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Effect of 2-(p-Methylphenoxy)-Triethylamine Hydrochloride on the Pigments in *Scenedesmus obliquus*

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Abstract

The presence of 2-(p-methylphenoxy)-triethylamine hydrochloride(MPTA) during growth of *Scenedesmus obliquus* caused the inhibition of growth (at concentrations above 1 ppm) and the induction of yellow mutant strain (1 ppm). When culture was treated with MPTA(0.5 ppm), δ-carotene, γ-carotene and lycopene were accumulated, suggesting MPTA to be a inhibitor of cyclases on carotenoid formation of *Scenedesmus obliquus*.

The yellow mutant strain indued by MPTA had a very low amount of chlorophyll and a high amount of ketocarotenoid (especially α-doradexanthin and astaxanthin) in comparison with the wild type of *Scenedesmus obliquus*.

Since the discovery of effect of CPTA (2-(4-chlorophenylthio) triethylamine hydrochloride) on the carotenoid pigmentation of citrus fruit, a large number of bioregulators have synthesized and studied[1-10].

MPTA, 2-(p-methylphenoxy)-triethylamine hydrochloride, one of these bioregulators was found to have an effect on the stimulating lycopene synthesis in flavedo of Marsh seedless grapefruit[8].

In the present study, we report the effect of MPTA on pigment compostition of *Scenedesmus obliquus*, one kind of unicellular green algae.

Our results show that MPTA inhibits the growth of *Scenedesmus* at a low concentration of 1 ppm, accumulates δ-carotene, γ-carotene and lycopene that are not detected in untreated *Scenedesmus*, and induces the mutation of *Scenedesmus obliquus*. Comparing with the pigment composition of wild type *Scenedesmus obliquus*, the induced mutant strain has a high amount of ketocarotenoid and a very low amount of chlorophyll.

The mutant strain grows heterotrophically and mixotrophycally but not autotrophycally.

Materials and Methods

Cultures of Scenedesmus obliquus

The strain of *Scenedesmus obliquus* was obtained from ATCC(ATCC11457) and maintained on agar slopes consisting of per 1, yeast extract 1g, beef extract 1g, tryptone 2g, $FeSO_4$ trace, glucose 10g, agar 15g(pH adjusted to 7.2). The media used for liquid culture was the same composition of agar slopes media (agar eliminated and concentrated to 1/2 of the given quantities).

The organism was cultured in Erlenmeyer flasks in a gyrotary shaker at 27℃ and illuminated with 16 h. of 400 foot-candle light with 8 h. of darkness a day.

Extraction, isolation and quantitative determination of pigments

The algae were harvested by centrifugation at

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5000 rpm for 10 min, and extracted three times with ethanol and acetone. The equation of Mackinney[11] was used for quantitative determination of chlorophyll. The pigments were saponified and the chlorophyll removed by the usual method[15]. The quantity of separated carotenoids was culculated from the absorbance at λ_{max} using $E_{1cm}^{1\%}$ coefficients[12]. For the unidentified or unknown carotenoids, coefficient $E_{1cm}^{1\%}=2500$ was used. Each separated carotenoid was examined on the identity with authentic sample or the sample obtained for the identification.

Pigment identification

For the identification, the organism was cultured in 10-1 fermentor and carotenoid pigments were separated on a column of sucrose or MgO column with petroleum ether or acetone in petroleum ether as developing solvent. The pigments were identified by the UV and visible spectra, the order of adsorption affinities on TLC, and chemical tests (epoxide test and reduction test by $NaBH_4$ relative to known compounds. Antheraxanthin and α-doradexanthin were confirmed by ^{1}H-NMR spectra.

Isolation of mutant strain

From 10 days of culture treated by MPTA at a 1 ppm concentation, pale yellow cells were picked up with pipet and inoculated to the normal mdia.

The cells grew well and turned geenish yellow. After 5 days of culture, an aliquot of media was diluted with sterilized water and inoculated on agar slant by a wire loop technique. Among the green cells, yellow cells could be found and one of the yellow cells was selected and removed to agar slant with a wire loop. The yellow cell was completely isolated from green cells by repeating the wire loop procedure three times.

Results and Discussion

Effect of MPTA on the pigments in Scenedesmus obliquus

The growth rate in shake culture of *Scenedesmus* was inhibited by a 1 ppm concentration of MPTA. At early growth stage(3-4 days after inoculation), the growth of *Scenedesmus* treated by low concentrations below 5ppm was delayed and the growth was reached to the control level after 10 days. The untreated *Scenedesmus* (control) reflected their normal green colors. However, *Scenedesmus* treated with 1 ppm MPTA had white or pale-yellow cells in addition to green cells.

Table 1 Effect of MPTA (0.5 ppm) on pigment composition of *Scenedesmus obliquus*

Pigments	Control		MPTA	
	10 days %	30 days %	10 days %	30 days %
$\alpha+\beta$-Carotene	15.3	13.4	12.4	22.4
δ-Carotene	-	-	8.1	-
γ-Carotene	-	-	2.4	-
Lycopene	-	-	4.2	-
Echinenone	tr	4.7	tr	4.3
Canthaxanthin	0.9	5.7	tr	3.6
Astaxanthin	tr	-	tr	tr
α-Doradexanthin	2.8	1.7	1.0	0.9
Lutein	50.5	54.7	46.3	47.9
Antheraxanthin	3.8	3.2	3.2	3.3
Violaxanthin	6.7	2.6	6.0	3.1
Neoxanthin	8.6	5.4	7.8	6.6
Unidentified carotenoids	11.4	8.6	8.6	7.9
Total carotenoid (μg/g*)	2073.2	2062.2	2432.6	2111.3
Total chlorophyll (mg/g*)	13.37	12.21	11.54	11.23

* Dry weight of delipidised *Scenedesmus*.

