Journal of Microbiology and Biotechnology
Vol. 1, No. 1, 75-78, 1991

Effect of Carbon Source and Carbon to Nitrogen Ratio on Carotenogenesis of *Rhodotorula glutinis*

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Received 27 October 1990 / Accepted 21 January 1991

The carotenoid biosynthesis of a red oleaginous yeast, *Rhodotorula glutinis* was significantly changed when the yeast was grown on different carbon substrates. The highest carotenoid production was obtained on culture medium containing glucose when the carbon to nitrogen ratio (C/N ratio) was adjusted to 25.7. Galactose stimulated the biosynthetic rate of torularhodin, a xanthophyll component of the yeast. With decreasing C/N ratio of the medium, significant changes of γ-carotene and torularhodin were observed such that increase in the torularhodin concentration was nearly equal to the decrease in γ-carotene. It was speculated that the nature of carbon substrate affected the metabolic rate of the cell, and accompanied by the different pattern of carotenoid accumulation in the cell.

Carotenoids have been generally used as a food colorant, providing colors ranged from yellow to red with good colorfastness (6, 9). With the increasing demand for natural colorant, microbial carotenoid has been considered as its potential source (5, 10). An oleaginous yeast, *Rhodotorula glutinis* produces carotenoid pigments with its growth and four major carotenoids, *i.e.*, torularhodin, torulene, γ-carotene and β-carotene, have been identified (15). The carotenogenesis has been known to be affected by the environmental factors including temperature, pH, oxygen, carbon and nitrogen sources, and miscellaneous additives (2, 12, 16). Among these, both the carbon sources and the carbon to nitrogen ratio (C/N ratio) would affect the rates of substrate uptake and assimilation by the cells, hence the pool size of intracellular acetyl-CoA being essential for carotenoid biosynthesis. Then, the pattern of carotenoid accumulation in the cell may be affected by the metabolic rate of growth substrate together with cell age.

In this study, we studied the qualitative and quantitative changes of carotenoid biosynthesis of *R. glutinis* when the yeast was grown in different carbon sources and with various C/N ratios of the medium, and discussed the influence of carbon assimilation rate on the pattern of carotenoid accumulation in the cell.

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Key words: Carbon source, carbon to nitrogen ratio, carotenogenesis, *Rhodotorula glutinis*
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MATERIALS AND METHODS

Microorganism

Rhodotorula glutinis NRRL Y-1091 was used in this study. The organism was obtained from Northern Regional Research Laboratory, ARS, USDA, Peorida, Illinois, U.S.A.

Reagents

The carbon sources used were sugars graded as chemically pure and analytically pure. Acetonitrile, tetrahydrofuran, and water were of HPLC grade. All solvents used for carotenoid extraction were of analytical grade.

Cultivation

The composition of basal medium used was as follows (in gram per liter): $CaCl_2 \cdot 6H_2O$, 0.2; $MgSO_4 \cdot 7H_2O$, 1.5; Na_2HPO_4, 2.0; KH_2PO_4, 7.0; $(NH_4)_2SO_4$, 1.0; yeast extract, 1.0. The solutions of carbon sources were introduced into the basal medium at 3.5% after separate sterilization (121℃, 15 min). The carbon to nitrogen (C/N) ratio of the medium was adjusted with various glucose concentrations, maintaining 1.0 g $(NH_4)_2SO_4$ and 1.0 g yeast extract (Difco) as nitrogen sources. The nitrogen content in yeast extract used was determined to

be 9.9% (w/w) by the microKjeldahl method.

R. glutinis was grown in a 500 ml Erlenmyer flask containing 100 ml of medium at 30℃ on a rotary shaker for four or seven days. The shaking speed was maintained at 200 rpm.

Sample Preparation and Analytical Methods

Dry cell weight was used for the determination of cell concentration. Glucose concentration in the culture broth was determined by dinitrosalicylic acid method (11).

