UDC 547.912:535.212
ISSN 1330-9862

original scientific paper

(FTB-1069)

Effect of the Light on Carotenoid Profiles of *Xanthophyllomyces dendrorhous* Strains (formerly *Phaffia rhodozyma*)

Manuel Vázquez

Departamento de Química Analítica. Área Tecnología de Alimentos
Universidad de Santiago de Compostela (Campus de Lugo)
Escuela Politécnica Superior, 27002 Lugo, Spain

Received: January 17, 2001
Accepted: May 2, 2001

Summary

The influence of the light on the carotenogenesis in 6 strains (ATCC 24202, ATCC 24203, ATCC 24228, ATCC 24229, ATCC 24261 and NRRL Y-10921) of *Xanthophyllomyces dendrorhous (*formerly *Phaffia rhodozyma)* grown on media containing xylose was studied. Carotenoids produced in the light or in the dark were extracted from the biomass, identified and quantified by HPLC with diode-array detection. All strains produced greater amounts of carotenoids in the light than in the dark. However, the production of these carotenoids does not follow the same pattern. The strain ATCC 24228 produced more total carotenoids and astaxanthin (2.45 mg/L and 2.13 mg/L, respectively) if grown in the light. In darkness, the strains ATCC 24203 and ATCC 24229 produced a carotenoid, 3-hydroxy-3´,4´-didehydro-β-ψ-caroten-4-one (HDCO), that was not produced when grown in the light. HDCO was not produced either in the light or in the dark by the other strains studied.

Key words: carotenogenesis, astaxanthin, 3-hydroxy-3´,4´-didehydro-β-ψ-caroten-4-one, *Xanthophyllomyces dendrorhous*

Introduction

Carotenoids are natural fat-soluble photopigments used as additives in feed, food, and drug as well as cosmetic industries (*1*). Astaxanthin (3,3´- dihydroxy -β, β-caroten- 4,4´- dione) is a carotenoid with a recognized commercial value. It is used as an additive in fish feed because this pigment gives the characteristic pink colour to the meat of salmon and rainbow trout (*2-4*). The use of astaxanthin for the supplementation of layers' feed was also studied (*5,6*).

Biotechnological production of astaxanthin can be carried out using several microorganisms: bacteria (*Mycobacterium lacticola* and *Brevibacterium* spp. (*7*)), microalgae (*Haematococcus* spp. (*8*), *Neochloris wimmeri* and *Chlamydomonas nivalis* (*1*)), fungus (*Peniophora* spp. (*9*)) and the yeast (*Xanthophyllomyces dendrorhous (*formerly *Phaffia rhodozyma)* (*10*)). Among these microorganisms, *H. pluvialis* and *X. dendrorhous* are in commercial production.

Carotenogenesis is a photoregulated process that has not been defined yet. The light stimulates carotenogenesis in fungi such as *Phycomyces*, *Neurospora crasa*, *Aspergillus giganteus*, *Gibberrella fujikuroi* and *Rhodotorula*

* Corresponding author; Phone ++34 982 285 900; Fax: ++34 982 241 835; E-mail: vazquezm@lugo.usc.es

minuta, but inhibits it in *Trichophyton mentagrophytes* and *Blakeslea trispora* (*11*).

In recent years, the production of astaxantin by *X. dendrorhous* has been studied by many researchers, but just few studies have been published about the photoregulation of the carotenoid production by *X. dendrorhous* (*11-15*).

X. dendrorhous can grow on a variety of carbon sources such as hexoses, pentoses and disaccharides. The influence of carbon source on the carotenoid profiles has been studied (*16*). In this work, it was shown that xylose is the carbon source that allows obtaining higher astaxanthin concentrations. Xylose is a pentose that can be easily obtained by hydrolysis of lignocellulosic materials such as wood, straw and agricultural wastes.

This paper deals with the effect of the light on the content and carotenoids profile of several strains of *X. dendrorhous* grown on the media that contained xylose.

Material and Methods

Microorganisms

Freeze-dried broths of wild *X. dendrorhous* strains were obtained from the American Type Culture Collection (Rockville, Maryland, USA) or from the Agricultural Research Service Culture Collection (Peoria, Illinois, USA). Microorganisms were maintained on agar plates at 4 °C, and transferred monthly. The agar plates had the following composition: 5 g/L peptone, 3 g/L yeast extract, 3 g/L malt extract, 10 g/L xylose and 20 g/L agar.

