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Effect of Oxygen Radicals and Aeration on Carotenogenesis and Growth of *Phaffia rhodozyma* (*Xanthophyllomyces dendrorhous*)

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Mn(II)+succinate decreased the carotenoid formation of the yeast *Phaffia rhodozyma*, probably by scavenging $O_2^{\cdot}$. When duroquinone (DQ), an internal and external O_2 generator, was added to medium, *P. rhodozyma* produced more amount of carotenoids. The increased carotenoid production was destroyed by oxygen radical (OR) scavengers, ascorbate+Cu(II) and dimethylsulfoxide. When sub-lethal concentrations of H_2O_2, an external OR source, and antimycin, an internal OR inducer, were used, the effect of H_2O_2 on carotenoid formation and composition was less significant than that of antimycin. Addition of superoxide dismutase, an external OR remover, rescued cells from death caused by the high concentration of DQ. In this condition, the yeast culture showed an increase in carotenoid content. Addition of DQ into *P. rhodozyma* culture in the stationary phase did not increase carotenoid production. Therefore, carotenoid formation was stimulated by internal ORs in the growing yeast. It was probably due to release of catabolite repression on carotenogenesis in the yeast. Aeration was important for carotenoid production but was not as effective as the internal OR producer, DQ.

Phaffia rhodozyma contains astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione) as its primary carotenoid pigment (5). Vegetative cells are ellipsoidal and occur singly, in pairs, and occasionally in short chains. A major proportion of the *P. rhodozyma* cell wall is α-(1-3)-glucan (24). Monosaccharide components of the cell wall polysaccharides are D-glucose 90%, D-mannose 9% and D-xylose 1% (8, 19). The vegetative cell wall is multilayered and forms a multilayered bud scar (25) which is characteristic of heterobasidiomycetous yeasts.

Phaffia can ferment glucose and some other sugars, and this property differentiates it from other genera of carotenoid-producing yeasts. *Phaffia rhodozyma* synthesizes astaxanthin as its principal carotenoid (25) which is rarely produced by yeasts of other genera (28). It also produces phoenicoxanthin, 3-hydroxy-echinenone, echinenone, γ-carotene, neurosporene, lycopene, 3-hydroxy-3',4'-didehydro-β,ψ-carotene-4-one (HDCO) (5), β-zeacarotene (20), torulene and 4-hydroxy-3',4'-dihydro-β,ψ-carotene (1).

The production of carotenoid pigments by *P. rhodozyma* depends on culture conditions (19, 20). The optimum temperature for yeast growth and pigment formation is 20-22°C. Astaxanthin is produced mainly during the stationary phase of growth. The optimum pH is 5.0 (20). Oxygen is an important substrate for optimum yields of yeast and astaxanthin. An and Johnson (4) found that light decreased growth and pigmentation of the yeast.

Astaxanthin is common in the animal kingdom. It is conspicuously displayed in the plumage of many birds, including flamingoes and the scarlet ibis (15). It is also known to be produced by some plants and alga and has been reported in a few basidiomycetous fungi (16). Also, it is commonly associated with marine crustacea (i.e. shrimp, crab, lobster, etc.) and fishes such as trout and salmon (15). These fishes, when raised in pens, often lack the desirable red flesh color. The color of their flesh or carapace is an important factor affecting the consumer acceptance of cultivated salmonids or crustaceans. Pigmentation of salmon and trout flesh has been accomplished using the synthetic carotenoid canthaxanthin (β,β-carotene-4,4'-dione) as a feed additive (30). But this chemical is rather expensive (17) and does not produce a desirable color (29).

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Astaxanthin is one of the most expensive ingredients in salmon feeds. The cost of the pigment for feeds in 1995 was expected to be more than $100 million (2). The calculated need for astaxanthin for aquaculture was 20-30 tons. Since *P. rhodozyma* can synthesize a large quantity of astaxanthin, there has been considerable commercial interest in using *P. rhodozyma* as a pigment source for pen-reared aquacultured animals. Therefore, developing methods of increasing astaxanthin content in *P. rhodozyma* is critical to the aquaculture industry.

