# INVESTIGATIONS ON YEASTS PRODUCING RED PIGMENTS

## I. Effect of diphenylamine on the carotenoids produced by Yeasts

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Qualitative and quantitative investigations were carried out by the authors with the scope to study the production of carotenoids in nutrient cultures of yeast poisoned by diphenylamine, in comparison to that observed in untreated blanks.

Untreated blank cultures of yeast proved to contain torulin, $\beta$-carotene, $\gamma$-carotene and torularhodin whilst in nutrients poisoned by diphenylamine the content of torulin appreciably decreased and torularhodin was completely absent.

A remarkable decrease was also observed in the total concentration of carotenoid pigments produced. Namely, the concentration of carotenoid pigments was in cultures of media poisoned by diphenylamine lower by a whole order of magnitude than that of blanks without diphenylamine.

Already several authors observed [1]—[4] that the synthesis of carotenoid pigments in micro-organisms is inhibited by certain concentrations of diphenylamine. The strong effect of diphenylamine on carotenoid pigments is often visually perceptible, due to fading of the colour of micro-organisms. In the present paper, we deal with investigations of yeasts extremely rich in carotenoids and capable of fixation of atmospheric nitrogen, isolated from the root nodules of *Lupinus luteus* [7]. The scope of our investigations was to follow the effect of diphanylamine by the qualitative and quantitative analysis of carotenoid pigments produced. In order to identify in an unambigous manner a carotenoid pigment with pigments of already disclosed structure, isolation of crystalline substances of the cultures would be required. Howerer, we did not dispose of a quantity of substances needed for this completely exact method. Therefore, identification of carotenoid pigments was carried out on the basis of following data:

1. behaviour at distribution between solvents,
2. position in chromatograms,

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3. data of chromatogram of mixtures,
4. data of spectra in the visible region (absorption maxima).

On the basis of the mentioned data, it is our intention to present informative values on the changes in the production of carotenoids pigments by yeasts under the effect of diphenylamine.

## *Experimental*

GY. NÉMETH succeeded in 1953 in isolating from the root nodules of *Lupinus luteus* some yeasts strains that produce red pigment and capable of fixation of atmosperic nitrogen [5]—[6]. The present investigations were carried out with these strains [7].

The cultures were prepared in Roux flasks in the nutrients as follows:

*Nutrient I.:*

5 g of bean extract
2 g of sucrose
0,2 g of $KH_2PO_4$
0,3 g of NaCl
0,2 g of $CaCO_3$
2 g of agar-agar
100 ml of distilled water

*Nutrient II.:*

same as Nutrient I, complemented, however, by 5 mg of diphenylamine

*Nutrient III.:*

1 g of dried yeast
1 g of sucrose
0,5 g of $(NH_4)_2SO_4$
0,1 g of $KH_2PO_4$
0,05 g of $MgSO_4 \cdot 7\,H_2O$
0,01 g of $CaCl_2 \cdot 2\,H_2O$
0,01 g of NaCl
2 g of agar-agar
100 ml of distilled water
20 mg of diphenylamine

On sterilizing nutrients at 110°C for 20 minutes, they were inoculated by test tube cultures, followed by incubation for 10 days at 20°C. No differences were perceptible in the rate of growth of cultures containing diphenylamine and of the blanks. However, the colour of the cultures was different. Whilst the colony grown in nutrient I. was vivid red, that grown in nutrient II. disclosed a faded cream colour and the colony developed in nutrient III. was entirely colourless.

After the tenth day, the colonies were scraped of the surface of the nutrient, washed with a physiological solution of sodium chloride, centrifuged and the sedimented portion dried under an infrared lamp in the presence of $Na_2S_2O_4$.

The following method, which according to data of literature is mostly used when processing carotenoids [8], proved to be suitable for extracting, separating and identifying pigments.

The dried samples were finely rubbed with quartz sand in an ethanolic medium and the pigments extracted from the obtained pulp by ether. The ethanol containing ethereal solution was washed with water and saponified by allowing it to stand overnight at room temperature with 20% methanolic potassium hydroxide. The dry residue obtained in this way was then dissol-

ved in a 1 : 1 mixture of petroleum ether : methanol and some water added, in order to separate pigments into epiphasic and hypophasic ones (distribution between solvents). Epiphasic pigments were chromatographed in a solution of petroleum ether, using a column of calcium hydroxide whilst hypophasic components were chromatographed in a mixture of benzene and petroleum ether, applying a column of calcium carbonate. Absorption maxima (AM) of the spectra of pigments were determind in the visible region, with a Löwe—Schumm, spectroscope. After establishing the spectra, the fractions were further chromatographed until completely homogeneous pigments were obtained. These latter were identified by preparing a mixture of them with already known pigments and establishing the chromatograms and spectra, respectively.