Cultivation conditions

Proliferation experiments were carried out during 7 days at 22 °C in orbital shakers (agitation speed: 200 rpm) using 50 mL Erlenmeyer flasks with 20 mL culture medium. The culture medium contained: 5 g/L peptone, 3 g/L yeast extract, 3 g/L malt extract and 10 g/L xylose. Fermentations were conducted in the dark or under constant illumination (500 lux) provided by cool white fluorescent lamps (BRL840 11W, Mazda, Poland). Duplicate experiments and triplicate analysis were performed and the mean results are reported.

Analytical methods

Samples were withdrawn from the fermentation media and centrifuged (4500×g, 10 min). The supernatants were analysed by HPLC with RI detection for substrate and metabolic co-products (*17*). Pellets were washed twice with a solution of 9 g/L sodium chloride in deionised water and centrifuged again. A part of cells was dried at 102 °C for 48 h, in order to allow the calculation of the biomass concentration on dry weight basis. The remained fraction of cells was used for carotenoid analysis by means of sequential steps of DMSO treatments for disrupting cell walls (*18*) and hexane extraction (*19*). Samples from the hexane phase were analysed by HPLC with Diode-Array Detection (DAD) using the following analysis conditions; column: Merck LiChrosorb Si 60, oven temperature: 30 °C, gradient elution (flow rate = 1 mL min^{-1}; mobile phase: 100 % hexane during 1 min; change up to 50 % hexane-50 % ethyl acetate in 2 min, this last concentration remaining constant during 6 additional min). Carotenoids were identified by their retention times and by comparison of the spectral features with those of pure compounds or with reported data. *All-trans*-astaxanthin and echinenone standards were kindly provided by Hoffmann-LaRoche (Basel, Switzerland). All the carotenoids were integrated using *all-trans*-astaxanthin as a standard, providing a carotenoid concentration »equivalent in astaxanthin«.

Results and Discussion

X. dendrorhous strains ATCC 24202, ATCC 24203, ATCC 24228, ATCC 24229, ATCC 24261 and NRRL Y-10921 are potential candidates for the biotechnological production of commercially valuable carotenoids. A systematic experimentation was carried out in order to establish the concentration and the carotenoids profile of the strains mentioned cultured in the dark or in the light.

Table 1 shows the results obtained from cells of strain ATCC 24202 grown in the dark or in the light. Biomass concentration was lightly increased in the dark. Biomass yield ($Y_{x/s}$) was from 0.41 to 0.46 g/g.

Table 1. Effect of illumination on the strain ATCC 24202 grown in a medium containing xylose

	In the light (500 lux)	In the dark
Biomass (g/L)	8.2	9.2
Xylitol (g/L)	2.3	2.1
Astaxanthin (mg/L)	1.70	1.11
Echinenone (mg/L)	0.00	0.05
3-hydroxyechinenone (mg/L)	0.11	0.21
HDC (mg/L)	0.24	0.25
Canthaxanthin (mg/L)	0.04	0.05
Total carotenoids (mg/L)	2.09	1.70

Xylitol was found in the medium at the end of the fermentation because it is an intermediate of xylose metabolism. The co-production of xylitol and astaxanthin by *X. dendrorhous* on xylose-containing media has been previously reported (*16,20*). Xylitol was not affected by the light.

The main carotenoid produced was astaxanthin, followed by HDC and 3-hydroxyechinenone. Astaxanthin concentration was increased up to 65 % in the light, compared to the one obtained in cells grown in the dark. Other carotenoids, such as canthaxanthin (0.05 mg/L) and echinenone (0.05 mg/L), were detected in small concentrations in cells grown in the dark. Echinenone was not detected in cells grown in the light.

Table 2 shows the results obtained for the strain ATCC 24203. $Y_{x/s}$ for this strain was lower than for ATCC 24202. $Y_{x/s}$ was also affected by the light (0.39 in the light and 0.25 in the dark). Fig. 1 shows chromatograms of carotenoid extracts from ATCC 24203 cells

grown on xylose in the light or in the dark. Important differences in the profile of carotenoids can be observed. Astaxanthin, 3-hydroxyechinenone and HDC were produced in the light. However, the astaxanthin concentration was decreased up to 91 % in the dark. 3-hydroxyechinenone and HDC were not produced in the dark, but a new carotenoid 3-hydroxy-3´,4´-didehydro-β-ψ--caroten-4-one (HDCO) was generated.