Carotenoids have been shown to prevent oxidative injury to various organisms and may exert their strongest protection at low oxygen tensions (12). Carotenoid formation in microorganisms is often associated with strong oxidative metabolism. Carotenes were shown to prevent injury to the yeast *Rhodotorula mucilaginosa* on exposure to reactive oxygen species (26). The apparent lack of Mn-superoxide dismutase (SOD) in *R. mucilaginosa* may make the antioxidant activity of β-carotene very critical in this yeast (26). We previously reported that carotenoid synthesis in *P. rhodozyma* was stimulated by antimycin A (3), which probably promotes formation of ORs by blocking the primary respiratory chain at the cytochrome bc_1 segment. Schroeder and Johnson (31) reported that $O_2^{\cdot}$-generator duroquinone increased carotenoid production of *P. rhodozyma*. In this study, we found that internal ORs were more effective for the increase of carotenoid production than external ORs. We also found that the increased carotenoid production by ORs was available from growing yeast, not from stationary yeast. This result could also be applied to *P. rhodozyma* fermentation related with aeration.

MATERIALS AND METHODS

Chemicals

The followings were obtained from Sigma Chemical Co., St. Louis, MO: superoxide dismutase (from bovine erythrocytes, 15,000 units/mg), 2,3,5,6-tetramethyl-2,5-cyclohexadiene-1,4-dione (duroquinone), 3-amino-1,2,4-triazole (amitrole), L-ascorbic acid, succinic acid, manganese sulfate, copper sulfate, and potassium hydrogen phthalate. Purity and sources of other chemicals used in this study have been described (3).

Microorganisms

The sources of *P. rhodozyma* strains 67-385 (natural isolate) and Ant-1 were described (3). Strain 2F-1 (an astaxanthin hyperproducer) were derived from 67-385 by mutagenesis (1, 3). Strain HT-0N2G was independently isolated as a carotenoid hyperproducing mutant (CHM) from serial mutation of strain 67-385. Yeasts were maintained on slants of yeast extract/malt extract/peptone/glucose medium (YM broth, Difco Co., Detroit, MI) with 2% agar (YM agar) and stored in the refrigerator. Yeast strains were also stored in 40% glycerol/60% YM broth at -70°C.

Cultivation of Yeast

P. rhodozyma was usually grown at 20°C in YM broth or on YM agar plates at room temperature (24°C). To determine the effects of metalic ions on yeast growth and pigmentation, a defined medium (yeast nitrogen base, Difco Co.) buffered with hydrogen phthalate (0.05 M, pH 5.0) was used. Growth in liquid media was measured by turbidity as previously described (3).

Carotenoid Extraction and Analysis

For quantitative analysis of yeast carotenoid content, we used 1 ml of yeast culture. Harvested yeast cells were washed with distilled water and dissolved with dimethylsulfoxide (32). One ml of acetone, petroleum ether and 20% NaCl solution were serially added with vortex (3). Quantification of carotenoid in petroleum ether layers was described (3). Experiments for determining carotenoid content were repeated at least twice in independent trials and samples were processed in duplicate or triplicate in each experiment. Individual carotenoids were analyzed by thin-layer chromatography and absorption spectra as previously described (3).

RESULTS

Effect of an $O_2^{\cdot}$ Scavenger, Mn(II)+succinate on Carotenogenesis of *P. rhodozyma*

When *P. rhodozyma* strain 67-385 grew on plates containing various carbon sources (glucose, succinate, xylose, glycerol, or ethanol) and different concentrations of various metal ions (Fe, Cu, Zn, and Mn), Mn(II)+succinate showed distinct color deviation (Keng-Wei Chang and Eric A. Johnson, unpublished data). If Mn(II) was eliminated from the plates, the colonies were highly pigmented.

