*Rhodotorula glutinis*에서 카로티노이드 생합성에 미치는 페놀의 영향

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The Effect of Phenol on Carotenogenesis in *Rhodotorula glutinis*

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INTORDUCTION

The carotenoids as isoprenoid compounds are widespread natural pigments, occurring in animals, plants, microorganisms. The number of known naturally occurring carotenoids has risen to more than 600 up to now.[2] Carotenoids have the antioxidant and singlet oxygen quenching properties[2], and enhance immunity and anticarcinogenic action.[1] In this reason, carotenoids have been noticed as functional natural pigments.

The genera of carotenogenesis yeasts are *Crytococcus*, *Rhodotorula*, *Rhodosporidium*, *Sporidiobolus*, *Sporobolomyces* and *Phaffia*. The major carotenoids of the yeasts except *Phaffia* are very similar such as β-carotene(β,β-carotene), γ-carotene(β,γ-carotene), torulene(3',4'-didehydro-β,ψ-carotene), torularhodin(3',4'-didehydro-β,ψ-carotene-16'-oic acid).[3,4]

The yeast of *Rhodotorula* genus also can biodegrade phenol. Recently, there was the study of phenol biodegradation by *Rhodotorula rubra* in Japan.[6] *Rhodotorula glutinis* oxidized chlorophenols and compeletly biodegraded 5mM phenol and utilized phenol as a sole carbon source.[5,8]

The prupose of this study is to investigate the effect of phenol on carotenogenesis and the optimal conditions of carotenogenesis with biodegrading phenol in *Rhodotorula glutinis*.

MATERIALS AND METHODS

Microorganism : *Rhodotorula glutinis* K-501 was isolated from deciduous tree and identified by Korea Research Institute of Bioscience & Biotechnology.

Media : The medium for seed culture was YM medium(glucose 1%(w/v), peptone 0.5%(w/v), yeast extract 0.3%(w/v), malt extract 0.3%(w/v)). The medium for flask culture was YBN(yeast nitrogen base w/o ammino acids & ammonium sulfate) medium which was added glucose 1.5%(w/v) as carbon source, and ammonium sulfate 0.5%(w/v) as nitrogen source. The resting cell culture medium was YBN(yeast nitrogen base w/o ammino acids & ammonium sulfate) medium which was added phenol as a sole carbon source, and ammonium sulfate 0.2%(w/o) as nitrogen source.

Culture Conditions : All experiments were worked at 22℃, initial pH 5.5 in shaking incubator. The flask culture inoculated with 2%(v/v) of a 30h seed cultured cell was worked at 100ml medium in 500ml baffled flask and was buffered with 0.05M-potassium hydrogen phtalate buffer. The resting cell culture inoculated with a 0.07g washed cell was worked at 25ml medium in 500ml baffled flask.

Analytical Methods : Cell mass in flask culture was measured by the optical density at 660nm with UV-spectrophotometer. Total carotenoids content of the cells was calculated as the value for torulene and expressed as µg/g yeast, on the basis of the results reported by Simpson et al. that the absorption maximum and extinction coefficient($E^{1\%}_{1cm}$) of torulene in petroleum ether are 485nm and 2680, respectively.[7] Phenol concentration was measured by HPLC. The conditions of HPLC for determination of phenol were as follows: column, µ Bondapak C_{18} 3.9× 300 mm ; mobile phase, methanol : water = 1 : 1; flow rate, 1ml/min; detector, UV detector ; wavelength, 260nm; temperature, ambient.

RESULTS AND DISCUSSION

As shown in Table 1, the cell in stationary phase biodegraded phenol better than those in exponential growth phase and biodegraded up to 98.8% in 500ppm of phenol. This results showed that *Rhodotorula glutinis* K-501 had higher biodegradability than one of *Rhodotorula rubra*.[6] According to Keiko Katayama-Hirayama's experiment, *Rhodotorula glutinis* completely biodegraded 5mM(470ppm) of phenol after 8 hours.[5] But, the cell of both the cases may

Table 1. Residual phenol concentration in the cultured broth after 30h of cultivation.

	Residual phenol concentration (ppm)					
	Initial phenol concentration (ppm)					
	100	200	300	400	500	700
35h-old-cells	9	16	4	26	181	597
85h-old-cells	0	0	0	0	6	536

have been inhibited, as both the cases had less than 25% of biodegradation in 700ppm of phenol.

According to Fig 1, the cell growth was inhibited in the case of initial addition of phenol. But a phenol addition in exponential growth phase and stationary phase little inhibited cell growth.

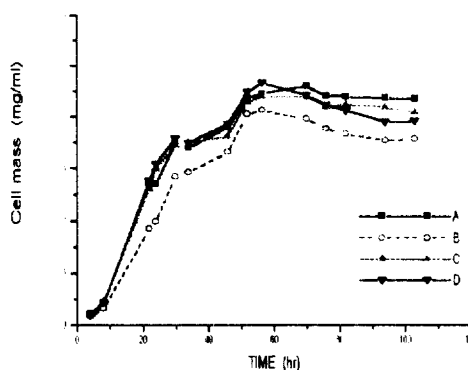

Fig. 1 The effect of 250ppm phenol addition on cell growth.
A : No addition of phenol, B : Initial addition of phenol,
C : Phenol addition after 30h of cultivation,
D : Phenol addition after 50h of cultivation

As shown in Fig 2, the phenol addition may have changed the composition

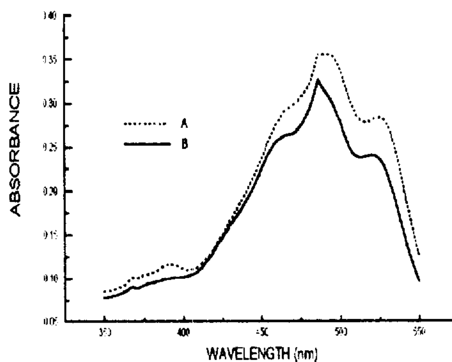

Fig. 2 Absorption spectra of carotenoids isolated in *Rhodotorula glutinis* K-501
A : No addition of phenol, B : Addition of 250ppm phenol.

of carotenoids. As shown in the spectrum of carotenoids which was isolated from cells cultured in phenol containing medium, absorbance of nearby 484nm was increased and absorbance of nearby 515nm was decreased. The absorption maximum for torulene and torularhodin in petroleum ether are 484nm and 515nm, respectively.[7] It was postulated that the step of torulene into torularhodin in carotenogenesis pathway was blocked and torulene was accumulated.

As shown in Table 2, the initial addition of phenol stimulated carotenoid biosynthesis but decreased cell mass. And the addition of phenol in exponential growth phase and stationary phase little affected carotenogenesis.

Table 2. The effect of phenol on growth and biosynthesis carotenoids in *Rhodotorula glutinis* K-501

	A	B	C	D
Cell mass(mg/ml)	4.60	4.12	4.39	4.67
Total carotenoids (μg/g yeast)	242	297	244	251
Yield coefficient (cell mass/glucose)	0.31	0.27	0.29	0.31
Final pH(after 100h of cultivation)	4.25	4.25	4.25	4.25

A : No addition of phenol, B : Initial addition of phenol,
C : Phenol addition after 30h of cultivation,
D : Phenol addition after 50h of cultivation

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