EFFECTS OF Mg^{2+}, NaCl, CITRIC ACID, AND OTHER FACTORS ON SYNTHESIS AND ACCUMULATION OF β-CAROTENE IN *DUNALIELLA SALINA* *

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Abstract The effect of Mg^{2+}, NaCl and citric acid on the accumulation of β-carotene in *Dunaliella Salina* was studied. The experimental results showed that 10.5 mmol/L Mg^{2+}, 5 mol/L NaCl, 3 μmol/L citric acid, and CO_2 are favorable for *Dunaliella Salina* cell growth and β-carotene accumulation. After 144 h culture under the above conditions, the *Dunaliella Salina* biomass increased by 7.18 times; β-carotene reached 9.61%.

Key words: *Dunaliella Salina*, β-carotene, accumulation

There are more than 400 kinds of carotenoids, about 100 of which can be obtained from oceanic microbes and algae, so oceanic algae are considered the most important resource of natural carotenoids (Becker, 1982).

Dunaliella Salina has drawn much attention from a number of countries recently, as more and more studies showed that this unicellular alga can accumulate large amounts of β-carotene under certain environmental conditions (Li, 1988).

Summarizing the biosynthetic mechanism of β-carotene, we know that acetyl CoA and CO_2 are the basic materials for sythesizing β-carotene, Mg^{2+} is the activator of phosphate MVA kinase, citric acid is the activator of acetyl CoA carboxylase. In theory, addition of these materials are favourable for biosythesizing of β-carotene.

However there are few reports on the effects of acetyl CoA, CO_2, Mg^{2+} and citric acid on cell growth and β－carotene accumulation.

MATERIALS AND METHODS

1. Micro alga

Dunaliella Salina cultured as experimental organism was kindly provided by Professor Zhao Xuewu (Qingdao Ocean University) who brought it from Australia.

2. Algal culture method

The alga was inoculated in new medium containing 5 mmol/L KNO_3, 0.2 mmol/L

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Na_2HPO_4, 1 mmol/L $MgSO_4$, 1.5 mmol/L $FeCl_3$, 30 mmol/L EDTA (Qian, 1985). The salinity was adjusted to 4 – 6 mol/L by adding NaCl. The culture solutions were incubated at 25 – 35℃ and agitated by bubbling intermittently with air or CO_2. pH value was maintained at 7.5 – 8.0. Illumination ranging from 15000 to 25000 lx/(m^2·s) 12 hours a day was provided by fluorescent lamps.

3. Extraction and determination of β-carotene

Organic solvent extraction and aluminium hydroxide column chromatography were used to extract and purify β-carotene. The content of β-carotene was determined by colorimetry at 450 nm. The number of alga was calculated with a globlimeter.

EXPERIMENT RESULTS

1. CO_2 effect

Table 1 shows that CO_2 is beneficial for β-carotene accumulation.

Table 1 The effect of CO_2 on β-carotene accumulation in *D. salina*

Item	Group	Dry weight	β-carotene	Percentage	Average	Deviation
1	2	(g)	(g)	(%)	(%)	
Treatment	1	0.76	0.0580	7.63	7.94 ± 0.45	obvious
(CO_2)	2	0.65	0.0550	8.46		
	3	0.78	0.0604	7.74		$\alpha = 0.05$
Control	1	0.78	0.0520	6.67	6.60 ± 0.19	
(atmospheric	2	0.85	0.0543	6.39		
air)	3	0.80	0.0540	6.75		

2. The effect of citric acid

Table 2 shows the effect of citric acid on β-carotene accumulation in *Dunaliella Salina*.

Table 2 The effect of citric acid on β-carotene accumulation

Item	Group	Dry weight (g)	β-carotene (g)	Percentage (%)	Average (%)	Deviation
Control	1	0.68	0.056	8.23	8.48 ± 0.28	very
	2	0.63	0.049	8.78		obvious
	3	0.68	0.054	8.44		
Treatment	1	1.42	0.155	10.91	10.88 ± 0.86	$\gamma = 0.01$
	2	1.20	0.126	10.50		
	3	1.31	0.147	11.22		

3. The effect of Mg^{2+} on β-carotene accumulation

Fig. 1 shows that Mg^{2+} concentration had great effect on β-carotene accumulation and that the best concentration was 10.5 mmol/L.

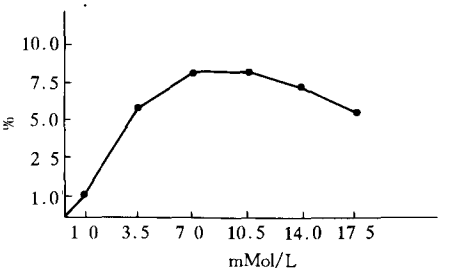

Fig. 1 The effect of Mg^{2+} concentration on β-carotene accumulation

4. The effect of NaCl concentration on β-carotene accumulation

Table 3 shows that 5 mol/L NaCl is best for β-carotene accumulation.