Table 2. Effect of the illumination on the strain ATCC 24203 in medium containing xylose

	In the light (500 lux)	In the dark
Biomass (g/L)	7.8	5.1
Xylitol (g/L)	1.7	2.3
Astaxanthin (mg/L)	1.75	0.15
HDCO (mg/L)	0.00	0.56
3-hydroxyechinenone (mg/L)	0.10	0.00
HDC (mg/L)	0.22	0.00
Total carotenoids (mg/L)	2.07	0.71

HDCO was identified by comparing the retention time and the UV-vis spectrum with data reported in the literature (*12*,*21*) because commercial HDCO is not available. Fig. 2 shows UV-vis spectra of astaxanthin and HDCO obtained from the isolated compounds. Important differences in maximum absorbance values of both astaxanthin and HDCO spectra can be observed. HDCO was detected first in *X. dendrorhous* by Johnson and Lewis (*10*), but these authors did not mention that the light affects the synthesis of this carotenoid. The values reported in Table 2 show that HDCO is synthesized only by ATCC 24203 in the dark.

Table 3 shows the results obtained for the strain ATCC 24228. High $Y_{x/s}$ was obtained in the light (0.46 g/g). The $Y_{x/s}$ obtained in the dark was lower (0.33 g/g). Low concentrations of xylitol were obtained as described in the above reported strains.

Table 3. Effect of the illumination on the strain ATCC 24228 grown in a medium containing xylose

	In the light (500 lux)	In the dark
Biomass (g/L)	9.3	6.7
Xylitol (g/L)	1.1	2.6
Astaxanthin (mg/L)	2.13	1.38
3-hydroxyechinenone (mg/L)	0.09	0.13
HDC (mg/L)	0.23	0.21
Total carotenoids (mg/L)	2.45	1.72

Astaxanthin, 3-hydroxyechinenone and HDC were synthesized. The light affected the synthesis of astaxanthin. The concentration of astaxanthin was 2.13 mg/L in the light and 1.38 mg/L in the dark. The astaxanthin concentration obtained for the strain ATCC 24228 in the light was the highest reported in this work. The value of astaxanthin concentration in the cells was also the highest reported in this work (263 mg carotenoids/kg biomass). 3-hydroxyechinenone and HDC were not affected by the light.

Table 4 shows the results for the strain ATCC 24229. The $Y_{x/s}$ scarcely changed during illumination (0.35 g/g in the light and 0.38 g/g in the dark). However, xylitol concentration was duplicated when the fermentation was carried out in the light.

Table 4. Effect of the illumination on the strain ATCC 24229 grown in a medium containing xylose

	In the light (500 lux)	In the dark
Biomass (g/L)	7.0	7.7
Xylitol (g/L)	4.2	2.1
Astaxanthin (mg/L)	0.70	0.00
HDCO (mg/L)	0.00	0.75
Echinenone (mg/L)	0.00	0.00
3-hydroxyechinenone (mg/L)	0.06	0.00
Canthaxanthin (mg/L)	0.08	0.00
Total carotenoids (mg/L)	0.84	0.75

Astaxanthin, 3-hydroxyechinenone and HDC were synthesized in the light. HDCO was the only carotenoid synthesized in the dark. Fig. 3 shows chromatograms of carotenoid extracts from the strain ATCC 24229 grown in the light or in the dark. HDCO is synthesized in the dark like in the strain ATTC 24203. This confirms that darkness is needed to synthesize HDCO.

Table 5 shows the results for the strain ATCC 24261. This strain was the least influenced by the light. Insignificant variations were observed in the values for biomass and xylitol concentrations. The carotenoid profile was constituted by astaxanthin, HDC and 3-hydroxyechinenone. The astaxanthin concentration in the light was doubled compared with the value obtained in the dark.