High concentrations of Mn(II) in liquid minimal medium containing succinate resulted in lesser carotenoid in strain 67-385 (Fig. 1). It was also observed with other strains of *P. rhodozyma* (data not shown). These results showed that Mn(II)+succinate inhibited carotenoid synthesis. With other carbon sources such as glucose or xylose, Mn(II) did not affect carotenogenesis (Fig. 2). Since Mn(II)+succinate is known to scavenge $O_2^{\cdot}$ (6, 13), $O_2^{\cdot}$ might have an effect on carotenoid production.

Effect of OR Scavengers, Ascorvate+Cu(II) and Dimethylsulfoxide with an OR generator, DQ, on Carotenogenesis of *P. rhodozyma*

Carotenogenesis of *P. rhodozyma* was strongly related to the respiratory chain (3). Carotenoid hyperproducing mutants were more susceptible to hydrogen peroxide, respiratory inhibitors and free radical formants (3, 4, 31). Therefore, we were interested in the effect of oxygen radicals on carotenogenesis. To study the effect of $O_2^{\cdot}$ on

carotenogenesis of *P. rhodozyma* with different concentrations of DQ, a redox-cycling quinone that generated intracellular $O_2^{\cdot}$ (26) was added into the YM broth culture. Carotenoid production was increased with the increased concentration of DQ (Fig. 3). A CHM, 2F-1, was more susceptible to DQ (Fig. 3b) than the wild strain, 67-385 (Fig. 3a).

It is known that OR scavengers, ascorbate+Cu(II), and dimethylsulfoxide, can protect cells against oxidative damage from $O_2^{\cdot}$ and hydroxyl radicals (33). Compared to DQ alone, carotenoid production was significantly decreased when OR scavengers were added to the media (Table 1).

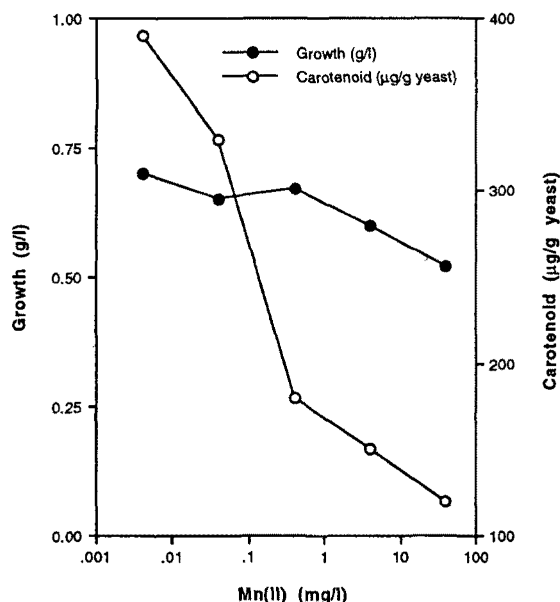

Fig. 1. Effect of Mn(II)+succinate on growth and carotenoid production of *P. rhodozyma* strain 67-385.

Mn(II) was supplied with $MnSO_4 \cdot 1H_2O$. No other carbon source other than succinate was added. Yeasts grew for 5 days in yeast nitrogen base.

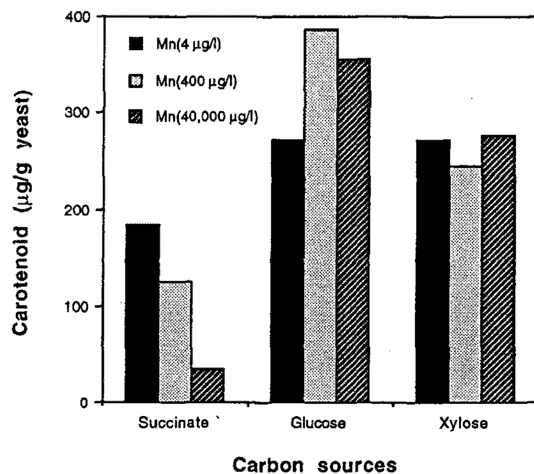

Fig. 2. Effect of Mn(II) with different carbon sources on carotenoid production of *P. rhodozyma* strain 67-385.