*Processing of sample I.* 10 g of dried red preparation was extracted as described previously and prepared for chromatography. Prior to chromatography, the concentrations of epiphasic and hypophasic pigments were determined.

Quantity of epiphasic pigments: 255 μg in 10 g
Quantity of hypophasic pigments: 30 μg in 10 g
Total: 285 μg in 10 g

*Chromatogram of epiphasic pigments*

| | |
|---|---|
| E1 | 30 mm dark pinkish |
| | 5 mm empty layer |
| E2 | 30 mm cyclamen coloured |
| | 10 mm light cyclamen coloured |
| E3 | 30 mm light yellow |
| E4 | 5 mm yellow |

*Chromatogram of hypophasic pigments*

H1 10 mm pink

| Epiphasic pigments | | μg in 10 g | AM in benzene | | AM in petroleum ether | |
|---|---|---|---|---|---|---|
| E1 | Torulin | 34 | 536 | 499 | 518,5 | 485 |
| E2 | Torulin | 97 | 536 | 499 | *518* | 485 |
| E3 | γ-Carotene | 55 | 508 | 476 | 492 | 460 |
| E4 | β-Carotene | 44 | 497 | 462 | 483 | 452 |
| Hypophasic pigment | | | | | | |
| H1 | Torularhodin | 25 | 558 | (519) | *539* | 503 |

*Processing of sample II.* 10 g of product of light cream colour was extracted and prepared for chromatography as described. Owing to the scar-

city of material, only the quantities of groups could be determined, the amount of separated pigments was not measured.

*Quantity of epiphasic pigments:* 95 μg in 10 g
*Quantity of hypophasic pigments:* 15 μg in 10 g
Total: 110 μg in 10 g

*Chromatogram of epiphasic pigments*

| | |
|---|---|
| E1 | 3 mm pink |
| E2 | 2 mm yellow |
| E3 | 3 mm yellow |

*Chromatogram of hypophasic pigment*

H1 5 mm pink

| Epiphasic pigments | | AM in benzene | | AM in petroleum ether | |
|---|---|---|---|---|---|
| E1 | Torulin | 536 | (498) | 518 | 484 |
| E2 | γ-Carotene | 508 | 476 | 492 | (460) |
| E3 | β-Carotene | 497 | (462) | 483 | 452 |
| Hypophasic pigment | | | | | |
| H1 | Torularhodin | 558 | (518) | 539 | 503 |

*Processing of sample III* 10 g of colourless product which contained no hypophasic pigment. The concentration of epiphasic pigments was extremely low.

Quantity of epiphasic pigments: 40 μg in 10 g

*Chromatogram of epiphasic pigments*

| | |
|---|---|
| E1 | 2 mm pink |
| E2 | 2 mm yellow |

| Epiphasic pigments | | AM in benzene | | AM in petroleum ether | |
|---|---|---|---|---|---|
| E1 | Torulin | 536 | (498) | 518 | 484 |
| E2 | β-Carotene | (496) | (462) | 483 | 452 |

**References**

[1] *Goodwin, T. W., H. G. Osman:* Biochem. J. **53**, 541 (1953); **56**, 222 (1954).
[2] *Turian, G.:* Helv. Chim. Acta **33**, 1988 (1950).
[3] *Cohen—Bazire, G., R. Y. Stanier:* Nature **181**, 250 (1958).
[4] *Schlechta, L., O. Gabriel, O. Hoffmann—Ostenhof:* Nature **181**, 268 (1958).
[5] *Németh, Gy.:* Nyírségi Mezőgazdasági Kísérleti Intézet Évkönyve 74 (1954).
[6] *Németh, Gy.:* Ibid. 102 (1955).
[7] *Németh Gy.* B. Matkovics: Naturwiss. **44**, 621 (1957).
[8] *Cholnoky, L., K. Györgyfy, E. Nagy, M. Pánczél:* MTA VII. Oszt. Közl. **5**, 419 (1955).