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MEVALONIC ACID INCREASES *trans*-ASTAXANTHIN AND CAROTENOID BIOSYNTHESIS IN *Phaffia rhodozyma*

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SUMMARY

Mevalonic acid has been tested as enhancer of pigment biosynthesis in wild-type *Phaffia rhodozyma*. The addition of 0.1% mevalonic acid to the culture media stimulated both *trans*-astaxanthin and total carotenoids biosynthesis, with average increases by ca 400%.

INTRODUCTION

The good quality of salmonids and crustaceans is first judged on the basis of their colour, the main red colour of these animals being the ketocarotenoid *trans*-astaxanthin (3,3'-ß-ß'-carotene-4-4'-dione) (Johnson *et al.* 1977). This carotenoid is abundant in nature, but only a few microorganisms can synthezise it, the yeast *Phaffia rhodozyma* being the most important one (Avalos *et al.*, 1986). Wild salmonids have enough sources of carotenoids (microalgae, small crustaceans and the like), but pen-reared salmonids must incorporate these pigments from their diets to acquire the desirable red colour. ß-Ionone (Lewis *et al.*, 1990), lycopene (Johnson and Lewis, 1979) and acetic acid (Meyer and du Preez, 1993) have been reported as exerting some effect on the synthesis of carotenoids by *P. rhodozyma*. Mevalonic acid, 3,5 dihydroxy-3-methyl pentanoic acid, is a key carotenoid precursor. In this paper we report on the

effect of the addition of mevalonic acid to the culture media of the pigmented yeast *P. rhodozyma* on the accumulation of *trans*-astaxanthin and other carotenoids of biotechnological interest.

MATERIALS AND METHODS

Strain and Medium. The *P. rhodozyma* strain used was the natural isolate UCD-FST-67-210 (Miller *et al.*, 1976). The medium used for yeast growth and maintenance was YM (yeast extract: 3 g/l; malt extract: 3 g/l; peptone: 5 g/l; glucose: 10 g/l; and agar: 30 g/l for solid media).

Reagents and Chemicals. All solvents were HPLC-grade from Romil Chemicals. Mevalonic acid, which was used as mevalonic acid-lactone (Sigma), was tested at 0.05 and 0.1% (w/v) concentrations. This compound was added to YM medium immediately before pouring the plates.

Sample preparation for HPLC. P. rhodozyma was grown in YM broth in a shaker at 230 rpm and 23°C for five days. Then cells were harvested by centrifugation at 7000 rpm for 15 min, washed with sterile water and dried in an oven at 37°C. Afterwards 0.2 g. of dried yeast were resuspended in 5 ml of dimethylsulfoxide (DMSO), preheated to 55°C and vortexed for 30 s (Sedmak *et al.*, 1990). Then 0.5 ml of phosphate buffer pH 7.0 and 10 ml of hexane-fraction from petroleum were added and mixed by vortexing for an additional minute. Finally, samples were filtered through 0.45 μm Millipore membranes and stored at -20°C until analyzed.

Carotenoid Analysis and Standards. Chromatographic separations were performed by high performance liquid chromatography (HPLC) on an Ultrasphere silica 5μ, 250 x 4.6 mm column (Beckman) protected by an Ultrasphere 5μ, 450 x 4.6 mm guard column (Beckman). The eluting solvent was hexane-fraction from petroleum/ethyl acetate 1/1 (v/v) and flow rate was 1 ml/min. The eluant was monitored at 476 nm. ß-carotene (Sigma) was used as standard and the concentrations of the other carotenoids were calculated in relationship to this compound. Standard stock solutions were diluted in hexane-fraction from petroleum. ß-carotene had linear calibration curves (peak area *vs* concentration) through the origin.

RESULTS AND DISCUSSION

Figure 1 shows chromatograms of the carotenoids extracted from *P. rhodozyma* wild-type grown in the presence of 0.05% and 0.1% mevalonic acid, with respect to the the control assay without this acid. Quantitative results from HPLC-analysis in the three assays are summarized in Table 1. The addition of mevalonic acid to the medium has a strong effect on the accumulation of carotenoids. Average

increases of over 300% were determined in the assays corresponding to the addition of 0.05% and 0.1% mevalonic acid to the medium.

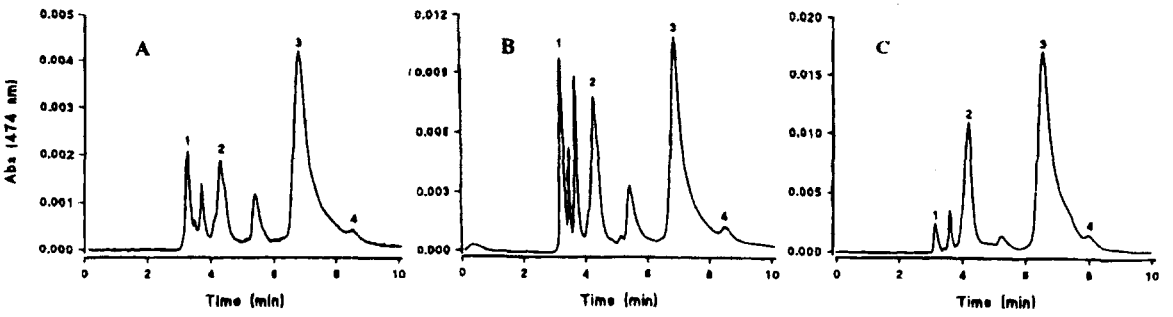

Figure 1. HPLC-carotenoid profiles of intracellular extracts from *P. rhodozyma* grown in A) usual media; B) 0.05% mevalonic acid-supplemented medium; and C) 0.1 % mevalonic acid-supplemented medium. Peaks: 1 = ß-carotene; 2 = 3-hydroxy-echinenone; 3 = *trans*- astaxanthin; and 4 = *cis*-astaxanthin.

Table 1. Effect of mevalonic acid on the biosynthesis and accumulation of intermediate and total carotenoids (µg/g. dried weight) by *P. rhodozyma.*

Mevalonic acid concentration (%)	ß-carotene	*ND_1	3-hydroxy -echinenone	*ND_2	*trans*- astaxanthin	*cis*- astaxanthin	Total
0 (Control)	20.8	9.0	39.9	14.4	181.6	1.9	274.9
0.05	127.5	88.2	124.9	62.4	399.9	9.6	889.7
0.1	28.2	25.8	238.2	16.6	758.2	4.0	1074.9

*ND_1 (retention time: 3.6 min) and ND_2 (retention time: 5.3 min) correspond to carotenoids which structure was not determined, possibly biosynthetic intermediates.

A direct relationship between mevalonic acid concentration and both *trans*-astaxanthin and 3-hydroxyechinenone biosynthesis was also observed. Thus, the overproduction of *trans*-astaxanthin in the presence of 0.1% mevalonate rose from 180 (control assay) to 760 µg/g yeast (dried weight). The accumulation of 3-

hydroxyechinenone in the presence of 0.1% mevalonate increased six-fold with respect to the control assay. Minor carotenoids, these including ß-carotene, *cis*-astaxanthin and others whose structure has not been determined so far, did not show the same proportionality pattern.

The overproduction of *trans*-astaxanthin by *P. rhodozyma* in the presence of 0.1% mevalonic acid may acquire practical interest in the biotechnological production of this ketocarotenoid for the fish and poultry industry.

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