Mn(II) was supplied with $MnSO_4 \cdot 1H_2O$. Yeast was grown in yeast nitrogen base with 5 g/l ammonium sulfate.

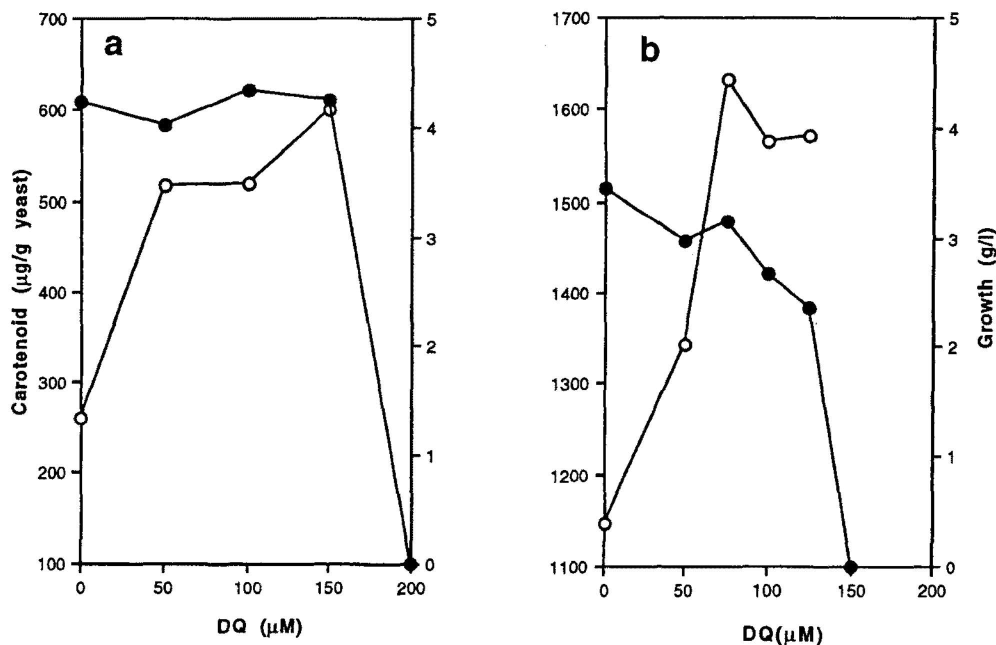

Fig. 3. Effect of duroquinone (DQ) on growth and carotenogenesis of *P. rhodozyma*.

Symbols: -●-, growth (g/l); -○-, carotenoid (µg/g yeast). Panels: a, Strain 67-385; b, Strain 2F-1.

Carotenogenesis Stimulation of *P. rhodozyma* by Internal ORs

To examine the effect of internal OR and external OR on carotenogenesis, we used hydrogen peroxide as an external OR source and antimycin as an internal OR inducer by inhibiting cytochrome bc_1 complex. Antimycin and hydrogen peroxide increased total carotenoid production by increasing HDCO (Table 2). However, the effect of H_2O_2 on carotenoid composition and content was less significant than that of antimycin. Therefore, the internal ORs activated carotenogenesis and the external ORs only sensitized cells. To prove this, we used SOD as an external O_2^- remover which also acts as a H_2O_2 producer.

SOD alone did not affect the growth or carotenogenesis of *P. rhodozyma* (Table 3). As we saw in table 1, DQ increased carotenoid production of 67-385 and 2F-1. However, when the concentration of DQ was high enough to kill the yeast cells, SOD rescued them by removing the external O_2^- (Table 3). Strain 2F-1 could not grow with 150 μM of DQ but the strain could grow when 60 units/ml of SOD was supplied. Under these conditions, the yeast produced increased carotenoids (Table 3).

Effectiveness of ORs on Growing Yeast

Carotenoid production was significantly increased when DQ was added at the lag phase (day 0, addition of DQ at the time of inoculation) or the early log phase (day 1, addition of DQ 1 day after inoculation) (Fig. 4). When DQ was added at the early stationary phase (day 2, addition of DQ 2 days after inoculation), the effect was

Table 1. Effect of oxygen radical scabengers on growth and pigmentation of *P. rhodozyma*.