Table 5. Effect of the illumination on the strain ATCC 24261 grown in a medium containing xylose

	In the light (500 lux)	In the dark
Biomass (g/L)	5.7	6.8
Xylitol (g/L)	4.6	4.5
Astaxanthin (mg/L)	1.17	0.61
3-hydroxyechinenone (mg/L)	0.06	0.14
HDC (mg/L)	0.17	0.22
Total carotenoids (mg/L)	1.40	0.97

Table 6 shows the results obtained for the strain NRRL Y-10921. Compared with the strains mentioned, this strain showed the smallest $Y_{x/s}$, 0.28 g/g in the light and 0.22 g/g in the dark. However, xylitol concentration is very high in the cells grown in the light (7.3 g/L). The value obtained in the dark was 4.2 g/L. Xylitol is also a product of interest for food industry. Further studies are needed to evaluate the co-production of astaxanthin and xylitol.

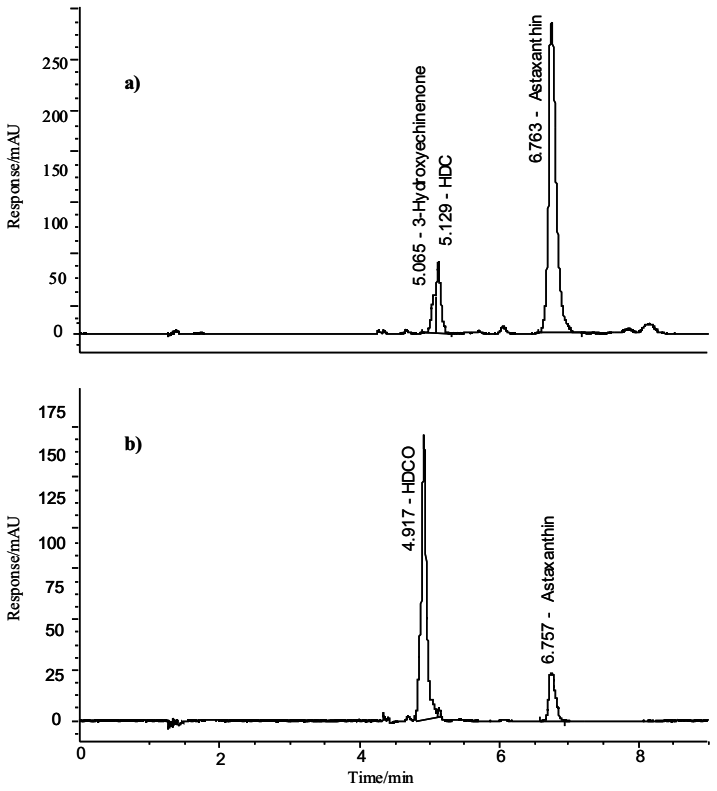

Fig. 1. HPLC chromatograms of carotenoids from ATCC 24203 cell extracts of the strain cultured in the media containing xylose: a) in the light; b) in the dark

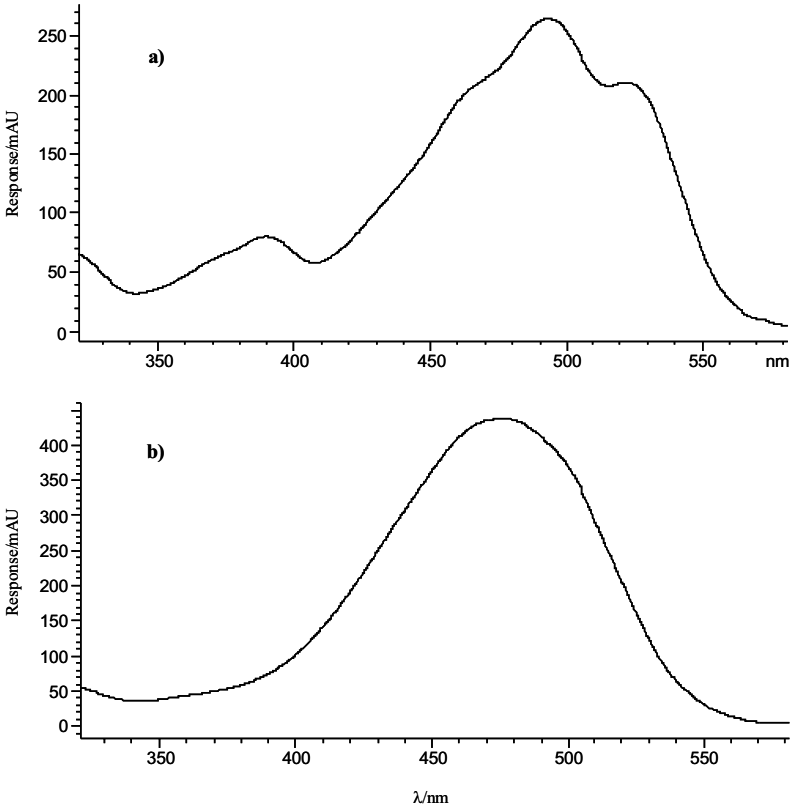

Fig. 2. UV-vis spectra of carotenoids from ATCC 24203 cell extracts: a) 3-hydroxy-3′,4′-didehydro-β-ψ-caroten-4-one (HDCO); b) astaxanthin