Pigment mixture from *R. glutinis* was prepared as described by Simpson *et al.* (17). The yeast cells were harvested, washed and then lyophilized. Two grams of seasand and 7 ml of acetone were added together with 0.5 g lyophilized yeasts into a mortar. The yeast cells were disrupted by grinding with a pestle. The ground sample mixtures were transferred into a test tube and 5 ml acetone was added. The pigment mixtures were extracted by a vigorous shaking. The extraction procedure was repeated until the cell debris was nearly colorless. The pigments in the combined acetone solution were transferred to an equal volume of petroleum ether (bp. 40-60℃) by the addition of water and washed free of acetone. The sample solution was poured into 1.8 ml vial and then filled with petroleum ether up to 1.8 ml. The carotenoid analysis was performed with a high performance liquid chromatographic method which has been already reported elsewhere (14).

Table 1. Effect of carbon sources on the carotenoid accumulation for four-day culture

Carbon sources	Glucose	Ribose	Xylose	Galactose
Biomass (g/l)	9.3	8.7	8.6	3.9
Total carotenoid content (μg/g)	257.7	214.4	223.2	124.9
Carotenoid composition (mol%)				
Torularhodin	11.5	17.6	16.4	36.2
Torulene	36.6	32.3	20.3	19.8
γ-Carotene	37.6	36.7	49.6	19.8
β-Carotene	14.3	13.4	13.7	24.2

Table 2. Effect of carbon sources on the carotenoid accumulation for seven-day culture

Carbon sources	Glucose	Ribose	Xylose	Galactose
Biomass (g/l)	11.2	10.4	9.4	5.8
Total carotneoid content (μg/g)	453.8	435.2	417.9	287.5
Carotenoid composition (mol%)				
Torularhodin	21.7	35.2	19.4	60.8
Torulene	34.3	30.8	36.1	9.2
γ-Carotene	14.5	10.9	17.3	2.3
β-Carotene	29.5	23.1	27.2	27.7

RESULTS AND DISCUSSION

Cellular growth and characteristics of carotenogenesis with various carbon sources for four-day cultures are given in Table 1. To confirm the results from four-day cultures, the cultures were further performed for seven days (Table 2). Cellular growth was shown to be nearly the same in glucose, ribose, and xylose media, but appeared to be low in galactose media. The best carbon source for carotenogenesis was glucose, whereas the worst galactose (Table 1, 2). Torularhodin, a xanthophyll component among four major carotenoid pigments of this yeast, was actively synthesized in galactose-grown culture, whereas both torulene and γ-carotene were remarkably accumulated in glucose and ribose cultures. For four-day culture, γ-carotene was substantially accumulated in xylose culture (Table 1), but the compound was converted into either torulene or β-carotene after seven-day cultivation (Table 2).

The reasons why galactose resulted in low cellular growth and low carotenoid biosynthesis could be explained by the low metabolic rate galactose compared with other carbon sources used. The available evidence suggests that the uptake of most monosaccharides is carried out by a single constitutive facilitated diffusion system which is responsible for glucose uptake (1). However, different sugars have different affinities for this transport system such that galactose has seven times lower affinity for the carrier than glucose does (1). Moreover, the catabolic pathway of galactose requires more conversion steps to glucose-6-phosphate in the glycolytic pathway than does that of glucose, referred to as the Leloir pathway (4). Since four more enzymatic conversion involving galactokinase, galactose-1-phosphate uridyltransferase, UDP-D-galactose-4-epimerase, and phosphoglucomutase would be required for the formation of glucose-6-phosphate, cells grown in galactose media tend to show low growth rate compared with cells in glucose media. It could be, therefore, speculated that low assimilation rate of galactose led to a low carotenoid production of the cells. High content of torularhodin in galactose-cultured cells, representing in high activity of the oxygenase converting torulene into torularhodin, has appeared to be unique except under special culture condition such as low cultivation temperature (13). The content of torularhodin was around 60 mol% of total carotenoids for seven-day culture (Table 2).