As shown in Table 1, the main carotenoids of control *Scenedesmus* were lutein (50.5%), epoxide carotenoids such as antheraxanthin, violaxanthin and neoxanthin (19.1%) and $\alpha+\beta$-carotene (15.3%), but ketocarotenoid such as echinenone, canthaxanthin, astaxanthin and α-doradexanthin were minor carotenoids (3.7%). These results were simlar to those of previous studies on carotenoid analysis of *Scenedesmus*[13-15]. In MPTA-treated culture (10 days), δ-carotene, γ-carotene and lycopene which were not detected in the control were accumulated to some extent (14.7%). The accumulation of these carotenoids in the MPTA treated culture suggested that γ-carotene and lycopene might be the substrate for β-carotene synthesis and δ-carotene and lycopene for α-carotene, and MPTA acted as an inhibitor of the cyclases as reported in flavedo of Marsh seedless grapefruit[8]. When compared with results of flavedo of Marsh seedless grapefruit, the

low level of accumulation of lycopene was seen. It can be assumed that the effect on lycopene accumulation in *Scenedesmus* should be small in the culture at a 0.5 ppm concentration.

The carotenoid composition of untreated culture after 30 days was almost the same as that after 10 days, but the ratio of ketocarotenoids such as echinenone and canthaxanthin was inceased slightly. On the other hand, the carotenoid composition of treated culture after 30 days was almost the same as that of untreated culture and acyclic carotenoids such as δ-carotene, γ-carotene and lycopene decreased to undetectable level.

Pigment composition of yellow mutant of Scenedesmus obliquus

When the wild type of *Scenedesmus obliquus* was treated with 1 ppm MPTA, pale yellow cells could be found in the media. These cells grew well in the normal culture media and turned geenish yellow. From this media, yellow mutant could be isolated by a wire loop technique. The yellow mutant strain growing on agar slant had yellow color at early stage (1 week after inoculation) and changed the color to orange at 10 days after inoculation. A similar change of color was observed in liquid cultures.

As shown in Table 2, the main carotenoids of the wild type of 10-day old *Scenedesmus* were lutein (50.1%), epoxide carotenoids (21.9%) and $\alpha+\beta$-carotene (17.1%), but such ketocarotenoids as echinenone, canthaxanthin, astaxanthin and α-doradexanthin constituted only 2.3%. The carotenoid composition of 10-day old yellow mutant was differed from that of wild type. Alpha-doradexanthin and astaxanthin were accumulated up to 27.2% and 10.8% of the total carotenoid, respectively; the total ketocarotenoid was accumulated up to 48.2%. On the contrary, the ratios of lutein and epoxide carotenoids were reduced.

Table 2 Pigment composition of yellow mutant and wild type of *Scenedesmus obliquus*

Pigments	Wild type		Yellow mutant	
	10 days %	30 days %	10 days %	30 days %
$\alpha+\beta$-Carotene	17.1	15.6	6.6	5.4
Echinenone	tr	3.8	3.5	5.2
Canthaxanthin	1.1	5.6	6.9	8.1
Astaxanthin	tr	tr	10.8	14.8
α-Doradexanthin	1.2	2.3	27.2	35.6
Lutein	50.1	46.8	32.9	22.5
Antheraxanthin	3.8	2.3	0.9	-
Violaxanthin	11.4	6.8	1.8	-
Neoxanthin	6.7	5.0	1.0	-
Unidentified carotenoids	8.6	11.8	8.4	8.4
Total carotenoid (μg/g*)	2014.1	1973.9	1863.2	2068.1
Total chlorophyll (mg/g*)	11.89	12.69	0.14	0.12

* Dry weight of delipidised *Scenedesmus*.

On 30-day culture, the carotenoid composition of wild type had the same tendency as the previous results and the ratio of echinenone and canthaxanthin was slightly increased. On the other hand, the carotenoid composition of yellow mutant of 30-day culture was almost the same as that of 10-day culture. But, ketocarotenoids was accumulated up to 63.7% and α-doradexanthin was replaced by lutein as the most abundant carotenoid.

The chlorophyll content of yellow strain was extremely low and this suggests that the yellow mutant might be chlorophyll deficient. Burczyk[16, 17] has studied the carotenoids of cell wall in *Scenedesmus obliquus* 633 and reported the relative abundance of carotenoids in cell wall, *i.e.*, β-carotene (tr.), echinenone (1.04%), canthaxanthin (37.93%), astaxanthin (18.18%), α-doradexanthin (32.71%), lutein (20.13%), violaxanthin (O-tr.) and neoxanthin (O-tr.). Judging from the data of this experiment, the carotenoid composition of cell wall was similar to that of yellow mutant with respect to ketocarotenoids. Burczk concluded that the prevailing cell wall pigments were ketocarotenoids, but the ketocarotenoids were only a minor component in the carotenoid moiety of the whole cell. If the function of ketocarotenoid formation was localized in cell wall of *Scenedesmus*, the ketocarotenoid fomation in cell wall of yellow mutant should be activated by induction of mutation.

Further characterization of yellow mutant was performed by comparative studies on the abilities of mutant growth hetrotrophic, mixotrophic or autotrophic.

Using a mutation techniqe, Bishop[18-21] isolated

several kinds of *Scenedesmus* mutants and studied on carotenoid compositions of these mutants[22, 23]. However, no ketocarotenoids were accumulated in these mutant is found among them. Thus, this yellow mutant might be characterized by accumulation of ketocarotenoids.

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