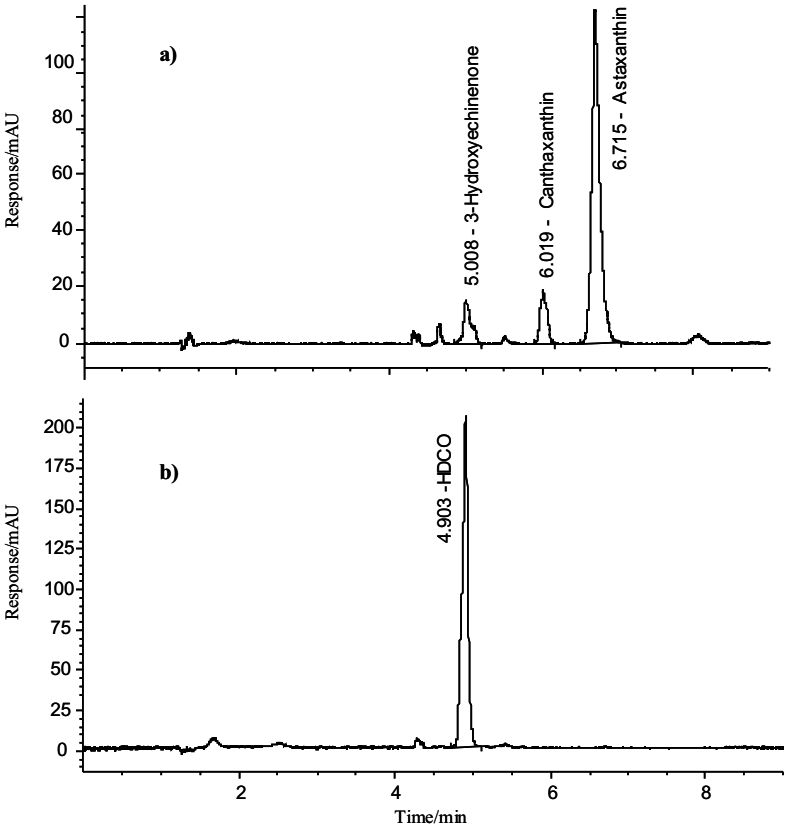

Fig. 3. HPLC chromatograms of carotenoids from ATCC 24229 cell extracts of the strain cultured in the media containing xylose: a) in the light; b) in the dark

Table 6. Effect of the illumination on the strain NRRL Y-10921 grown in a medium containing xylose

	In the light (500 lux)	In the dark
Biomass (g/L)	5.7	4.5
Xylitol (g/L)	7.3	4.2
Astaxanthin (mg/L)	1.38	1.00
3-hydroxyechinenone (mg/L)	0.04	0.05
HDC (mg/L)	0.07	0.08
Total carotenoids (mg/L)	1.49	1.13

The carotenoid profile was constituted by astaxanthin, HDC and 3-hydroxyechinenone. Astaxanthin synthesis was also affected by the light in the strain NRRL Y-10921. Astaxanthin concentration in the light was 1.38 mg/L and 1.00 mg/L in the dark. 3-hydroxyechinenone and HDC were not affected by the light.

Conclusions

Our study indicates that all strains are not affected by the light in the same way. However, all strains synthesize higher amounts of total carotenoids in the light than in the dark.

The illumination influences not only total carotenoid concentrations, but also the carotenoid profile, biomass and xylitol concentrations.

Among the studied strains, ATCC 24228 grown in the light produced more carotenoid and astaxanthin, 2.45 mg/L and 2.13 mg/L, respectively.

The strains ATCC 24229 and ATCC24203 can produce HDCO. HDCO is a carotenoid with potential use as food pigment. The fact that HDCO was the only carotenoid produced by a yeast is an important advantage for industrial production since no separation of carotenoids is needed.