Table 3 The effect of NaCl concentration on β-carotene accumulation

NaCl (mol/L)	Group	Dry weight (g)	β-carotene (g)	Percentage (%)	Average (%)	Deviation
4.0	1	1.84	0.094	5.10	5.56 ± 0.53	very
	2	1.25	0.077	6.16		obvious
	3	1.55	0.089	5.74		
5.0	1	0.76	0.074	9.74	9.59 ± 0.50	
	2	1.03	0.093	9.03		
	3	0.90	0.089	10.00		$\alpha = 0.01$
6.0	1	0.20	0.015	5.50	5.28 ± 0.48	
	2	0.71	0.034	4.79		
	3	0.45	0.025	5.56		

5. The effect of different culture conditions on alga growth and β-carotene accumulation

From the above-mentioned results, six different culture conditions groups were studied to determine optimal culture conditions.

Table 4 The effect of different culture conditions on alga growth and β-carotene accumulation

Group	NaCl (mol/L)	CO_2	$MgSO_4$ 5 mmol/L	Citric acid 0.3 μmol/L	Inoculation quantity[a] (10^4 stain)	Growth factor after 48 h	144 h	216 h	288 h	β-carotene (%)
1	4.0	−	+	−	14.00	26.00	98.25	85.50	80.25	4.80
2	4.0	+	+	−	17.75	11.50	19.75	19.25	18.25	5.58
3	5.0	−	−	−	17.55	24.75	66.75	29.00	25.00	3.75
4	5.0	+	+	−	52.50	13.25	17.75	32.50	19.25	8.55
5	5.0	+	+	+	14.25	31.25	102.00	58.00	52.50	9.64
6	6.0	−	+	−	21.75	18.50	36.00	45.00	45.00	4.76

a) The unit was 10^4 cells/ml

Table 4 shows that the culture conditions of group 5 were the best; β-carotene yield was the highest (9.64%); growth rate was optimum. After 144 h growth, the alga number increased by 7.18 times. Moreover, the peak growth period's early occurrence makes possible early harvest under optimal conditions to yield early maximum economic return on investment in alga culture to obtain β-carotene.

6. β-carotene determination

The product was a deep purple crystal of β-carotene which dissolved in carbon disulfide, phenyl, chloroform, and oil, dissolved slightly in hexanoate, hardly dissolved in water and methanol, melted at 183℃, and had highest absorption peak in phenyl, chloroform and acetone at about 466 nm (Fig 2). The β-carotene characteristics determined in our experiment were consistent with those reported in the literature.

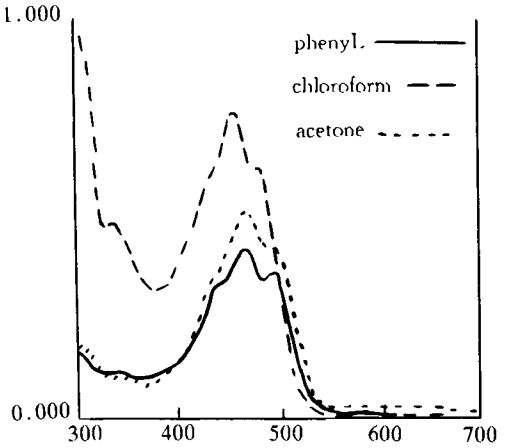

Fig. 2 Ultraviolet absorption spectra of β-carotene in phenyl, chloroform, and acetone

DISCUSSION AND CONCLUSIONS

1. When we began the research based on the synthesis mechanism of β-carotene, we hypothesized that β-carotene content in cultured alga could possibly be increased by adding substrate acetyl CoA or CO_2 into the culture solution, and using high concentration of Mg^{2+} (activator of phosphate MVA kinase) and citric acid (activator of acetyl CoA carboxylase) (Shen, 1991). The effect of single factors on β-carotene accumulation validated our hypothesis.

2. As acetyl CoA is very costly, we did not add it directly to the culture solution, but used an indirect method which induced fatty acid oxidation into acetyl CoA through β-oxidation.

3. Appropriate NaCl concentration in the culture medium could enhance the ability of alga to accumulate β-carotene, but could also inhibit its growth. Based on this point, we used relatively high concentration of NaCl in our experiments. The results indicated that 5 mol/l NaCl concentration inhibited alga growth, but enhanced β-carotene content, and was optimum compared to 6 mol/L and 4 mol/L NaCl concentrations. At 6 mol/L NaCl concentration, alga growth was inhibited and β-carotene content dropped. 6 mol/L NaCl concentration probably exceeded the requirement for normal alga growth. So it is very important to determine and apply in actual production the optimum NaCl concentration, above which growth is inhibited and below which β-carotene yield decreases.

4. Application of the experimental culture conditions yielded satisfactory results. Algae grew rapidly (up to 7.16 times), and β-carotene content was high (9.18%). This indicated that aerating with CO_2, adding citric acid and increasing Mg^{2+} and NaCl concentration are proper measures for culturing this algae for maximum β-carotene yield. But the β-carotene yield (9.14%) in the culture condition experiment was slightly lower than that (10.36%) in the strain selection experiment. This showed that our conditions (NaCl 5 mol/L, aerating with CO_2 intermitently, 5 mmol/L $MgSO_4$, 0.3 μmol/L citric acid) were possibly not the best, so further study is needed to choose ideal culture conditions for better alga growth and higher β-carotene.

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