Condition	Growth (mg/ml)	Carotenoid (μg/g yeast)
Control	4.34	335
AC[a] (20 mM)+Cu[b] (1 mM)	5.27	362
DMSO[c] (10 mM)	3.83	308
DQ[d] (100 μM)	4.56	425
DQ (100 μM)+AC (30 mM)+Cu (1 mM)	2.59	212
DQ (100 μM)+AC (30 mM)+Cu (1.5 mM)	2.71	144
DQ (100 μM)+DMSO (20 mM)	4.52	332

P. rhodozyma strain 67-385 grew in YM broth for 5 days. [a]AC, ascorbic acid. [b]Cu, supplied with $CuSO_4 5H_2O$. [c]DMSO, dimethylsulfoxide. [d]DQ, duroquinone.

Table 2. Effect of antimycin and hydrogen peroxide on carotenogenesis.

	Carotenoid (μg/g yeast)					
	Strain 67-385			Ant-1		
	Control	H_2O_2	Antimycin	Control	H_2O_2	Antimycin
DCD[a]	2	10	9	37	57	29
cis-Astaxanthin	23	28	26	63	68	74
trans-Astaxanthin	344	363	362	730	659	641
Phoenicoxanthin	2	5	6	5	23	23
HDCO[b]	34	48	117	116	222	286
HE[c]	37	25	23	37	28	40
β-Zeacarotene	11	5	6	4	8	3
β-Carotene	5	8	3	16	8	11
Total	460	500	570	1050	1140	1140

Yeasts grew in YM broth for 7 days at 20°C. The yields of yeasts were 3.5-4.5 g/l. Concentration of antimycin A was 0.1 μM and that of hydrogen peroxide was 2 mM. [a]DCD, 3,3'-dihydroxy-β,ψ-carotene-4,4'-dione. [b]HDCO, 3-hydroxy-3',4'-didehydro-β,ψ-carotene-4-one. [c]HE, 3-hydroxy-echinenone.

Table 3. Effect of superoxide dismutase (SOD) and duroquinone (DQ) on growth and pigmentation of *P. rhodozyma*.

		Duroquinone (μM)					
		0		100		150	
	SOD[a]	Growth[b]	Car.[c]	Growth	Car.	Growth	Car.
Strain 67-385	0	3.98	300	3.33	600	ND[d]	ND
	12	3.20	280	4.03	540	ND	ND
2F-1	0	3.05	1180	2.67	1570	0.00	ND
	60	2.71	1200	2.38	1510	3.32	1770

Yeasts grew in YM broth for 5 days at 20°C. [a]SOD, superoxide dismutase (units/ml). [b]Growth, (gram yeast/liter medium). [c]Car., Carotenoid (μg/gram yeast). [d]ND, not determined.

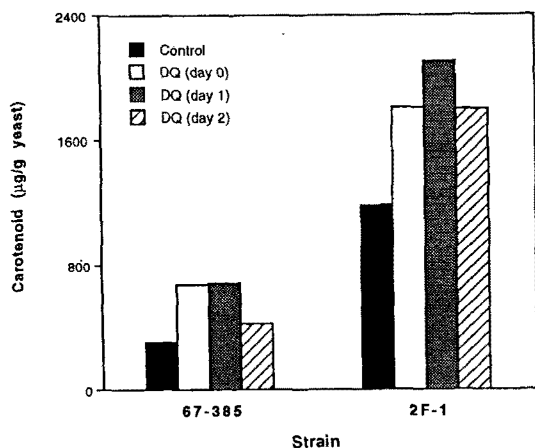

Fig. 4. Effect of duroquinone (DQ, 100 μM) on carotenoid production related with cell ages.
Strains 67-385 and 2F-1 were grown for 5 days in YM broth.

dimished. It is possible that superoxide and/or hydroxyl radicals induced enzymes for the biosynthesis of carotenoids. For strain 2F-1, the optimum time for addition of DQ was the early log phase (1 day after inoculation) (Fig. 4). This was probably due to the higher susceptibility of carotenoid hyperproducing mutants to DQ than that of wild-type yeast.