Acknowledgements

Authors are grateful to Hoffmann-LaRoche (Basel, Switzerland) for samples of astaxanthin and echinenone.

References

1. E. A. Johnson, W. A. Schroeder, *Adv. Biochem. Eng. 53* (1995) 119-178.
2. E. A. Johnson, T. G. Villa, M. J. Lewis, *Aquaculture, 20* (1980) 123–134.
3. B. Bjerkeng, T. Storebakken, S. Liaaen-Jensen, *Aquaculture, 91* (1990) 153–163.
4. S. Sigurgisladottir, C. C. Parrish, S. P. Lall, *Food Res. Int. 27* (1994) 23–32.
5. A. O. Dike, F. Lettner, W. Zollitsch, *Archiv fur Geflügelkunde, 5* (1992) 205–210.
6. E. A. Johnson, M. J. Lewis, C. R. Grau, *Poultry Sci. 59* (1980) 1777–1782.
7. K. L. Simpson, T. Katayama, C. O. Chichester: Carotenoids from microorganisms. In: *Carotenoids as Colorants and Vita-*

min A Precursors, J. C. Bauernfeld (Ed.), Academic Press, London (1981) pp. 463-480.

8. P. Bubrik, *Bioresource Technol. 38* (1991) 237-239.
9. T. W. Goodwin: *The Biochemistry of the Carotenoids*, 2nd ed., Chapman and Hall, London (1980).
10. E. A. Johnson, M. J. Lewis, *J. Gen. Microbiol. 115* (1979) 173-183.
11. P. S. Meyer, J. C. du Preez, *Syst. Appl. Microbiol. 17* (1994) 24-31.
12. M. Vázquez, V. Santos, *Biotechnol. Lett. 20* (1998) 181-182.
13. G. H. An, *Appl. Biochem. Biotechnol. 66* (1997) 263-268.
14. W. A. Schroeder, E. A. Johnson, *J. Ind. Microbiol. 14* (1995) 502-507.
15. G. H. An, E. A. Johnson, *Antonie Van Leewenhoek, 57* (1990) 191-203.
16. M. Vázquez, V. Santos, J. C. Parajó, *J. Ind. Microbiol. Biotechnol. 19* (1997) 263-268.
17. J. C. Parajó, V. Santos, M. Vázquez, J. M. Cruz, *Food Chem. 60* (1997) 347-355.
18. J. J. Sedmak, D. K. Weerasinghe, S. O. Jolly, *Biotechnol. Techn. 4* (1990) 107-112.
19. P. Calo, J. B. Velázquez, C. Sieiro, P. Blanco, E. Longo, T. G. Villa, *J. Agric. Food Chem. 43* (1995) 1396-1399.
20. J. C. Parajó, V. Santos, M. Vázquez. *Biotechnol. Lett. 19* (1997) 139-141.
21. G. H. An, D. B. Schuman, E. A. Johnson, *Appl. Environ. Microbiol. 55* (1989) 116-124.

Utjecaj svjetla na vrste karotenoida u sojevima *Xanthophyllomyces dendrorhous* (prije *Phaffia rhodozyma*)

Sažetak

Ispitan je utjecaj svjetla na karotenogenezu u šest sojeva *Xanthophyllomyces dendrorhous* (ATCC 24202, ATCC 24203, ATCC 24228, ATCC 24229, ATCC 24261 i NRRL Y-10921) koji su rasli u podlozi sa ksilozom. Karotenoidi proizvedeni na svjetlu ili u tami ekstrahirani su iz biomase te identificirani i kvantitativno određeni pomoću HPLC s diodnom detekcijom. Svi sojevi proizveli su na svjetlu veću količinu karotenoida nego u tami. Međutim, proizvodnja tih karotenoida ne teče na isti način. Soj ATCC 24228 proizveo je na svjetlu više ukupnih karotenoida (2,45 mg/L) i astaksantina (2,13 mg/L). Sojevi ATCC 24203 i ATCC 24229 proizveli su u tami karotenoid 3-hidroksi-3'4'-didehidro-β-ψ-karoten-on (HDCO) koji se nije sintetizirao tijekom rasta na svjetlu. Ostali sojevi nisu proizveli HDCO niti na svjetlu niti u tami.