on overall carotenogenesis, diphenylamine showed the opposite effect, depressing carotenogenesis to almost 50 per cent of the control. The favourable effect of lower alcohols on carotenogenesis has been shown earlier by Van-Lanen and Tanner.[14]

The conversion of torulene to torularhodin seems to be rather sensitive to various inhibitors and physiological conditions. Simpson *et al.*[2] reported a considerable shift

[12] T. W. GOODWIN, *Advan. Enzymol.* **21**, 295 (1959).
[13] D. M. THOMAS, R. C. HARRIS, J. T. O. KIRK and T. W. GOODWIN, *Phytochem.* **6**, 361 (1967).
[14] J. M. VAN-LANEN and F. N. TANNER, *Vitamins Hormones* **6**, 163 (1948).

towards the biosynthesis of β-carotene when *R. glutinis* was grown at 5° in comparison to 25°. It would be interesting to establish which of the enzymes concerned with the oxidation of torulene are involved.

EXPERIMENTAL

Organism

Rhodotorula mucilaginosa strain R33 from the Technion culture collection was employed throughout this work. The organism was maintained on agar slants containing 0·2 per cent yeast extract (Difco), 0·5 per cent peptone (Difco), 2 per cent glucose, and 2 per cent agar in distilled water; pH 6·8–7·0.

Culture

The organism was cultured in 500-ml Erlenmeyer flasks with 100 ml media on a gyrorotary shaker (200 rev/min) at 30°. 2 ml of a standardized yeast suspension from a fresh slant were transferred into 100 ml yeast extract–glucose–peptone (as for slants) broth and shaken for 48 hr. Again, 2 ml were transferred to other flasks containing the following medium (g per l.): Ammonium lactate 11·5, glucose 50, $MgSO_4 7H_2O$ 0·5, K_2HPO_4 0·5, $FeCl_3$ 0·001, thiamine hydrochloride 0·001, in distilled water. The pH was adjusted to 6·8–7·0. All media were autoclaved at 121° for 20 min. Thiamin hydrochloride was Seitz-filtered; $FeCl_3$ was autoclaved separately and both ingredients were added aseptically after autoclaving.

The culture was shaken for 6 days and harvested by centrifugation.

Analysis

Dry weight determinations and analysis of the carotenoids via solvent extraction, column chromatography, and spectrophotometric determinations were carried out following the procedure of Peterson *et al.*[5]