Aeration was important for carotenoid production (Table 4). Growth and carotenoid production increased ~2-fold in wild-type yeast 67-385 and in a CHM HT-ON 2G by increasing shaking speed from 50 to 100 rpm. However, increased aeration was not significantly effective for the increase of carotenoid production (Table 4). Increase of shaking speed from 100 to 150 rpm did little increase carotenoid content or growth.

Table 4. Effect of aeration on carotenoid production and growth.

	Strain 67-385		HT-0N2G	
Shaker speed (rpm)	Growth (g/l)	Carotenoid (μg/g yeast)	Growth (g/l)	Carotenoid (μg/g yeast)
50	4.7±1.3	0.17±0.04	2.1±0.2	0.61±0.13
100	8.3±0.5	0.30±0.02	4.0±0.3	1.11±0.13
150	6.8±0.1	0.33±0.04	4.3±0.1	1.17±0.03

Yeasts grew in YM broth with 2% glucose for 5 days at 20°C. Numbers following "±" indicate standard deviations.

Table 5. Effect of H_2O_2 on carotenoid production and growth under dark condition.

	Strain 67-385		Ant-1-4	
	Control	H_2O_2 (0.1 mM)	Control	H_2O_2 (0.1 mM)
Carotenoid (μg/g yeast)	480	470	1240	1090
Growth (g/l)	3.6	3.1	3.4	3.2

Yeasts grew in YM broth for 5 days at 20°C.

DISCUSSION

It is intriguing that the ripening and aging of certain fruits and differentiation and aging in fungi are associated with decreases in catalase activity and correlated increases in endogenous peroxides and ORs (10, 11, 27). Munkres *et al.* (27) showed that conidial longevity was positively correlated with the activities of oxygen-metabolizing enzymes in *N. crassa*. Differential activities in catalase have been demonstrated to be associated with developmental changes in *N. crassa* (14). Aged cells of *P. rhodozyma* are distinctive because of the unusual bud scars observed in heterobasidiomycetous yeasts (25).

Many light derepressible genes, including those regulating carotenogenesis in fungi, require molecular oxygen and a photosensitive pigment, in addition to blue light for activation (18). It has been postulated that carotenogenesis is induced by photooxidative destruction of a regulatory protein (18). We previously reported that carotenoid formation in *P. rhodozyma* is stimulated by antimycin and light (4). Addition of H_2O_2 (Table 5) or antimycin (4) without light, decreased carotenoid production slightly. Probably, light inactivates a regulatory protein associated with ORs, and it activates the carotenogenesis of *P. rhodozyma*.

Free Mn(II) is a poor scavenger of O_2 (21, 22). However, in the presence of succinate its symmetry is distorted and the scavenging activity is increased (6, 7, 13). Therefore, Mn(II)+succinate decreased carotenoid production (Fig. 1), probably by scavenging O_2. DQ increased carotenoid production of *P. rhodozyma* (Fig. 3). When OR scavengers, ascorbate+Cu(II) and dimethylsulfoxide (33), were used with DQ, they also decreased the carotenoid production of *P. rhodozyma* (Table 1). The higher susceptibility of carotenoid hyperproducing mutants to DQ than that of wild-type yeast (Fig. 3), might be due to mitochondrial mutation producing ORs (1, 3). SOD rescued yeast cells from external O_2 when a high concentration of DQ was applied (Table 3). SOD permitted the yeast to grow at the high concentration of DQ and it further increased carotenoid production of the yeast. Therefore, only cellular (internal) ORs were effective in increasing carotenoid production.