As the C/N ratio of the medium increased, cell concentration was found to be increased, but glucose remained unused at C/N ratio above 45 (Fig. 1). The best C/N ratio for carotenoid production was 25.7 (Fig. 1). In growing cells at C/N ratio above 25.7, carotenoid content decreased as C/N ratio increased. The result

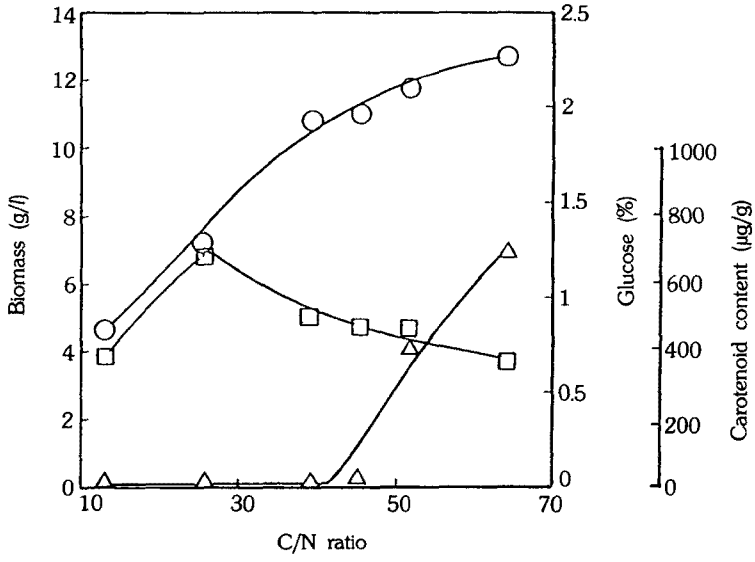

Fig. 1. Effect of C/N ratio on the cell growth and carotenoid formation during shaking culture.
—○—; Biomass, —□—; Carotenoid content, —△—; Glucose.

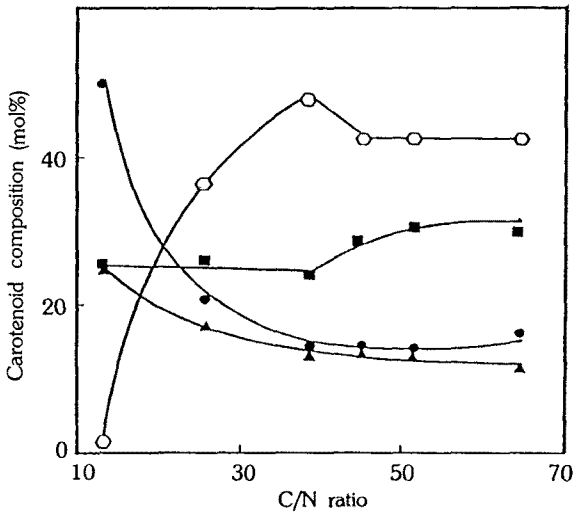

Fig. 2. The changes of the composition of carotenoid pigments during shaking culture at various C/N ratios.
—●—; Torularhodin, —■—; Torulene, —◇—; γ-Carotene, —▲—; β-Carotene.

showed that not only the availability of carbon source as a substrate for carotenoid biosynthesis (7), but also the age of culture would be the important factors for the carotenogenesis (3, 8). Low amount of carotenoid pigments of the cell grown at the C/N ratio of 15.3 might result from the exhaustion of available glucose for the carotenoid biosynthesis.

Significant changes of γ-carotene and torularhodin in carotenoid pigments were observed at the C/N ratio below 45 (Fig. 2), and the result is consistent with the previous one (17); increase in the torularhodin concentration with time was very nearly equal to the decrease in γ-carotene concentration. When glucose was exhausted in culture medium, γ-carotene, which is convertible either to torulene and hence torularhodin, or to β-carotene in the carotenoid biosynthetic pathway, was decreased. This indicates that γ-carotene was exclusively converted to torularhodin and the oxygenation leading to torularhodin was faster than the cyclization to β-carotene. At C/N ratio above 45 (glucose-excess condition), the content of the four major carotenoids appears to be constant (Fig. 2), with the torularhodin, torulene, γ-carotene, and β-carotene constituting around 15, 30, 43, and 12 mol% of total carotenoids respectively.

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