Dismutation of O_2 by SOD generates H_2O_2, and O_2 has been shown to inhibit catalase activity (23). Therefore, O_2 accumulation in the cells could lead to the increased intracellular H_2O_2. Amitrole, a catalase inhibitor, has been demonstrated to affect carotenoid synthesis in cultured plant cells (9). Amitrole alone did not sig-

nificantly affect carotenoid formation in *P. rhodozyma*, but it did further enhance the stimulation with DQ (Keng-Wei Chang and Eric A. Johnson, unpublished data). Since the combination of $O_2^{\cdot}$ and H_2O_2 can generate OH·, it is possible that OH· also plays an important role in carotenoid formation.

Carotenoids are produced as secondary metabolites in *P. rhodozyma* after cell growth has mostly terminated, and may function to preserve viability in the face of continued production of ORs in the non-growing cells (1, 20). DQ was effective for the increase in the carotenoid content of *P. rhodozyma*, only when it was added into a growing culture (Fig. 4). Therefore, ORs might release catabolite repression on carotenogenesis of the yeast.

Simple aeration might not produce ORs, because vigorous agitation did not increase carotenoids in *P. rhodozyma* (Table 4). Therefore, methods to produce internal ORs in the growing *P. rhodozyma* other than just aeration would increase carotenoid production of *P. rhodozyma*.

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REFERENCES

1. An, G. H. 1991. *Improved astaxanthin production from the red yeast Phaffia rhodozyma*. Ph. D. thesis, University of Wisconsin, Madison, WI.
2. An, G. H. 1995. *Case studies on the potential demand estimation of biotechnological products*. MBA thesis, Yunsei University, Seoul, Korea.
3. An, G. H., D. B. Schuman, and E. A. Johnson. 1989. Isolation of *Phaffia rhodozyma* mutants with increased astaxanthin content. *Appl. Environ. Microbiol.* **55**: 116-124.
4. An, G. H. and E. A. Johnson. 1990. Influence of light on growth and pigmentation of the yeast *Phaffia rhodozyma*. *Antonie van Leeuwenhoek* **57**: 191-203.
5. Andrewes, A. G., H. J. Phaff, and M. P. Starr. 1976. Carotenoids of *Phaffia rhodozyma*, a red-pigmented fermenting yeast. *Phytochem.* **15**: 1003-1007.
6. Archibald, F. S. and I. Fridovich. 1981. Manganese and defenses against oxygen toxicity in *Lactobacillus plantarum*. *J. Bacteriol.* **145**: 442-451.
7. Archibald, F. S. and I. Fridovich. 1981. Manganese, superoxide dismutase, and oxygen tolerance in some lactic acid bacteria. *J. Bacteriol.* **146**: 928-936.
8. Arx, J. A. von and A. C. M. Weijman. 1979. Conidiation and carbohydrate composition in some *Candida* and *Torulopsis* species. *Antonie van Leeuwenhoek* **45**: 547-555.
9. Ashtakala, S. S., G. T. Dodds, and S. W. Lamoureux. 1989. Effect of 3-amino-1,2,4-triazol on the stimulation of chlorophyll and carotenoid biosynthesis in the presence of exogenous cytokinins and d-aminolevulinic acid. *J. Plant Physiol.* **135**: 86-93.
10. Brennan, T. and C. Frenkel. 1977. Involvement of hydrogen-peroxide in the regulation of senescence in pear. *Plant Physiol.* **59**: 411-416.
11. Brennan, T., A. Rychter, and C. Frenkel. 1979. Activity of enzymes involved in the turnover of hydrogen-peroxide during fruit senescence. *Bot. Gaz.* **140**: 384-388.
12. Burton, G. W. and K. U. Ingold. 1984. β-carotene: an unusual type of lipid antioxidant. *Science* **224**: 569-573.
13. Chang, E. C. and D. J. Kosman. 1989. Intracellular Mn(II) - associated superoxide scavenging activity protects Cu, Zn superoxide dismutase-deficient *Saccharomyces cerevisiae* against dioxygen stress. *J. Biol. Chem.* **264**: 12171-12178.
14. Chary, P. and D. O. Natvig. 1989. Evidence for three differentially regulated catalase genes in Neurospora crassa: effects of oxidative stress, heat shock, and development. *J. Bacteriol.* **171**: 2646-2652.
15. Goodwin, T. W. 1984. *The biochemistry of the carotenoids*. vol. **II**. *Animals*. 2nd ed. Chapman and Hall, NY.
16. Goodwin, T. W. and W. Lujinsky. 1952. Studies in carotenogenesis. 2. Carotene production by *Phycomyces blakesleeanus*: the effect of different amino acids when used in media containing low concentrations of glucose. *Biochem. J.* **50**: 268-274.
17. Hildingstam, J. 1976. Economics of research and development in the sea farming of salmonid species. *Fish Farming Int.* **3**: 16-19.
18. Hoober, J. K. and D. G. Phinney. 1988. Are "light-derepressible" genes controlled by metal-protein complexes? *TIBS* October, 371-374.
19. Johnson, E. A. 1978. *Astaxanthin production by the yeast Phaffia rhodozyma and its use as a pigment source in animal feeding*. Master Thesis, University of California, Davis, CA.
20. Johnson, E. A. and M. J. Lewis. 1979. Astaxanthin formation by the yeast *Phaffia rhodozyma*. *J. Gen. Microbiol.* **115**: 173-183.
21. Kenten, R. H. and P. J. G. Mann. 1955. The oxidation of manganese by illuminated chlorolpast preparations. *Biochem. J.* **61**: 279-286.
22. Kono, Y., M. A. Takahashi, and K. Asada. 1976. Oxidation of manganous pyrophosphate by superoxide radicals and illuminated spinach chloroplasts. *Arch. Biochem. Biophys.* **174**: 454-462.
23. Kono, Y. and I. Fridovich. 1982. Superoxide radicals inhibit catalase. *J. Biol. Chem.* **257**: 5751-5754.
24. Meyer, M. T. 1975. Ph.D. Thesis. University of California, Davis, CA.
25. Miller, M. W., M. Yoneyama and M. Soneda. 1976. *Phaffia*, a new yeast genus in the Deuteromycotina (Blastomycetes). *Int. J. Syst. Bacteriol.* **26**: 286-291.
26. Moore, M. M., M. W. Breedveld, and A. P. Autor. 1989.

The role of carotenoids in preventing oxidative damage in the pigmented yeast, *Phodotorula mucilaginosa*. *Arch. Biochem. Biophys*. **270**: 419-431.
27. Munkres, K. D., R. S. Rana, and E. Goldstein. 1984. Genetically determined conidial longevity is positively correlated with superoxide dismutase, catalase, glutathione peroxidase, cytochrome c peroxidase, and ascorbate free radical reductase activities in *Neurospora crassa*. *Mech. Age. Develop*. **24**: 83-100.
28. Nakayama, T., G. Mackinney and H. J. Phaff. 1954. Carotenoids in asporogenous yeasts. *Antonie van Leeuwenhoeck* **20**: 217-228.
29. Saito, A. and L. W. Regier. 1971. Pigmentation of brook trout (*Salvelinus fontinalis*) by feeding dried crustacean waste. *J. Fish. Res. Board Can*. **28**: 509-512.
30. Schmidt, P. J. and E. G. Baker. 1969. The indirect pigmentation of salmon and trout flesh with canthaxanthin. *J. Fish. Res. Board Can*. **26**: 357-360.
31. Schroeder W. A. and E. A. Johnson. 1993. Antioxidant role of carotenoids in *Phaffia rhodozyma*. *J. Gen. Microbiol*. **139**: 907-912.
32. Sedmak, J. J., D. K. Weerasinghe, and S. O. Jolly. 1990. Extraction and quantitation of astaxanthin from *Phaffia rhodozyma*. *Biotech. Tech*. **4**: 107-112.
33. Simpson, P. J., J. C. Fantone, and B. R. Lucchesi. 1987. Myocardial ischenia and reperfusion injury: oxygen radicals and the role of the neutrophil. p. 63-77. *In Oxygen Radicals and Tissue Injury*. Upjohn Company. NY.

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